RECENT ADVANCES IN
MEDICINE AND HEALTH SCIENCES

Concepts, Researches and Practice

Editors

Nizami DURAN

Feray AYDIN

Health Sciences

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Recent Advances in Medicine and Health Sciences: Concepts, Researches and Practice

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Cover Design • Motion Graphics
Book Layout • Motion Graphics
First Published • March 2023, Lyon

ISBN: 978-2-38236-551-9

Publisher • Livre de Lyon
Address • 37 rue marietton, 69009, Lyon France
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PREFACE

Dear Reader,

This book is a fundamental source where studies in 3 different elementary fields of medicine and dentistry are collected. The book contains significant investigations of medical biology, biochemistry, and physiology from the field of basic medical sciences. In addition, there are studies in the field of physiotherapy, midwifery, and family medicine from the field of health sciences in the book. The book also includes studies in the scopes of Pharmacology, Emergency Medicine, and Nuclear Medicine from the field of Internal Medicine. In addition, there are studies from the departments of gynecology and neurosurgery, urology, oncological surgery, and general surgery within the scope of surgical medical sciences. Apart from all these areas, this book also contains two substantial studies from pharmacy and dentistry.

In short, this book is a valuable resource for both students studying in the field of health and scientists working in this field, which includes current studies from different scopes of medicine. I believe that this book will be a very practical handbook for readers.

Prof. Dr. Nizami DURAN

Assoc. Prof. Dr. Feray AYDIN

CONTENT

CHAPTER I

ACUTE ABDOMEN

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1. Introduction

The acute abdomen diagnosis could be made on the patient who is displaying abdominal pain and having positive abdominal examination findings. The majority of the diseases using acute abdomen symptoms are surgical diseases, and a part of them are non-surgical diseases. Some of the surgical diseases require emergency surgery, while others require which are followed conservatively and/or interval operation.

Considering that there may be a large number of patients requiring urgent surgical intervention in this variety, it is very important to examine patients with symptoms of abdominal pain without delay and to make a rapid diagnosis if there are signs of acute abdomen.

Non-surgical diseases; they can be examined in three groups as endocrine and metabolic diseases, hematological diseases and poisonings.

After a detailed anamnesis and physical examination in terms of differential diagnosis, necessary laboratory tests and radiological imaging methods are requested. Today, although the advances in radiological imaging methods make our work easier, sometimes anamnesis and physical examination are the most important diagnostic methods for diagnosis. Despite all diagnostic tests, there

will be patients whose diagnosis cannot be made clearly. In patients whose diagnosis cannot be clarified, laparoscopy or laparotomy can be performed, taking into account the clinical condition of the patient, or the patient can be hospitalized and followed up. The primary aim should be not to miss the pathologies that require emergency surgery.

2. Pathophysiology of Abdominal Pain

Since the diagnosis of acute abdomen is associated with abdominal pain, it is very important to understand the pathophysiology of abdominal pain. Abdominal pain has three components: visceral pain, parietal pain, and referred pain.

Visceral (splanchnic) pain is transmitted from the intra-abdominal organs through C-type fibers. In addition, nerve fibers are less in number in the visceral organs. Therefore, the pain originating from the visceral organs is more blunt, slow and aching pain. The localization of the pain is not clear and a widespread pain is described. If the cause of the pain is an incision, minimal pain is felt due to the small number of nerve fibers stimulated, while intense pain is described in cases affecting a large area such as mesenteric ischemia. The causes of visceral pain can be counted as hematoma causing stretching of the capsule or peritoneum, mass, obstructions in luminous organs, ileus, urinary stones, spasms in smooth muscles and vascular obstructions.

Nerve fibers from the visceral organs and some fibers from the skin may overlap at the synapse sites. In this case, the pain originating from the visceral organ is felt in the skin dermatome where the synapse-forming fibers reach, this is called reflected pain. Reflected pains are localized according to the embryological formation. The pain of foregut organs is felt in the epigastric region, the pain of midgut organs is felt in the periumbilical region, and the pain of hindgut organs is felt in the suprapubic region. For example; It can be said that ureteral pain can be felt in the scrotum, hepatobiliary pain in the right shoulder region, appendix pain in the periumbilical region, and pancreatic pain in the back region.

Parietal (somatic) pain occurs as a result of the progression of inflammation in the internal organs and affecting the parietal peritoneum. Since there are more nerve fibers in the parietal peritoneum and a faster impulse is transmitted between the fibers, the pain is more distinctly localized, more intense and sharp.

If we explain with an example; When the appendix is blocked by a fecalith, visceral pain develops due to dilatation of the appendix lumen. This visceral

pain is a mild blunt pain that may be accompanied by nausea and vomiting. The feeling of this pain in the periumbilical region is an example of referred pain. Later, as the inflammation reaches the parietal peritoneum, the pain turns into a more pronounced and severe pain localized to the right lower quadrant. This type of pain is an example of somatic pain.

In addition, when the form, duration and severity of the pain are observed, comments can be made about the diseases. A pain that starts mildly and gradually increases, and then continues to be severe and continuous, suggests an inflammatory disease such as acute appendicitis or acute cholecystitis. Pain that increases and decreases with certain short intervals is defined as colic pain. Colic pain suggests obstruction of luminous organs such as ileus and renal colic. First, a sudden onset of severe pain in a specific localization and then pain spreading to the entire abdomen should be suspected luminous organ perforation. In addition, vascular occlusions such as mesenteric ischemia should be considered in the case of very severe pain that cannot be localized with a sudden onset.

3. Anatomy

In abdominal examination, the abdomen is usually divided into 4 quadrants and sometimes 9 quadrants. The patient's complaints and examination findings should also be evaluated according to these quadrants. It should be known which organs are located in which quadrant and their relationship with the peritoneum. The examination of these quadrants guide the differential diagnosis.

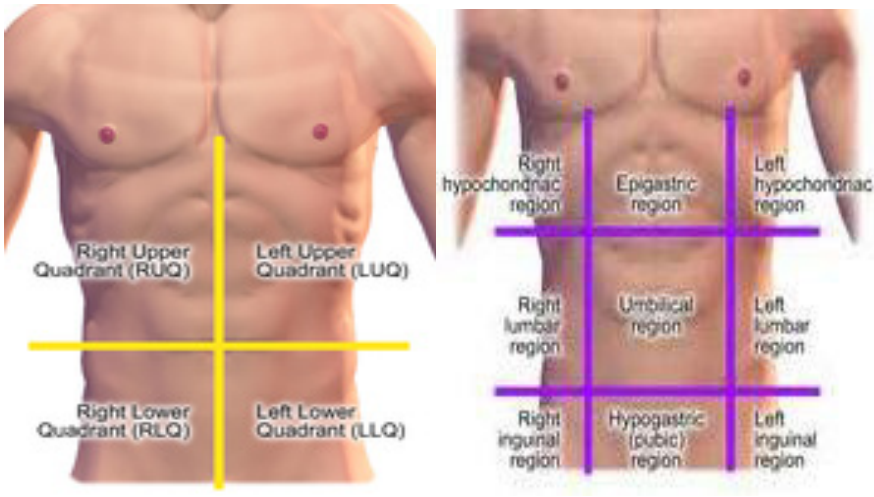

Figure 1: 4 quadtants and 9 quadrants for abdominal examination

The right upper quadrant includes the liver, gallbladder, biliary tract, distal stomach, duodenum, hepatic flexure of the colon, ascending colon and transverse colon, pancreas, right adrenal gland, and right kidney. The left upper quadrant includes the spleen, stomach, left lobe of the liver, pancreas, splenic flexure of the colon, transverse and descending colon, left adrenal gland, and left kidney. In the right lower quadrant includes the right kidney, ascending colon, appendix, right ureter, and in women, the right ovary, uterus, and right fallopian tube. In addition, the left lower quadrant includes the descending colon, left kidney, left ureter, bladder, sigmoid colon, left ovary, and left fallopian tube.

Since the small intestine is widely located in the abdomen, it is associated with all dials. It will be useful to evaluate the epigastric region pain and suprapubic region pain in detail. In the epigastric region there is stomach, pancreas ; in the suprapubic region there is bladder, uterus and rectum.

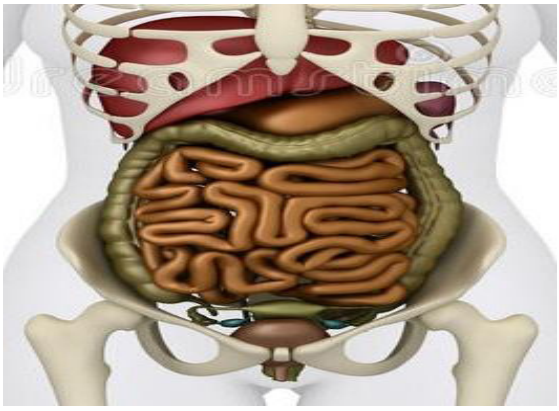

Figure 2: İntra-abdominal organs

It should not be forgotten that; It is not enough to know the anatomical localization of the intra abdominal organs alone. Because pain will be felt outside of its anatomical localization due to visceral pain and radiating pain.

4. Anamnesis

Pre-diagnosis of the patient who presents with abdominal pain occurs after anamnesis. For this reason, if the patient's history, the nature of the pain, the time of the pain, the spread of the pain and the findings accompanying the pain are questioned in detail, the diagnosis of the patient will be cleared more accurately and quickly. Thus, the examinations to be performed will be diagnostic. Taking the detailed anamnesis of the patient is important in terms of revealing the patient's accompanying complaints.

It should be questioned when the abdominal pain started and how long it has continued. Pain lasting for a few hours suggests an acute illness, while pain lasting for a month suggests a chronic illness.

It is important whether the pain is of slow onset or sudden onset. A slow onset and increasing pain suggests visceral pain first and then parietal pain. Diseases such as acute appendicitis, acute cholecystitis, diverticulitis, acute pancreatitis, PID (pelvic inflammatory disease) with progressive inflammation can be given as examples. Pain that rises rapidly, disappears within a few minutes, and then recurs in the same way is called colic pain and will suggest obstruction of the luminous organs. Ileus is an example of kidney stone colic pain. Sudden, severe and persistent pain suggests either a perforation or ischemic events, such as gastric perforation or mesenteric ischemia.

The displacement of the pain over time or the feeling of it in other areas also provide information about the source of the pain. In acute appendicitis, the pain that starts in the periumbilical region is felt more strongly in the right lower quadrant, suggesting first visceral pain originating from the appendix and then parietal pain originating from peritonitis. Pain felt in the right shoulder in cholecystitis, in the left shoulder in splenic trauma, and in the scrotum and labium in genitourinary diseases suggests referred pain.

In patients with peritonitis, abdominal pain increases with movement and stretching of the peritoneum. Therefore, patients want to remain motionless and knees flexed. In renal colic, the patient is observed as unable to stand still.

Enteral nutrition increases pain in ileus, biliary colic, cholecystitis, pancreatitis, diverticulitis and intestinal perforations. Eating increases pain in duodenal ulcers and reduces pain in peptic ulcers. Findings such as nausea and vomiting and jaundice accompanying right upper quadrant or epigastric region pain suggest cholecystitis, cholangitis, and pancreatitis. Periumbilical or suprapubic pain accompanied by diarrhea, constipation, nausea and vomiting suggests an ileus at the level of the small intestine or colon. Epigastric pain and hematochezia or hematemesis suggest duodenal ulcer bleeding. Hematuria urinary stone with colic flank pain; Suprapubic pain with vaginal discharge suggests PID. Abdominal pain with bloody diarrhea and accompanying nausea and vomiting suggest gastroenteritis, IBD (inflammatory bowel disease).

The relationship between pain and other symptoms is also important in terms of diagnosis. When pain first, then nausea and vomiting occurs, inflammatory diseases such as acute appendicitis and cholecystitis are considered

in the foreground, while the onset of pain after nausea and vomiting suggests gastroenteritis.

The patient's history should also be questioned in detail. The patient's surgeries, comorbidities, and medications can guide us. For example; While the recent sepsis picture of a patient who had a colon surgery may suggest a fistula, the complaints of inability to pass gas and stool, nausea and vomiting in the subacute period suggest ileus. Having a history of renal stone in a patient presenting with flank pain may suggest urinary stone; abdominal pain in a patient with unregulated diabetes may suggest diabetic ketoacidosis; abdominal pain and fever peritonitis in a patient with peritoneal dialysis; abdominal pain in a patient using narcotic drugs may suggest cholestasis or paralytic ileus. While the risk of gastric perforation increases with non-steroidal anti-inflammatory drugs, the risk of bleeding has increased in patients using anticoagulants and antiaggregants. It should be kept in mind that complaints, examination findings and laboratory tests may not yield results as expected in patients with immunosuppressive disease or using immunosuppressant drugs.

Gynecological history should be questioned in detail in female patients. Because gynecological causes of acute abdomen are not rare, especially in women of childbearing age, and treatment protocols differ. Therefore, checking β-hCG in patients at this age is very important in terms of pregnancy or ectopic pregnancy. It will also guide the radiological imaging methods that are planned to be performed after β-hCG is examined. Learning the menstrual cycle should be questioned in terms of Mittelschmerz, vaginal discharge should be questioned in terms of PID, and if there is a history of ovarian cyst, it should be learned.

5. Physical Examination

After a good examination, a good examination is next. In fact, the patient's examination begins from the first moment he is seen. Because the first step of the physical examination is inspection. Standing motionless with the knees pulled to the abdomen suggests peritonitis, while writing without stopping may suggest more renal colic or early ischemia.

The patient's abdomen should be fully opened and then inspection should be performed. Surgical scars or other scars, hernia, distention, abscess, cellulitis, hematoma, ecchymosis, frog belly appearance, an existing deformity, etc. situations can be easily understood by inspection. Considering that patients can

sometimes forget or hide their surgeries, the importance of inspection findings can be better understood.

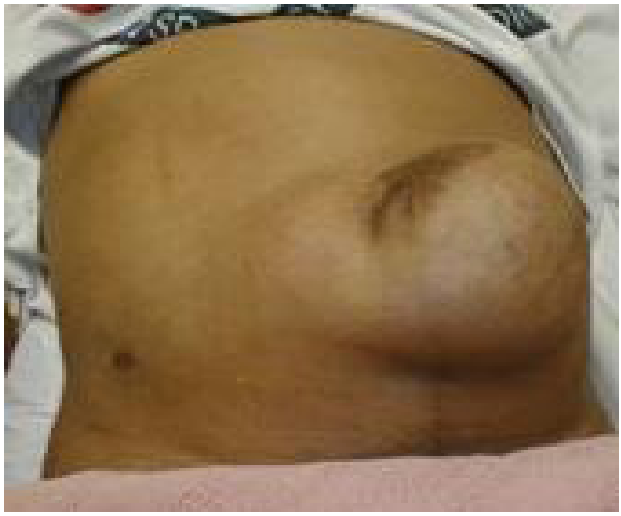

Figure 3: Hernia image

Auscultation is then performed. During auscultation, the abdomen should be divided into 4 quadrants and each quadrant should be rested for 1 minute. In a patient with normal bowel motility, an average of 4-10/min bowel sounds is expected. While hypoactive or absent bowel sounds suggest ileus, mesenteric ischemia, narcotic drug use; Being hyperactive is seen in early obstruction or in conditions such as gastroenteritis. The character of bowel sounds is important. High frequency of bowel sounds, metallic sound and sound of intermittent flow of fluid as if emptied from a glass are findings suggestive of obstruction.

Percussion is applied after auscultation. The middle part of the abdomen is filled with intestines. The normal percussion finding in the middle parts of the abdomen is tympanic voice. If there is a mass, organomegaly or ascites in the abdomen, dullness is felt. In the case of ileus, where the gas in the intestinal lumen increases, or in cases of perforation characterized by free air in the abdomen, tympanic seems to increase. If the intestine comes to the anterior of the liver in the ileus or if air comes to this area in the perforation, tympanic voice can be taken instead of the dullness that should normally be heard.

The last and most important part of the physical examination is palpation. It is necessary to start palpation from the pain-free quadrant and with superficial

palpation. Thus, this condition does not cause voluntary defense in patients. With superficial palpation attempts to examine whether there is a palpable mass or organomegaly. Then, it is examined by deep palpation from the place where there is no pain. The patient should be distracted to prevent voluntary defense. In addition, while the patient with volitional defense can easily perform deep inspiration, the patient with peritonitis cannot do it because of pain. In order to understand the pain experienced by the patient, it is necessary not to ask the patient, but to follow the facial expressions and reactions. If the patient has defense in all quadrants, it is understood that he has diffuse peritonitis and it is called wooden abdomen. In the case of localized peritonitis, defense is seen only in that quadrant. If the hand is suddenly withdrawn from the abdomen after deep palpation, the feeling of pain is called rebound. Rebound is also a sign of peritoneal irritation and supports peritonitis.

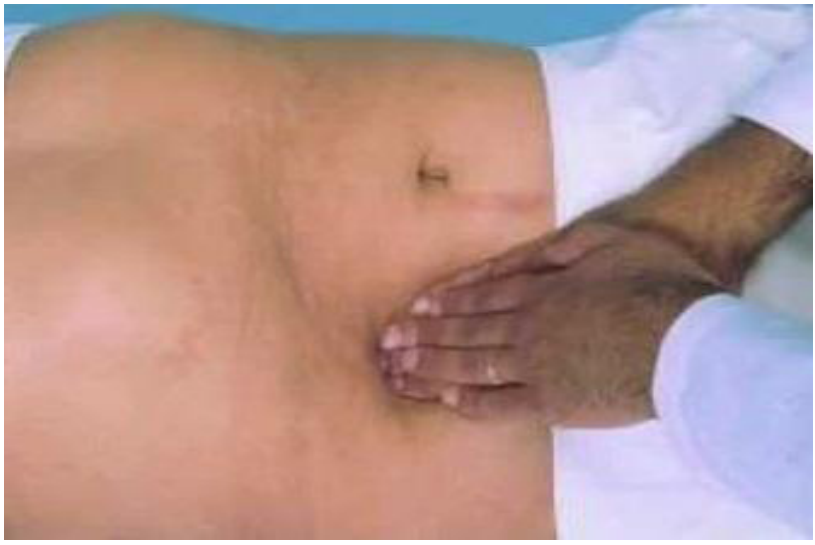

Figure 4: Deep palpation image

6. Laboratory Tests

If acute abdomen is considered in the anamnesis and physical examination of a patient, there are tests that should be requested. We can divide these into routine analyzes and what should be requested according to the clinic.

Neutrophil dominance and leukocytosis are seen in almost all diseases causing acute abdomen. As mentioned before, the aim in acute abdomen is to identify patients who need surgery and to treat them as soon as possible.

Therefore, if we are considering a pathology requiring emergency surgery in the etiology of a patient with an acute abdomen, the tests required for surgery should be performed quickly. These tests can be listed as complete blood count, biochemistry, INR, blood group, EKG and PAAG.

In addition, AST, ALT, bilirubin values should be measured in patients with cholecystitis, choledocholithiasis, cholangitis presenting with right upper quadrant pain, nausea, vomiting, fever or jaundice. If pancreatitis is suspected, amylase and lipase should be added to them. If there is a suspicion of mesenteric ischemia in a patient with a pre-diagnosis of ileus, blood gas is requested and the lactate level is evaluated. Urine analysis (TIT) should be requested in patients with urinary complaints, hematuria and dysuria. And the thing that should not be forgotten is to ask for β-hCG in female patients of childbearing age. β-hCG test should be requested to detect both healthy and unhealthy pregnancy status or to prevent harm to the pregnant patient before radiological imaging is performed.

7. Radiological Imaging

Radiological evaluation should be started with the simplest, and if necessary, further investigations should be requested. Today, with the widespread use of CT, direct radiographs are less frequently used for diagnostic purposes. However, with a high quality and carefully examined direct X-ray, information about many surgical diseases can be obtained very quickly. It is useful in cases where urgent intervention is required, but the limited knowledge gained is also a disadvantage.

The presence of free air under the diaphragm in a chest X-ray taken in the standing position makes the diagnosis of perforation. In standing direct abdominal radiographs, the appearance of the colon and small intestine haustra is distinguished by the localization of the colon. In addition, while air is not seen in the small intestines on a normal X-ray, some gas is expected to be seen in the colon. The presence of air-fluid level in the small intestine in ADBG suggests ileus. Coffee bean appearance sigmoid volvulus spreading from the left quadrants to the right; A comma-shaped colonic and spreading from the right lower quadrant to the left suggests cecal volvulus. An excessively increased diameter of the colon annus suggests obstruction or toxic megacolon. There is a possibility of opaque gallstones or urinary stones or an appendicolith in ADBG. Depending on the inflammation, an air-fluid level may develop in the adjacent

intestinal loop, which is called the sentinel loop (guard ans. The presence of this loop supports the diagnosis when seen together with the clinic. For example, monitoring of sentinel loops in the upper quadrants of the pancreas adjacent to the pancreas in a patient with pancreatitis, and in the right lower quadrant of a patient with acute appendicitis supports the diagnosis. X-rays taken in the lateral decubitus position will be guiding in intubated patients with poor general condition who cannot stand up.

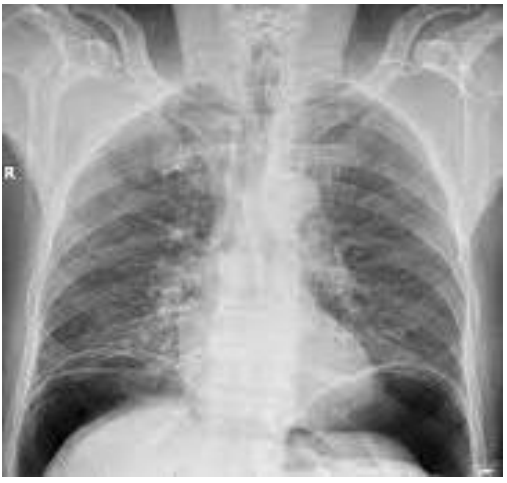

Figure 5: Free air under the diaphragm

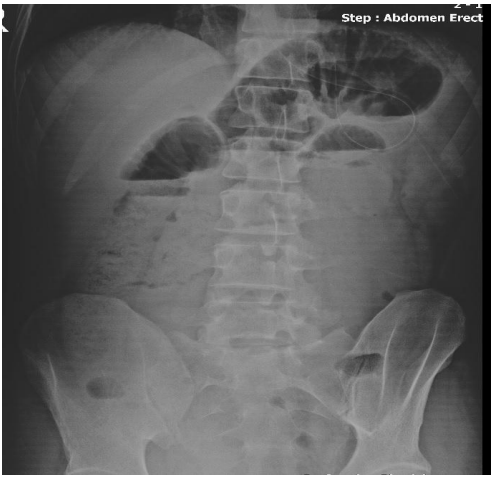

Figure 6: Air-liquid level

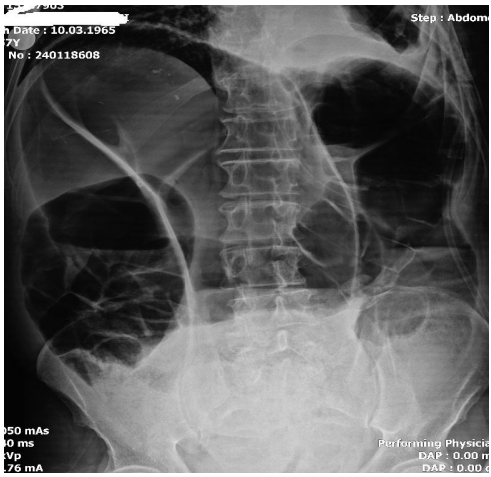

Figure 7: Sigmoid volvulus image

The advantages of ultrasonography can be counted as that it does not contain x-rays, displays non-opaque stones, can be easily repeated in case of clinical necessity, and is much more inexpensive. Disadvantages are the need for a radiologist who is taking the shots, the result is dependent on the person, and the inability to evaluate intestinal structures very well. İn the evaluation of solid organs such as liver, spleen, kidney, gallbladder pathologies, acute appendicitis, urinary stones and infections,also especially in pediatric and pregnant patients whom we do not want to receive radiation are among the first-choice tests. If there is a space-occupying lesion in solid organs, its localization, size, solid-cystic distinction can be made. However, these conditions are rarely guiding in patients with acute abdomen. In acute cholecystitis, the gallbladder is hydropic, with increased wall thickness, and fluid accumulation and contamination around it. At the same time, the patient's sensitivity when pressed with the ultrasound probe is also a sonographic Murphy positivity, which helps in the diagnosis. While the width of the intrahepatic bile ducts can be easily evaluated, the extrahepatic biliary tract, especially the distal common bile duct, is limited due to intestinal structures and adjacent organs. In a patient with acute appendicitis, the diameter of the appendix, contamination around it and fluid accumulation, thickness of the appendix wall, and abscess, if present, may be seen. The absence of compression in the appendix in compression with the ultrasound probe also supports acute appendicitis. Ultrasound also shows the blood or fluid accumulated in the abdomen very well and can be used as an aid for aspiration for treatment purposes.

Nowadays, It has developed a lot and its accessibility has increased a lot. The fact that radiology technicians are sufficient for shooting also contributes to this accessibility. In addition, it can be taken for screening purposes in patients whose preliminary diagnosis is unclear or in unconscious patients with poor general condition, since the entire abdomen enters the image area in CT, and it can provide information about additional pathologies if they are present. While CT is being taken, it should be taken by determining the protocol suitable for the preliminary diagnosis. IV contrast should be given in the patient whose mass lesion, tissue integrity or inflammation will be investigated, and oral and/or rectal contrast should be given in the patient whose intestinal perforation will be investigated. While imaging should be performed in the angio phase for vascular evaluations, CT without contrast should be planned in the evaluation of renal colic. CT, which is not taken in the appropriate protocol, may provide clinical information or may be insufficient. Although diseases such as ileus and perforation can be diagnosed with direct radiographs, a surgeon planning an operation will want to make surgery knowing the cause of ileus and the focus of perforation. Therefore, CT should be performed in these patients, if possible. In addition, CT provides a great opportunity for the evaluation of complications in patients who develop post-operative acute abdomen.

Inflammation seen on CT and contamination in surrounding tissues support the diagnosis in acute abdominal causes such as acute cholecystitis, acute appendicitis, acute diverticulitis, and acute pancreatitis. In addition, possible complications such as perforation, abscess, and pseudocyst can also be seen with CT. Wall thickening or wall bleeding in the intestines can be seen on CT. Accordingly, comments can be made about inflammatory bowel diseases, tumoral formations, mesenteric ischemia. Again, in ileus, air-fluid level and luminal obstructions can be seen in the intestines. In patients with volvulus, dilatation in only a certain intestinal loop, obstruction in the proximal and distal parts, and vortex findings in the intestinal meso are observed. In patients with perforation, free air outside the lumen and if oral/rectal contrast is given, contrast material leaks into the extraluminal area. In cases of strangulated hernia and mesenteric ischemia, lack of blood in the intestinal walls, air monitoring in the intestinal wall, and even air monitoring in the portal vein in mesenteric ischemia support the diagnosis. Bleeding, abscesses and infections in intraperitoneal or retroperitoneal areas can also be easily recognized by CT.

MRI is not generally used for the diagnosis of acute abdomen. Because it is a long and costly examination that is not available in all centers. In addition,

CT already shows the areas evaluated with MR. However, it is preferred as an alternative to CT in pregnant women who should not undergo CT due to excessive radiation.

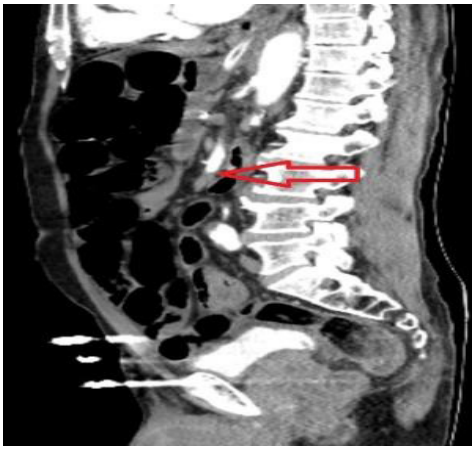

Figure 8: Embolism in SMA

MRI is not generally used for the diagnosis of acute abdomen. Because MRI may not be available in all centers, it is long term and costly examination. CT may show the areas evaluated with MRI. However, it is preferred as an alternative to CT in cases where CT should not be performed due to radiation and in pregnant women.

It should not be forgotten that the two most important diagnostic methods for the diagnosis of the patient are a good anamnesis and physical examination. No laboratory examination and imaging method can precede these diagnostic methods. It may be difficult to access laboratory tests and imaging methods in situations such as war and natural disasters. In such cases, the importance of anamnesis and physical examination for diagnosis will be understood more clearly.

8. Diagnostic Laparoscopy

DPL (diagnostic peritoneal lavage) was performed to find out if the patient had an acute abdomen requiring surgery. However, today, DPL is not applied due to the decrease in the need for DPL due to the development of non-invasive imaging methods and the active use of laparoscopy, which is more useful for diagnosis. Diagnostic laparoscopy is a minimally invasive method that can be applied in patients with acute abdomen, but sometimes in the absence of adequate

non-invasive examinations, and sometimes in the absence of diagnosis despite all current detailed examinations. In addition, if surgical pathology is observed during the operation, the operation can be continued for therapeutic purposes and the patient can be treated. It is advantageous both because of direct seeing of intraperitoneal organs and access to retroperitoneal areas when necessary. It is very useful and beneficial in terms of being able to return to laparotomy if necessary, and being a minimally invasive alternative diagnosis and treatment method before laparotomy.

9. Abdominal Compartment Syndrome

The abdomen is a closed box surrounded by the ribs anteriorly, the vertebrae posteriorly, the pelvis inferiorly, the diaphragm superiorly, and the anterior abdominal wall anteriorly. Intra-abdominal pressure may increase as a result of a pathology that causes acute abdomen. Likewise, if the intra-abdominal pressure increases for any reason, it may cause acute abdomen. Because when the intra-abdominal pressure increases, the venous return decreases. As a result, cardiac output decreases and the patient develops circulatory disorder. Respiratory distress may also develop as a result of compression on the diaphragm. As a result, ischemia, acute renal failure, and a systemic disease affecting all systems develop in the abdomen due to impaired tissue perfusion. What needs to be done is to reduce the intra-abdominal pressure and if there is an event that causes it, to treat it.

Causes that increase intra-abdominal pressure intraperitoneal bleeding, packing application, abscess or peritonitis development, intestinal obstructions, acute gastric dilatation, mesenteric vascular obstructions, visceral edema due to high-volume resuscitation, large intra-abdominal masses, massive ascites, retroperitoneal hematoma, abscesses, severe acute aortic rupture pancreatitis, tight closure of the abdomen, giant hernia repairs, morbid obesity, extensive burn eschars.

To measure intra-abdominal pressure, a catheter or a catheter that reaches the drain or vena cava or a foley catheter can be used. The most commonly used method today is the measurement of bladder pressure with a Foley catheter. While the patient is in the supine position and lying flat, after 50 cc of sterile isotonic is sent to the bladder, the intra-bladder pressure is measured in water and converted to mmHg with the help of a CVP measurement catheter connected to the catheter.

Intra-abdominal hypertension (IAH) is classified as:

- 5-7 mmHg normal
- 12-15 mmHg grade I IAH
- 16-20 mmHg grade II IAH
- 21-25 mmHg grade III IAH
- >25 mmHg grade IV IAH

When the intra-abdominal pressure rises above 20 mmHg, the development of organ failure is called abdominal compartment syndrome.[1]

In the treatment of intra-abdominal hypertension (IAH), the etiology is addressed. NG decompression, rectal decompression or surgery if an intestinal obstruction, acute gastric dilatation is present; paracentesis if massive acid; Surgical intervention may be required in cases such as intra-abdominal or retroperitoneal mass, bleeding, abscess. In addition to the treatments, respiratory support with intubation, diuresis and attempts are made to solve the intra-abdominal edema, and the patient is closely followed up in the intensive care unit. If necessary, it can be followed in this way by turning it to the open abdomen.

If the abdominal compartment syndrome is not treated, it results in impaired perfusion of the organs. Even if there is no acute abdomen present, it can cause acute abdomen. Abdominal compartment syndrome is a serious disease that can result in multiorgan failure and causes mortality when treatment is delayed.

10. Differential Diagnosis

There are many diseases that cause acute abdomen. Some of these diseases are surgical and some are non-surgical diseases. What a surgeon should do is to make a differential diagnosis after examining the patient and to minimize the etiological causes. In this way, it can separate surgical and non-surgical patients and quickly guide the treatment of surgical patients.

Diseases that cause acute abdomen can be listed as follows:

- Acute appendicitis
- Acute cholecystitis
- Acute diverticulitis
- Mesenteric ischemia
- Acute peptic ulcer
- Acute pancreatitis
- Acute peritonitis

- Biliary colic
- ileus
- İntestinal volvulus
- Spleen rupture
- Hemoperitoneum
- Abdominal aortic aneurysm
- Acute pyelonephritis
- Acute ureteral colic
- Ectopic pregnancy rupture
- Ovarian torsion
- Kidney stone
- Adrenal crisis
- Carcinoid
- Familial mediterranean fever
- Sickle cell anemia[2]

The primary goal should be to identify the causes of surgical acute abdomen and to treat the patients in need of emergency surgery without delay. In this case, the most important thing is the anamnesis and examination of an experienced surgeon. Diagnosis is supported by laboratory and radiological examinations.

Despite all this, there will be diseases that cannot be diagnosed. In such patients, if the patient's condition is good, the patient can be hospitalized and followed up. These patients are followed up with serial examinations, laboratory follow-ups and, if necessary, control imaging methods. However, diagnostic laparoscopy or laparotomy can be planned for patients with a poor general condition and septic condition.

11. Atypical Patients

One of the atypical patient groups is the pregnant patients. As known, pregnancy physiology includes varieties from normal physiology. Especially in the early stages of pregnancy, almost all patients may complain of nausea and vomiting. In the following weeks of pregnancy, displacement develops in the intra-abdominal organs due to the enlargement of the uterus. At the same time, increased hormones affect some laboratory tests. White blood cell increase and even neutrophil dominance are considered physiological in pregnancy. Alkaline phosphatase elevation can be seen due to increased estrogen. There may be rapid weight gain, which increases the incidence of biliary diseases.

Abdominal examination may change due to pregnancy and may cause limited abdominal examination. In terms of radiological imaging, restrictions in the use of x-ray and CT containing radiation are required and should not be used unless necessary. Instead of these, USG and MR are preferred. All these may cause delay in diagnosis in patients with acute abdomen during pregnancy. In terms of the decision of surgery in patients with suspected diagnosis, physicians prefer more conservative follow-up, and patients and their relatives give consent later due to fetal loss concerns. This causes an increase in mortality and morbidity.

Acute abdomen during pregnancy may develop due to obstetric and non-obstetric reasons. The need for surgery due to non-obstetric causes of acute abdomen is 5-20 per 1000 pregnancies.[3] The incidence of acute appendicitis, which is the most common cause of non-obstetric acute abdomen in pregnant women, was reported as 0.04-0.2%.[4] The nausea and vomiting symptoms expected for typical acute appendicitis can already be observed during pregnancy. Leukocytosis and an increase in neutrophils are also considered physiological. In addition, with the enlargement of the uterus in the advancing weeks of gestation, the appendix is displaced superolaterally. For these reasons, the suspicion of acute appendicitis can be overlooked if care is not taken. In suspected cases, the first applied radiological method is USG. However, an enlarged uterus, displacement of organs, and intestinal gas may prevent visualization of the appendix or prevent compression from being applied. In these cases, MRI is used. Although it is more difficult to access compared to CT, takes longer to take and needs interpretation, it is an advantage for the diagnosis that it can distinguish both normal and inflamed appendix. The most feared complications in appendectomy are fetal loss, premature birth, etc. Studies have reported the incidence for fetal loss as 1.5% in acute appendicitis and 20% in complicated appendicitis. As it can be understood from here, it is not the appendectomy that affects the fetal loss, but whether it is complicated or not. It has been reported that there is no difference between laparoscopic appendectomy and open appendectomy, and that laparoscopic appendectomy can be safely performed in all trimesters of pregnancy.[5]

The second most common cause of non-obstetric acute abdomen in pregnancy is gallbladder and biliary tract diseases. Gallstones may increase due to the physiology of pregnancy. He presents with complaints of nausea, vomiting, pain in the right upper quadrant or epigastric region. Physical examination is similar to normal patients. The elevation of white blood cells and alkaline phosphatase is not very instructive, but if present, elevations of AST,

ALT, bilirubin, and amylase support the diagnosis. This situation is associated with an increase in morbidity and mortality due to complications such as cholangitis and pancreatitis. It has been observed that the risk of recurrence is higher in patients who were followed up conservatively due to uncomplicated biliary colic and cholecystitis, and that fetal loss was higher than in patients who were operated on. Therefore, laparoscopic cholecystectomy is especially recommended for patients in the first and second trimesters.[3] Despite this, non-surgical follow-up is mostly preferred by both patients and physicians.

Another atypical patient group is poor general conditioned, intubated, sedated, multi-trauma, coronary bypass etc. patients who have undergone major surgery. Pathologies that cause acute abdomen may develop due to drugs used, surgeries and vital signs in these patients. However, due to the absence of examination findings of the patients, the diagnosis is made and treatment is planned with clinical suspicion, laboratory tests and radiological images. In these patients, the risks of mesenteric ischemia, acalculous cholecystitis, pancreatitis, paralytic ileus, peptic ulcer and perforation increase. When the patient has an unexplained general condition disorder and an infective process, it should be considered that there may be additional pathology. The patient's laboratory values are checked for lactic acidosis, leukocytosis, elevation in acute phase reactants, deterioration in liver function tests, and renal failure. If necessary, imaging with USG or CT is performed.

Patients with immunodeficiency can be classified in the patient group that is difficult to manage. Because although there is acute abdominal pathology due to immunosuppression at the time of diagnosis, symptoms and physical examination findings may be suppressed, and the expected response may not develop in laboratory values. Plus, due to immunosuppression during the treatment phase, severe infective process, opportunistic infections and septic picture may accelerate and the expected response to treatment may not be obtained. This patient group includes advanced age, malnourished patients, patients using immunosuppressive drugs for transplantation or other reasons, AIDS patients, and malignant patients. In cases where these patients are suspected, CT scan will be very useful in terms of diagnosis. It is very important not to delay for laparoscopy or laparotomy in cases that are diagnosed or cannot be excluded.

12. Conclusion

Acute abdomen is a preliminary diagnosis made by examination findings in a patient presenting with acute abdominal pain. The diagnosis of acute abdomen

has many etiological pathologies. Most of these etiological causes are surgical pathologies and require emergency surgery. Correct treatment of the patient and minimizing morbidity and mortality depend on accurate and rapid diagnosis. Laboratory tests and imaging methods are not the main diagnostic tool, but are supportive examinations. A well taken anamnesis and a good examination are the two most important steps of diagnosis.

There may be encountered situations where not all patients will present with typical findings, they may be complicated, atypical and difficult to manage, and the patient's condition or hospital facilities may be insufficient for the examinations to be performed. It should not be forgotten that in these cases, emergency surgery can be taken if necessary after clinical evaluation.Tüm hastaların tipik bulgular ile gelmeyebileceği, komplike, atipik ve yönetimi zor hastalar olabileceği, tetkiklerin yapılabilmesi için hastanın durumunun ya da hastane imkanlarının müsaade etmeyebileceği durumlarla karşılaşılabilir.

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CHAPTER II

APPENDIX DISEASES AND EMERGENCIES

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Acute appendicitis is one of the most common causes of abdominal pain. A thorough medical history and physical examination are needed for diagnosis. In around half of patients, the history and physical examination show evidence of 'classic acute appendicitis,' but in the other patients, the signs and symptoms may not be suggestive since they are mild. Consider appendicitis in nearly every episode of acute abdominal pain. The cost-benefit analysis between the development of perforation and the removal of the normal appendix as a result of a delayed or erroneous diagnosis must be performed effectively. Despite the introduction of sophisticated diagnostic techniques such as CT, US, and laparoscopy, misdiagnosis and delayed diagnosis continue to be a prevalent issue.

1. Appendix Anatomy and Function

The appendix is a vermiform extension of the cecum and a blunt tube. It is around 5-10 centimeters long. Normal appendix diameter is less than 6 mm (1). The appendicular artery, which originates from the iliocolic artery, supplies it. Its veins empty into the ileocolic vein, which empties into the superior mesentery vein. In the past, the appendix was removed during nearly every abdominal surgery, whether or not it was required; today, the appendix is only removed when necessary. Because it serves as a repository for gut bacteria. When a sickness (such as dysentery, cholera, or clostridium difficile) disrupts the gut flora, helpful bacteria proliferate from the appendix. Moreover, the appendix is a lymphoid organ that aids in the development of B cells and the manufacture

of antibodies (Ig A). Its function is at its peak in youth and gradually declines with age (2).

2. Acute Appendicitis

• This is the inflammation that results from the occlusion of the appendix's lumen.

Patients frequently report right lower quadrant ache.

• It is the most prevalent cause of acute abdomen.

• It is prevalent between ages 10 and 30.

• If left untreated, perforation or plastron will occur.

• The diagnosis is still determined clinically, that is, based on an examination, notwithstanding the introduction of imaging techniques (3).

2.1. Pathophysiology

Appendicitis is caused by a partial or whole blockage of the appendix mouth. According to various age groups, the cause of blockage may vary. Despite the fact that lymphoid hyperplasia is always present, this inflammation results in the formation of a restricted abscess or a substantial hole, which leads to regional ischemia, perforation, and peritonitis. This obstruction may have several reasons. Among them include lymphoid hyperplasia, parasite infections, fecalitis, and benign or malignant tumors. When a blockage is the source of appendicitis, intraluminal pressure rises. Small vessel blockage and lymphatic stasis develop as a result. Once the appendix is obstructed, mucus fills it. The appendix becomes ischemic and necrotized as the process progresses. Necrosis increases the chance of appendix perforation. This results in a localized abscess and occasionally severe peritonitis (4).

Locations of the appendix:

- Retrocecal in the abdominal cavity (65%)
- Pelvic (30%)
- Subcecal
- Ileocecal (preileal or postileal) (preileal or postileal)
- Retroperitoneal

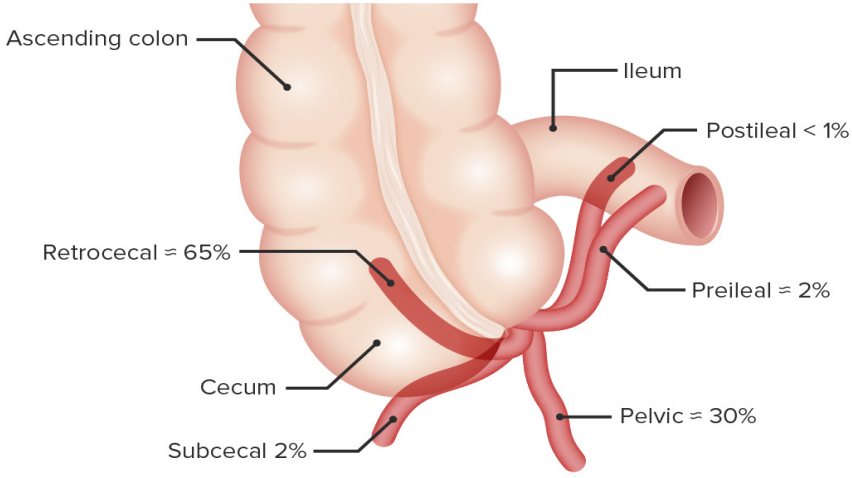

Figure 1: Positions of the appendix

2.2. Symptoms

Typically, appendicitis is characterized by pain in the lower right quadrant. Visceral discomfort from T8 to T10 creates non-localized pain around the umbilicus. Eventually, when the parietal peritoneum gets inflamed, a parietal discomfort develops in the right lower quadrant. Accompanying pain may be the following symptoms:

- Anorexia
- Nausea
- Fever
- Diarrhea
- Weakness
- Increased urinary frequency (5)

2.3. Physical Examination Findings

- Localized sensitivity, resistance, and rebound
- Obtrator sign: On the right, the obturator internus muscle crosses the appendix. This muscle can be strained by acute appendicitis. To perform this test, the right hip of the patient is flexed and subsequently placed into internal rotation. This strains the muscle inside.
- Rovsing sign: During this examination, the hand is abruptly withdrawn following palpation of the lower left quadrant. The test is deemed positive if there is discomfort in the right lower quadrant at the conclusion of the examination.

• Psoas sign: The leg is pushed back (extension), and the test is good if the patient experiences discomfort in the right lower region.

• Maximum point of tenderness; when asked where he is experiencing pain, the patient points to the McBurney point.

• Right lower quadrant pain with coughing.

• Hill drop sign; the patient rises to his toes and falls to his heels, experiencing pain in the lower right quadrant.

• Abdominal mass palpable in plastron and periappendicular abscesses. It is not observed in simple appendicitis (6).

2.4. Laboratory and Imaging

With appendicitis without complications, there is often a modest leukocytosis. Yet, in one-third of patients it is normal. In the event of a leukocytosis more than 17,000/mm3, perforated appendicitis should be suspected. PNL is dominant in peripheral smear. CRP should also be sought in addition to these. Both WBC and CRP readings are normal, rejecting the diagnosis of acute appendicitis with a specificity of 98%. (7). Normal urine microscopy results are seen. If erythrocytes and leukocytes are found, an infection or stone in the urinary tract should be ruled out.

The clinical diagnosis of appendicitis is supported by imaging techniques. The diagnostic accuracy of abdominal CT for appendicitis exceeds 95 percent. CT findings for appendicitis include an enlarged appendix (more than 6 mm in diameter), an enlarged appendix wall (greater than 2 mm in diameter), lubricant surrounding the appendix, an enlarged appendix wall, and the presence of appendicitis (in around 25% of patients). In most instances of appendicitis, air or contrast should not be visible in the appendix lümen (8).

Ultrasonography of the abdomen is one of the most popular procedures used to examine patients with acute abdomen. Diameter of the appendix less than 5 mm and decompression features are used for diagnosis. Its specificity and sensitivity are, respectively, 90% and 85%. Although it is an effective tool, people with significant subcutaneous fatty tissue may not always acquire good pictures. Furthermore, individuals with acute abdomen and peritoneal irritation may not be able to tolerate ultrasonic compression well (9).

MRI has a sensitivity of 95% and a specificity of 92%, respectively. While it delivers great sensitivity and specificity, it is rarely used due to its expensive cost and the necessity for a highly experienced interpreter. It may be recommended in pregnant individuals with negative USG results (10).

Acute appendicitis is also diagnosed using a variety of grading methods. The Alvarado score and the appendicitis inflammatory response score are the two most often utilized of these. Many recent investigations have demonstrated that the appendicitis inflammatory response score has a greater diagnostic value (11).

2.5. Complications

Perforation is the most prevalent medical problem. The treatment for perforated appendicitis is depicted in Figure 2. It is more prevalent among children and the elderly. The spread of ruptured abdominal contents might result in widespread peritonitis and adynamic ileus. Plastron appendicitis must be considered if there is a palpable lump in the lower right quadrant and a sensation of fullness. Plastron appendicitis without complications can be treated with intravenous antibiotics and intravenous fluids. After 6-8 weeks, an interval appendectomy can be performed. If abscess development occurs in a patient without peritonitis symptoms, drainage and intravenous antibiotic treatment are administered. If plastron or abscess is suspected, abdomen CT is the most informative examination (12).

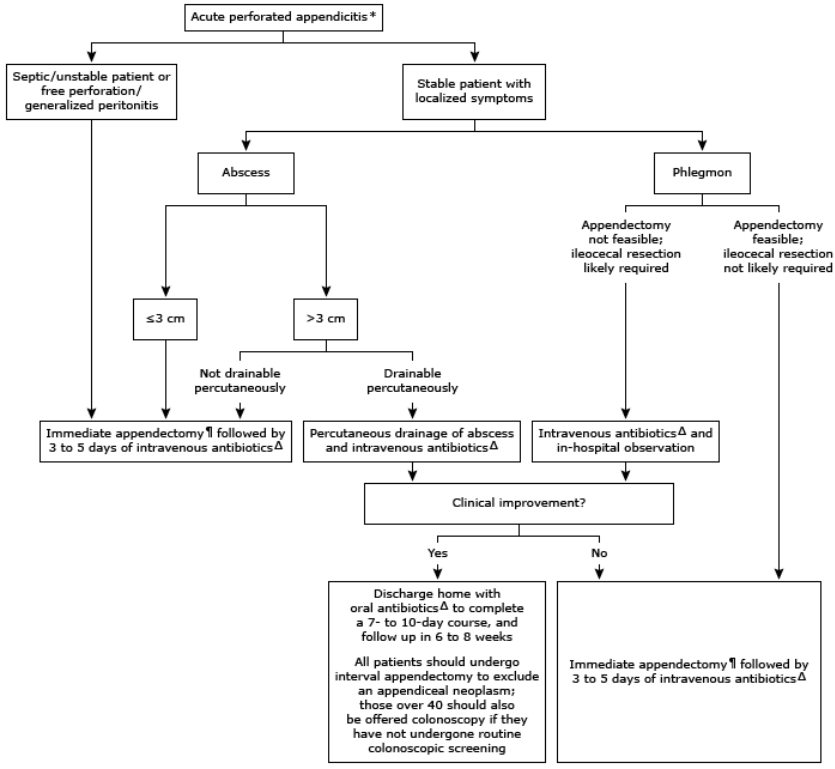

Figure 2: Approach to perforated appendicitis

2.6. Differential Diagnosis

• In children
• mesenteric adenitis (often after viral illness)
• acute gastroenteritis
• intussusception
• Meckel's diverticulitis
• inflammatory bowel disease
• Testicular torsion in men
• Nephrolithiasis
• Urinary tract infection
• In the elderly
• acute diverticulitis
• malignant disease
• Gynecological problems
• ruptured ovarian cysts
• endometriosis
• ovarian torsion
• Ectopic pregnancy
• pelvic inflammatory disease (PID)
• in neutropenic patients, typhlitis (neutropenic enterocolitis)

2.6. Therapeutic

Appendectomy is the major therapy for acute appendicitis. In recent years, laparoscopic appendectomy has been favored over open appendectomy. There are several studies in the medical literature that compare laparoscopic and open appendectomy. According to the findings, laparoscopic appendectomy is associated with less wound infection, less postoperative discomfort, and shorter hospital stays. The extended duration of laparoscopic appendectomy is a drawback (13). The most frequent surgical consequence is wound infection.

In rare instances, antibiotic treatment alone can be administered without surgical intervention. As an antibiotic, a combination of fluoroquinolone and metronidazole or moxifloxacin alone may be recommended. Seventy to seventy-five percent of simple appendicitis patients may not require surgery if antibiotics are administered (14,15).

3. Acute Appendicitis in Pregnant Patients

Acute appendicitis is the most common condition requiring general surgery during pregnancy. Owing to the relatively high prevalence of abdominal/gastrointestinal discomfort, the structural changes associated with a dilated uterus, and the physiological leukocytosis of pregnancy, diagnosis during pregnancy is very difficult. Appendix rupture is more likely in pregnant women, especially during the third trimester. This is likely attributable to the difficulty and reluctance to operate on pregnant women, which delays diagnosis and treatment. Acute appendicitis is suspected in 1 in 600 to 1 in 1000 pregnancies; it is confirmed in 1 in 800 to 1 in 1500 pregnancies (16).

Pregnant women, particularly those in late pregnancy, are less likely than non-pregnant persons to display the signs of appendicitis. The most common symptom of appendicitis (i.e., right lower quadrant pain) occurs near the McBurney point in most pregnant women, regardless of trimester; however, as the uterus grows, the appendix shifts a few centimeters away from the head, so in the third trimester the pain may be localized in the middle or even upper right side of the abdomen (16).

The most painful McBurney point is located 1.5 to 2 inches from the anterior superior iliac spine (ASIS) on a direct line from the ASIS to the navel. During pregnancy, as the uterus raises and strains the anterior abdominal wall away from the inflamed appendix, this pain may lessen. When direct contact between the inflamed site and the parietal peritoneum is avoided, rebound pain and protection are reduced. Moreover, the pregnant uterus may prevent the omentum from touching the inflamed appendix (16).

When a patient arrives with non-classical symptoms, which are prevalent throughout pregnancy, imaging is required. The primary purpose of imaging is to reduce surgical intervention delays brought on by diagnostic uncertainty. A secondary goal is to reduce, but not eliminate, the rate of appendectomy. In such cases, ultrasonography may reveal the likely underlying source of the patient's symptoms (eg, ovarian cyst or torsion, degeneration or torsion of the fibroid, nephrolithiasis, cholecystitis). During pregnancy, graded compression ultrasonography is the imaging technique of choice for appendix diagnosis. Magnetic resonance imaging (MRI) is the next-best diagnostic test for pregnant women whose ultrasound examination for appendicitis is insufficient, as it avoids the ionizing radiation of computed tomography (CT) and is cost-effective. Gadolinium is not commonly administered during MRIs performed

during pregnancy due to safety concerns for the fetus and inconsistent findings; nevertheless, gadolinium administration may be considered if necessary for the evaluation of the mother. Pregnancies exposed to gadolinium-containing MRI are associated with an increased risk of rheumatologic, inflammatory, and infiltrative skin diseases, as well as stillbirth and neonatal death, according to at least one study. MRI has a high degree of sensitivity and specificity for diagnosing appendicitis during pregnancy (16).

A curative appendectomy is the conventional treatment for acute appendicitis in pregnancy. Perioperative antibiotic therapy must defend against Gram-negative and Gram-positive organisms (e.g., a second-generation cephalosporin) in addition to anaerobes (e.g., clindamycin or metronidazole). Antibiotic treatment alone is contraindicated because it is associated with both short- and long-term failure, and there are insufficient safety data for pregnant women (16).

4. Appendix Cancer

Its incidence is around 1 in 100,000. Gastroenteropancreatic neuroendocrine tumors (GEP-NETs) and adenomas are the most prevalent (17). The appendix is the most prevalent location of carcinoid tumors. It is often positioned at the appendix's tip. Treatment differs according on size. The overall method to therapy is depicted in Figure 3.

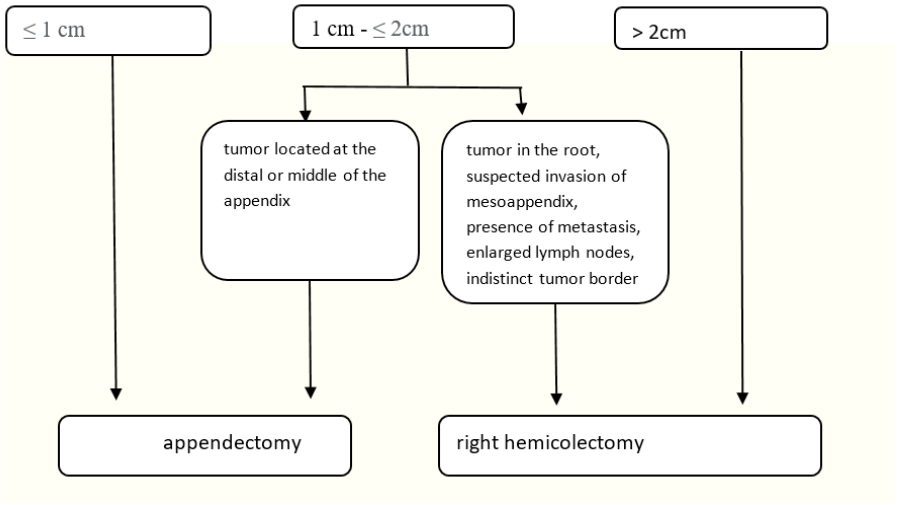

Figure 3: Approach to carcinoid tumors

Lymphoma is an uncommon tumor of the appendix. The only surgical therapy available is an appendectomy. After appendectomy, a comprehensive assessment is required (18). Adenocarcinoma is a second cancer of the appendix. Regardless of size and lymph node status, right hemicolectomy must be done (19). Mucocele of the appendix can be benign or cancerous. Even though it is often observed on preoperative imaging, intraoperative examination and histological confirmation are necessary. When pseudomyxoma peritonei is present, the prognosis is often dismal. If peritoneal involvement is confirmed, HIPEC is the acknowledged standard of care (20).

Adenocarcinoma is rare in appendix (0.08%-0.1% in all appendectomies). Treatment includes right hemicolectomy, regional lymphadenectomy, and chemotherapy for cecal tumors. Appendiceal adenocarcinomas perforate early and have a poor prognosis. Overall 5-year survival 55% varies by stage and grade. Patients with appendiceal adenocarcinoma are associated with synchronous and metachronous neoplasms. 50% of them originate from the gastrointestinal tract (20).

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CHAPTER III

COMPLICATIONS RELATED TO THYROID SURGERY

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1. Introduction

Thyroid surgery is widely practiced all over the world for reasons such as goiter due to iodine deficiency, widespread use of ultrasonographic examination, and easier access to health services (1). The proximity to vital neck structures can make thyroid surgery a challenge for low-volume surgeons. In addition, metabolic disorders after endocrine surgery may cause problems in patient follow-up. Dr. Emil Theodor Kocher was awarded the Nobel Prize for his work in the field of thyroid surgery for his contribution to reducing mortality and morbidity rates (2). Rapidly developing technology in the 20th century has further reduced the complication rates in thyroid surgery. In such procedures, where the mortality risk is very low, the quality of life after surgery becomes even more important.

2. Nonspecific Complications

2.1. Wound Infection

Surgical site infections due to clean surgical procedures such as thyroidectomy are rare. According to the literature, this rate remains below 3% in most series (3). The development of wound infection (WI) after neck surgery can be very morbid compared to infections developing in other parts of the body, and it can also cause more cosmetic problems. WI rates

after thyroidectomy are directly proportional to the size of the surgery (4). The higher rate of WI after thyroid operations performed with lymph node dissection confirms this situation. Again, precipitating factors such as obesity, smoking and diabetes are mentioned in the literature (5,6). In the study of Moskalenko et al., when the groups that received and did not receive antibiotic prophylaxis before thyroid and parathyroid surgery were compared, the rate of WI did not exceed 0.2% in both groups. Based on this series of 534 patients, antibiotic prophylaxis is not recommended in low-risk patients prior to such operations (7). Apart from the patient's factors, shortening the hospital stay before the operation, proper antiseptic skin preparation, and the surgical team's compliance with sterility and optimum operation technique are the main methods of preventing surgical site infections. There is evidence that using fewer drainage catheters reduces hospital stay and morbidity (8). Symptoms appear on average 3 days after thyroidectomy. The most frequently isolated microorganism is staphylococcus aureus. It can be understood with the help of ultrasonographic examination whether there is a condition that only concerns the skin and subcutaneous fat tissue, or whether deeper cervical structures are affected. It is recommended to use an antibiotic suitable for the soft tissue and specific to the microorganism for an average of 7-10 days (9).

2.2. Seroma Formation

Seroma is a general term for fluid accumulation in the subcutaneous space after surgery. This situation, which is familiar to surgeons dealing with breast and axillary surgery, may develop after many procedures and may require drainage due to uncontrolled accumulation. The incidence of seroma after thyroid surgery, which was previously reported to be around 5%, has decreased to 2% with the widespread use of electrical sealing devices (10). There are articles reporting that the incidence of seroma formation is reduced to 1.7% with endoscopic and even robotic minimally invasive approaches (11). In addition, the fact that seroma can cause surgical site infection often does not make it a simple cosmetic problem, it can even be shown among the causes of skin flap necrosis in some series (12). This situation, whose mechanism of formation is unclear, may force surgeons to routinely use drainage catheters. There are authors who recommend using a drainage catheter to prevent seroma only in cases with large dead space (13). As Ramouz et al. stated, advanced age, increased BMI and severely decreased ionized calcium level are independent factors that facilitate seroma

formation in the surgical field. Calcium replacement by focusing on the most easily modifiable of these factors can reduce seroma formation. Improvement of surgical technique and better sealing may also be recommended. The most common method for treatment is evacuation of the seroma with repetitive aspirations (10).

2.3. Bleeding & Hematoma Formation

Bleeding during surgery is a complication that should not be underestimated because it is adjacent to major vascular structures such as the jugular vein and carotid artery in the neck of the thyroid gland. It can be overcome with appropriate intervention. The main problem is bleeding that may occur after surgery. Although this situation does not exceed 1% in highly experienced centers, it can be seen up to 4-5% on average according to the literature (14). According to Wojtczak et al., the major risk factors for postoperative bleeding were the size of the surgical procedure, toxic goiter, and male gender. Retrosternally located thyroid and surgeon's experience were not significant in terms of bleeding. Again, according to the same author, the origin of bleeding was listed equally (18% each) as skin-subcutaneous tissues, strap muscles, and thyroid stump. When a more comprehensive literature review is performed, it is seen that patient-related factors are hematological disorders, cirrhosis, smoking, anticoagulant use, high blood pressure and chronic kidney failure, apart from the factors mentioned by Wojtczak et al. Although there are those who claim to the contrary, most authors state that advanced age is a risk factor for bleeding (15-17). Some articles mention that malignancies other than toxic goiter may also cause bleeding. Also postoperative retching and coughing may also pose a risk for bleeding. In the first 4-6 hours after the operation, swelling and pressure sensation in the neck, pain, bleeding at the wound site, difficulty in breathing, dysphagia, and abundant hemorrhagic contents from the drains suggest bleeding. Careful hemostasis during surgery and the Valsalva maneuver before skin closure is currently recommended by many authors. According to Khadra, the use of hemostatic agents in thyroid surgery provides minimal advantages for the management of perioperative bleeding risk. Close monitoring of the patient for the first 4-6 hours is very important, as early intervention is known to be life-saving.

Bleeding is often unpredictable today, despite the use of sealing devices and other precautions due to advancing technology. Therefore, every patient should be approached with the same care. It should be kept in mind that it may

cause recurrent nerve paralysis due to bleeding, the need for tracheostomy and even mortality. Therefore, it requires urgent intervention.

3. Specific Complications in Conventional Thyroidectomy

3.1. Scar Formation

Scar formation almost always develops after traditional thyroidectomy with a horizontal neck incision. This situation may cause psychological problems in some patients because the majority of the patients are young women and they are in a part of the body that cannot be concealed by clothing. Because of such concerns, making smaller cuts can result in more tension on the skin and a higher rate of scarring. According to Chung et al, subcuticular suturing using barbed suture material and a combination therapy using non-ablative fractional laser and intralesional injection of triamcinolone demonstrated a favorable aesthetic outcome for both patients and operators (18). Today, with the development of technology, transoral or axillary approach can be preferred in suitable patients.

3.2. Hypoparathyroidism

Parathyroid glands are endocrine units that have important roles in the regulation of calcium metabolism. There are 4 parathyroid glands with a rate of 87%, according to their relationship with the thyroid gland two of them are known as the right and left upper glands, and the other two are known as the right and left lower glands. It can be found in an unusual location in 16% of patients (19). Many authors consider a decrease in serum calcium levels beyond normal limits within the first 48 hours after surgery as a sign of postoperative hypoparathyroidism (PHP) (18,20). During thyroid surgery, loss of function in the parathyroid glands secondary to ligation of the inferior thyroid artery or due to heat damage can be expected. In addition, undesired parathyroidectomies and postoperative hypocalcemia may be inevitable in large surgeries for malignancy. It is important for the prediction of PHP that serum parathormone levels fall below 10 pg/mL at the 12th hour after surgery. In this way, it is possible to prevent serious symptoms with oral calcium and active vitamin D replacement. Routine monitoring and protection of the 4 parathyroid glands during thyroid surgery is the gold standard for the prevention of postoperative hypoparathyroidism. In addition, the accidentally removed parathyroid gland should be transferred between the strap muscles. Although some authors argue the opposite, it has been proven in many series that there is a negative correlation between the

surgeon's experience and postoperative hypoparathyroidism (21. Although hypoparathyroidism has been reported as 37% in some series after thyroid surgery, the difference in rates may be due to the lack of a serious standard for the definition of this condition (22. Continuation of hypocalcemia for more than 6 months can be considered as permanent hypoparathyroidism and can be seen in 2-3 % of average cases. Toxic goiter, large thyroid gland and recurrent surgeries can be considered as risk factors for permanent hypocalcemia (4. The majority of PHP patients recover within a few months, with serum PTH levels above 10 pg/ml, calcium levels within the normal range, and disappearance of hypocalcemia symptoms (23. Although it is accepted that the cheapest and easiest method in treatment is oral calcium and vitamin D replacement, routine blood tests and follow-up can be seen as a serious problem in terms of patient compliance. Although synthetic parathormone replacement is safe, both its cost and unpredictability in some patients make it far from being the ideal treatment. Auto-transplantation of parathyroid tissue and allo-transplantation are other treatment options. In particular, allotransplantation creates the need for immunosuppression.

3.3. Recurrent Laryngeal Nerve & Superior Laryngeal Nerve Injury

Recurrent laryngeal nerve (RLS damage is one of the most feared and even the first problems that come to mind of every physician dealing with neck surgery. Except for the cricothyroid muscle, the motor function of all intrinsic muscles of the larynx depends on these nerves. The vagus, a multifunctional nerve fiber called the 10th cranial pair, gives off branches of the recurrent laryngeal nerve under the aortic arch on the left and the subclavian artery on the right. Recurrent nerves are considered to be 1-1.5%, this variation can be seen a little more on the right side. Both RLNs run along the tracheoesophageal groove. It is known that while the left RLN has a longer and deeper course due to its different anatomical structure, the right RLN has a more oblique and superficial course. The close association of both nerve fibers with the Bery ligament increases the likelihood of injury at this level. While hoarseness, aphonia, shortness of breath, and aspiration during feeding may occur in unilateral nerve injury, sudden airway obstruction and the need for tracheostomy may occur in addition to previous symptoms in bilateral injuries.

Nerve injury rates ranging from 1-5% depend on the type of disease, the extent of the surgical procedure, the presence of non-recurrent nerves, the surgeon's experience, postoperative radiotherapy, and the technique used (24.

The best measure to reduce permanent nerve palsy is to expose the nerve along its course and manipulate it as little as possible. Today, routine use of intraoperative nerve monitoring (IONM) is recommended. According to Maowei et al., the use of IONM significantly reduces the detection rate of RLN and the frequency of surgeon-related nerve injury, especially in recurrent surgeries (25). In addition, recognizing the loss of signal during the operation is very important in preventing bilateral paralysis by protecting the opposite nerve.

In unilateral RLN injuries, if the patient is asymptomatic, no intervention other than routine follow-up is considered but voice therapy should be considered anyway. In case of heavy aspiration, there is no need to wait for vocal cord medialization. If the patient has mild symptoms, it can be waited for 6-12 months. If symptoms persist after this period, thyroplasty, cord injection, or arytenoid adduction may be considered. In patients with bilateral nerve incision, lateralization procedures can be started without delay. If there is no nerve incision, it may be considered to keep the patient intubated for a few days accompanied by steroid therapy. If respiratory distress develops after extubation, tracheotomy or lateralization may be considered.

Physicians performing thyroid surgery are more concerned with the external branch (EBSLN) rather than the superior laryngeal nerve. The internal branch is a sensory fiber and is generally not in the field of view in thyroid surgery. The external branch of the superior laryngeal nerve originating from the vagus is the motor nerve of the cricothyroid muscle. After the EBSLN crosses the superior thyroid artery, it enters the cricothyroid muscle posterior to the insertion site of the sternothyroid muscle. Cernea classification (type-1, 2a, 2b) has been developed according to the way this branch crosses the artery.

Cernea type 2b is the most common variant with the possibility of nerve injury. The Friedman classification, on the other hand, is not that popular, although it may be an alternative to cernea. It is known that there is an anastomosis between the EBSLN and the RLN. In this way, it is possible to receive signals from EBSLN using IONM. Therefore, EBSLN can be found and preserved during the use of IONM, but today, many surgeons prefer to ligate the superior pole vessels separately to preserve the nerve fiber without finding it. If EBSLN paralysis lasts longer than 3 months, it is considered to be permanent. These patients have difficulty in making high-pitched sounds and a tired tone is understood when speaking. While no treatment is considered most of the time, various thyroplasty and reinnervation options should be kept in mind, especially for professionals who use their voice. Although it is unlikely to

be encountered during thyroid surgery, it should be known that there is a serious risk of aspiration in internal branch injuries.

4. Rare Complications

4.1. Thyroid Storm

Sudden elevation of thyroxine and triiodothyronine in the blood circulation, especially in the first 12 hours after thyroid surgery, may lead to a serious clinical picture with tachycardia and hyperthermia. As a result of the development of the clinical approach and making the patients euthyroid with appropriate treatment before surgery, the incidence of thyroid storm has decreased considerably (26). Its incidence is currently estimated to be 0.6-0.7 per 100000 per year (27). It should be treated with sedation under intensive care follow-up, and fluid and electrolyte balance should be tried to be achieved.

If there is a concomitant infection, antibiotic treatment may be given. Beta-blockers, anti-thyroid drugs, and steroids may often be required. Lithium and thyroid hormone binding molecules can be tried. Plasmapheresis and dialysis should be among the options.

4.2. Other Rare Complications

Trachea and esophageal injuries that develop during thyroid surgery and stenosis that develop in later periods are available in the literature, albeit very rarely (28). Early diagnosis and immediate surgical intervention are recommended for such injuries. Pleural and brachial nerve plexus injuries may develop in cases where thyroidectomy was performed with the transaxillary approach (29). Thoracic duct injuries that can develop in any type of neck surgery. Cervical plexus injuries and other nerve injuries are rare complications that can also be seen in thyroid surgery.

5. Complications Related to Transoral Endoscopic Thyroidectomy Vestibular Approach (TOETVA)

TOETVA has been accepted as an ideal surgical approach for patients who want to prevent scar formation due to aesthetic concerns in recent years. However, this surgical intervention can be performed in a carefully selected group. As many authors have stated, almost all of the complications seen in conventional thyroidectomy can be seen at similar rates in TOETVA. However,

Anuwong et al. He claimed that TOETVA was associated with less postoperative pain and less hypocalcemia compared to longer operative time (30).

Cervical emphysema has been reported to disappear spontaneously within 24-48 hours in other series (31). A 2018 article noted that TOETVA can cause thermal skin lesions and mental nerve damage (32). Most publications examining the TOETVA series emphasize that surgical site infection due to port site contamination is more common than conventional thyroidectomy.

6. Conclusion

Thyroid surgery is a place full of difficulties and pitfalls. Workshops organized at regular intervals can be useful for surgeons to gain experience. At the same time, patience and attention should remain the 2 constant criteria of thyroid surgery. In order to reduce the complication rates and increase the quality of life, technology should be followed closely and the indication for surgery should not be too daring.

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CHAPTER IV

SURGICAL APPLICATIONS IN THE SURGICAL TREATMENT OF COLON CANCER

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Like all cancers requiring surgery, the basic principle in colon cancers is to remove the malignant mass within surgically clean limits and to remove the invading lymph nodes, which is the regional lymphatic drainage.

It should be noted that the region where the tumor is located, the regional lymphatic drainage and the vascular structures feeding the related colon section should be carefully approached and these constitute the main cornerstones. However, palliative surgery, which is known as relaxing surgical procedures in medicine, can be applied in order to make the rest of the patient's life more comfortable rather than surgical treatment in locally advanced or systemically advanced tumors. These palliative procedures can also be performed with deviative procedures such as ileostomy and colostomy to prevent different complications (ileus, perforation, infection, etc.) during the physiological recovery of the patient after the first surgery and other medical treatments (1,2). Adenocarcinomas constitute 95% of primary colorectal cancers. 60% of colon cancers are located in the distal colon, about 30% of all colorectal cancers are located in the rectum, 20% are located in the sigmoid colon, and the frequency decreases gradually proximal, but increases slightly in the cecum, reaching 25% (3-5).

1. Basic Surgical Procedures Applied in Large Bowel Cancers:
It can be classified as;

a) Right hemicolectomy
b)Extended right hemicolectomy

c)Left hemicolectomy
d)Extended left hemicolectomy
e)Transvers colectomy
f) Sigmoid colectomy
g) Subtotal or total colectomy

*Today, surgical procedures can be performed either openly or laparoscopically. The important issue here is; it is necessary to comply with the principles of cancer surgery

The main factors to consider are;

In surgery;

*Progression and dissection in the right plane thanks to mastery of anatomy

*Appropriate resection from the correct localization and maintenance of gastrointestinal continuity

*and it can be summarized as a technically correct anastomosis.

If there is synchronous tumor or adenomas in colon cancers, familial colorectal history, the entire large intestine should be reviewed. On the other hand, it should always be kept in mind that metachronous tumors may develop in the follow-ups after cancer surgery (6).

1.1. Right Hemicolectomy

The so-called right colon; Cecum, ascending colon, hepatic It is a classical and well-known resection for tumors detected in the area containing the flexure and the proximal half of the transverse colon (6).

Excision of the lymphovascular pedicle feeding them must be included in radical complete block resection in order to both provide local control and prevent lymphatic splash in colon cancers. Since lymphatic drainage accompanies the arterial pathway for the resection of the cancerous area in the large intestine, it is very important to divide the arteries supplying the large intestine close to the superior or inferior mesenteric arteries. Right hemicolectomy requires division of the ileocolic and right colic arteries originating from the superior mesenteric artery (7).

In this surgery, ileo-colic artery (a.ileo-colica, right colic artery (a. colica dextra and the right branch of the middle colic artery (a. colica media are ligated espectively distal 10 cm of the terminal ileum, cecum, ascending colon, hepatic flexure and one-third of the transverse colon (8).

1.2. Extended Right Hemicolectomy

In some cases, depending on the surgeon's preference, a more radical surgery can be performed by removing the entire lymphatic network of the right colon. In this case, it is preferred to connect the middle colic artery close to the place where it exits from the superior mesenteric artery (a. mesenterica superior: SMA). In this procedure, of course, because the amount of resected transverse colon increases, one-third of the residual distal transverse colon is left intact (9). According to this technique; A significantly longer transverse segment of large intestine (covering the 2/3 proximal part) is removed than with right hemicolectomy under normal conditions, due to the deactivation of the left branch of A. colica media (6,9).

In addition, some authors prefer Turnbull's "no-touch" isolation technique in surgery; This technique is preferred to begin dissections by ligating the lympho-vascular structures first. Technically, it is performed primarily for a small peritoneal incision in the root of the mesentery, without removing the hepatic flexure (10).

1.3. Left Hemicolectomy

Respectively; Left hemicolectomy is the gold standard operation for cancers of the distal part of the transverse colon, splenic flexure and descending colon.

As a procedure; While the right branch of A. colica media is preserved, only the left branch is ligated. In addition, another important issue; A. colica sinistra is ligated at the level where it exits the IMA, taking care not to injure the inferior mesenteric artery (IMA).

Subsequently, the distal part of the transverse colon fed by these vascular structures, which are ligated and cut, and the splenic flexure on the left, descending colon and proximal sigmoid colon are resected. In distal descending colon tumors, some authors prefer to tie only the first branches of a. colica sinistra and a. sigmoidea without tying the left branch of A. Colica media.

After the resection is completed, an anastomosis is made between the middle of the transverse colon and the proximal of the sigmoid colon. For many patients, the length of the sigmoid colon allows for a tension-free anastomosis. However, subtotal or total colectomy should always be kept in mind as a viable surgical alternative if there is an anatomically unsuitable condition for a tension-free anoscomosis, or if the patient has had a previous colon resection operation (2,8,11).

1.4. Extended Left Hemicolectomy

Extended (radical) left hemicolectomy may be considered for tumors in the distal transverse colon or splenic flexure, or in the descending colon and sigmoid colon (6).

In cases where extended left hemicolectomy is required; With a careful incision made in the peritoneum of the posterior abdominal wall, the inferior mesenteric vessels are exposed by opening longitudinally between the duodenojejunal flexure and the bifurcation of the aorta. The IMA is cut at the level where it emerges from the aorta and in the inferior V. mesenterica (IMV) close to the splenic vein by ligating a double layer separately. Some authors recommend ligating the IMA 2 cm distal to the aorta to avoid damage to the autonomic neural network at the level where the IMA exits the aorta (2,8,11).

1.5. Transvers Colectomy

Transverse colectomy is performed in tumors involving the middle and distal parts of the colon transversum (12). In transverse colectomy especially, it is aimed to remove the lymphatics in the greater part of the transverse colon, omentum majus and arteria colica media together with the tumor. The first step as surgery; Omentum majus involves dissection by separating from the greater curvature of the stomach to both flexures along a line passing under or over the gastro-epiploic arch. At this stage the dissection must be done with care to avoid injury to the stomach. On the other hand, both colon flexures usually need to be liberalization in order for the anastomosis to be tension-free after resection (6).

1.6. Sigmoid Colectomy

In sigmoid colon tumors; Resection is performed between the descending colon and the upper part of the rectum (13). For tumors located in the sigmoid colon, some The routinely performed surgery is the sigmoid colectomy, although surgeons prefer extended left hemicolectomy. However, it has not been demonstrated that extended left hemicolectomy performed in cases with lymphatic involvement at the root of the IMA provides a significant survival advantage over sigmoid colectomy (6).

1.7. Subtotal or Total Colectomy

Leading indications of subtotal and total colectomy in terms of malignancy and premalignancy; It can be listed as multiple colon cancer, diffuse rectocolonic

polyposis, multiple colon polyposis (14). If we take a look at the surgical definition of subtotal or total colectomy; If, after the resected colon, the distal sigmoid colon or the proximal third of the rectum is surrounded by peritoneum, this procedure is subtotal colectomy, on the other hand, the removal of intra-abdominal colon sections. Total colectomy is also possible if it is completely removed and only the rectum below the peritoneal reflection remains. is defined as (8).

Subtotal or total collectomies are more expensive than limited resections. complex surgical applications. However, it is also an advantage to provide continuity with ileo-rectal or ileo-sigmoid anastomoses, which are easier technically than the anastomoses in the surgical applications mentioned above (6). It can be said that a second advantage is the removal of all lymphatic spread areas in subtotal or total collectomies (6). C. Tohme et al.; They reported that emergency subtotal or total colectomy with primary anastomosis in the case of acute obstructive neoplasm of the left colon is both a safe and effective procedure and allows the treatment of cancer and obstruction in a single step (15). Additionally in the literature, in the presence of synchronous tumors (benign or malignant) in the right and left colon, in the presence of previous colon resection, in tumors obstructing the distal colon, if there is no technical possibility to perform less resection, FAP (familial ademato us polyposis) or HNPCC (hereditary It has been reported that subtotal or total colectomy may be preferred in cases of nonpipoid colorectal carcinoma) accompanied by symptomatic diverticular disease(3,16,17).

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CHAPTER V

USE OF BETA 3 AGONISTS IN THE MEDICAL TREATMENT OF OVERACTIVE BLADDER

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1. Voiding Physiology

The neural control of urination is by the peripheral nervous system, somotic and autonomic systems. The somatic system is responsible for the activity of voluntarily controlled structures.The autonomic system is responsible for functions such as contraction and relaxation of the bladder. The autonomic nervous system consists of the sympathetic and parasympathetic nervous systems. Parasympathetic nerves originate from the cranial and sacral segments, while sympathetic nerves originate from the thoracic and lumbar spinal segments. The voiding cycle consists of filling and voiding phases. Sympathetic activity predominates in the filling phase. With stimulation of the sympathetic system, contraction of the smooth muscles of the bladder floor and proximal urethra occurs through α-adrenergic receptors in the bladder. With the stimulation of β-adrenergic receptors in the bladder, relaxation of the detrusor occurs. Somatic activity by stimulation of the pudendal nerve causes contraction of the external urethral sphincter, which is composed of striated muscles. (1). Parasympathetic activity is dominant in the voiding phase. It begins with voluntary stimuli from the cerebral cortex. By inhibiting somatic efferent activity, the external urethral sphincter relaxes.. Parasympathetic activity in the bladder and urethra increases simultaneously with the inhibition of sympathetic activity. Bladder contraction occurs with muscarinic receptors in the bladder, and smooth muscle relaxation occurs in the urethra with nitric oxide release. The

continuation of the voiding reflex is due to the complex relationship between the spinal cord, the pons, and the periaqueductal gray area in the midbrain (2). There are 5 subtypes of muscarinic receptors and M1,2,3 receptors are located in the bladder. Although there are more M2 receptors in the bladder, M3 is thought to be more active. The bladder is in the filling phase, that is, under the influence of the sympathetic system, 98-99.7% of the day, and under the influence of the parasympathetic system, i.e., the excretion is under the influence of the maximum 2% of the day(3).

2.1. Definition of Overactive Bladder (OAB)

Overactive bladder(OAB) is a disease with urgency symptoms associated with frequent daytime urination (≥8 per day) and nocturia (≥1 at night) without urinary tract infection or other pathologies(4)

2.2. Incidence of OAB

According to the prevalence studies, it is seen at a rate of 9-43% in women and 7-27% in men(5). OAB is seen in people of all age groups, and its prevalence increases with age (6).

2.3. Diagnosis of OAB

2.3.1. Local Causes

Infection Bladder stone, bladder tumor, interstitial cystitis, Outflow obstruction

2.3.2. Metabolic Causes

Diabetes, Polydipsia, Pregnancy, Psychological, Other

2.3.3. Medicines

Diuretics, antihypertensives, antidepressants, hypnotics, sedatives, narcotic analgesics

3. History and Physical Examination in OAB

A careful history taking is the basis of the clinical process. A detailed medical, gynecological and surgical history should be taken. Sexually transmitted diseases, menstrual history and bowel habits should be questioned. It is very

important that the diseases in the differential diagnosis and the drugs they use are questioned and excluded. Physical examination (scar, mass, atrophy, prolabsus, tone), bladder distension in terms of urinary retention, pretibial edema, prostate examination in men and pelvic examination in women should be done in terms of the disorder between fluid intake and excretion(5).

A voiding diary and questioning forms and a complete urinalysis are sufficient in the first stage, and no additional examination is required(5). The voiding diary should be between 3-7 days. The voiding diary provides very useful information, especially in cases where a healthy anamnesis cannot be obtained. It is included in the EAU 2022 guidelines. Tables 1 and 2

Table 1. EAU 2022 guidelines

Evidence summary	**LE**
A voiding diary is kept for three to seven days. It is a reliable tool for objective measurement of mean voiding volume, frequency of day and night, and frequency of incontinence episodes.	2b
The voiding diary is sensitive to changes and is a reliable measure of outcome.	2b

Table 2. EAU 2022 guidelines

Recommendations	**GR**
Ask patients with urinary incontinence to fill out a voiding diary.	A
Use between three and seven days	B

4. Laboratory

In patients with symptoms of overactive bladder, urinary sediment should be checked first. Urine culture has no place in the routine evaluation of patients without signs of urinary tract infection. According to the AUA/SUFU OAB guidelines, it is not recommended in uncomplicated patients at initial evaluation. However, in patients who are refractory to therapy or who cannot differentiate between neurogenic and non-neurogenic, urodynamics may provide additional information that may alter therapy. In the presence of hematuria in complete urinalysis, urinary system USG is recommended(5).

5. Treatment

5.1.1. Behavioral Therapy

According to the AUA/SUFU OAB guidelines, treatment is classified into 5 steps. Usually the first-line treatment for controlling OAB is behavioral therapy; It has been effective in clinical trials. However, its long-term usefulness has been limited due to the lack of continuation of treatment outside the study(7). In this treatment, diet and fluid restrictions are recommended first, and fluid intake is avoided 2-4 hours before bedtime. It is recommended to avoid diuretic and bladder stimulating foods and drinks. Pelvic muscle exercises should be applied for at least 8 weeks. It is aimed to suppress the feeling of compression by strengthening the pelvic floor muscles. A voiding diary, timed voiding, delayed voiding, and double voiding are recommended to the patient(8). According to the EAU Guidelines, pelvic floor exercises are recommended grade A for urge incontinence and mixed incontinence. It has been reported that 8% weight loss in obese women in a 6-month period provides a 16% more reduction in urgency urinary incontinence attacks compared to the control group(9)

5.1.2. Medical Treatments

Table 3. Antimuscarinic drugs

Active Drug	**Dose**	**Usage**
Short acting forms		
Oxybutynin	5mg	2-4 times a day
Tolterodine	1mg,2mg	2-4 times a day
Trospiyum	30 mg	45-60 mg daily (3 times 1/2 tb, 1 tb in the morning, 1/2 in the evening or 1 tb in the morning and evening)
Propiverin	15 mg	2 times a day
Long acting forms		
Darifenacin	7.5 mg, 15 mg	once daily
Fesoterodine	4 mg, 8 mg	once daily
Solifenacin	5 mg, 10 mg	once daily
Tolterodine SR	4 mg	once daily
Propiverine SR	30 mg	once daily
Transdermal form of Oxybutynin	3,9 mg/day	Transdermally every 3-4 days

The AUA/ SUFU OAB guideline recommends combining behavioral treatments with second-line treatments (Pharmacological Treatments). Although drug treatments do not completely correct OAB, they reduce the episodes of incontinence and the frequency of urinary urgency. Oral anticholinergics or β3 adrenoreceptors should be recommended as standard therapy at this stage. In the selection of drugs, it is necessary to act according to the effect and side effect profile of the patient Table 3(10).

In antimuscarinic drug therapy, bladder muscles are relaxed and bladder contraction is prevented by blocking the muscarinic receptor level(11). Muscarinic receptors are cholinergic receptors and are distributed throughout the body. M3 receptors are responsible for bladder contraction. It is thought that simultaneous stimulation of M2 and M3 receptors may increase the effects of M3 receptors. Antimuscarinic therapy increases the bladder capacity before the first voluntary contraction and decreases the severity of the first voluntary contraction (12).

In antimuscarinic use, they produce mild side effects (dry mouth, constipation, dry eyes, blurred vision, dyspepsia, urinary retention, cognitive disorders). Very rarely, severe cardiac arrhythmia can be seen. The incidence of these side effects is different for each antimuscarinic drug. Extended release forms are preferred to reduce the possibility of undesirable effects(13).

6. β3 adrenergic Receptors

The distribution and density of β3-receptors varies with species. β3 adrenergic receptors in humans; It is located in adipose tissue, brain, eyes (conjunctiva and choroid), heart, lungs, liver, gall bladder, intestines, uterus, ureter, prostate, penis and bladder(14,16,17). The diversity of density, affinity, or sensitivity of β3 receptors in these tissues is not fully understood. However, it is reported that more than 95% of beta m-RNA in the bladder is in the β3 subtype. β3-adrenergic receptors are the most effective receptors for relaxation and are found in the smooth muscles of the bladder body, base, and proximal urethra (15).

As a result of β3 receptor agonists stimulating the receptors, the adenylate cyclase enzyme is activated and cAMP accumulation occurs in the tissue. By activating cAMP protein kinase, it directly relaxes the detrusor smooth muscle (18).

6.1. Mirabegron Pharmacokinetic Properties

Mirabegron is metabolized in the liver after absorption following oral administration. It reaches peak plasma concentrations (Cmax) in 3 to 4 hours. Absolute bioavailability increased from 29% at the 25 mg dose to 35% at the 50 mg dose. In phase 3 studies, the use of mirabegron with or without food showed the same effect in terms of safety and efficacy. Mirabegron binds to human plasma proteins. Renal excretion of mirabegron is by glomerular filtration and active tubular secretion. Urinary excretion of mirabegron is dose depende (19).

6.2. The Mechanism of Action of Mirabegron

Mirabegron relaxes detrusor smooth muscle through activation of β3-adrenoreceptors during the urinary retention phase of the urinary bladder filling-voiding cycle. In addition, mirabegron increases the storage capacity of the bladder without affecting the contraction size during bladder emptying(20).

In adult patients with overactive bladder (OAB) syndrome, it is recommended as second-line therapy for symptomatic treatment of urgency, increased urinary frequency, and/or emergency urinary incontinence that may occur. Use of mirabegron in children under the age of 18 is not recommended(20).

Tablo 4. EAU 2022 Guidelines

Recommendations	**LE**
Mirabegron is used in patients with OAB and unless they have uncontrolled hypertension or inadequate response to conservative treatment.	A

6.3. Mirabegron Posology and Application Method

Adults (including elderly patients): The recommended dose of mirabegron is 50 mg once daily with or without food. Tablets should be taken once a day with liquid, swallowed whole, not chewed, split or broken(21). Use of mirabegron in children under the age of 18 is not recommended. Safety and efficacy were similar between patients aged ≥65 years and younger, but higher variation in QT interval was detected in patients ≥65 years compared to younger patients. The pharmacokinetics of mirabegron are not significantly affected by age. No dosage adjustment is required in the elderly. There are potential cardiovascular side effects with mirabegron treatment; that is, appropriate follow-up is required, especially in elderly patients with cardiovascular comorbidities(22-23).

6.4. Side Effects of Mirabegron

Table 5. Side effects

	Placebo (%)	25 mg Mirabegron (%)	50 mg Mirabegron (%)
N	1380	432	1375
Hypertension*	7.6	11.3	7.5
Nasopharyngitis	2.5	3.5	3.9
Urinary infection	1.8	4.2	2.9
Headache	3.0	2.1	3.2
Constipation	1.4	1.6	1.6
Upper respiratory tract infection	1.7	2.1	1.5
Arthralgia	1.1	1.6	1.3
Diarrhea	1.3	1.2	1.5
Tachycardia	0.6	1.6	1.2
Abdominal pain	0.7	1.4	0.6
Fatigue	1.0	1.4	1.2

* They showed that in patients with hypertension, patients with high blood pressure (BP) had a greater increase in BP than baseline. The most common side effects for the 25 mg or 50 mg dose are; hypertension, nasopharyngitis, urinary tract infection were detected. Table 5(23).

Based on laboratory data, mirabegron has no clinically significant effect on haematological, serum chemistry or urinalysis parameters. Chemistry laboratory data from the clinical program support the safety of hepatic, renal, glucoregulatory, and thyroid function of 50 mg mirabegron in the treatment of OAB patients (23).

No clinical studies have been conducted in patients with end-stage renal disease (GFR < 15 mL/min/1.73 m2 or patients requiring hemodialysis). Therefore, its use in these patient groups is not recommended. Severe renal failure (GFR15-29 mL/min/1.73 m2); Information on patients with diabetes mellitus is limited. Patients in this group are recommended to reduce the dose to 25 mg. No dose adjustment is recommended in patients with mild or moderate renal impairment (CrCl 30-89 ml/min). (21). It has not been studied in patients with severe hepatic impairment (Child-Pugh Class C) and therefore its use in these patient groups is not recommended. Caution and dose adjustment

are recommended in patients with moderate hepatic impairment (Child-Pugh Class B). Dose adjustment is not recommended in patients with mild hepatic impairment (Child-Pugh Class A). (21).

All three β-adrenoreceptor subtypes are expressed in the cardiovascular system. β1-mediated effects increase heart rate and force of contraction. β2-adrenoreceptors regulate vasodilation in vascular smooth muscle. β3-adrenoreceptors induce positive inotropic effects in human atrial tissue and negative inotropic effects in ventricular tissue. In three 12-week, placebo-controlled safety and efficacy studies in overactive bladder (OAB) patients receiving 25 mg, 50 mg, or 100 mg once daily mirabegron, systolic blood pressure and diastolic blood pressure (SBP/DBP) were approximately 0.5 to 1 compared to placebo. Mean increases of mmHg were observed. In patients taking mirabegron 50mg, an average increase in pulse rate of 1bpm was detected. Both SBP and DBP increases, and pulse rate were reversible after discontinuation of therapy. Mirabegron did not cause more cardiovascular-related adverse events than patients treated with placebo or tolterodine. Mirabegron application; sex, race, ethnicity, age, baseline blood pressure status, or treatment-based blood pressure (BP) central tendency in different subgroups with or without alpha or beta blockers. Mirabegron may increase blood pressure. Regular monitoring of blood pressure is recommended, especially in hypertensive patients. In patients with severe uncontrolled hypertension (Systolic TA≥180mmHg and Diastolic TA≥110mmHg), the use of mirabegron is contraindicated(24-25).

In the central nervous system (CNS), β3-adrenoreceptors are almost absent. For this reason, SSS side effects are negligible. No cognitive function assessment tools were used to examine the effect of mirabegron on cognitive function in the phase 3 clinical program. CNS findings in the form of decreased activity have been observed in mirabegron animal models using human equivalent doses of 29-38.4 times the maximum recommended human dose. These observations were obtained in more than one species. However, it has been suggested by researchers that mirabegron has an acceptable CNS safety profile in the chronic treatment of OAB patients because of the transient nature of the findings, spontaneous resolution, and the need for high dose levels to induce these responses, at least in monkeys (26).

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CHAPTER VI

RADIONUCLIDE IMAGING METHODS IN A PATIENT WITH PAIN IN THE PERIPROSTHETIC AREA AFTER TOTAL KNEE AND TOTAL HIP ARTHROPLASTY

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1. Common Complications in Total Knee Arthroplasty

Total knee arthroplasty (TKA) is considered a widely used, safe, and effective procedure for patients with end-stage osteoarthritis or inflammatory arthritis of the knee. However, complications may occur during and after TKA (1,2). Efforts should be made to minimize the risk of complications with appropriate patient selection and optimization, meticulous surgical technique, and careful postoperative management. As with other major non-cardiac surgeries, patients undergoing TKA are at increased risk of myocardial infarction, and this risk is highest between two and four weeks following the procedure (3). The risk is highest in patients aged 80 and over (4). Perioperative blood loss during TKA can be minimized by using protective techniques such as tourniquets and topical agents (5). The incidence of asymptomatic pulmonary embolism after TKA operation is 10-20%, while the incidence of symptomatic pulmonary embolism is 0.5-3% and the incidence of mortality is approximately 2%. The development of deep vein thrombosis with the potential for pulmonary embolism is reduced by prophylaxis (6). The most common serious neurological complication after TKA is peroneal nerve palsy (7). Clinical manifestations of peroneal nerve injury include paresthesia, numbness, and extensor weakness. Patients at the highest risk are those with severe valgus deformity or flexion

contracture. After surgery, the patient may develop peroneal nerve palsy due to swelling, hematoma, or direct compression of the nerve. Patients with previous spinal pathology may be more likely to develop peroneal nerve palsy due to the "double crush phenomenon". Initial treatment includes loosening tight dressings and flexing the knee up to 30 degrees to reduce pressure on the nerve (8). The use of tourniquets used to reduce intraoperative blood loss in TKA has been associated with ischemic injury. However, the tourniquet is quite safe when used at the lowest pressure and in the shortest possible time (9). Vascular injuries are rare in TKA (10). Wound healing problems may occur around the knee. Immediate diagnosis and intervention are required to prevent serious complications such as infection and possible implant loss. High-risk patients may require changes in wound closure technique and dressing type used. Surgical site infection or periprosthetic joint infection is a serious complication of TKA (11). Infections are considered acute if they occur within three to six weeks following the surgical procedure. Diabetic patients are at higher risk for superficial and deep surgical site infection compared to nondiabetic patients. Other risk factors for infection include obesity, smoking, malnutrition, and inflammatory arthritis (12). Intraoperative fractures and ligament injuries are rare complications of TKA (13). Implant loosening, which may occur in the postoperative period or later, is more common in patients under 50 years of age (14). Aseptic failure of TKA can occur through a variety of mechanisms, including osteolysis due to polyethylene wear and component loosening. When aseptic loosening is suspected, the most critical aspect of the examination is to rule out the possibility of prosthetic joint infection. Laboratory studies including serum erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are obtained. If any of these are elevated or there is a strong suspicion of infection despite normal acute phase reactants, a joint aspiration for cell count, Gram stain, cultures, and crystals is performed. Once the infection is ruled out, a patient with symptomatic aseptic prosthesis failure may be a candidate for revision TKA.

Instability after knee replacement is another important factor that can lead to revision surgery. instability; It may be associated with rheumatoid arthritis, connective tissue diseases, severe osteoporosis, neuropathy, muscle diseases, and obesity (15). Instability may also occur due to difficulty in stabilizing the intraoperative knee. Infections may be of hematogenous origin, typically months to years after TKA. Patellofemoral complications include patellar instability, loosening of the patellar component, patellar component failure, patella fracture, patella clunk syndrome, rupture of the extensor mechanism, and anterior knee

pain. Loosening of the patellar component may be associated with osteolysis or displacement of the prosthesis and is often secondary to another condition. Associated disorders include instability, fracture, component malposition, osteoporosis, avascular necrosis, and poor cement technique. Rupture of the extensor mechanism is a feared complication but fortunately rare (16). MARS, MRI scan, or CT scan can be used to diagnose extensor mechanism rupture. Surgical repair is the preferred treatment method (17). Patella clunk syndrome is clinically diagnosed and refers to a click that is felt while actively extending the knee at 60 to 30 degrees, resulting from the formation of fibrous tissue under the quadriceps tendon (18). Pain originating from the anterior part of the knee may be due to patella or patellofemoral joint pathology. Fractures may occur around the femoral component, a tibial component, or the patella. Displaced fractures are reduced and fixed with plates and screws or intramedullary rods. If the component is loose, it is revised. Tibial fractures around the TKA are rare. Like supracondylar femur fractures, these are treated either non-surgically or operatively, depending on the degree of displacement, alignment, and condition of the implant. While wear is expected due to friction between the femoral component and the polyethylene of the tibial and patellar components, faster wear may occur due to patient-related factors, surgical technique, prosthetic design, and quality control issues. Osteolysis or bone loss can occur as a result of polyethylene wear and is one of the causes of aseptic loosening. Arthrofibrosis refers to the restriction of the postoperative range of motion resulting from scar tissue formation that may result in functional impairment. Treatments include manipulation under anesthesia, arthroscopic lysis of adhesions, and revision knee replacement. Revision knee replacement is not often done to treat arthrofibrosis unless the stiffness is secondary to improper positioning of the implants. In a systematic review of high-quality prospective studies of patients with osteoarthritis undergoing TKA, approximately 20 percent of patients report moderate to severe knee pain after surgery (19). It is stated that some patients may have metal sensitivity and this may cause implant failure (20). It is important to manage the treatment quickly in wound problems to prevent infection and implant loss. Although the incidence of infection is low, it is a serious complication of TKA that can occur in the perioperative period or months to years later. Risk factors include diabetes, obesity, smoking, malnutrition, and inflammatory arthritis. Other rare complications include perioperative tourniquet-related ischemic injury and arterial injury. Intraoperative or postoperative periprosthetic fractures and ligament injuries are also rare. Periprosthetic fractures, which

are more common in patients with osteoporosis or rheumatoid arthritis, may occur around the femoral component, a tibial component, or the patella. Aseptic loosening, which can occur early or late, is one of the most common causes of failure and often requires revision surgery. Osteolysis or bone loss can occur as a result of polyethylene wear and is one of the causes of aseptic loosening. Joint instability is another important factor that may lead to revision surgery. Patellofemoral complications are another common reason for reoperation after TKA. These include patellar instability, loosening of the patellar component, patellar component failure, patella fracture, patella clunk syndrome, and rupture of the extensor mechanism. Arthrofibrosis refers to the restriction of the postoperative range of motion resulting from scar tissue formation and resulting in functional impairment. Treatments include manipulation under anesthesia, arthroscopic lysis of adhesions, and revision knee replacement. Manipulation under anesthesia is safest and most effective if done within the first three months postoperatively.

2. Common Complications in Total Hip Arthroplasty

Various femoral and acetabular implants are available for use in total hip arthroplasty (THA). There are differences in fixing type, design features, and materials. Bone ingrowth into or on the porous implant surface without the use of cement, in general, to fix prosthetic components to the bone, or alternatively, methylmethacrylate cement can be used to fixate the femoral and/or acetabular component in patients with poor bone quality. Most of the acetabular components used today are cementless, porous, and modular. Typically, a hemispherical porous metal shell is placed inside the acetabulum, and then a modular lining is placed inside the shell. The porous surface of the implant varies from manufacturer to manufacturer and may have a surface coating (such as hydroxyapatite) that promotes the ingrowth or growth of bone. An alternative to a cementless acetabular component is an acetabular component fixed to the acetabulum with cement. This type of acetabular implant has a higher overall relaxation rate than cementless implants. For elderly patients with poor bone quality, the use of all-polyethylene cemented acetabular implants is advocated (21) Due to osteolysis, a metallic (cobalt-chromium) femoral head covered with traditional high-density polyethylene has been replaced by acetabular lined ceramic femoral head prostheses made of highly cross-linked polyethylene today. Laboratory results demonstrate superior wear performance of highly cross-linked polyethylene compared to a metallic femoral head prosthesis

covered with conventional high-density polyethylene (22). In addition, clinical results have shown that highly cross-linked polyethylene has a longer lifetime compared to conventional high-density polyethylene in vivo (23-24). Ceramic femoral heads prevent the risk of conical corrosion that can occur with a metal head. Femoral components can be classified as cementless or cemented. A cementless femoral component is the implant of choice for younger patients and any patient with good bone stock. However, in patients with poor bone stock, such as elderly patients, a cemented implant is an appropriate choice. The porous coating may be limited to the proximal portion of the femoral implant or may extend over the entire length of the implant. The use of bone cement during arthroplasty may rarely cause bone cement implantation syndrome (BCIS), a poorly understood condition that can cause hypoxia and hypotension and result in cardiac arrest. It may be necessary to take measures such as providing a vent in the bone or applying minimal pressure to the cement. Today, it usually uses a ceramic or cobalt chrome femoral head. Ceramics can be preferred to reduce wear. The surgical approach may affect the type and incidence of some complications. There are methods such as the posterolateral approach, a direct lateral approach, an anterior approach, surgical navigation, and robotic surgical assistance (25). THA can involve significant intraoperative and perioperative blood loss. Tranexamic acid, an antifibrinolytic agent, is increasingly used in joint arthroplasty to help minimize blood loss (26). A deteriorating hip joint can cause a reduction in leg length. Most surgeons accept a small leg length disparity in exchange for a more stable and less dislocated hip. Intraoperative radiographs or fluoroscopy may be useful for measurements. The hospital stay following total hip arthroplasty is typically one to three days. However, the number of surgeons now safely performing this procedure as an outpatient is increasing (27). Postoperative management includes pain management, prophylaxis against venous thromboembolism, appropriate attention to medical comorbidities, and physical therapy. Many patients can be discharged home after surgery, but some may require temporary inpatient rehabilitation. Mobilization and physical therapy are initiated as soon as possible to facilitate recovery of function and help prevent deep vein thrombosis.

To restore normal hip motion and strength and gradually return to daily activities, exercises are started in the hospital and continued after discharge. This may include 20 to 30 minutes of exercises two or three times a day during early recovery. Typically, weight bearing is allowed as tolerated and an assistive device is used to assist balance and stability. Depending on the surgical approach

and the surgeon's protocol, some patients may need to take certain dislocation precautions. For example, patients given posterior hip dislocation precautions may be asked to avoid low chairs, not to bend their hips more than 90 degrees, and not to cross their legs. The exact recovery time varies greatly, but patients are typically in fairly good shape with minimal pain within three months of surgery. For patients at high risk for heterotopic ossification, prophylactic nonsteroidal anti-inflammatory drugs are recommended. External beam radiation is a reasonable alternative for patients who cannot tolerate NSAIDs or for whom NSAIDs are contraindicated. Both methods have been shown to reduce the incidence of heterotopic ossification in high-risk patients (28). Prophylactic use of a proton pump inhibitor may reduce the risk of NSAID-induced gastroduodenal injury and subsequent gastrointestinal bleeding. Although indomethacin is relatively inexpensive and readily available, some patients cannot tolerate the drug due to gastrointestinal side effects. Indomethacin is not a good choice for prophylaxis in patients with kidney problems such as chronic renal failure. These patients may be more suitable for external beam radiation (29). The treatment results of total hip arthroplasty show excellent clinical and functional results. TKA can be successfully applied in patients ranging from young to older adults (30). Patient satisfaction is generally high following the procedure. Most studies show that the risk of early revision due to infection increases in obese patients (31). Complications following TKA include complications that can occur with any major surgery, such as anesthesia, blood loss, or transfusion reactions, and complications specific to the hip arthroplasty procedure. Total hip arthroplasty consists of the resection of diseased articular surfaces of the hip and subsequent replacement with prosthetic hip components. For the correctly selected patient, the procedure results in significant pain reduction as well as improvement in function and quality of life. The most common indication for THA is for the relief of pain associated with hip osteoarthritis in patients for whom non-operative treatments have failed. Other conditions that cause pain and loss of function and may lead to the need for TKA include inflammatory arthritis, femoroacetabular impingement syndrome, developmental hip dysplasia, childhood hip disorders, trauma, neoplasms, and osteonecrosis. Active infection is perhaps the most important contraindication of THA. Other contraindications include pre-existing or significant medical problems, skeletal immaturity, quadriplegia, and muscle weakness. Careful consideration should be made to ensure the appropriateness of the surgery and to assist in surgical planning. Preoperative prophylactic antibiotics are given to reduce the risk of surgical site infection and prosthetic

joint infection. Postoperative thromboprophylaxis should also be applied in patients undergoing TKA. Postoperative care includes pain management, venous thromboembolism prophylaxis, and appropriate physical therapy. Mortality following THA is generally low and is primarily associated with pre-existing medical comorbidities.

3. Imaging Methods

Radiography; It is the imaging study of choice for routine evaluation of fractures, inflammatory and degenerative arthritis, metabolic bone diseases, and developmental disorders. CT can be useful in detecting cortical bone lesions and is widely used for fracture detection, especially in complex areas such as the cervical spine. Joint internal derangement and soft tissue injuries are best evaluated with MRI. MRI can evaluate all tissues associated with osteoarthritis and detect synovitis, erosion, and bone marrow edema in patients with rheumatoid arthritis. Positron emission tomography (PET) is important for evaluating the metabolic activity of tumors. Musculoskeletal ultrasonography can be used to evaluate soft tissues, cartilage, bone surfaces, and fluid-containing structures. It can also be used clinically to evaluate and monitor inflammatory arthritis, view tendons and bursae, and guide aspiration and/or injection of joints or soft tissues.

3.1. Radionuclide Imaging Methods in Total Knee and Total Hip Arthroplasty

Nuclear imaging modalities such as bone scans, labeled leukocyte scintigraphy, gallium scan, and positron emission tomography (PET) can be used to assess joint pain. PET imaging is performed with 2-(fluorine-18) fluoro-2-deoxy-D-glucose (FDG) or fluorine-18 sodium fluoride (NaF). Technetium-99m (Tc-99m) labeled diphosphonate in the form of methylene diphosphonate (MDP) or hydroxy methylene diphosphonate (HDP) for bone scintigraphy are the most widely used radiopharmaceuticals. The diphosphonate is adsorbed on the surface of the hydroxyapatite crystals.

3.1.1. 3-Phase Whole Body Bone Scintigraphy

3-phase bone scintigraphy shows signs of increased blood flow and blood pool activity in the acute phase of infection or inflammation. However, the intensity in the late phase of the scan is less than the degree of inflammatory activity. Other studies such as FDG-PET or labeled WBC scans may be more appropriate to

evaluate serial changes in inflammatory activity. Although the blood pool phase provides some information about the extent of inflammation, bone scanning is generally not useful for assessing the activity level of arthritis, as it cannot distinguish partially active inflamed joints from chronically damaged joints. Bone scintigraphy is useful in the evaluation of patients with a painful knee and hip arthroplasty. SPECT/CT is increasingly used in the evaluation of painful joint replacement. Labeled leukocyte scintigraphy is performed to evaluate infectious processes such as osteomyelitis or infected joint replacement. The patient's own white blood cells are labeled with indium-111 or Tc-99m and reinjected. FDG-PET is very sensitive in prosthetic joint infection. Aseptic loosening, instability, and infection are the main causes of TKA failure. Radionuclide imaging is helpful to evaluate a painful total joint arthroplasty. Radionuclide imaging plays an important role in evaluating total knee arthroplasty, but its specificity is low in distinguishing aseptic loosening from infection. Leukocyte/bone marrow scintigraphy is accepted as the gold standard in demonstrating infection. In periprosthetic infections, laboratory tests, serology, aspiration joint fluid, and microbiological tests are the most common ways to differentiate these conditions. Early and accurate diagnosis is very important for the timely initiation of antimicrobial therapy (32). The most accurate method to diagnose prosthetic infections is to isolate one or more organisms from tissues (33). Various nuclear medicine procedures can be used in patients with hip and knee arthroplasty to obtain information about painful joint replacement and its specific complications (34-35). It is important to distinguish between aseptic and septic loosening. Bone scintigraphy is more useful in total hip replacement (36). Three-phase bone scintigraphy using intravenously applied 99m TC-MDP can be easily applied in cases where the infection is suspected in total joint arthroplasty (37). Increased involvement is due to increased blood flow and new bone formation. Fractures, heterotopic ossification, neoplasm, arthritis, aseptic loosening, and infection cause an increased bone turnover. Bone scans may be positive for at least 2 years and 5 years due to physiological bone remodeling after total joint arthroplasty. It has been reported that more than 60% of the femoral components and approximately 90% of the tibial components show permanent periprosthetic activity in bone scintigraphy even more than 12 months after implantation (38). In 3-phase whole body bone scintigraphy, the first phase shows perfusion, the second shows relative vascularity, and the third shows osteoblastic activity. A positive bone scan may indicate loosening, infection, or a stress fracture. Three-phase bone scan has high sensitivity but poor specificity. Increased intake in

the first and second stages indicates hyperemia and increased blood, but these findings are not specific (39). Aseptic loosening is an inflammatory reaction to prosthetic components (40). This reaction damages bone and cartilage and activates immune cells. The increased inflammatory response leads to osteolysis and eventually to relaxation. In the case of infected TKA, characteristic findings show an increased involvement in all three phases of the disease (41). In 3-phase bone scintigraphy, the sensitivity is 88% and the specificity is around 90% in demonstrating infection in THA (36), these rates are lower in demonstrating infection in TKA; sensitivity is around 75% and specificity is around 55%. In asymptomatic patients, increased periprosthetic radiopharmaceutical uptake around the TKA usually persists for several years (36). The presence of dense focus after 6 months suggests postoperative loosening or infection, but false positive rates are high (42). 3-phase bone scintigraphy is often combined with other functional radionuclide imaging techniques such as 67Gacitrate scintigraphy and leukocyte scintigraphy, resulting in better specificity.

3.1.2. 67-Ga citrate Scintigraphy

Three-phase bone scintigraphy combined with 67-Ga citrate scintigraphy reflects inflammation and osteoblast activity, respectively. If gallium uptake is more intense in 67-Ga citrate scintigraphy, it is in favor of infection. If gallium uptake is less than the uptake in the bone scan or if there is no uptake in gallium, periprosthetic infection is excluded (43). Findings on gallium scans are secondary to the accumulation of leukocytes and may be positive in both infectious and non-infectious inflammatory lesions (44). Combined bone/gallium imaging increases the accuracy rate compared to bone scintigraphy alone.

3.1.3. Labeled Leukocyte Scintigraphy

Leukocyte labeling; is carried out with Indium-111 (111-In) or 99m Tc-hexamethyl propylene amine oxime (99m Tc HMPAO). The majority of labeled leukocytes are neutrophils. Neutrophils are usually absent in aseptic loosening. The major disadvantage of leukocyte scintigraphy is that leukocytes do not accumulate only in the infected. It is also found in the bone marrow. He reported approximately 100% sensitivity and 50% specificity in leukocyte-marked scintigraphy(45). Bone marrow imaging is performed with 99m Tc-sulfur colloid or 99m Tc-nanocolloid for fixed macrophages of the marrow to prevent uptake by the reticuloendothelial (46). Sensitivity increases with combined

imaging. Bone marrow scintigraphy combined with 111-In- or 99m Tc-labeled leukocyte scintigraphy has disadvantages such as time consumption, high cost, need for well-trained technicians, special facility and direct processing of blood and therefore the risk of contamination, potential misapplication among patients(47). Screening using mouse-derived antibodies and human antimurine antibodies can be triggered (48). It recommends the use of triphasic bone scintigraphy after culture negative in cases with suspected TKA infection. This rational approach avoids unnecessary radiation exposure. The mean sensitivity and specificity values of leukocyte/bone marrow scintigraphy in detecting infection were 70.57% and 94.6%, respectively. (49). Today, leukocyte/bone marrow scintigraphy is accepted as the gold standard for this purpose (49).

3.1.4. PET-CT

Fluor-18 fluorodeoxyglucose positron emission tomography (18F-FDG-PET) is primarily used for the localization of malignancy. It is also a glucose analog taken up by neutrophils and monocytes in the detection of infection or inflammation (50). In demonstrating periprosthetic infection, the specificity of PET-CT has been reported to be lower than WBC/Bone marrow SPECT/CT (79%, and 98%, respectively) (51.52).

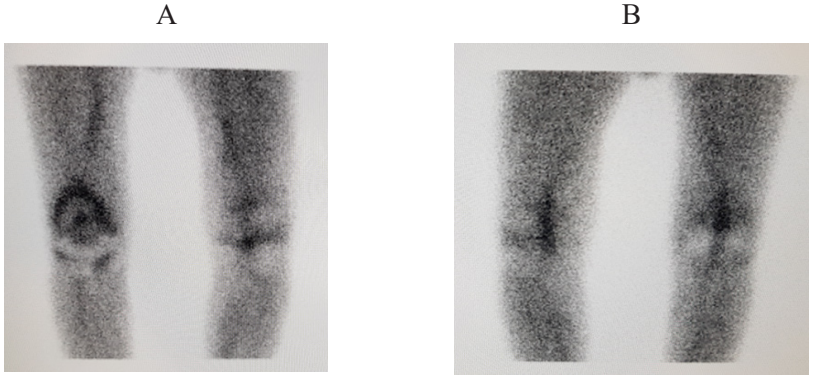

Figure 1: Increased Activity Uptake In the Blood Pool Phase Around The Right Knee Prosthesis In 3-Phase Bone Scintigraphy A: Anterior B: Posterior

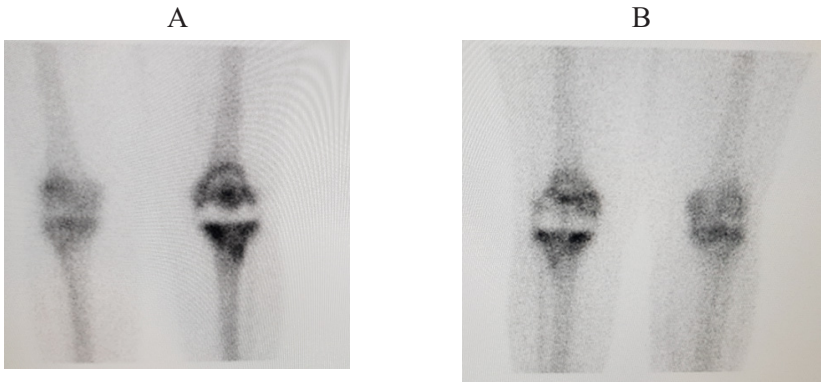

Figure 2: Increased Osteoblastic Activity Uptake Around The Left Knee Prosthesis In Bone Scintigraphy A: Anterior B. Posterior

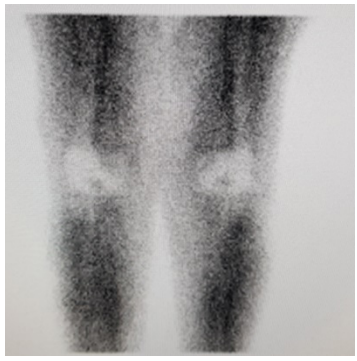

Figure 3: Photopenic Appearance Of Bilateral Knee Prosthesis In Blood Pool Phase In 3-Phase Bone Scintigraphy

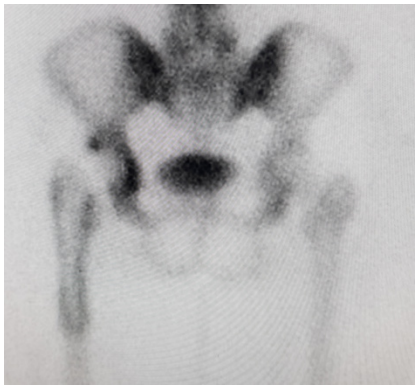

Figure 4: Increased Osteoblastic Activity Uptake Around The Right Hip Prosthesis On Bone Scintigraphy

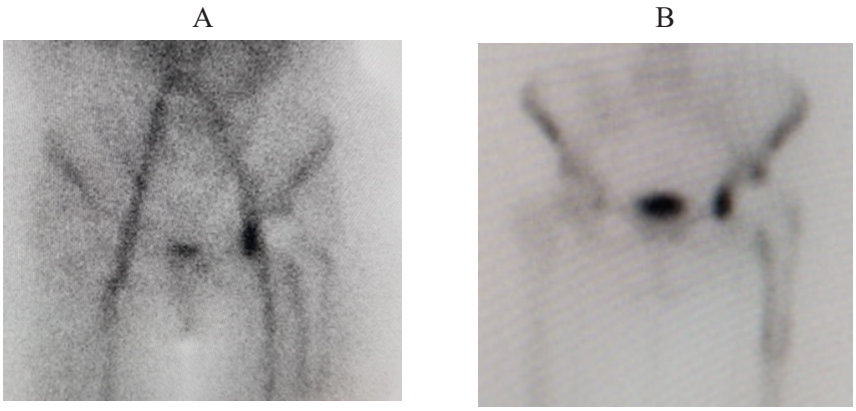

Figure 5: 3-Phase Bone Scintigraphy In Left Hip Prosthesis
(A: Blood Flow Phase B: Late Bone Phase)

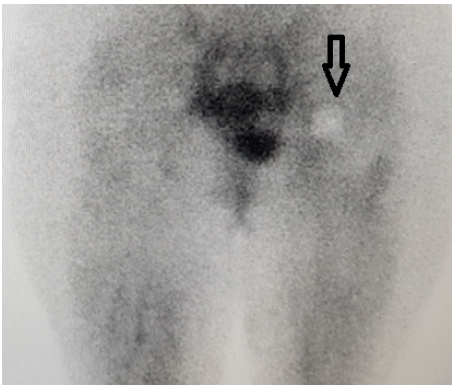

Figure 6: Photopenic Area Of The Left Hip Prosthesis In
The Blood Pool Phase In 3-Phase Bone Scintigraphy (black arrow).

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CHAPTER VII

CARBON MONOXIDE INTOXICATION

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1. Introduction

Carbon monoxide (CO) is a colorless, odorless, tasteless, and non-irritating gas that is the product of the incomplete combustion of hydrocarbons. (1,2) It is a life-threatening gas that can quickly diffuse in the air. It is lighter than the air, and according to some authors, it has a lavender-like odor. In addition, since CO has 210 to 280-fold more affinity to hemoglobin than oxygen, it can lead to cellular anoxia.

2. Epidemiology

CO intoxication (COI) is the most frequent intoxication in the United States (US). (4) Worldwide, COI accounts for more than half of all intoxication cases. The annual rate of emergency department (ED) presentations due to COI is approximately 5000/year in the US. (5) It is estimated that 5 to 6 thousand of these presentations result in mortality. (4) However, the mortality rates of patients presenting to EDs reduced starting from the 2000s. On the other hand, the annual mortality rate of suicide-related COIs is 15000/year corresponding to more than two third of all deaths. (5)

The COI results from the incomplete combustion of substances, including carbon. Charcoal, natural gas, gasoline, and petroleum are the most commonly used carbon-based fuels. Intoxication due to combusting charcoal in stoves occurs in winter months due to insufficient chimney aeration. Exposure to smoke during forest fires in summer, exposure of car mechanics to exhaust smoke in indoor facilities, incomplete combustion of charcoal during a barbeque, and use of water heaters with inadequate bathroom aeration systems are the other

routes of COI. Additionally, COI can occur in dye industry workers and those removing the surface paint via methylene chloride inhalation. The comparison of the impact of COI between smokers and non-smokers revealed that smokers had a more severe impact. (6)

Table 1. Carbon monoxide Resources

CO resource	
Endogenous	Normal heme catabolism by heme oxygenase
	Hemolytic anemia, sepsis, increase in the rates of severe respiratory disease
Exogeneous	Incomplete combustion of carbon fossil-based fuels
	Fires
	Exhaust smoke
	Vehicles operated by propane (forklifts, ice rink resurfacing tools)
	Bakery ovens operated by natural gas, countertop stoves and fireplaces
	Heaters
	Indoor barbeques
	Space heaters used for tent camping
	Exhaust smoke (motorboat)
	Cigarette smoke
	Methylene chloride

*CO: Carbon monoxide

Reference: 7

3. Pathophysiology

3.1. Binding of CO to Hemoglobin

It was previously postulated that COI's pathophysiology was based cellular hypoxia resulting from relative anemia due to CO-Hemoglobin (CO-Hb). (4) However, it is known that CO has a high affinity to Hb, which is much higher than that of oxygen. In addition, the affinity of CO to myoglobin is 60-fold higher than that of oxygen. Therefore, the oxyhemoglobin dissociation curve deviates to the left; oxygen cannot adequately penetrate the tissues resulting in tissue hypoxia. (6)

3.2. Direct Cellular Toxicity

It has been recently revealed that the pathophysiology of COI is more complicated than previously hypothesized, and more complex mechanisms

than CO-Hb-related hypoxia was involved. For example, an experimental study conducted with dogs showed that dogs inhaling 13% CO died within an hour with a mean CO-Hb level of 65%. On the other hand, transfusion of red blood cells containing 80% CO-Hb to healthy dogs did not lead to intoxication despite a blood CO-Hb level of 50%. (8 Today, COI's pathophysiology and clinical manifestations are explained by CO-Hb formation, direct CO toxicity at the cellular level, hypoxia, and ischemia. (4) (Figure 1)

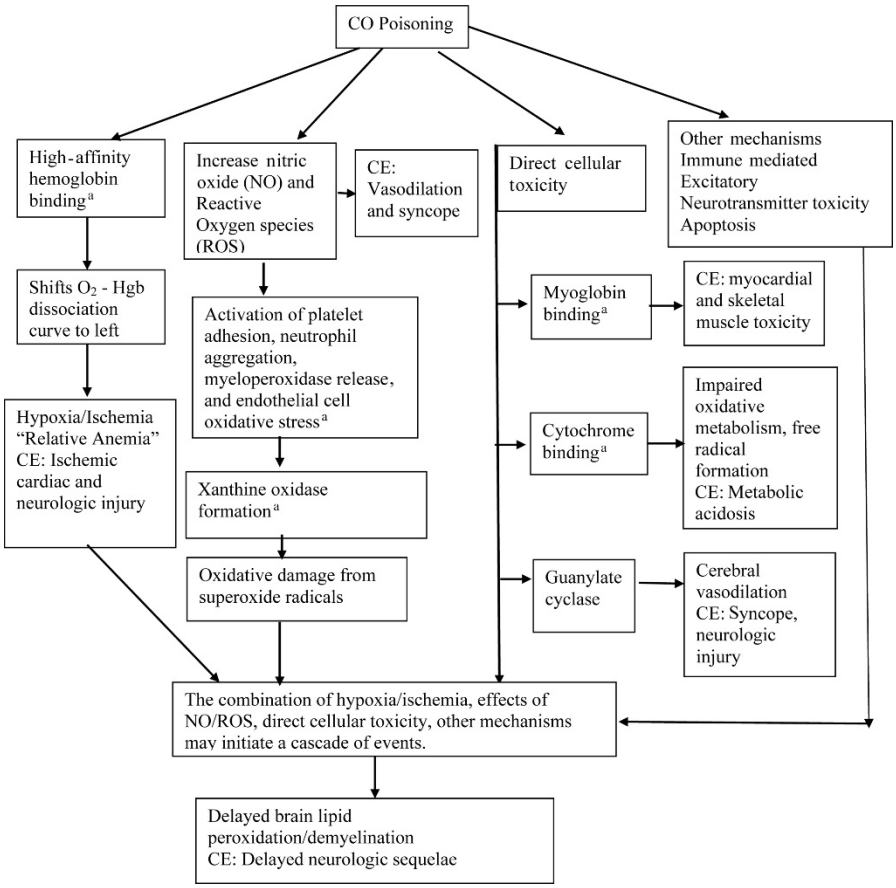

Figure 1. Pathophysiology of CO Intoxication

*CO: Carbon monoxide, CE: Clinical effect

3.3. Binding to Proteins (Cytochromes, myoglobin, guanyl cyclase)

The CO binds to heme-containing proteins such as cytochromes, myoglobin, and guanyl cyclase in addition to hemoglobin. In vitro binding of CO to cytochrome a3 leads to damage in the oxidative metabolism and formation

of free oxygen radicals. The inactivation of the mitochondrial enzymes and the impact of the free oxygen radicals on the electron transport chain can damage cellular respiration. Although the CO-Hb levels are normalized, cellular energy metabolism can remain inhibited. This finding explains the prolonged clinical effects despite normalized CO-Hb levels (4). The CO has a 60-fold higher affinity to myoglobin than oxygen. (9) The binding of CO to myoglobin can lead to arrhythmia and cardiac dysfunction by lowering cardiac oxygen levels. It can also result in rhabdomyolysis and myotoxicity in the skeletal muscles. An experimental animal study showed that CO induced the guanyl cyclase enzyme, increased the cGMP levels, and led to cerebral vasodilatation-related confusion. (4)

3.4. Nitric Oxide

The roles of nitric oxide (NO) and free oxygen radicals in COI were comprehensively investigated. (4-9) In experimental animal studies, it was shown that post-CO exposure vasodilatation-related confusion was associated with increased NO levels. Also, the syncope was suggested to be related to cerebral vasodilatation and cerebral blood inflow. The NO can also lead to systemic hypotension by causing peripheral vasodilatation. However, there is no study regarding the role of this effect in the setting of CO intoxication.

Nevertheless, systemic hypotension correlates with various cerebral lesions, such as watershed infarction. The NO has a significant role in the process of cerebral oxidative damage. In addition, it can be related to delayed neurological sequelae development. The post-CO intoxication cerebral lipid peroxidation is caused by changes in cerebral blood flow and oxidative free radical-related damage. Hypotension and confusion may facilitate lipid peroxidation.

In experimental studies, it was shown that NO synthetase inhibitors could inhibit both cerebral vasodilatation and oxidative damage. It has recently been suggested that delayed neurological sequelae occur due to immune-mediated mechanisms. Furthermore, it was shown that the experimental rats with immunological tolerance to myelin basic protein (mbp) before COI, started to develop difficulty in learning without any associated cerebral histopathological changes. According to this hypothesis, COI can lead to biochemical and antigenic changes in mbp, and the subsequent reactions with the lipid peroxidation products can trigger the immunological cascades. However, further studies are needed to fully explain COI's complex pathophysiology.

4. Clinical Findings

4.1. Acute Carbon Monoxide Intoxication

The COI is an intoxication that can imitate almost all other diseases and act like a “thousand faces player”. The CO intoxication cases, usually encountered in the winter months, imitate the symptoms of viral infections, which usually peak during the same period. (4) These cases can present with nausea, headache, dizziness, dyspnea, and tachycardia. (2) The most common symptoms are listed in Table 2.

Table 2. Signs and Symptoms of Acute Carbon Monoxide Intoxication

Signs and symptoms	
Severity	Signs and symptoms
Mild	Headache Nausea Vomiting Dizziness Blurred vision
Moderate	Confusion Syncope Chest pain Dyspnea Weakness Tachycardia Tachypnea Rhabdomyolysis
Severe	Palpitations Dysrhythmias Hypotension Myocardial ischemia Cardiac arrest Respiratory arrest Noncardiogenic pulmonary edema Seizures Coma

The COI usually starts with headache, dizziness, and nausea, and the organs sensitive to low oxygen levels, such as the heart and brain, are also affected if exposure persists (10).

Headache and dizziness are early neurological symptoms. Relatively prolonged exposure can lead to confusion, syncope, seizures, acute ischemic syndromes, and coma. (11) In brain imaging studies of these patients, bilateral globus pallidus lesions can be detected. (12) It was suggested that hypotension could be a sign of central nervous system involvement in CO intoxication cases. (13)

The spectrum of cardiac involvement is vast in COI cases. Patients may present with hypotension and tachycardia in the early periods or be afflicted with arrhythmia, myocardial infarction, or sudden cardiac death. (14) Hypotension can result from myocardial damage due to hypoxia and ischemia. Cardiac ion channels, peripheral vasodilatation, or their combination may be involved in this process. (15) In addition, COI can have a toxic effect on the skeletal muscles and lead to rhabdomyolysis and acute renal failure. (16) It has been reported that cutaneous bullae and non-cardiogenic pulmonary edema could be detected in several CO intoxication cases. On the other hand, “cherry-red” skin color is rarely encountered. (4)

The CO has a higher affinity to fetal hemoglobin than adult hemoglobin. Therefore, the binding of the CO to fetal hemoglobin in a pregnant woman puts the fetus at significant risk. (17) If left undiagnosed, this clinical condition can be life-threatening for the infant. (18) In addition, since the basal metabolic rates and oxygen consumptions of pediatric patients are relatively high, these patients are more sensitive to COI, and they usually present with non-specific symptoms. Therefore, the rate of misdiagnosis is relatively high in this patient population. (19)

In pregnant patients, COI leads to different clinical pictures based on the severity of maternal CO exposure. Stillbirth, anatomical malformations, and neurological sequelae can be encountered. (20) However, even in pregnant patients with mild COI symptoms, there may be severe fetal complications. While anatomical malformations can be encountered in the infants of pregnant patients exposed to CO during early pregnancy, exposure during later periods can lead to mild neurological developmental deficits and functional abnormalities. (21)

4.2. Delayed Intoxication

The impact of CO intoxication is not limited to the immediate post-exposure period. Especially neurological effects can also be encountered during the late periods. In the convalescence period of acute COI, behavioral and neurological

dysfunction can occur between the 2nd and 40th days of the latency period. This worrisome clinical picture is defined as “delayed neurological sequelae” (DNS). Symptoms encountered in patients with DNS are neurological and psychiatric symptoms such as memory loss, confusion, ataxia, seizures, urinary and fecal incontinence, emotional lability, loss of orientation, hallucinations, parkinsonism, gate disturbance, and motor dysfunction. While radiological brain imaging studies usually reveal pathological findings in patients with DNS, there are cases with normal imaging findings. (4) It is estimated that the incidence of post-CO intoxication DNS is between 1-47%. This wide range can be explained by the differences in the definitions of DNS. (22) In general, the rate of DNS is relatively higher in symptomatic patients. This rate is even higher in elderly patients with long-lasting unconsciousness and CO exposure. (23)

Different authors use various DNS definitions. While making these definitions, these authors refer to abnormal physical findings, neuropsychometric test findings, and their combination in addition to clinical symptoms. (24) Thus, no basic clinical definition can be used to compare COI patients. On the other hand, neuropsychometric tests provide clinicians with a tool to objectively identify and follow COI patients. (26)

4.3. Diagnosis

Diagnosis of patients with CO exposure depends on clinical suspicion. In prospective studies, it was shown that patients presenting to the emergency departments with non-specific symptoms without a known CO exposure had relatively high CO-Hb levels. (27) Since conventional pulse oximetry cannot differentiate CO-Hb from oxyhemoglobin, the oxyhemoglobin percentage can be falsely high. (5) Although finger-tip pulse oximetry devices can measure CO-Hb levels, they can only be used for screening since they cannot give absolute results compared to co-oximetry devices. (28)

In acute CO intoxications, co-oximetry should be used to measure blood CO-Hb levels. (5) The co-oximetry device measures the oxyhemoglobin, total hemoglobin, CO-Hb, and methemoglobin values. (29) There is no need to take an arterial blood sample for CO-Hb level measurements. In prospective studies, a strong correlation was found between arterial and venous CO-Hb levels. (29)

Since the CO-Hb level is Reduced after oxygen treatment following CO intoxication, it does not always correlate with the severity of the CO-Hb level. On the other hand, the basal CO-Hb level of non-smokers is between 1% and 3%, while it is anticipated to be in the range of 5-38% in smokers. (30) Also,

there is no correlation between the symptom severity and CO-Hb levels in CO intoxication patients. Therefore, periodic CO-Hb level measurements are not required during patient management. (32) The other tests that should be done in CO intoxication patients are complete blood test, electrolytes, blood urea nitrogen, cardiac biomarkers, creatinine, creatine phosphokinase, lactate, chest X-ray, electrocardiography (ECG), and brain tomography (Table 3).

Table 3. Clinical Care Points

Clinical care points	
Acute evaluation of the CO intoxication patient	Basic metabolic panel Complete blood count with differentials Liver function tests Blood carboxyhemoglobin level Troponin Creatine phosphokinase Lactate Chest radiography ECG CT scan of the brain
Evaluation for delayed neurologic sequelae	Neuropsychometric testing MRI of the brain

*CO: Carbon monoxide, ECG: Electrocardiogram, CT: Computerized tomography, MRI: Magnetic resonance imaging
Reference: 4

It was suggested that metabolic acidosis correlates with the severity of the clinical symptoms and development of sequelae in COI patients. (33) The lactate level is a better predictor of clinical severity than the CO-Hb level in severe intoxication cases. (34)

A high neutrophil count is also a marker of clinical severity in CO intoxication. (35) In CO intoxication, concurrent cyanide intoxication can occur if there is smoke inhalation. (36) In pregnant patients with CO intoxication, prenatal follow-up can aid in identifying potential anomalies. (37) However, cerebral damage biomarkers such as neuron-specific enolase and S-100 beta are rarely used in clinical practice in COI. (38)

The myocardium can be severely affected in COI since it is oxygen-dependent. Cardiac involvement can manifest as cardiomyopathy in the absence of coronary artery disease, arrhythmia, infection, and cardiogenic shock. (39)

Troponin is a better predictor of clinical severity than CO-Hb in CO intoxication patients. Therefore, ECG and troponin level analysis should be performed in patients suspected of COI. (40) Cardiac involvement is associated with mortality in COI patients. (41) In the brain tomography of CO intoxication patients, cerebral infarction signs and bilateral globus pallidus lesions can be detected. These lesions can be accompanied by white matter lesions. (42,43) It should be noted that globus pallidus lesions are not specific to COI cases, and they can also be detected in methanol and hydrogen sulfide intoxication cases. (44) The globus pallidus and white matter lesions can also be detected by magnetic resonance imaging (MRI). (45) The MRI findings in patients diagnosed with DNS include demyelinating lesions in the globus pallidus and white matter. (4)

In COI, neuropsychiatric tests are used for the determination of cognitive dysfunction. These tests include subtitles such as general intellect, reading comprehension, executive functions, visuospatial skills, motor speed, and dexterity. (46) These tests are commonly used in emergency departments. In COI, neuropsychometric tests are used to assess DNS rather than diagnose COI during the acute phase. (47)

5. Treatment

The current therapeutic management of CO intoxication includes ventilation with 100% oxygen or hyperbaric oxygen treatment (HBOT). (48) Initially, the patient should be removed from the CO source, and oxygen support should be started. For this reason, 100% oxygen should be given at a rate of 10-15L/minute by a non-rebreather mask. In severe CO intoxication cases, referral to the intensive care unit and mechanical ventilation should be considered. The vital signs should be followed closely, and the patient should be monitored regarding cardiac parameters and potential cardiac complications. The complications should be treated as early as possible. The patient's urine output should be followed, and fluid replacement should be performed accordingly. The patient should be given Trendelenburg position if there is hypotension. Intravenous normal saline infusion should be initiated in these patients, and vasopressors such as dopamine should be given if necessary. Norepinephrine can be preferred in patients with persistent hypotension. Benzodiazepines should be given to patients with neurogenic seizures. Other anti-epileptics, such as phenobarbital, can be added to the treatment regimen if the patient is unresponsive or the seizure recurs.

The half-life of CO is 5 hours (h) with spontaneous respiration in room air, 1.5 h while taking 100% oxygen, and 25 minutes while under HBOT with a 3 Barr pressure. (49) One of these treatment methods should be performed based on the patient's clinical status until the CO-Hb level is lower than 5%. The HBOT should be performed mainly in patients with confusion due to neurogenic damage, those with acidosis, and those with myocardial involvement. (50)

Since lactic acidosis facilitates the penetration of oxygen into the tissues, it should be corrected before the pH value is below 7,15. Also, some studies reported that hypothermia could reduce cortical cell damage and neuronal damage in patients with CO intoxication. (51)

Patients with CO intoxication can be treated with oxygen replacement given by a mask. This treatment should be given until the clinical findings resolve or the Hb level is below 5% in patients without cardiopulmonary complications. On the other hand, this treatment should be given until the CO-Hb level is under 2% in those with cardiopulmonary complications. In general, this treatment lasts for 4 to 6 hours.

It was shown that HBOT given during the first 6 hours could lead to a rapid symptomatic improvement and reduce mortality. This treatment is beneficial in the acute phase and is a practical approach for preventing neuropsychiatric symptoms. (52) Therefore, the HBOT should be started within the first hour of patient management. (53) In patients with persistent confusion, the HBOT should be repeated after 6 to 8 hours. (54)

The HBOT is not recommended if the patient is hemodynamically unstable, needs cardiopulmonary resuscitation, and has comorbidities such as chronic bronchitis or emphysema. (55,56)

Conclusion

The COI is one of the most common intoxications leading to ED presentations. It should be considered that COI cases can present with non-specific symptoms. The therapeutic management depends on the clinical severity and starts with taking the patient away from the CO source. The contraindications of HBOT should be kept in mind while managing these patients.

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CHAPTER VIII

SEPSIS: PATHOPHYSIOLOGY, DIAGNOSIS, AND TREATMENT

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1. Introduction

Sepsis is a life-threatening condition defined by an inflammatory response triggered by microorganisms and toxins in the body. The uncontrolled inflammatory response, which plays a role in developing and progressing sepsis, results in severe hypotension, metabolic acidosis, tissue damage, multiple organ failure, and even death (1). The medical term sepsis was first derived from the word "sepo," meaning "decay," about 2,700 years ago (2). The first definition of sepsis was made at the American College of Chest Physicians/Society of Critical Care Medicine (ACCP/SCCM) consensus conference in 1991. In this definition, sepsis was called"Systemic Inflammatory Response Syndrome (SIRS) developing with infection."In subsequent consensus conferences, many new concepts have been added to the definition of sepsis. In the latest definition, sepsis was defined as a life-threatening organ dysfunction induced by a dysregulated response of the host to the infection (3-6).

If the conditions are appropriate for both the host and microorganism, all existing microorganisms can initiate systemic infection and lead to sepsis in the host (6). Bacteria, fungi, viruses, and parasites can cause sepsis, with bacteria

being the most common cause. Although gram-negative bacteria are thought to be the primary cause of sepsis, studies have shown that gram-positive bacteria have a more significant impact on sepsis and that this impact increases over time (7-10).

It has been reported that sepsis affected an estimated 48.9 million people worldwide in 2017 and caused the deaths of more than 11.0 million people (11). According to data from a study conducted by the Turkish Statistical Institute (TUIK) between 2009 and 2016, sepsis cases constitute 60.46% of all infectious diseases in Turkey(12). Therefore, it is important to implement effective treatment to reduce mortality and morbidity in sepsis.

This book chapter covers general information about sepsis's epidemiology, etiology, and pathophysiology based on the terminology defined in 1991 and the expanded criteria for sepsis diagnosis at the 2016 conference. Additionally, essential points regarding sepsis prevention, diagnosis, and treatment are discussed in detail.

2. General Information

2.1. History and Definitions

Sepsis evidence dates to ancient times, and in the earliest written medical documents, sepsis was one of the leading causes of death (6). The term sepsis, first used in a medical context 2700 years ago, is derived from the word "sepo," meaning "decay." The term sepsis also appears in the Corpus Hippocraticum, a book by Hippocrates. Hippocrates defined sepsis as a dangerous biological change that can occur in the body (2). A Greek physician, surgeon Claudius Galenus (Galen of Pergamon), also conducted studies on sepsis.

In the past, it was believed that the decay of a wound caused sepsis due to contact with air and the resulting decay contaminating the blood. However, with the knowledge gained from the works of Koch, Semmelweis, Lister, and Pasteur, it was revealed that infection caused sepsis (13). In the 20th century (1914), bacteriologist Schottmuller's research showed that sepsis develops due to bacterial infection (14).

The first definition of sepsis was made at the ACCP/SCCM consensus conference in Chicago in 1991 (3). Subsequent conferences were held in 1992, 2001, 2005, 2008, 2012, and 2016, and sepsis-related definitions were made (Table 1) (4,6). Sepsis is a significant health problem that affects millions of people worldwide each year, and one-fourth of these people die

as a result. The frequency of sepsis is increasing daily (5). Therefore, the broad definitions made in various consensus conferences guide us in the early diagnosis and treatment of the disease (3). The definitions and criteria within the sepsis spectrum made in these consensus conferences are as follows (6,13):

***Infection*:** Invasion of sterile host tissues by microorganisms. An inflammatory response is not obligatory in infection (3,13).

***Bacteremia*:** Condition in which live bacteria are present in the blood. This issue should be demonstrated by culture (3).

***SIRS*:** It is the systemic inflammatory response to various clinical conditions not caused by infection or infection. Examples of clinical conditions not caused by infection are pancreatitis, ischemia, and hemorrhagic shock (3). In order to diagnose SIRS, two or more of the following clinical findings must be present in the patient (6).

- Heart rate > 90/min
- Body temperature > 38 °C or < 36 °C
- Respiratory rate > 20/min or PaCO2 < 32 mmHg
- Circulating leukocyte count > 12000 / µL or < 4000 / µL or greater than 10% of immature neutrophil granulocytes

Sepsis: If it occurs due to SIRS infection, it is called sepsis, and the presence of at least two of the four criteria given in the definition of SIRS is a sign of sepsis.

***Severe sepsis*:** It is sepsis-related hypoperfusion, hypotension, or organ dysfunction. Acute changes such as lactic acidosis and oliguria may occur with hypoperfusion.

***Septic shock*:** It is the development of hypotension, lactic acidosis, oliguria, and mental changes resulting from perfusion abnormalities. Hypotension does not improve despite fluid replacement therapy.

***Multiple Organ Dysfunction Syndrome (MODS)*:** It is a condition that develops in a patient with signs of sepsis, where homeostasis is impaired, and organs cannot perform their functions. Homeostasis does not improve without intervention (3). The changing definitions of sepsis between 1991 and 2016 are given in Table 1.

The changing definitions of sepsis between 1991 and 2016 are given in Table 1.

Table 1. Changing definitions of sepsis (5).

1. Definitions related to sepsis in 1991		
Terms	**Definitions**	**Comments**
Sepsis	SIRS by infection	The etiology of SIRS may be non-infectious. Regardless of organ dysfunction, the patient has SIRS in the presence of severe infection. Just SIRS + infection is called sepsis.
Severe sepsis	Presence of acute organ dysfunction with sepsis	There is a false impression that there should be sepsis, severe sepsis, and septic shock infection—organ failure with SIRS.
Septic shock	Sepsis with resistant hypotension despite fluid replacement therapy	Blood pressure is essential, but the metabolic component is not considered.
2. Definitions related to sepsis in 2001 (Definitions have not changed, the list of signs and symptoms of sepsis has been expanded.)		
3. Definitions related to sepsis in 2016		
Sepsis	Life-threatening organ dysfunction caused by the host's dysregulated response to infection	The infection may cause local organ failure without triggering a dysregulated host response.
Septic shock	Sepsis with persistent hypotension requires vasopressors to maintain mean arterial pressure above 65 mmHg and serum lactate level above 2 mmol/L despite adequate fluid replacement therapy.	The term "severe sepsis" was renewed considering circulatory and metabolic abnormalities. It should be confirmed that the sepsis criteria established in 2016 improve clinical outcomes.

2.2. Epidemiology

Due to the increasing incidence of mortality and morbidity and high treatment costs, sepsis is a severe health problem (6). In studies conducted between 1975-2015, it was estimated that the incidence of sepsis treated in hospitals was 288 per 100,000 people annually. When it is looked at 2005-2015, it was estimated that there were 437 cases of sepsis per 100,000 people annually. Based on these figures, the annual average of sepsis cases treated in

hospitals worldwide is 31.5 million. Moreover, most patients receive treatment in intensive care units (ICUs). Severe sepsis and septic shock occur in 10-20% of these patients. However, the total incidence of sepsis is highly variable, and this variability is due to data scarcity. Most of the available data comes from high-income countries, and there is limited data from middle- and low-income countries (15). According to a study in Turkey in 2018, 15.8% of 1,499 patients had an infection without SIRS, 10.8% had an infection with SIRS, 17.3% had severe sepsis without shock, and 13.5% had septic shock. Additionally, it was observed that the mortality rates of patients with severe sepsis and septic shock were higher than those with only an infection or infection with SIRS. According to data from a study conducted by the Turkish Statistical Institute (TUIK) between 2009-2016, sepsis cases accounted for 60.46% of infectious diseases in Turkey (12).

2.3. Risk Factors

Risk factors for sepsis, which are associated with both the patient's susceptibility to infection and the likelihood of acute organ dysfunction when an infection occurs, include the following:

- Presence of underlying malignancy,
- Age (Newborns and patients over 65 are in the risk group.),
- Chronic disease (Diabetes Mellitus, Chronic Kidney Failure, Congestive Heart Failure, Liver Cirrhosis),
- Drugs and diseases that weaken the patient's immune system (Hematological malignancies, neutropenia, dysproteinemias, corticosteroid, and immunosuppressive drug use.),
- The widespread body burns and trauma cases,
- Existing infections,
- Postpartum and septic abortion,
- History of hospitalization in intensive care,
- Excessive administration of blood, blood product, or parenteral fluid. (6,10,17)

2.4. Etiology

If the conditions are suitable for the host and microorganism, all existing microorganisms can initiate systemic infection and cause sepsis in the host (6). Bacteria are the leading cause of organisms that cause sepsis, followed by

fungi, viruses, and parasites. It was believed that gram-negative bacteria were predominantly responsible for sepsis between 1960 and 1980. By the mid-1980s, it was observed that sepsis caused by gram-positive bacteria emerged and gradually increased. Gram-negative and gram-positive bacteria that cause sepsis are listed in Table 2 (6-10).

Table 2. Bacteria causing sepsis. (6)

Gram-negative bacteria	**Gram-positive bacteria**
Escherichia coli	*Enterococcus sp.*
Klebsiella sp.	*Staphylococcus sp.*
Pseudomonas aeruginosa	*Streptococcus sp.*
Aerobic Gram-negative Bacilli	

The leading cause of fungal sepsis is Candida species. The mortality rate of these species is very high and accounts for 40% of all pathogens (8,9).

Urinary system infections, respiratory system infections, intravenous catheters, and intra-abdominal infections are important sources of sepsis, and urinary system infections are seen as the most common cause of sepsis. Intra-abdominal infections, on the other hand, occur with surgical intervention on the gastrointestinal tract or perforation at any point of the gastrointestinal tract, and sepsis arising from this gastrointestinal system are infections that cause severe sepsis in intensive care units. Anaerobic and aerobic bacteria are blamed for intraabdominal infections; *Bacteroides fragilis, Fusobacterium sp., and Lactobacillus sp.* are examples (6,8).

3. Sepsis Pathophysiology

Sepsis is a severe illness causing many deaths for a long time. A good treatment practice will be critical in reducing sepsis-related deaths. In order to implement this treatment effectively, a good understanding of the pathophysiology of the disease is required. Researchers have conducted numerous studies on the pathophysiology of sepsis and gained many insights. However, there are still unresolved issues in the pathophysiology of sepsis today (10,18).

Researchers have found that the host creates an effective defense against the pathogen, ultimately producing endogenous inflammatory mediators that harm the body. The pathophysiology of sepsis involves microbial pathogens and inflammatory response. The sepsis triad includes systemic inflammation, coagulation, and impaired fibrinolysis (18,19). Studies have shown that

traumatic injury and infection activate the humoral system and release cytokines in tissues. As a result, SIRS, hemostatic changes, and organ damage occur (19).

The first stage in the development of sepsis is the infection of the individual by a microorganism. The microorganism's entry into circulation cannot trigger sepsis alone; for this to happen, the pathogen's entry must activate the humoral system, and endogenous mediators must be released and activated (17,20). Endogenous mediators are the main factors in determining the course of the disease. Endogenous mediators cause organ failure and death due to their hemodynamic and vascular permeability-increasing effects on the entire system. Therefore, they are also known as endogenous toxins (21).

The immune system comprises the skin, mucosa, immunoglobulins, cytokines, phagocytic cells, and lymphocytes. The balance between the immune system and the virulence factor of the microorganism is important during the development of sepsis (6). The immune system's initial response to the pathogen entry occurs through phagocytic cells. Subsequently, immunoglobulins and immunocompetent cells are also activated (10).

Various antigenic structures and toxins of microorganisms initiate inflammation (19,22). Table 3 shows the etiologic agents of inflammation and their antigenic structures and toxins.

Table 3. Inflammatory agents, antigenic structures, and toxins initiate inflammation (19).

Inflammatory agent	**Antigenic structure and toxins**
Gram-negative bacteria	Endotoxin [Lipopolysaccharide (LPS), Lipid A]
Gram-positive bacteria	Peptidoglycan (PG), teichoic acids, capsule antigens, exotoxins
Fungus	Cell wall antigens
Other microorganisms and structures	Lipopeptides, flagellin, viral RNA, parasitic antigens

The endotoxin effect is the best-known bacterial antigen, and it activates endothelial cells, mononuclear phagocytes, and other cells, activating the complement system and the coagulation cascade. Among the inflammatory agents, endotoxins from gram-negative bacteria are the molecules that have been most extensively researched and are the triggers for sepsis (10,19). In patients with sepsis, the target organ is the vascular endothelium, and mediators such as tumor necrosis factor-alpha (TNF-alpha), platelet-activating factor (PAF),

thromboxane A2, endotoxin, interleukin-1 (IL-1), and nitric oxide (NO) are released. All these mediators affect the vascular system and increase endothelial permeability.

In contrast, toxic oxygen radicals and some lysosomal enzymes released from neutrophils also increase endothelial permeability (23). Activation of the complement system and increased vascular permeability lead to endothelial damage. The increase in endothelial permeability causes fluid to leak out of the endothelium, resulting in microthrombus formation and further damage to the endothelium. If the damage cannot be controlled, multiple organ failures can occur, with the lungs, heart, liver, and kidneys most commonly affected in sepsis. If the damage is not controlled, metabolic imbalances begin to occur (6,24,25).

Coagulation disorders are common in sepsis patients, so understanding the coagulation cascade is important for understanding the pathophysiology of sepsis (26). In sepsis, cytokines, especially IL-1 and IL-6, trigger coagulation. At the same time, a decrease in naturally occurring anticoagulants such as antithrombin, protein C, and tissue factor also triggers coagulation in sepsis. LPS activates coagulation pathways through tissue factors in mononuclear and endothelial cells and other microbial products. Then, tissue factor activates the proteolytic cascade, prothrombin converts to thrombin, and fibrin is formed from fibrinogen. However, in addition to fibrin formation, the fibrinolytic mechanism is insufficient, and there is an increase in fibrin production while a decrease in fibrin degradation is seen. As a result, fibrin clots form in blood vessels, leading to tissue perfusion disorders and organ failure (19,27-29). Complement-derived anaphylatoxin, complement 5a (C5a), protects the host against microorganisms by chemotaxis, activation of granulocytes and macrophages, and release of antimicrobial products from phagocytes with pro-inflammatory effects. However, excessive release of this anaphylatoxin can harm the host and lead to death. Excessive release of C5a occurs in the early stages of sepsis. Increased production of C5a leads to many harmful consequences of sepsis, such as apoptosis of thymocytes and adrenal medullary cells and cardiomyopathy (30).

Although microorganisms or toxins are the structures that cause sepsis, the development of sepsis ends with the host's excessive immune response to the microorganism, widespread inflammation, and multiple organ failure. Hemostatic imbalance, endothelial dysfunction, cardiovascular disorders, and intracellular hemostasis disorders accompany sepsis. The condition that

causes multiple organ dysfunction and failure, and ultimately death, which is common in sepsis, is cellular hypoxia and apoptosis (6,31).

4. Sepsis Diagnosis

There are no standardized diagnostic criteria for sepsis. Diagnosing a patient suspected of sepsis is based on laboratory findings, clinical conditions, and various scoring techniques. The scoring systems used include:

- Acute Physiology and Chronic Health Evaluation (APACHE),
- Simplified Acute Physiology Score (SAPS),
- Multiple Organ Dysfunction Score (MODS),
- Mortality Predict on Model (MPM),
- Logistic Organ Dysfunction Score (LODS),
- Sequential Organ Failure Assessment Score (SOFA),
- Quick-Sepsis Related Organ Failure Assessment Score(qSOFA).

Due to its ease of use and higher success rate, the qSOFA scoring system is used among these scoring systems. Many sepsis guidelines mention an early diagnosis to reduce sepsis mortality. The qSOFA scoring system is used to facilitate early diagnosis (6,32-34).

According to the consensus conference of the American College of Chest Physicians/The European Society of Intensive Care Medicine (ACCP/ESICM) held in 2016, if there is a suspected or proven infection, and the qSOFA score and SOFA score are ≥ 2, the patient is considered to have sepsis (4,6,35,36).

4.1. SOFA Score

SOFA (Sequential Organ Failure Assessment) score scoring criteria are listed in Table 4.

Table 4. SOFA score (37-39).

System	0	1	2	3	4
Respiratory PaO 2 /FiO 2 (mmHg)	≥ 400	< 400 MV with or without	< 300 MV with or without	< 200, and there is MV	< 100, and there is MV
Coagulation Platelet 10 3 / mm 3	≥ 150	< 150	< 100	< 50	< 20
Liver Bilirubin (mg/dL)	< 1.2	1.2-1.9	2.0-5.9	6.0-11.9	> 12
Cardiovascular Hypotension	MAP ≥ 70 mmHg	MAP < 70 mmHg	Dopamine < 5 or dobutamine (any dose) *	Dopamine 5.1-15 or adrenaline ≤ 0.1 or noradrenaline ≤0.1*	Dopamine > 15 or adrenaline> 0.1or noradrenaline >0.1*
Neurological GCS	15	13-14	10-12	6-9	< 6
Kidney Creatinine mg/dL or urine output	< 1.2	1.2-1.9	2.0-3.4	3.5-4.9 Flow rate < 500 ml/day	> 5 Flow rate < 200 mL/day

*It should be given at a dose of µg/kg/min for at least 1 hour. MV: Mechanical ventilation, MAP: Mean arterial pressure, GCS: Glasgow coma scale score.

If the SOFA score is ≥ 2, the patient is considered to have sepsis.

4.2. qSOFA Score

qSOFA (Quick Sequential Organ Failure Assessment) scoring criteria are listed in Table 5.

Table 5. qSOFA score (33).

Respiratory rate ≥ 22/min
Change in mental status (GCS ≤ 13)
Systolic arterial blood pressure ≤ 100 mmHg

Having at least two of the criteria is considered sepsis.

5. Sepsis Treatment

Sepsis treatment is multifaceted and requires eradicating the infection that causes sepsis, repairing organ dysfunction, and preventing tissue hypoperfusion (10). The 2016 Society of Critical Care Medicine / The European Society of

Intensive Care Medicine (SCCM / ESICM) sepsis-3 guidelines specify what needs to be done in the first 3 and 6 hours of sepsis treatment. In the latest 2018 update, sepsis has been regarded as a severe emergency, and it is recommended that what needs to be done in the first 3 hours should be done in the first 1 hour. In addition, it has been added that vasopressor support should be provided to patients who do not respond to fluid resuscitation and have a MAP > 65 mmHg (33).

Things to do in the first hour for the sepsis treatment are listed below (10,33):

- Lactate level should be measured.
- Blood culture should be taken in less than 45 minutes, and antibiotic treatment should not be delayed.
- Broad-spectrum antibiotics should be started.
- If hypotension is present or lactate > 4 mmol/L, crystalloid therapy should be started immediately (at least 30 mL/kg).
- Patients not responding to fluid resuscitation should be provided vasopressor supplementation to keep MAP > 65 mmHg.

Things to do in the first six hours are as follows (10):

- Vasopressor should be applied to stabilize MAP ≥ 65 mmHg.
- If hypotension persists despite fluid resuscitation or the first measured lactate is ≥ 4 mmol/L, tissue perfusion, and volume should be reassessed.
- If the first measured lactate is high, it should be measured again.

One of the most important steps in sepsis treatment is to ensure sufficient blood flow to the organs and perfuse them. In cases of hypotension where fluid resuscitation is ineffective without perfusion problems, vasopressor therapy should be applied within the first hour to raise the MAP above 65 mmHg. Dopamine should not be preferred in vasopressor therapy, and norepinephrine should be the first choice. The use of epinephrine to prevent resistant hypotension and the low-dose use of vasopressin to reduce the dose of norepinephrine is recommended (6).

It has been observed that the appropriate selection of antibiotics and early treatment reduce mortality in sepsis. If the infection is suspected in the patient, a culture should be taken first, and early prophylactic antibiotic treatment should be started. Empirical antibiotic treatment for sepsis varies depending on the suspected site and origin of the infection. If pneumonia is suspected and community-acquired, second or third-generation cephalosporin + macrolide

treatment is applied; if it is hospital-acquired, antipseudomonal beta-lactam/carbapenem + aminoglycoside treatment is used. If a urinary system infection is suspected and community-acquired, sulbactam-ampicillin/third-generation cephalosporin + aminoglycoside treatment is applied; if it is hospital-acquired, antipseudomonal beta-lactam + aminoglycoside treatment is used. If the skin and soft tissue infection are suspected and community-acquired, penicillin G + antistaphylococcal beta-lactam treatment is applied; if it is hospital-acquired, antipseudomonal beta-lactam/third-generation cephalosporin + aminoglycoside treatment is used. If intraabdominal and biliary system infection is suspected and community-acquired, metronidazole/clindamycin + quinolone/third-generation cephalosporin treatment is applied; if it is hospital-acquired, carbapenem + antifungal treatment is used. If the neutropenic infection is suspected, regardless of its origin, antipseudomonal beta-lactam/cefepime + aminoglycoside treatment is applied (6,40-42). Sepsis is mainly caused by gram-positive bacteria, followed by gram-negative bacteria, but fungal agents should also be considered in patients. Fluconazole, voriconazole, caspofungin, or amphotericin-B are used in sepsis with fungal-acquired (10,42).

Sepsis management involves antibiotic therapy, hemodynamic support, and adjunctive therapies. Vasoactive drugs such as adrenaline, noradrenaline, phenylephrine, and dopamine support blood pressure in septic patients, with noradrenaline being the first-line choice. Inotropic therapy with dobutamine may also be used in some cases. Corticosteroids should only be used in septic shock and not routinely (37-39).

Fever and coagulation disorders seen in sepsis pose a risk for bleeding. If the risk of bleeding is very high, platelet replacement therapy is recommended. In patients with low blood pressure and inadequate tissue perfusion, the hemoglobin (Hb) level is important. Erythrocyte suspension should be given to maintain the Hb level within a specific range (10).

In sepsis, confusion, metabolic acidosis, or shock increase the breathing workload and cause respiratory distress, in which case mechanical ventilation should be applied to the patient. Patients in the ICU are also at risk for deep vein thrombosis (DVT). For this, subcutaneous low molecular weight heparin (LMWH) should be preferred (10).

5. Conclusion

The terminology that was defined for sepsis in 1991 remains valid today. However, in subsequent conferences held in 1992, 2001, 2005, 2008, 2012, and

2016, further definitions of sepsis were provided, and the criteria for diagnosing sepsis were expanded. Despite all these expanded criteria and treatment protocols, it is still observed that sepsis, which can start from bacteremia and lead to septic shock, organ failure, and even death, continues to have a high mortality and morbidity rate. The scientific community continues researching the unknown aspects of sepsis pathophysiology and daily adds new information to the literature. The added information plays an important role in the early diagnosis of sepsis. It is seen that early diagnosis is crucial for prognosis in sepsis, and it is necessary to monitor the possible signs and symptoms of sepsis closely.

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CHAPTER IX

AQUASOMES: A NOVEL CARRIER FOR IMPROVED DRUG SOLUBILITY AND PERMEABILITY

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1. Introduction

Aquasomes are a type of nanoparticle that can be used as a carrier system for drugs or other biomolecules (1). They are made up of three layers: an inner core layer, which helps to maintain the molecular conformation and therapeutic activity of the drug or biomolecule, and provides additional protection during transportation, coated with a carbohydrate layer, allowing for targeted drug delivery and improved stability, and an outer layer that contains the drug or biomolecule (2,3). A diagrammatic representation is shown under the preparation section. Aquasomes can be likened to "water bodies," as their water-like characteristics safeguard and maintain biological molecules (2). Additionally, the use of aquasomes reduces the risk of side effects and ensures optimal pharmacological activity (1). Due to increased demand for safer and more effective drug delivery systems, aquasomes are a promising option not only for oral delivery which represents over half of the drugs on the market (4) but for other routes also.

2. Preparation of Aquasomes

The method of preparing Aquasomes involves three steps: the formation of an inorganic core, coating the core with a polyhydroxy oligomer and loading the desired drug. The first step involves the fabrication of a ceramic core, which can be made from materials such as calcium phosphate or diamond. These cores are usually fabricated by methods such as colloidal precipitation, sonication, inverted magnetron sputtering or plasma condensation. Ceramic materials are widely used for core fabrication due to their high level of structural regularity and preservation of bulk properties when surface modification is done. The precipitated cores are centrifuged, washed, resuspended in distilled water and passed through a fine membrane filter to collect the particles of desired size (5).

In the second step of the aquasomes preparation, ceramic cores are coated with a carbohydrate (polyhydroxy oligomer) by adding it to an aqueous dispersion of the cores and subjecting them to sonication. This is followed by lyophilization to promote the irreversible adsorption of the carbohydrate onto the ceramic surface. The unadsorbed carbohydrate is then removed by centrifugation (5). In the final step, the drug is loaded onto the coated particles by adsorption. This is done by creating a solution of the drug in a suitable pH buffer and adding the coated particles to it. The mixture is then left overnight at a low temperature for drug-loading or lyophilized to obtain the drug-loaded formulation (6).

Aquasomes were first prepared by a technique called the "reverse phase evaporation" method. This method involves mixing a water-soluble polymer, like polyethylene glycol (PEG), with a lipophilic compound, such as a phospholipid, to form a water-in-oil emulsion. High-pressure homogenization is then applied to the emulsion to create small vesicles, which are stabilized by the PEG coating on their surface (7,8).

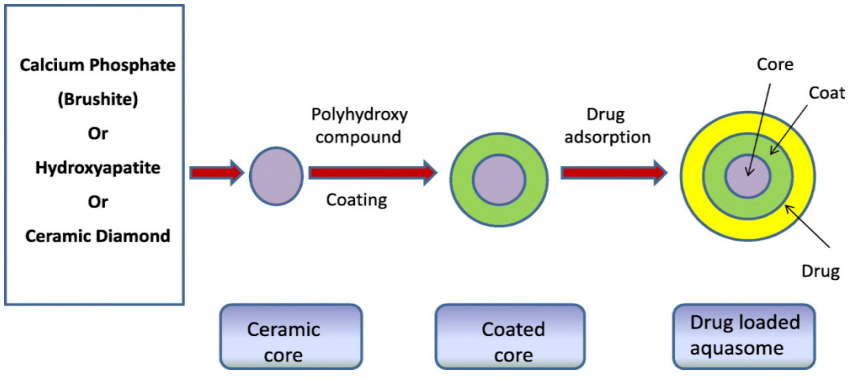

Figure 1. Illustration of the preparation of Aquasomes (9).

3. Characterisation of Aquasomes

Aquasomes are primarily defined by their structure and shape, the distribution of particle sizes, the ability to load drugs, the composition of the ceramic core, and the distribution of core sizes (10). The morphological properties and size distribution of aquasomes can be analysed using various techniques such as scanning electron microscopy (SEM) and transmission electron microscopy (TEM). These methods are employed to study both the uncoated ceramic core and the drug-loaded aquasomes. In addition, photon correlation spectroscopy can be used to determine the mean particle size and zeta potential of the particles, which provides information about their stability and behaviour in suspensions. The combination of these analytical techniques enables the comprehensive characterization of aquasomes and provides valuable information for optimizing their use in drug delivery systems (11,12).

Fourier-Transform Infrared (FTIR) spectroscopy is a technique used to analyse the structure of aquasomes. By using the potassium bromide (KBr) sample disk method, the structure of the core material can be analysed and compared to reference peaks. FTIR not only provides a structural analysis of the core but also helps in the identification and confirmation of coating sugar and loaded drugs. The presence of polysaccharide coatings over the core particles causes shifts in the observed IR peaks, indicating the formation of hydrogen bonds between the ceramic core particles and the polyoligomer coating (13,14).

The glass transition temperature (Tg) of the coating material used in aquasomes can be accurately determined through the use of differential scanning calorimetry (DSC). This technique is commonly used for evaluating the Tg of carbohydrates and proteins and is highly applicable for the analysis of aquasomes formulations. During a DSC study, a sample cell filled with the aquasomes formulation, and a reference cell filled with a buffer is placed in a DSC analyser. The results of the DSC analysis provide valuable information regarding the purity, compatibility, and effect of different components on the aquasomes, allowing for a comprehensive evaluation of the system's properties (15).

The crystalline nature of the ceramic core of aquasomes is evaluated using X-ray diffraction (XRD). This technique matches the XRD patterns of samples with reference diffractograms to determine the crystalline lattice arrangement or amorphous characteristics of the core. The XRD pattern of the calcium phosphate core displays intense and sharp peaks, indicating its crystalline state. However, after coating with different polysaccharides such as trehalose, cellobiose, and

pyridoxal-5-phosphate, the sharp peaks reduce in intensity and deform into an amorphous form (12).

3.1. Drug Loading Capacity

The drug loading capacity of aquasomes is evaluated to determine the amount of drug bound to the particles. This is done by incubating plain aquasomes formulations with a precisely weighed amount of the drug for 24 hours at 4°C. The excess drug is then removed through high-speed centrifugation for one hour, and the remaining amount of drug in the supernatant can be assessed using appropriate analytical techniques, such as High-Performance Liquid Chromatography (HPLC), UltraViolet (UV) spectrophotometry, etc (16).

3.2. In Vitro Drug Release

The results of in vitro release studies on aquasomes can vary greatly depending on various factors. For example, a previous study found that about 90% of ovalbumin was released from aquasomes coated with trehalose after 50 minutes (17). However, another study found that over 95% of recombinant human interferon-α-2b was released from aquasomes coated with trehalose, cellobiose, and pyridoxal-5-phosphate after 4, 6, and 8 hours respectively. These differences in release patterns could be attributed to the unique properties of the drugs, as well as the materials used in the preparation of the aquasomes (13).

4. Pharmacokinetics of Aquasomes

The small size and unique properties of the aquasomes enhance their ability to permeate cell membranes and reach target sites in the body. Aquasomes have been found to improve uptake compared to other drug delivery systems (18). The improved permeation of aquasomes leads to a more efficient distribution of the drugs to target sites in the body (19). This can help to increase the efficacy of the drugs and reduce unwanted side effects. The effect of aquasomes on drug metabolism is complex and depends on various factors such as the chemical structure of the drug, the activity of the enzymes, and the physiological conditions of the body (20). In some cases, the use of aquasomes can even help to reduce the metabolism of drugs by protecting them from enzymatic degradation. This can help to increase the therapeutic effect of the drugs and reduce toxicity. Elimination of aquasomes is dependent on renal excretion, biliary excretion, and elimination through the faeces (21). The biodegradation of calcium phosphate

ceramic in vivo is mediated by the coordinated activity of several cell types, including monocytes, macrophages, and fibroblasts. These cells play a crucial role in the initial inflammatory response and the subsequent resorption of the biomaterial and are responsible for the degradation of ceramics (3). The use of aquasomes in clinical practice is still limited, and more research is necessary to evaluate their safety and efficacy in different applications.

5. Innovative Applications of Aquasomes

Cherian *et. al*. developed aquasomes for insulin delivery, utilizing a calcium phosphate ceramic core. The core was then coated with a range of disaccharides including trehalose, cellobiose, and pyridoxal-5-phosphate, followed by drug loading through adsorption. The efficacy of these formulations was evaluated in albino rats, and the results showed that the pyridoxal-5-phosphate-coated particles were the most effective in reducing blood glucose levels. This superiority can be attributed to the high structural stability provided by the pyridoxal-5-phosphate coating, leading to a slow release of the drug and maintaining the structural integrity of the insulin peptide (22). These findings demonstrate the potential of aquasomes as a promising drug delivery system for proteins (23).

In antigen delivery, aquasomes have been shown to elicit a strong and specific immune response by minimizing the surface-induced denaturation of adsorbed antigens (24). In another study, the formulation of bovine serum albumin (BSA) prepared with aquasomes showed a significantly stronger immunological response compared to plain BSA following subcutaneous injection. These results highlight the potential of aquasomes in preserving the structural integrity of protein antigens, thereby enhancing their presentation to immune cells and eliciting a stronger immune response (11). For gene therapy, aquasomes have been proposed as a potential delivery system that can protect and maintain the structural integrity of the gene segment, while avoiding the risks associated with viral vectors (25).

Vengala *et al*. developed ceramic nanoparticles of piroxicam and evaluated their release profile in vitro. The results showed that trehalose-coated piroxicam nanoparticles demonstrated controlled release, while uncoated particles released 90% in 1 hour (26). In the same study, lactose-coated ceramic nanoparticles were also developed for the oral delivery of the hydrophobic drug pimozide. The calcium phosphate ceramic core was prepared using various methods, and the best method was selected based on the percentage yield and preparation

time. The in vitro dissolution of the drug-loaded formulation was compared with the pure drug, and it was found that the dissolution results were improved in the former. The release of pimozide from the aquasomes followed first-order kinetics. In another study, Nanjwade *et al*., 2013, formulated and evaluated etoposide-loaded aquasomes. The results showed that the drug release from the aquasomes increased with an increase in carbohydrate concentration. In vivo studies were conducted and it was found that the maximum percentage of the injected dose was in the liver, followed by the spleen, lungs, and kidney, indicating that the drug can be targeted to specific organs (27).

Table 1. Other Applications of Aquasomes

Biomolecule	Application	Results	Reference
Haemoglobin	Oxygen carrier	High potential as an artificial blood substitute	(28)
Hepatitis B serum Antigen	Vaccine delivery	Eliciting combined immune response	(29)
Lornoxicam	Lipophilic drug delivery	Enhanced the dissolution with a better release profile	(14)
Interferon	Protein delivery	Prolonged release	(13)

Improving solubility & permeability with Aquasomes

Approximately ~70% of new drug candidates have shown poor aqueous solubility, and ~40% of marketed drugs for oral use are identified to be practically insoluble in aqueous media (<100 μg/mL) (30). Aquasomes are self-assembled structures that are held together by various types of non-covalent bonds, such as ionic bonds and Van der Waals forces (31). The carbohydrate coating on the surface of the aquasomes creates a water-like environment that protects the bioactive molecules from dehydration, by keeping them hydrated (22). This unique feature of aquasomes not only improves the stability of drugs but also increases the solubility of hydrophobic drugs. The water-soluble carbohydrate coating on the surface of aquasomes allows hydrophobic drugs to be dissolved in the aqueous environment (9). Furthermore, the coating protects the drugs from precipitation, aggregation, and degradation, thus increasing their stability and bioavailability (1). Lastly, the steric hindrance of drugs in aquasomes enables them not to be recognised by the reticuloendothelial system, prolonging their

time in the body, and increasing penetration into target tissues and organs (32). This makes aquasomes an attractive drug delivery system, especially for poorly water-soluble drugs, as they can help to improve the bioavailability and efficacy of the drugs.

6. Enhancing Molecule Stability with Aquasomes

Aquasomes as a drug delivery system have shown promising results in protecting bioactive molecules. Unlike prodrugs and liposomes, aquasomes are less susceptible to destructive interactions between the drug and the carrier. This is due to the solid carrier being treated with a carbohydrate film that acts as a protective barrier (3).

Furthermore, aquasomes are efficient in maintaining the optimal three-dimensional conformation and pharmacological activity of the active ingredient (33). Active ingredients require the preservation of their unique conformation, internal molecular rearrangement, and bulk movement for optimal efficacy. Dehydration, degradation, and decomposition can alter these spatial qualities, leading to degradation, denaturation, and altered chemical composition (34).

Incorporating biological molecules on aquasomes with natural stabilizers such as sugars and polyols helps to preserve the molecular conformation and prevent adverse or allergic reactions. These natural sugars act as dihydro protectants and stabilize proteins against denaturation by acting similarly to water molecules and preserving the aqueous environment of proteins. The high concentration of hydroxyl groups in these disaccharides replaces water around the polar residues, thus maintaining their integrity (35).

7. Limitations and Challenges

Despite the many benefits and advantages that aquasomes offer as a drug delivery system, there are still some limitations and challenges that must be addressed to make the widespread use of this technology a reality. One of the major limitations of the aquasomes is their stability under storage conditions, as they can easily be degraded or altered by environmental factors such as temperature, pH, or exposure to light (3). Additionally, the scalability of production can also be a challenge, as the process of synthesizing and producing large quantities of aquasomes can be time-consuming and costly (2). Furthermore, the regulatory approval process for using aquasomes as a drug delivery system can also be lengthy and challenging, as many safety and

efficacy standards must be met to receive approval for use (1). Despite these limitations, research is ongoing to improve the stability and scalability of aquasomes, and to find new and innovative ways to overcome the challenges posed by this technology.

8. Conclusion

Aquasomes are a type of nanoparticulate carrier system that has been developed as a delivery system for bioactive molecules such as peptides, proteins, hormones, antigens, and genes to specific sites. Aquasomes comprise three main self-assembled structures, consisting of a solid phase nanocrystalline core coated with an oligomeric film to which biochemically active molecules can be adsorbed, with or without modification. These structures are self-assembled by non-covalent and ionic bonds. The solid core provides structural stability to the Aquasomes, while the carbohydrate coating protects against dehydration and stabilizes the biochemically active molecules. They have a larger surface area, volume, and mass ratio which enables the drugs to penetrate cells and provides a sustained release profile. Additionally, aquasomes possess high mechanical strength, and minimal biodegradability during storage. These features make aquasomes a particularly noteworthy and compelling option for drug delivery. The development and large-scale manufacture of Aquasomes present some challenges. Despite these challenges, Aquasomes have demonstrated promising potential in various fields of pharmacy.

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CHAPTER X

CERVICAL SPINE TRAUMAS AND TREATMENT

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1. Introduction

The cervical spine is divided into two sections as the craniovertebral junction (CVB) and subaxial cervical spine. The region that includes C1, C2, and the occiput is called the CVD and the lower cervical region from C3 to C7 is called the subaxial cervical spine. C1 and C2 spines have atypical anatomy. The C1-C2 joint is responsible for 40 degrees of rotation and 20 degrees of flexion/extension of the neck. The vertebrae, anterior and posterior bone elements, intervertebral discs, joint capsules, ligaments and surrounding neurovascular structures in the lower cervical region are similar to each other. The subaxial cervical spine provides an average of 6 degree rotation at every level (3-7 degrees) and an average of 16 degree flexion/extension at every level (9-20 degrees). The structural and functional differences may cause different damages in the regions in traumas.

Plain radiography is the first choice in diagnosis. Computed tomography (CT) provides detailed evaluation in the detection of central canal compression, lamina fractures, pedicle fractures and facet fractures. With the development of technology, the presence of CT in every hospital allows us to use CT as the first choice in daily practice. Magnetic resonance imaging (MRI) is used to determine whether surgical treatment is necessary in fractures. The posterior ligamentous complex is evaluated with MRI to determine if surgery is required. In fractures involving the vertebral artery, CT angiography must be performed to evaluate vascular injury.

2. Upper Cervical Traumas (Craniovertebral Junction Fractures)

Craniocervical junction fractures are usually common in young adults and in motor vehicle accidents (1). The main purpose in the treatment of fractures in this region is to prevent neurological deficits and provide spine stability by protecting the motion functions of vertebrae as much as possible (2). Among the post-trauma pathologies that may occur in this region are atlanto-occipital dislocation, occipital condyle fractures, atlas and axis fractures, odontoid fractures, hangman fracture, and atlantoaxial subluxation (3).

2.1. Atlanto-Occipital Dislocation

The ligament located in the craniovertebral junction creates a very stable structure that connects atlas and occipital bone (4). The cardiovascular structure of adults is more stable than that of pediatric patients (5). CVB injuries are more common in motorcycle accidents, especially in pediatric patients (5). In radiological diagnosis, the Powers ratio BC (Basion-Atlas Posterior Arch)/AO (between Atlas anterior arch and opisthion) ratio must be between 0.7 and 1 (6). Anterior subluxation has occurred if the ratio is >1 and there is posterior subluxation if >0.7. Clinical examination may show signs of brainstem lower cranial nerve involvement. Vincent et al. classified the atlanto-occipital dislocation in 3 groups (7).

2.2. Occipital Condyle Fractures

These fractures are very rare with a rate of 0.4-0.7% (4). In 1988, Anderson and Montesano classified these fractures into 3 groups. Type I and II fractures are considered stable fractures. And followed up by a Philadelphia collar. Type III fractures are unstable and require surgical treatments (5).

2.3. Atlas Fractures

2% of spinal fractures accounts for 10% of cervical fractures. These fractures occur as a result of axial loading. Landells and Van Peteghem divided these fractures into 3 groups (6). In these fractures, a transverse ligament tear may have occurred if the lateral displacement of the C1 lateral mass over the C2 lateral mass is longer than 6.9 mm (7). In such cases, transverse ligament must be evaluated with Stir sequence Magnetic Resonance MRI. According to the Landell classification, Type II (burst or Jefferson's fractures) fractures may require surgery if there is any damaged to the transverse ligament. Type I and III are followed up by a collar.

2.4. Atlanto-Axial Instability

The atlantoaxial joint, located between the C1 atlas and C2 axis vertebrae, is one of the most mobile joints. The mobility is ensured by odontoid process, atlas, axis and ligaments. A possible pathology in the bones, joints and ligaments that provide this movement prevents the rotation of neck. Idiopathic, traumatic, inflammatory, tumoral or congenital causes may disrupt this joint structure (8). Under normal conditions, transverse ligament is damaged and there is instability in this region if the distance between the posterior cortex of the anterior arch of the atlas and the anterior cortex of the densin (Atlantodental interval-ADI) is longer than 5 mm in children and 3 mm in adults (8). Deterioration of the atlantoaxial joint and torticollis occurs clinically after ligamentous laxity. Posttraumatic AAS is a common cause of childhood torticollis. The Hawkins-Fielding classification is the most widely used classification in clinical practices (9).

2.5. C2 Fractures

C2 fractures are discussed in 2 parts as odontoid and pars fractures (Hangman Fractures). The odontoid process on the C2 axis plays an important role in the rotation of the head by making a complex connection with the C1 atlas. Due to the strong ligamentous support in this region, C2 fractures occur as a result of high-energy trauma (10). Odontoid fractures constitute 20% of the entire spine (11). The Anderson-D'Alanzo classification is the most commonly used grouping tool for such fractures. This classification divides odontoid fractures into 3 groups. Type I fractures are considered stable fractures. Type 2 fractures are the most common. Surgical intervention is recommended for Type 2 and Type 3 fractures.

C2 vertebra pars interarticularis-istmus fractures are also called Hangman's fractures. This fracture causes C2 to slide forward onto the C3 spinal bone. Levine and Edward classification, which is a modification of Effendi's classification, is the most used grouping tool (3).

3. Subaxial Cervical Traumas

The classifications for subaxial spinal traumas have been available since 1970 (12). These classifications are based on the anatomical description of injury mechanism (13). The classification of subaxial cervical spine injuries (SLIC), which was first defined by Vaccaro et al., is today the most preferred

classification (16). The condition is evaluated and scored under 3 sections as morphology of injury, state of disco-ligamentous complex and neurological status. Conservative treatment is given to patients who score 3 points or less according to the SLIC classification. The prospective conditions of patients with score 4 are surgeon-optional. Conservative and surgical treatment can be done. Surgical treatment is recommended for patients with ≥ 4.

4. Conclusion

Type 3 condyle fractures in the upper cervical spine, Atlanto-occipital dislocations (Type 1-3), the fractures with total Atlas lateral masses ≥ 6.9 mm, A-D distance ≥5 mm, Odontoid Type 2 fracture, displaced (3 mm≥) C2 listezis (Levin&Edward) Type 2a, 3), Atlanto-axial combined fractures and tear fracture (flexion type) require surgical treatment. In the subaxial cervical spine (C3-C7), surgery must be performed in patients with a >4 scores according to the SLIC classification.

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CHAPTER XI

POLYCYSTIC OVER SYNDROME

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1. Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous group of diseases with unclear etiology and is the most common cause of menstrual irregularity and androgen elevation in women. Clinically, ovulatory dysfunction, excess androgen, an ultrasonographic finding of polycystic ovary, and obesity are the most prominent symptoms. PCOS is a condition that affects 6% to 8% of women of reproductive age (1). It is a complex disease that develops due to endocrine dysfunction between the central nervous system, pituitary, ovaries, adrenal glands, and extra glandular tissues; it can occur in any period of reproductive life, has a chronic course, and paves the way for diseases such as endometrial carcinoma, hyperlipidemia, cardiovascular disease (CVD), type 2 diabetes mellitus (DM) that may adversely affect the quality of life in the future (2). PCOS patients have an increase in many cardiovascular disease risk factors, including diabetes mellitus, hypertension, and dyslipidemia (3). PCOS is also the most common cause of infertility associated with anovulation (4). 6-14% of the typical population displays polycystic ovary appearance (PCOM). PCOM is not related to lower fertility, although it may be linked to changes in insulin sensitivity, glucose metabolism, and androgen production (5).

2. General Information

2.1. Definition and History

PCOS, which can arise at any age throughout the reproductive cycle, has a varied clinic and a chronic course characterized by ovarian hyperandrogenism, polycystic ovaries, hyperinsulinemia resulting from insulin resistance, and reduced fecundity (6). PCOS and polycystic ovary are distinct and ultrasonographically; polycystic ovary structure can be observed in women with no clinical or laboratory disease. Oligo-amenorrhea, anovulation, hirsutism, acne, and oily skin are indicators of hyperandrogenism associated with PCOS. 32% ultrasonographic prevalence of polycystic ovaries is reported in the literature (7).

Multicystic and sclerotic ovaries have been linked to pelvic pain and menorrhagia since the mid-18th century. In the 20th century, it was thought that polycystic ovaries were caused by inflammation resulting from an infection. In those years, it was believed that polycystic ovaries resulted from a decline in ovarian blood flow caused by partial torsion. In 1935, Stein and Leventhal reported seven patients with amenorrhea, hirsutism, and polycystic ovaries for the first time. Four of these patients were found to be obese. These two witnesses witnessed the resumption of menstruation in patients who had undergone ovarian biopsies and then discovered the ovarian wedge resection procedure. They thought that ovarian surface thickening hindered ovulation. In 1958, McArthur et al. demonstrated an increase in luteinizing hormone (LH) in the urine of PCOS patients (8).

2.2. Diagnostic Criteria for Polycystic Ovary Syndrome

2.2.1. AES+PCOS Society Diagnostic Criteria-2009

In 2009, the Androgen Excess Society PCOS Phenotype Task Force released a report announcing the most recent agreement. Under the titles ovulatory and menstrual dysfunction, hyperandrogenemia, clinical features of hyperandrogenism, and polycystic ovaries, the features of the syndrome are summarized. It was emphasized that conditions such as adrenal hyperplasia with androgen excess, severe insulin resistance syndromes, and androgen-secreting neoplasms; cases of idiopathic hirsutism and hyperprolactinemia and thyroid disorders leading to ovulatory dysfunction should be excluded. Some of the established features of PCOS, such as gonadotropin abnormalities, insulin resistance, and obesity, were not included in the diagnostic criteria (9).

2.2.2. AES Diagnostic Criteria -2006

After the Rotterdam criteria were implemented in 2003, two patient profiles arose. 1- Patient profile devoid of clinical and/or biochemical hyperandrogenism but with ovulatory dysfunction and ultrasonographically polycystic ovaries; 2- Patient profile with clinical and/or biochemical hyperandrogenism and ultrasonographically polycystic ovaries. In order to clarify the diagnosis of PCOS, the Rotterdam criteria were examined again in 2006, and the AES Diagnostic Criteria were released (10). AES has researched the epidemiology and patient groups of PCOS. Therefore, at least two of the following must be present for the diagnosis of PCOS, whose etiology is predominantly hyperandrogenism:

1. Hyperandrogenism (clinical manifestations of hyperandrogenemia such as biochemical hyperandrogenemia and/or hirsutismus)
2. Ovarian dysfunction (oligo-anovulation and/or ultrasonographically polycystic ovaries)
3. Exclusion of other diagnoses such as prolactinoma, non-classical congenital adrenal hyperplasia (9)

2.2.3. Rotterdam ASRM/ESHRE Diagnostic Criteria -2003

At a 2003 meeting of experts convened in Rotterdam, the 1990 NIH diagnostic criteria were revised and renamed the ASRM/ESHRE Diagnostic Criteria. Similar to the 1990 NIH Criteria, after excluding other etiological conditions such as congenital adrenal hyperplasia, prolactinoma, or androgen-secreting tumor with no classical onset, it was decided to diagnose PCOS in the presence of at least two of the following three criteria (11).

1. Oligoovulation and/or anovulation
2. Clinical and/or biochemical findings of hyperandrogenism
3. Presence of polycystic ovaries ultrasonographically

2.2.4. National Institutes of Health (NIH) Diagnostic Criteria-1990

Stein and Leventhal first described polycystic ovary syndrome in 1935 as a characteristic symptom complex with amenorrhea, hirsutism, anovulation, and large polycystic ovaries (12). In 1990, the National Institute of Health (NIH) criteria were determined for the diagnosis of PCOS. These criteria are;

1. Hyperandrogenism and/or hyperandrogenemia
2. Chronic anovulation

3. Excluding other etiologies that may cause PCOS-like clinics, such as Cushing's Syndrome, hyperprolactinemia, and non-classical congenital adrenal hyperplasia.

Ultrasonographic identification of polycystic ovaries is not among the NIH's diagnostic criteria, mainly focused on the differential diagnosis of other diseases. This is likely since ultrasonography was common in the 1990s (13).

At the Rotterdam conference, PCOM was described in depth. Demonstrating these findings in a single ovary was considered an acceptable requirement. These ultrasonography criteria should not be applied to oral contraceptive-using women. Peripheral, sequential placement of the follicles and enhanced echogenicity in the ovarian stroma are not among the ultrasonographic diagnostic criteria (14,15). All four diagnostic criteria share the requirement of an elevated androgen level and the exclusion of other common anovulation-causing factors. PCOS is a diagnosis of exclusion. To exclude hyperthyroidism and hypothyroidism as potential causes of anovulation, thyroid function tests must be performed. The clinical progression of hyperandrogenism, caused by androgen-secreting ovarian tumors, is typically quick and severe. There is a thickening of the voice, clitoromegaly, and male pattern baldness. Women with Cushing's syndrome may also experience high serum androgen levels, menstrual abnormalities, and central adiposity, similar to PCOS. Cushing's syndrome is a rare disease; hence, routine screening is not advised. Screening should be limited to patients with signs of hypercortisolism alone (16).

2.3. Etiopathogenesis

For ovulation to occur usually, all units that contribute to the menstrual cycle must function correctly and in concert. The hypothalamohypophyseal axis, feedback signals, and local responses in the ovary should all be normal. If these units differ from the usual, ovulation loss may result. As a result, a dysfunctional condition develops, and a picture of anovulatory polycystic ovaries appears. The origin of polycystic ovary syndrome is not known for definite.

The most prominent hypotheses concerning the pathogenesis of PCOS are (17):

1. Primary neuroendocrine disorder leading to an increase in the frequency and amplitude of LH secretion (Hypothalamo-pituitary dysfunction)

2. Prenatal or postnatal exposure to androgens

3. Disorder of enzyme activity resulting in increased ovarian androgen production

4. Insulin resistance that develops as a result of a disorder in insulin secretion and function

5. Disturbance in cortisol metabolism leading to an increase in adrenal androgen production

6. Genetic transmission

7. Inflammation

8. Certain drugs

2.3.1. Hypothalamo-Pituitary Dysfunction

In the years when the disease was initially diagnosed, the worsening of hormone secretion in the hypothalamus and pituitary was thought to be the primary cause of PCOS. Recent years have rendered this viewpoint invalid. It has been demonstrated that a defective steroid hormone feedback system causes an increase in pituitary LH production. In a normal menstrual cycle, the hypothalamic release of gonadotropin-releasing hormone (GnRH) triggers pulsatile follicle-stimulating hormone (FSH) and LH release from the anterior pituitary. In patients with polycystic ovary syndrome, on the other hand, GnRH sensitivity to the negative feedback effect of estradiol and progesterone is lowered, and the increased GnRH release frequency induces an increase in LH release. LH stimulates androgen production in theca cells. These changes in the dynamics of central gonadotropin found in patients with PCOS may arise predominantly or after peripheral hormonal abnormalities (18). Unlike in ovulatory women, LH secretion in patients with PCOS is not cyclic. It occurs approximately once an hour and has a fixed secretion frequency (19).

Over time and with technological developments, ovarian pathology, which is seen as the primary pathological etiology of the syndrome, has migrated towards the hypothalamo-pituitary axis and primary abnormalities in insulin action. Particular primary problems can result in the same pathology, as there is compelling evidence that these three variables are involved in the pathogenesis of PCOS and govern ovarian function through interplay (20).

2.3.2. Changes in Steroidogenesis

It is believed that an abnormality in ovarian and adrenal steroid synthesis is the primary cause of PCOS pathophysiology (21). Even after chronic

gonadotropin suppression, in-vivo studies have demonstrated that ovarian-derived steroid synthesis and enhanced sensitivity to gonadotropins continue. Steroid synthesis in the adrenal glands has shown comparable results. In-vitro studies have demonstrated that enhanced androgen production in isolated theca cells persists even after LH suppression with GnRH agonists (22).

The fact that some patients who underwent ovarian biopsy and wedge resection regained their ovulatory cycles and reduced their androgen levels highlighted the significance of ovarian-derived androgen production in the pathophysiology of PCOS. Similarly, when the amount of ovarian tissue removed by Wedge resection grows, the ovulatory function has been reported to improve at the same pace (23).

The increase in ovary androgens causes the growth of non-dominant follicles within the ovary until they reach a size where the dominant follicle is picked (24). In addition to causing early luteinization of follicles, an increase in intraovarian androgen leads to thecal, stromal, and cortical hyperplasia of follicles. In addition, it causes anovulatory symptoms and the appearance of polycystic ovaries (25).

In healthy women, an LH increase causes LH receptors desensitization on theca cells. In response, ovarian androgen production is inhibited by the increased LH. Due to ovarian-derived steroidogenesis problems, there is insufficient downregulation of LH in patients with PCOS (26).

2.3.3. Genetics

The increased incidence of polycystic ovary syndrome and related traits among members of the same family has prompted research into the condition's genetic characteristics (27). Significant genetic influences contribute to the development of the syndrome's reproductive and metabolic characteristics. In addition to the increased frequency of hyperandrogenism and menstrual disruption in the mothers and sisters of patients with the polycystic ovarian syndrome, serum androgen levels appear to be increased in the dads and brothers. In addition, the risk of insulin resistance and varied degrees of glucose homeostasis disorders increased in all first-degree relatives compared to healthy controls of the same age and body mass index (28). A twin investigation established a correlation between fasting insulin and circulating androgen levels in PCOS-affected twin women (29). Multiple studies addressing probable genetic diseases that may contribute to the development of polycystic ovary syndrome demonstrate that the syndrome is a complicated polygenic disorder (30).

When gene linkage analyses are evaluated, they indicate a location on chromosome 19p13.3 close to the insulin receptor gene. Insulin resistance is more prevalent in families with PCOS, and this illness also affects men. It has enhanced the evidence in studies indicating that under activity of the pancreatic -cell is genetically inherited in families of women with PCOS.

2.3.4. Exaggerated Adrenarche

Adrenarche is when hair growth commences in the axillary and pubic regions with adrenal androgen exposure. According to exaggerated adrenarche, teenagers with excessive adrenarche are likely to develop PCOS. In people with PCOS, all androgenic hormones and their precursors are increased. In 92% of cases in which the dexamethasone suppression test was conducted to suppress the adrenal cortex of women with PCOS, serum testosterone levels remained elevated (31).

2.3.5. Intraovarian Factors

Local androgens prevent the development of dominant follicles, one of the primary causes of chronic anovulation. When androgen levels in the ovaries are elevated, they are converted to 5-alpha metabolites, which inhibit estrogen synthesis through aromatase activity (32). In addition to preventing normal follicular development in the ovary, excessive androgen levels also induce premature follicular atresia. Consequently, stromal tissue in the ovary increases. It promotes androstenedione and testosterone synthesis in the ovary by increasing stromal tissue and LH stimulation (33).

During the early follicular phase of polycystic ovary syndrome, the number of tiny preantral and antral follicles in the ovary has increased, with autocrine and paracrine factors playing a significant role in this process. Activin, epidermal growth factor(EGF), transforming growth factor-beta(TGF-beta), insulin-like growth factor(IGF-1), growth differentiation factor-9 (GDF-9), and oocyte-derived growth factor (ODGF) are believed to influence this process (34). In the theca cells of the ovarian follicle exist insulin, IGF-1, and IGF-2 receptors, and it has been discovered that activation of these receptors affects ovarian androgen production. Although the precise impact of insulin is unknown, a drop in serum androgen levels without a change in LH has been observed with the control of hyperinsulinemia (35).

2.3.6. Inflammation

According to studies, numerous acute phase proteins and inflammatory markers are elevated in PCOS patients (36). PCOS is a proinflammatory

syndrome and based on the available data, it is believed that the fundamental cause of metabolic problems and ovarian dysfunction in PCOS is mild, persistent inflammation (37). Insulin resistance is held accountable for the elevated androgen levels increased in PCOS. The picture of hyperandrogenism in PCOS patients without insulin resistance cannot be explained in this instance. Inflammation may be the primary cause of insulin resistance in PCOS patients. This hypothesis suggests that inflammation may produce hyperandrogenism directly (37).

3. Clinical Findings

3.1. Chronic Anovulation, Menstrual Irregularities

Menstrual irregularity is the most prevalent complaint of patients with polycystic ovary syndrome. Peripubertal women experience anovulation and menstrual irregularities, including oligomenorrhea and amenorrhea. The age of menarche in these patients is not delayed. Menstrual abnormalities are observed in fifty to ninety percent of these patients (38).

3.2. Infertility

In patients with polycystic ovary syndrome, FSH deficiency, hypersecretion of LH, hyperandrogenemia, insulin resistance, and a hyperinsulinemic environment cause anovulation or problems in the development and implantation of the oocyte due to the disruption of many mediator balances in the follicle fluid (39).

3.3. Hirsutism

One of the most predominant clinical characteristics of polycystic ovary syndrome is hirsutism of varying severity. Hirsutism is assessed using a modified version of the Ferriman-Gallwey method. Hair distribution in 9 areas, including the upper lip, chin, chest area, lower and upper parts of the back, lower and upper abdomen, upper arms, and legs, is scored between 0-4. If this value is less than 8, hirsutism is diagnosed.

3.4. Obesity

It is estimated that 30-75% of polycystic ovary syndrome patients are obese. 30% to 50% of patients are of average or underweight weight. Insulin

resistance is present in lean and obese PCOS patients; however, its presence is proportional to obesity. It has been discovered that polycystic ovary syndrome patients begin menstruating after they drop 10-15% of their body weight (40).

3.5. Acanthosis Nigricans

They are dark, velvety plaques most frequently observed on the nape, skin folds, elbows, and vulva. It has epidermal hyperkeratosis and dermal fibroblast proliferation as its pathology. Despite enhanced pigmentation, neither melanocyte count nor melanocyte deposition increased.

3.6. Ultrasonography

Ultrasonographic criteria for polycystic ovary syndrome define polycystic ovary (PCO) as 12 or more follicles with a diameter between 2 and 9 mm in each ovary and/or an increase in ovarian volume (more than 10 ml) (25). Even though increased stromal volume or echogenicity is unique to PCO, ovarian volume is more relevant in clinical practice. Diagnosis is sufficient with the presence of a single PCO. The optimal period to examine the ovaries in a woman with regular menstrual cycles is between days three and five of the cycle. Because oral contraceptive use might affect ovarian morphology, these definitions do not apply to women using oral contraceptives. In the presence of a dominant follicle (>10mm) or corpus luteum, the examination must be repeated the following cycle (25).

4. Laboratory Findings

No biochemical marker can identify polycystic ovary syndrome on its own. Typically, plasma testosterone levels are high. The decrease in SHBG causes a level of free testosterone. High LH, normal FSH and LH/FSH >2 are diagnostically valuable markers. Not only are laboratory tests required for diagnosis, but also differential diagnosis. For amenorrhea causes, beta HCG, prolactin, and thyroid stimulating hormone (TSH) values should be measured. Check DHEA-S, androstenedione, and 17-OH progesterone levels for other causes of hyperandrogenism.

5. Differential Diagnosis in Pcos

When diagnosing polycystic ovary syndrome, it is essential to rule out certain diseases that may cause a similar clinic. Pituitary and adrenal

diseases, which may cause hyperandrogenism, menstrual irregularity, and hirsutism, require a differential diagnosis. Certain medications can also cause hyperandrogenism or hyperandrogenic alterations (such as androgens, steroids, antiepileptics, and progestogen agents). In situations of quickly increasing hirsutism and virilization, androgen-secreting tumors should be investigated in the differential diagnosis. A testosterone level greater than 200 ng/dl and a dehydroepiandrosterone-sulfat level greater than 7,000 ng/dl indicates an adrenal or ovarian malignancy (25).

6. Pcos Treatment

Currently, the recommended treatment for PCOS is tailored to the patient's symptoms. Patients with polycystic ovary syndrome typically experience hirsutism, oligo-amenorrhea, and reproductive issues. Utilized treatment protocols aim to reduce the clinical manifestations of hyperandrogenism, including hirsutism, regulate menstruation, and promote conception.

6.1. Combined Oral Contraceptives

Combined oral contraceptives are the foundation of treatment for the correction of hyperandrogenism and the regulation of menstruation in patients with PCOS who are not pregnant or planning to become pregnant.

6.2. Antiandrogens

Among the antiandrogen drugs, spironolactone, CPA, finasteride, and flutamide are the most commonly used (41).

6.3. Insulin Sensitizing Drugs

Metformin, thiazolidinediones (TZD).

6.4. Other Treatments

Finasteride, eflornithine hydrochloride 13.9%, glucocorticoids, ketoconazole, and myoinositol.

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CHAPTER XII

THORACOLUMBAR SPINE TRAUMAS AND TREATMENT

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1. Introduction

Thoracic vertebral fractures are less common because they are more stable than other parts of the spine. The presence of the sternum anteriorly and the ribs on the sides and the muscles surrounding the chest wall in the thoracic region contributes to the biomechanical strength of the thoracic spine (1). The breakdown of such a stable structure is only possible with high-energy trauma. High-energy trauma that breaks a fairly stable spine, such as the thoracic spine, causes more neurological damage. Thoracolumbar junction fractures include the T10-L2 vertebral borders. The fractures in this region are more common as there is a transition from a region with less range of motion stabilized by the ribs and sternum to the lumbar region with greater range of motion. The fractures in this region constitute 10-20% of all spinal fractures (2). Lower lumbar spine fractures (L4, L5) are rare compared to other spine levels. They constitute 1-4% of all spinal fractures (3). Treatment approaches to this region are still controversial today due to its rarity. In the treatment of lower lumbar spine fractures, the mechanical stability of the fracture and the potential to cause neural damage are important as in other spine levels. Classifications are used to determine appropriate treatment approaches.

2. Diagnosis

Neurological examination and radiological imaging are assessed together in the evaluation of spinal fractures. Spinal cord injury is also frequently observed with post-trauma spinal cord fractures. Spinal cord injuries cause motor,

sensory and autonomic disorders. The ASIA classification is used in patients with spinal cord injury (1). After neurological and detailed physical evaluation is complete, the appropriate imaging method must be selected for patients. Detection and diagnosis of thoracic spine fractures with direct radiography is difficult. Detection of fractures is difficult, especially in the upper thoracic spine. Computed tomography is very valuable in the detection of central canal compression, lamina fractures, pedicle fractures and facet fractures (3).

Magnetic resonance imaging is used to determine whether surgical treatment is necessary in fractures. Evaluation of the posterior ligamentous complex is necessary to determine whether surgical treatment is required. Posterior ligamentous complex includes ligamentum flavum, interspinous ligament, facet capsule, supraspinous ligament. Particularly fat-suppressed (STIR) sequence MRI enables the evaluation of posterior ligamentous complex structure. In cases where STIR sequence MRI cannot be performed, high signal intensity in T2 sequences indicates that the posterior ligaments complex is damaged (4) (5).

3. Classifications and Treatment of Thoracic and Lumbar Traumas

In order to create a common language in the classification of spinal fractures, classifications were made by using the technologies of the time. The purpose of these classifications is to assess whether the spine is stable. Classifications that started with Denis' 3-column theory in 1980 became more detailed and functional with the development of technology and possibilities (6). Early classifications did not include the clinical condition of the patient and the condition of the posterior ligamentous structures. These classifications provided no recommendations for the surgical treatment to be performed. Among these classifications, the most commonly used ones are the thoracolumbar injury and severity score (TLICS) and the AO spine Thoracolumbar Injury Classification (ATLICS).

3.1. Thoracolumbar Injury and Severity Score (TLICS)

In 2005, Vaccaro et al. classified the thoracolumbar injury and severity score (TLICS) (7). The 3 main variables in surgical decision making in this classification are the morphology of the fractured vertebra (Compression, Burst, Translation/Rotation, Distraction), Integrity of the Posterior Ligamentous Complex, and Neurological Status. The posterior ligamentous complex is grouped as intact, indistinct and suspicious and scored accordingly. The damage

to the posterior ligamentous complex is detected by increased signal on STIR sequence MRI or T2 sequence MRI. On radiography and computed tomography, increased interspinous distance, facet dehiscence, facet subluxation, vertebral translation and rotation show damage to the posterior ligamentous complex. The presence of a space between the interspinous process on physical examination also indicates damage to the posterior ligamentous complex. Neurological status is important in terms of surgical indication.

Neurological status is scored under 3 main headings: Nerve root injury, complete spinal cord injury (ASIA-A) and incomplete spinal cord injury (ASIBA B, C, D) or cauda equina injury. Presence of neurological deficit is an indication for surgical decompression. Conservative treatment is given to patients who score 3 points or less according to TLICS classification. Patients with a score of 4 are in-between. Surgical indication of these patients is surgeon-optional. Conservative and surgical treatments can be administered. Surgical treatment is recommended for patients who score above 4 points (see Table 1).

Injury Morphology	Compression	1
	Burst	2
	Translational/Rotational	3
	Distraction	4
Integrity of Posterior Ligamentous Complex PLC distrupted in tension, rotation or translation	Intact	0
	Suspected/İndeterminate	2
	Injured	3
	Intact	0
	Nerve Root	2
	Complete Spinal Cord (ASIA-A)	2
	İncomplete Spinal Cord (ASIA-B,C,D	3
	Cauda Equina	3

Table 1. TLICS Classification (7)

3.2. AO spine Thoracolumbar Injury Classification (ATLICS)

In 2013, TLICS classification was revised due to inadequacies and a new classification was introduced as the AO spine Thoracolumbar Injury Classification (ATLICS) (8). This classification is based on 3 parameters: Morphology of fractured vertebra, neurological status and modifying factors (see Table 2).

Morphologic classification of the fracture	Type A Compression Injuries	AO	0
		A1	1
		A2	2
		A3	3
		A4	5
	Type B Injuries Tension Band Injury	B1	5
		B2	6
		B3	7
	Typce C Injuries:Displacement/Translational Injury		8
Neurological Status	N0	Neurologically intact	0
	N1	Transient Neurological Deficit	1
	N2	Radiculopathy	2
	N3	Incomplete spinal cord injury or cauda equina injury.	4
	N4	Complete Spinal Cord Injury	4
	NX	Cannot be examined	3
Clinical Modifiers	M1	injury to the tension band based on spinal imaging such as MRI or clinical examination.	1
	M2	designate a patient-specific comorbidity,	0

Table 2: AO Spine TLICS Classification (8)

In the ATLICS classification, the title of modifying factors has been added in addition to TLICS. The title is under the heading of under this title. Possible comorbidities in patients include ankylosing spondylitis, rheumatologic diseases, diffuse idiopathic skeletal hyperostosis, osteoporosis and skin burns during spinal injury. Suspicion of injury in the posterior tension band, which is also included in the TLICS classification, makes it difficult to decide in favor of surgery. The presence of comorbidity in the patient supports the conservative approach. Conservative treatment is given to patients who score 3 points or less according to the ATLICS classification. Those with 4 and 5 points are in-between patients. Surgical indication of these patients in-between is surgeon-optional. Conservative and surgical treatment can be administered. Surgical treatment is recommended for patients who score above 6 points.

4. Conclusion

Thoracic vertebral fractures occur as a result of high-energy trauma. Conservative or surgical treatment may be administered via TLICS or AO spine TLICS classifications in treatment. Minimally invasive methods such as anterior, posterior, combined surgeries, vertebroplasty-kyphoplasty are preferable for patients that are considered for surgical treatment based on the classifications.

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CHAPTER XIII

FROM HIVES TO HEALING: THE POWER OF HONEY BEE PRODUCTS AND APITHERAPY IN THE NUTRITION AND HEALTH OF DOMESTIC ANIMALS

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1. Introduction

Honey bee products (in the following sections, they will be referred to as bee products only) are natural substances produced by bees that have various health benefits. Honey contains glucose, fructose, enzymes, and antioxidants that contribute to its antibacterial, antifungal, and anti-inflammatory properties (1). Propolis is a complex mixture of resin, essential oils, waxes, and other compounds that have antioxidant and immune-boosting effects due to its flavonoids, phenolics, and terpenoids content (2). Royal jelly is rich in proteins, vitamins, minerals, fatty acids, and antioxidants, and may have anti-inflammatory, antimicrobial, and anti-tumor properties (3). Bee pollen is a mixture of nutrients, including amino acids, vitamins, minerals, and antioxidants, and may have anti-inflammatory, immune-boosting, and allergy-relieving properties (4). Beeswax is composed of esters of fatty acids, and may have antibacterial and anti-inflammatory effects (5). Although it lacks scientific research to support its widespread use as a validated therapeutic method, bee venom (BV) therapy has been recognized in traditional medicine for centuries. BV has many medicinal properties due to its complex chemical composition, including anti-inflammatory, antioxidant, antibacterial, antiviral, and antifungal

properties (6). Although numerous studies have investigated the uses of BV in human medicine, few have explored its potential in veterinary medicine (7).

1.1. Medical Applications of Bee Products

Bee products have been shown to affect the production performance and health of animals (8). Apitherapy, which involves the use of bee products for medicinal purposes, has been used since the early days of beekeeping. BV is the most commonly used product in apitherapy, and it has been used to treat various acute and chronic diseases, including inflammation, arthritis, neurological degenerative diseases, chronic pain conditions, autoimmune diseases, and dermatological diseases (9). The medical community and the general public should be aware of the potential risks and lack of research on some alternative therapies. Further studies are needed to determine the efficacy and safety of apitherapy.

Apitherapy is a cost-effective alternative to conventional therapies and honey has been demonstrated to effectively combat drug-resistant pathogens in hospitals (10). However, there are risks associated with apitherapy, including anaphylactic reactions, which can be fatal in some cases. There have been reports of serious complications, such as bilateral thalamic and mesencephalic hemorrhage, caused by BV as a result of apitherapy (11,12).

Propolis, also known as bee glue, bee putty, hive dross, and propolis resin, is a natural substance produced by honey bees. Propolis has a complex chemical composition that includes flavonoids, phenolics, and terpenoids. It is available in various forms, including capsules, extracts, and creams. In addition to its use in humans, several scientific studies have investigated the effects of propolis on the performance, immunity, intestinal flora, and hematological parameters of poultry, including broiler chickens, laying hens, quail, and ducks (13).

This book chapter examines the nutritional and therapeutic effects of bee products on domestic animals. Although studies conducted on experimental animals have been excluded, this chapter provides a mechanistic review for a better understanding of the topics. We hope that this book chapter will serve as a source for veterinary students, practitioners, and researchers.

Throughout the chapter, the nutritional and therapeutic properties of bee products on various animal species have been described under subheadings within the main headings.

2. Nutritional Properties of Bee Products

2.1. Nutritional Properties of Propolis

Bee products are useful as nutritional supplements for livestock, improving animal health and productivity. Studies have mainly concentrated on the effects of propolis supplementation in different conditions and age groups of broilers and layers (8).

2.1.1. Broilers

A study investigated the effects of different levels of propolis in broiler chickens. The study used 224 Ross 308 chicks. The treatments included four different levels of propolis powder, ranging from zero to 2000 ppm in the diet for 42 days. The results showed that the use of 2000 ppm propolis powder in the diet improved BWG, feed intake, feed conversion ratio (FCR), and production index. Incorporating propolis into poultry diets was found to have a beneficial impact on growth performance, carcass characteristics, and blood parameters according to the study (14).

The effects of different levels of alcoholic extract of propolis on the performance of Ross 308 broiler chicks were studied. The average BWG, feed consumption, feed efficiency, and mortality rate of the propolis-fed birds were higher. The inclusion of propolis in the diet reduced the mortality rate. Higher doses of propolis (200-250 mg/kg of fodder) resulted in higher feed intake and daily BWG, while lower doses had less pronounced effects (15).

According to a study on 120 Ross 308 broiler chickens, propolis supplementation in their diets resulted in an increase in *Lactobacillus* genera in the ileum and a decrease in *Enterobacteriaceae* genera in the crop, indicating that it could positively influence the gastrointestinal tract colonization pattern and enhance production performance (16).

A study was conducted on 120 Ross 308 broiler chickens, which found that feeding them propolis resulted in improved health and vitality, as well as better broiler fattening (17).

To investigate the impact of propolis on the gastrointestinal tract of chickens, a study was conducted where feed mixtures were supplemented with propolis extracts at varying concentrations. The group that added 600 mg of propolis to 1 kg of feed mixture had the highest count of fecal *enterococci*, while the group that added 800 mg of propolis to 1 kg of feed mixture had the highest count of *lactobacilli*. The ethanolic propolis extract showed the

most effective antimicrobial activity against *Citrobacter braakii* at all tested concentrations (18).

In a research study, two hundred male Ross 308 broiler chicks were divided into five groups, including a basal diet group and four groups that were fed with different levels of propolis (600, 700, 800, and 900 mg/kg) added to their basal diet. The results revealed that the broilers fed with 800 mg/kg of propolis had the highest body weight, daily feed intake, carcass weight, and carcass yield, along with improved FCR. Moreover, the inclusion of propolis (700-900 mg/kg) in the broiler diet led to higher serum IgG and IgM levels and improved antibody response against sheep red blood cells. Additionally, the inclusion of 900 mg/kg of propolis in the broiler diet reduced total triglycerides, cholesterol, LDL, and LDL/HDL ratio while increasing the relative weight of the spleen and bursa. Furthermore, broilers fed with 800 and 900 mg/kg of propolis had a greater calculated European broiler index and crop percentage (19).

In a study, chicks were fed diets supplemented with OEP ranging from 0 to 1000 mg/kg from hatching and immunized with *Newcastle Disease Virus* (NDV) at days 20 and 32. The addition of OEP stimulated the immune system without affecting performance. Chicks fed 70 and 100 mg/kg of OEP showed an increase in antibody response to NDV at 30 days of age. Histological sections revealed an increase in proliferating cells in the bursa of fabricius in chickens fed with 1000 mg/kg of OEP. Higher levels of OEP resulted in a higher number of leukocytes in the lamina propria of the intestine and an increase in lymphoid cells in the periportal area of the liver (20).

An experiment used 672 Ross 308 chickens. Chickens were fed a normal soybean meal-corn diet supplemented with 0, 40, 70, 100, 400, 700, and 1000 mg/kg of OEP, and were immunized against *infectious bronchitis virus* (IBV), NDV, and infectious bursal disease (IBD). Blood samples were collected on days 21 and 42 of age, and antibody concentrations against IBV, NDV, IBD, and avian influenza (AI) were measured. Results showed that OEP supplementation increased antibody titer against AI, NDV, and IBD, without affecting IBV. However, higher concentrations of OEP had a relatively negative effect on the broiler's humoral immunity (21).

2.1.2. Laying Hens

A study was conducted on laying hens fed varying levels of propolis. The results showed that the hens who received propolis had improved immune responses, as evidenced by a higher lymphocyte count and lower

heterophil count. Additionally, the hens showed a hyper-responder reaction to phytohemagglutinin-P injection. These findings suggest that propolis supplementation can improve the immune status of laying hens, potentially by reducing residual feed intake (22).

2.1.3. Bovines

In vitro studies have confirmed that propolis possesses bactericidal properties, while in vivo studies have demonstrated its positive impact on both the health of cattle and their production outcomes (23).

In a study aimed at evaluating the effects of different diets on the performance and carcass characteristics of feedlot-finished bulls, the results showed that bulls fed with propolis extract had higher final weight, hot carcass weight, and average daily gain. Dry matter conversion was also better. However, carcass characteristics were not affected by the diets (24).

Another study investigated the effects of propolis ethanolic extract (PEE) added to the diet of dairy cows on milk production and characteristics. The cows were given 19.2 g of propolis extract as a balanced feed additive daily. The results indicated that the addition of propolis extract to the diet can improve ruminal conditions, leading to an increase in milk production and an increase in milk protein content (25).

Propolis has demonstrated promising results as a natural substitute for feed antibiotics, which can contribute to enhancing animal health and productivity. Propolis shows potential as a beneficial feed additive in the animal industry (23–25).

2.1.4. Pigs

In two studies the effects of propolis on the functional activity and histology of the ileum in young pigs were investigated. In the first study, the researchers examined the effect of biologically active substances of propolis on the ileum of young pigs. The study found that the functional activity of the organ changed after the administration of propolis (26).

The second study aimed to investigate the effects of a propolis water-alcohol emulsion on the functional activity of the ileum in young pigs. The study found that the height of the intestine villi was higher, with a more differentiated villi shape, allowing for better absorption of nutrients and faster BWG. The most significant histological changes in the structural components of the intestinal wall were also observed in the ileum (26).

2.2. Nutritional Properties of Venom

2.2.1. Broilers

Two studies have investigated the impact of BV on broiler chickens. The first study investigated the effect of water supplementation with BV on broiler performance and liver function. Two different doses of BV, 0.5 mg/l, and 1 mg/l were added to the drinking water for 28 days. The results showed that BV supplementation increased BWG and feed intake, with no adverse effects on liver function and hematological parameters. BV also increased antioxidative activity (27).

The second study investigated the potential of adding purified BV to a maize-soybean meal-based diet in broiler chickens. The study found that dietary BV had a positive effect on FCR and BWG, with higher levels of BV found to lower the relative weight of certain organs. BV also increased the lightness value for meat but decreased ileal villus height and width. Additionally, BV led to an increase in sIgA concentration and a decrease in nitric oxide (NO) contents in serum samples, as well as a reduction in short-chain fatty acids in caecal digesta (28).

Kim (2019) investigated the impact of dietary BV on serum characteristics, antioxidant activity, and hepatic fatty acid composition in broiler chickens. The study included 875 male broiler chicks, the results showed that dietary BV inclusion increased the concentration of stearic acid, while decreasing other types of fatty acids. Additionally, BV tended to lower hepatic malondialdehyde contents, suggesting that it may improve antioxidant capacity and affect fatty acid metabolism (29).

2.2.2. Pigs

A study investigated the effect of administering BV in piglets. The injection of BV and apipuncture resulted in an increase in BWG and survivability by 26.6% and 21.8%, and 7.9% and 6.7%, respectively, while not affecting blood parameters like total protein and albumin but increasing IgG levels, indicating that BV can enhance the immune system in pig farming by utilizing its primary bioactive components. Administering BV via injection or acupuncture did not affect the growth performance of young pigs (30).

2.2.3. Rabbits

In a study comparing the effects of BV and oxytetracycline (OXY) supplementation on weaning rabbits. BV was given at doses of 2, 4, and 8 mg/kg body weight/day (35).

Treatment with OXY or BV resulted in increases in total plasma protein and globulin levels, while levels of AST and ALT decreased, except for the OXY group where ALT levels rose. Both treatments also reduced levels of triglycerides, total cholesterol, and VLDL, with no changes in HDL and LDL. The group treated with BV showed increased levels of IgG, total antioxidant capacity (TAC), superoxide dismutase, catalase, and glutathione peroxidase (GPX). In addition, the group treated with 2 mg BV/kg body weight/day had a reduced total bacterial count, as well as lower counts of *salmonellae, Escherichia coli, Proteus,* and *Clostridia* (31).

2.3. Nutritional Properties of Pollen

2.3.1. Bovines

Previous laboratory research has shown that bee pollen has potential benefits as a supplement in practical breeding and production, especially in ruminant nutrition. In particular, during the first three weeks of life when the rumen is developing, pollen may be advantageous for young calves due to its positive effect on the digestive system, which includes regulating ingesta passage, increasing appetite, and promoting faster growth. Pollen can improve milk protein synthesis in cows (8,32). However, there is limited research on the application of bee pollen in animal feeding, particularly in cattle nutrition (32).

3. Therapeutic Properties of Bee Products

3.1. Therapeutic Properties of Propolis

3.1.1. Dogs

Propolis has shown potential in dentistry for treating caries, plaque, chronic periodontitis, oral candidiasis, and pulp therapy. It has antimicrobial properties and lower associated risks, making it a promising alternative for controlling oral diseases (33).

Siceanu (2008) investigated the therapeutic effects of api-phyto-therapeutical products in dogs with dermatological, parasitological, and surgical diseases. A new product called proactivator, containing propolis extract and purified aloe extract, was developed. The study demonstrated proactivator's superior wound healing and anti-microbial and anti-parasitological effects, with a 50% reduction in healing time (34).

Another study found that using propolis to store teeth prior to reimplantation in healthy mixed-breed dogs was effective in increasing the success rate of the

procedure (35). Propolis exhibits antibacterial properties that prevent bacteria from adhering to the tooth surface, which not only inhibits bacterial resistance but also highlights its potential as an oral antiseptic (36,37).

3.1.2. Broilers

A study showed that even low doses of propolis (10 mg/kg) in the diet protected the liver against pathological lesions. Propolis also exhibited a protective effect on the cardiovascular system and liver. The higher dose (50 mg/kg) of propolis showed greater protective effects (38).

3.1.3. Bovines

Neonatal diarrhea is a common disease in young calves. The diarrhea is predominantly bacterial and poses a risk to the calves' health (32). The propolis extract (400 mg/day) led to a reduction in diarrhea symptoms, partial compensation of respiratory acidosis, an increase in BWG, and a beneficial effect on the total protein content and γ-globulins in the blood serum of the calves (39).

In a study involving young calves with clinical signs of bacterial diarrhea, propolis was found to have a positive effect on the animals' health (40). Higher doses of propolis were found to result in more significant improvements in the health of calves in a study investigating the effects of propolis on calf diarrhea. Calves fed with propolis also demonstrated higher daily BWG. The level of total bacteria, specifically *Escherichia coli*, was found to decrease in fecal bacteriological studies (32).

A study comparing propolis extract, and two antibiotics on lactating cows' udders found that the propolis extract caused temporary changes in milk that disappeared after 1.5-2 days, and there was no biological activity of propolis extract in cow's milk after 72 and 120 hours from the last infusion. The study concluded that propolis extract does not pose a risk to the quality of milk (41).

A 20-year study compared the effectiveness of antibiotics and propolis gel in healing mastitis during lactation and involution. The study found that propolis gel was more effective in treating mastitis. The study proposed a method of treatment that involved using propolis gel in all lobes of the mammary gland and targeted antibiotics based on microbiological tests for infected areas only (42).

3.1.4. Pigs

In a study involving pigs with burn wounds, the efficacy of ointments containing 1% propolis and 1% nanosilver was assessed. The 1% propolis ointment

exhibited broad-spectrum antibacterial activity, hastened neoangiogenesis and epithelialization, enhanced the cosmetic appearance of scars, and did not cause any adverse effects. On the other hand, the 1% nanosilver ointment was effective in reducing free radicals, but it did not show any synergistic effects with propolis. The researchers recommended the use of 1% propolis ointment as a topical treatment for burn wounds owing to its antimicrobial characteristics, quicker healing time, and favorable influence on scar formation (43).

3.1.5. Other Animals

Propolis preparations are commonly used in veterinary surgery to prevent infections and complications in various skin and mucous membrane injuries. They have a wide regenerating effect on micro- and macro-traumas, scratches, and postoperative wounds, and provide a protective effect against skin damage during the mass processing of animals. Sensitivity tests of microflora to propolis preparations are necessary for effective animal therapy. Propolis protective gel, hoof cleaning concentrate of 1%, and hoof cleaning concentrate of 3% are found to be the most effective options for antimicrobial hoof care products (44).

3.2. Therapeutic Properties of Venom

According to research, BV has anti-inflammatory properties due to various components, including melittin, apamin, and adolapin. Melittin, in particular, inhibits the production of pro-inflammatory cytokines and has demonstrated a reduction in inflammation in several experimental models. Therefore, BV may have the potential to be used as a natural anti-inflammatory agent to treat conditions such as asthma, multiple sclerosis, and arthritis (45).

In addition to its anti-inflammatory effects, studies have reported on the anti-cancer effects of BV components apamin and melittin. Different components of BV stimulate various cell signaling pathways, which have been shown to modulate cell apoptosis and have anti-angiogenic properties in cancer therapy. The capacity of melittin to interact with phospholipid membranes is associated with additional mechanisms that lead to the demise of cancer cells. However, these studies are currently based on research involving in vitro cell cultures and animal experiments (46).

The results of various studies suggest that the components present in BV have the potential to be used as natural therapeutic agents for the treatment of inflammatory and cancerous diseases. However, more research is needed to fully comprehend the mechanisms of action and the possible clinical uses of BV as a natural therapeutic agent (45,46).

3.2.1. Dogs

Several studies have investigated the potential therapeutic effects of BV on various arthritic-like conditions and neurological dysfunction in dogs. In one study, 24 mixed-breed dogs were divided into four groups and received injections of either sterile saline or whole BV. The results showed that BV increased plasma cortisol levels and daily cage activity in arthritic dogs, suggesting that it may be a potential therapy for arthritic-like conditions in dogs (47).

In a study conducted on 17 arthritic dogs, BV treatment was administered. Out of 17 dogs, 14 dogs showed considerable recovery after receiving BV treatment. Moreover, all dogs with disc complications recovered to normal or nearly normal conditions. Among five dogs treated for joint complications and six dogs treated for poor surgical recovery, four dogs in each group showed an improvement in mobility (48).

A study was conducted on 40 adult dogs suffering from neurological dysfunction caused by intervertebral disk disease (IVDD). The study found that treatment with BV injections at acupoints was more effective in treating IVDD-induced neurological dysfunction and pain compared to treatment without BV injections. The results suggest that BV injections at acupoints can improve the treatment of canine neurological dysfunction caused by IVDD, especially in dogs with moderate to severe IVDD (49).

Facial paralysis is a common neurological condition in dogs that can be caused by a variety of factors. One potential treatment option for this condition is the use of BV therapy. In a study investigating the effects of BV on facial nerve paralysis, twelve dogs with the condition were administered an injection of 100 μg BV, which effectively resolved the paralysis. The study compared the outcomes of the BV group with those of the dexamethasone group, but no discernible difference was observed between the two groups (50).

A dog was tentatively diagnosed with idiopathic facial paralysis. BV acupuncture therapy was administered, resulting in a gradual improvement of clinical signs. Eight weeks after the initial acupuncture, the dog's sensory and neurological facial signs had recovered (51).

Two studies investigated the effectiveness of BV in treating otitis externa in dogs. In the first study, ten dogs with otitis externa were divided into two groups, one treated with antibiotics and the other receiving a subcutaneous injection of BV into the tragus. After two weeks, the BV group showed a reduction in bacterial cell counts, with no observed adverse reactions (52).

A study involving 15 dogs with otitis externa found that injection acupuncture with BV alone or in combination with antibiotics reduced bacterial cell count (53).

3.2.2. Horses

Several studies have explored the efficacy of BV in treating various conditions in horses. One study investigated the use of BV to treat chronic obstructive pulmonary disease (COPD) in racehorses. Horses were injected with BV at three bilateral acupuncture points, and the study found that 76% of the horses had improved performance as their COPD decreased (54).

Another study evaluated the effects of BV injections on eight arthritic horses aged 8 to 17. Six horses were included, and three of them showed an improvement, with six showing full recovery (55). The study suggested that BV could be an effective treatment for arthritis in horses.

A 13-year-old Arabian horse with laminitis was treated with BV injections at multiple sites in a case study. The patient demonstrated almost normal walking after the third session (56). Another Arabian horse with conjunctivitis was treated with a solution containing BV injected at multiple sites. By the third treatment session, the horse showed improvement with no signs of excessive tearing, eye discharge, or redness (57).

3.2.3. Bovines

Researchers isolated 44 bacteria strains from cows with mastitis and found that BV, along with two other products, effectively inhibited the growth of *Staphylococcus aureus* and *Coagulase-negative Staphylococcus*, and partially inhibited the growth of *Escherichia coli*. While no pores were observed forming on the cell membranes of sensitive strains during the time-kill assay, the findings suggest that BV may have potential as an alternative treatment for mastitis in cows (58).

3.2.4. Pigs

In a study, researchers aimed to investigate the therapeutic effect of BV in sows with hypogalactic syndrome postpartum. The treatment involved bee acupuncture using honey bees, which was administered once a day for three consecutive days. Following the treatment, 85.0% of the penicillin G-treated control group and 90.9% of the BV-treated group showed recovery from hypogalactia syndrome (9).

3.2.5. Rabbits

BV injections have been found to have positive effects on reproductive performance, immune response, and blood antioxidant content in V-line bucks during the summer season (59). In this study involving 48 bucks, three groups received BV injections twice a week for 20 weeks in different doses (0.1, 0.2, and 0.3 mg/rabbit). The treated groups showed improvements in sperm quality, fertility percentage, and blood biochemical parameters such as testosterone concentration, total protein, albumin, and glucose levels. The BV doses also increased antioxidant indices and immune response markers such as IgA and IgM. The bucks that received BV injections had a shorter reaction time and increased libido, and the effect was dose-dependent.

Another study investigated the long-term effect of BV on the reproductive performance, immune function, and health of Spanish V-line rabbit does, as well as its effect on their litters. Sixty mature does were assigned to four groups, with each group receiving different doses of BV (60). The study found that treating does with BV resulted in higher litter size, litter weight, and survival rate at weaning. Milk yield also increased. Serum estradiol 17-β levels were higher, while serum progesterone levels decreased. Conception and fertility rates improved. Furthermore, BV caused a gradual reduction in liver enzyme activities and an increase in TAC, glutathione S-transferase (GST), GPx, and serum IgG, IgM, and IgA levels. There was also a decrease in malondialdehyde (MDA) and thiobarbituric acid reactive substances (TBARS) in BV groups.

3.2.6. Other Animals

Animal studies over the past two decades have explored the potential uses of BV, particularly in the fields of apitherapy, allergology, and experimental biology. A significant amount of research has focused on the anti-inflammatory effects of BV in arthritis and other inflammatory conditions (7).

In a rat study, BV was found to have anti-inflammatory effects on chondrocytes by inhibiting the synthesis of TNF-α and IL-6, which may lead to pain relief and reduced articular destruction. Another study on monkeys showed that BV or its component, melittin, can stimulate the production of cortisol from the adrenal gland, potentially explaining the positive effects of BV in treating conditions that respond to adrenal steroid therapy (61).

3.3. Therapeutic Properties of Honey

Honey, a sweet liquid produced by bees from flower nectar, has clinical significance in dentistry for various oral conditions (33).

One potential use for honey in dentistry is in the treatment of periodontitis. However, its application in gingival margins can be challenging. Despite this, honey has an advantage over other antimicrobial agents due to its natural properties, which reduce the risk of antimicrobial resistance (62).

Overall, honey has the potential the treatment of various oral infections, ulcers, periodontal disease, stomatitis, and halitosis. While its antibacterial properties are well-known, more research is needed to fully explore its therapeutic potential in dentistry (33).

Food honey and medicinal honey should not be confused. Using food honey on wounds can cause inflammation and infections. Therefore, medicinal honey is sterilized using gamma rays before use to preserve the enzymes responsible for the healing effect. Unlike thermal sterilization, gamma ray sterilization doesn't destroy these enzymes (63).

Honey is considered beneficial for wound dressing due to its multiple mechanisms of action, such as being an antibacterial, debriding, and anti-inflammatory agent. Honey promotes the growth of granulation and facilitates the acceleration of epithelial tissues to promote faster healing (64).

3.3.1. Dogs

Researchers used honey dressings to treat a 3-month-old dog with traumatic injuries and tissue necrosis on the front limbs due to their low cost and multiple healing properties. They found that honey dressings are effective in the initial stages of wound healing until epithelialization begins. The study concluded that wound management should be a comprehensive approach that combines systemic and nutritional support with topical healing agents (65).

3.3.2. Equines

Two studies on wound treatment in equines were conducted with natural remedies. The first study examined the use of a mixture of cod liver oil and honey in treating old wounds in horses and donkeys (66). The study included ten animals with wounds in the metacarpus or metatarsus, and fetlock and carpal joints. The wounds were treated with the mixture, resulting in a decrease in wound size and the formation of healthy scars within a month. The study also

compared the use of honey, cod liver oil, and the mixture on surgically-induced wounds in donkeys. The results showed that treated wounds had a decreased size and complete epithelization, with wounds treated with the mixture showing a higher degree of maturity in granulation tissue.

The second study investigated the antibacterial efficacy of manuka honey on contaminated and non-contaminated wounds in horses (67). Manuka honey and manuka honey gel were found to reduce wound retraction and healing time, according to the study. Applying manuka honey daily for 12 days or manuka honey gel throughout the healing process was effective in promoting healing and reducing wound retraction by 66%. Manuka honey gel was found to be more suitable for distal limb wounds due to its consistency, eliminating the need for bandaging.

3.3.3. Rabbits

In a study conducted on 17 male New Zealand white rabbits, the effects of honey as an antibacterial agent on open wounds were evaluated. Under general anesthesia, two full-thickness skin pieces were removed from the backs of the rabbits, and the wounds were divided into three groups. The first group was treated with honey applied twice daily, the second group with silver sulfadiazine 1% cream and the third group served as the control and received saline dressings. The treated wounds were covered with sterile plaster twice a day. The wounds were assessed by histological study eight times every four days, and the results showed that honey accelerated the healing of open wounds in rabbits. Specifically, honey increased the activation of fibroblasts, leading to an increase in the formation of granulation tissue and collagen fiber formation, while decreasing edema and dehiscence (68).

3.4. Therapeutic Properties of the Mixture of Bee Products

3.4.1. Dogs

Two studies have investigated the therapeutic effects of bee products on dogs. The first study aimed to investigate the effects of beeswax ointment on dogs with acute otitis externa. The study found that the use of beeswax ointment helped to normalize lymphocyte, erythrocyte, and leukocyte levels in sick dogs and also activated their nonspecific resistance. Additionally, the study found an increase in some indicators of antimicrobial properties of blood serum in treated dogs (69).

The second study evaluated the effectiveness of an apitherapeutic product in treating hepato-biliary diseases in dogs. The product was a mixture of bee products, plant extract, microelements, and vitamins. Clinical tests were carried out on dogs with various hepato-biliary diseases, including liver insufficiency, acute hepatitis, liver cirrhosis, and cholestasis. The animals were given the product alone or in combination with other therapies, and their conditions were monitored through clinical, echographic, and biochemical examinations. The results showed that the product was as effective as standard treatment methods, as evidenced by improvements in the biochemical values before and after treatment (70).

3.4.2. Broilers

The effects of feeding broiler chickens with fodder containing 0.025% propolis and/or 0.5% pollen during the first two weeks of fattening were studied. The macroscopic and microscopic examination of the liver and kidneys was carried out, and the impact of these substances on breeding results and pathological changes of the liver and kidneys during natural infection with *Salmonella Enteritidis* was determined. The study found that propolis had a protective effect on the liver of broiler chickens by reducing the intensity of regressive lesions. It was noted that the most advanced lesions were found in chickens that were fed with pollen or pollen with propolis (71).

3.4.3. Pigs

In a study, the therapeutic effects of propolis and honey in burn wound treatment were investigated. The study compared scar formation processes in burn wounds treated with 1% and 3% balms containing standardized extracts of propolis and honey with those of a cream containing 1% silver sulfadiazine, a commonly used treatment for burns. The use of apitherapeutics, such as propolis and honey, has gained popularity in burn wound treatment due to their regenerative and analgesic properties. The study showed that the balm reduced the time for granulation and regenerated epithelium formation, and increased the collagen level in wounds. The 3% balm was found to be the most effective, resulting in faster healing than the standard cream therapy (72).

In a study conducted on burn wounds in pigs, the antibacterial activity of honey balm was compared with silver sulfadiazine. The study found that honey balm exhibited higher antimicrobial activity than sulfadiazine, based on clinical and antimicrobial examinations (73).

4. Other Properties of Bee Products

4.1. Other Properties of Propolis

A study was conducted to explore the impact of various concentrations of PEE on rumen microbial fermentation by using the rumen simulation technique (74). Study with six fermenters and three treatments: control, 0.5 ml/day of 20% PEE, and 0.5 ml/day of 60% PEE. Results showed no effect on ruminal pH, short-chain fatty acid production, acetate production, acetate-to-propionate ratio, total protozoa count, or dry matter digestibility. High PEE concentrations decreased propionate production, while both low and high concentrations increased butyrate production. Total ruminal bacteria count decreased with PEE addition. Ammonia-nitrogen concentration decreased in a dose-dependent manner by 24% and 39% with low and high concentrations of PEE, respectively.

In another study, the researchers extracted propolis using water and various concentrations of ethanol (75). They found that the specific compounds extracted varied with the concentration of ethanol used. The 60 to 80% ethanolic extracts of propolis exhibited strong inhibitory effects on microbial growth, and the 70 and 80% ethanolic extracts showed the greatest antioxidant activity. Additionally, the 80% ethanolic extract strongly inhibited hyaluronidase activity.

Another study investigated the efficacy of geopropolis sourced from the Jataí bee (*Tetragonisca angustula*) against *Staphylococcus sp. coagulase-negative*, in vitro (76). The results indicated that the geopropolis exhibited effective antimicrobial properties against the tested pathogenic bacteria.

A recent research study examined how in ovo injection of propolis water extract affects the hatchability, embryonic mortality, starter live performance, and livability of Japanese quails (77). The study included 500 fresh hatching eggs, and the eggs were injected on day 14 of incubation with distilled water (control), 1%, 2%, or 3% propolis water extract. Results indicated that hatchability and embryonic mortality were lower in the 2% and 3% propolis groups. However, there were no differences in BWG, feed intake, FCR, or livability among treatment groups.

4.2. Other Properties of Venom

Studies have been conducted under this heading to assess the effects of crude BV and/or its components on various in vitro outcomes, including antioxidant, anti-diabetic, anti-inflammatory, antimicrobial, and anticancer effects, among others.

The antioxidant pharmacological effects of Apis mellifera venom have been studied (6). In vitro assays were conducted to determine the optimum antioxidant activities of standard crude venom (SV) and crude venom from a breeder (YV) dissolved in different solvents at concentrations of 1.95-500 µg/ml. The results showed that the venoms had different optimum activities for radical scavenging and metal chelating depending on the solvent and concentration used. The highest radical scavenging activity (RSA) was observed in SV dissolved in distilled water, while the highest metal chelating activity (MCA) was observed in SV and YV dissolved in saline. The findings suggest that the use of venom at a concentration of 500 µg/ml (1.95 µg/ml YV for RSA) dissolved in saline is optimal for antioxidant activity. The use of PBS or distilled water resulted in decreased antioxidant activity (75).

A study examined the impact of BV on diabetic rats' reproductive systems. The diabetes was induced with streptozotocin, and BV was administered to some of them. Results showed that BV treatment had a protective effect on the reproductive system, particularly in improving sperm motility and reducing abnormal sperm rates. Therefore, BV was found to have a beneficial effect in reducing the negative effects of diabetes (78).

Several studies have investigated the anti-inflammatory effects of BV and its major component, melittin, on different types of cells. Moon (2007) found that BV and melittin suppress lipopolysaccharide (LPS)-induced NO and inducible NO synthase (iNOS) expression in BV2 microglia, without causing cytotoxicity. BV and melittin also suppressed the activation of nuclear factor kappa B (NF-κB) by blocking the degradation of IκBα and phosphorylation of c-Jun N-terminal kinase (JNK) and Akt, which resulted in the inhibition of iNOS expression. In addition, BV and melittin suppressed the transcription of cyclooxygenase (COX)-2 genes and proinflammatory cytokines, such as interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α. BV and melittin also attenuated the production of prostaglandin E2 (PGE2). Park (2007) suggested that BV may act on IKKs to reduce IKK activities, IκB release, NF-κB activity, and the generation of inflammatory mediators (79).

Jeong (2017) conducted a study to explore the potential anti-inflammatory effects of BV on bovine mammary epithelial cells in response to LPS-induced inflammation, which is a major contributor to bovine mastitis. The study findings revealed that BV has the ability to reduce the expression of COX2 and pro-inflammatory cytokines like IL-6 and TNF-α. BV was also observed to suppress the activation of NF-κB by dephosphorylating ERK1/2 and inhibiting

the production of intracellular reactive oxygen species. The results indicate that BV can potentially alleviate LPS-induced inflammatory responses in bovine mammary epithelial cells by inhibiting oxidative stress, NF-κB, ERK1/2, and COX-2 signaling (80).

These studies suggest that BV and melittin may offer therapeutic potential for the treatment of neurodegenerative diseases and bovine mastitis, respectively (79,81). Additionally, BV may act on IKKs to reduce inflammation (82).

Melittin has been found to inhibit the replication of a wide range of viruses, including *coxsackievirus, enterovirus, influenza A viruses, HIV, HSV, JV, RSV, VSV,* and *TMV*. In addition to melittin, non-cytotoxic amounts of BV have been shown to inhibit the replication of enveloped viruses such as *influenza A virus, VSV, RSV,* and *HSV*, as well as non-enveloped viruses such as *EV-71* and *H3*. The antiviral properties of BV and melittin are primarily attributed to a virucidal mechanism. Moreover, melittin has been shown to protect mice from lethal doses of the pathogenic *influenza A H1N1 viruses* (83,84).

A recent research study examined the effectiveness of chitosan/alginate nanoparticle encapsulated BV (CH/AL-BV) in inducing a systemic immune response and improving the clearance of porcine reproductive and respiratory syndrome virus (PRRSV) in pigs through nasal administration. The study findings showed that CH/AL-BV delivered through the nasal route was successful in stimulating non-specific immune responses, particularly those associated with Th1 responses and viral clearance activities against PRRSV infection (85).

A study investigated the potential antiviral effect of BV on cervical carcinoma cell lines, including CaSki, HeLa, C33A, and TC-1. The results showed that BV treatment led to a more significant suppression of cell growth in *HPV16*-infected cells (CaSki) compared to *HPV18*-infected cells (HeLa). In contrast, there was less suppression observed in *HPV*-negative C33A cells. These findings highlight the antiviral and anticancer properties of BV in *HPV*-infected cervical cancer cells and suggest that BV downregulates E6/E7 protein, which plays a differential role in suppressing *HPV16*- and *HPV18*-infected cells (86).

Borrelia burgdorferi, the bacterium responsible for causing Lyme disease, has demonstrated resistance to various metabolic inhibitors. However, in vitro studies have shown that the use of melittin can effectively inhibit the motility of *B. burgdorferi* within seconds, even at low concentrations. This sensitivity to melittin may provide useful insights into the development of new drugs against Lyme disease (87).

While antibiotics are the primary treatment for Lyme disease, relapse can occur after the discontinuation of antimicrobial agents. BV and melittin, exhibit remarkable efficacy against all forms of *B. burgdorferi*, comprising the biofilm form that is attached to surfaces. In contrast, antibiotics showed limited effects on this form (88).

In vitro experiments demonstrated that both BV and melittin had antibacterial effects, particularly against *methicillin-resistant Staphylococcus aureus* (MRSA) strains. The minimum inhibitory concentrations (MICs) of BV and melittin were determined against *Escherichia coli* and *S. aureus*, and postantibiotic effects (PAEs) were also assessed. The PAEs of whole BV and melittin against both bacterial strains were observed to be comparable. Additionally, melittin was found to be effective in treating MRSA infections in vivo, with rescued animals exhibiting recovery from MRSA-infected skin wounds (89,90).

Another study investigated the antibacterial properties of BV against bacteria isolated from pigs and chickens with the disease. The MICs of BV against *Staphylococcus aureus, Staphylococcus hyicus*, and *Staphylococcus chromogenes* were 8, 128, and 128, respectively. The MICs of BV against 11 strains of *Escherichia coli* ranged from 8 to greater than 512, while the MICs of BV against 8 strains of *Salmonella sup* were all greater than 512. Multiple drug-resistant patterns were observed in most strains isolated from pigs and chickens (91).

A study tested the effects of different doses (3, 6, 12, and 24 mg) of BV administered subcutaneously to 15 lactating mastitic cows. There was a non-linear dose-dependent relationship observed between BV and the reduction of somatic cell count (SCC) in milk samples. The most effective BV therapy was administered at a 12 mg dose, with a reduction seen on days 3 and 6. An additional trial of BV treatment for 14 days showed an increase in clinically cured quarters with less than 0.2 million/ml SCC. After two weeks of BV treatment, a decrease in the identification of *Staphylococcus aureus* and other Gram-positive microorganisms was observed (92).

Studies have found that BV and melittin can enhance the bactericidal effects of several antimicrobial agents when used together, particularly against multi-drug-resistant pathogens. This suggests the potential development of new or complementary antibacterial drugs (93).

Vitellogenin, a compound found in bees, also has antimicrobial properties and can damage bacterial cell membranes (94). Phospholipase A2, another

component of BV, has antibacterial activity and has shown potential as an anti-parasitic and antifungal agent (95).

In addition to its antibacterial activity, BV has been investigated for its potential to combat *Toxoplasma gondii*, as it has been found to cause damage to living tachyzoites (96).

4.3. Other Properties of Honey

Honey has been studied for its anticancer properties in animal experiments with transplantable tumors induced by carcinogenic substances. Honey and black cumin seeds in the diet protected against colon cancer. It was shown that honey inhibited the development of human and mouse bladder cancer cells. Honey has demonstrated anticancer activity in animal studies and can enhance the effects of oncology treatments (97).

Several studies have investigated the antibacterial properties of honey and its potential use as a therapeutic agent for infections. However, the potency of the antibacterial activity of honey varies greatly and can be easily lost through improper handling and storage. Therefore, honey intended for therapeutic use should be assayed for its antibacterial activity as a form of quality assurance (98).

In a study, different concentrations of honey obtained from various regions exhibited antibacterial activity against *Escherichia coli, Listeria monocytogenes, Salmonella Typhimurium,* and *Staphylococcus aureus*. Each microorganism exhibited different sensitivity to the honey tested (99).

Different research conducted an experiment to assess the efficacy of different types of honey obtained from both beekeepers and supermarkets against 10 bacterial isolates obtained from wounds in horses. Out of the 28 types of honey examined, 18 were found to have bacterial and/or fungal contamination. Among the 11 types of uncontaminated honey, 8 demonstrated effectiveness against all 10 isolates at concentrations between 4% to 16%. The most impressive outcomes were observed with Manuka 20+ and heather honey, indicating their potential application in the treatment of wound infections (100).

In a separate study, researchers evaluated the activity of chitosan and apiphytoextract, a chitin-containing drug produced from biologically active products of bee breeding. The apiphytoextract was found to have high antibacterial activity and safety, making it a potential alternative to antibiotics-decontaminants in cell cultivation (101).

5. Conclusion

In conclusion, honey bee products and apitherapy have shown great potential in improving the nutrition and health of domestic animals, including bovines, equines, pigs, and others. From wound healing and skin health to reproductive and immune system function, the therapeutic benefits of honey, propolis, royal jelly, bee venom, and other bee products are being increasingly recognized and researched. While more studies are needed to fully understand the mechanisms behind these benefits and to establish proper dosages and administration methods, the growing body of evidence suggests that apitherapy can be a valuable addition to the toolkit of veterinarians and animal owners in promoting the health and well-being of their animals.

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CHAPTER XIV

ARTERIAL BLOOD GAS ANALYSIS: CURRENT PRACTICES

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1. General information

Blood gas analysis is a standard diagnostic method for determining the partial pressures of blood gases and acid-base concentration. Blood gas analysis enables medical professionals to diagnose respiratory, circulatory, and metabolic issues (1). Blood gas analysis is the most prevalent diagnostic method employed in critical care. In reality, comprehensive knowledge and use of arterial and pulmonary/central venous blood gas and electrolytes analysis enable the correct interpretation of most respiratory, circulatory, and metabolic abnormalities that may arise in critically sick patients. Blood from any part of the circulatory system can be subjected to a "blood gas analysis" (artery, vein, or capillary). An arterial blood gas (ABG) test specifically analyzes blood drawn from an artery. ABG testing determines a patient's partial pressure of oxygen (PaO2) and carbon dioxide (Co2 partial) (PaCO2). PaO2 indicates oxygenation status, whereas PaCO2 indicates the ventilation condition (chronic or acute respiratory failure). Hyperventilation (rapid or deep breathing), hypoventilation (slow or shallow breathing), and acid-base state affect PaCO2. Although pulse oximetry and end-tidal carbon dioxide monitoring can measure oxygenation and ventilation non-invasively, ABG analysis is the gold standard.

Most ABG analyzers immediately measure pH and PaCO2 when determining the acid-base balance. A Hasselbach equation derivative determines the serum bicarbonate (HCO3) and base deficiency or excess. This approach frequently produces a mismatch between the measured and calculated values due to the blood CO2 that needs to be accounted for by the equation. The measured HCO3 utilizes a powerful alkali that releases all CO2 in the blood, including dissolved CO2, carbamino compounds, and carbonic acid. This calculation solely accounts for dissolved CO2; a standard chemical study would likely refer to this quantity as "total CO2." Therefore, the difference will be around 1,2 mmol/L. However, there may be a more considerable disparity between the ABG and the measured value, particularly in severely sick individuals (2). The computation has been contested as correct or wrong based on the research, equipment, or calibration, and it must be evaluated appropriately according to the institution's criteria (3). Although typically requested by emergency medicine, intensivist, anesthesiology, and pulmonology doctors, arterial blood gases may also be required in other therapeutic contexts. An ABG is used to examine several conditions, such as acute respiratory distress syndrome (ARDS), severe sepsis, septic shock, hypovolemic shock, diabetic ketoacidosis, renal tubular acidosis, acute respiratory failure, heart failure, cardiac arrest, asthma, inborn metabolic disorders. Multiple clinical diseases can be identified by collecting an ABG, examining the pH and partial pressures, and comparing it to the measured serum bicarbonate in a sick patient. Patients with a ventilation-perfusion mismatch often have an irregular alveolar-arterial oxygen gradient, a helpful indicator of lung gas exchange.

2. Specimen Requirements and Procedure

The needed specimen for an arterial blood gas sample is whole blood. The specimen is collected either by an arterial puncture or a catheter placed in the artery. These operations are outside the scope of this page; for further information, please see the StatPearls article "arterial lines" and other sources. Once acquired, the arterial blood sample should be immediately placed on ice and tested to minimize the likelihood of erroneous findings. Blood gas samples are often analyzed using automated blood gas analyzers, and conclusions are acquired within 10 to 15 minutes. Directly and indirectly, automated blood gas analyzers assess particular components of an arterial blood gas sample [4].

ABG Components:
pH = blood's determined acid-base balance
PaO2 = partial pressure of oxygen in arterial blood as measured.
PaCO2 = partial pressure of carbon dioxide in arterial blood, as measured.
HCO3 = estimated bicarbonate concentration in arterial blood
Base excess/deficit = relative excess or deficiency of base in arterial blood, as determined by calculation.
SaO2 = arterial oxygen saturation computed unless co-oximetry is provided, in which case it is measured.

3. Blood Drawing Procedures

A modified Allen test is a must before an ABG is drawn from either upper extremities to check for adequate collateral flow. Alternatively, pulse oximetry and duplex ultrasound can be used too. The arterial site commonly used is the radial artery, which is superficial and easily palpable over the radial styloid process. The following most common site is the femoral artery. The test is performed on the unilateral upper extremity chosen for the procedure (Please look at the attached image for a graphical illustration). The selected upper extremity is flexed at the elbow, and the patient requested to clench the raised fist for 30 seconds. Then pressure is applied over the ulnar and radial arteries to occlude the blood flow. After five seconds, unclench the raised fist. The palm will now appear pale, white, or blanched. Then pressure over the ulnar artery is released while the radial artery compression is maintained. In 10 to 15 seconds, the palm returns to its original color, indicating adequate Ulnar collateral blood flow. If the palm does not return to its actual color, it is an abnormal test and unsafe to puncture the radial artery. Similarly, the radial collateral blood flow is assessed by maintaining ulnar artery pressure and releasing the radial artery pressure [5].

4. Interpretation of Results

Noting that the acceptable normal range of ABG values of ABG components may vary among laboratories and age groups from infants to geriatrics [6,7], the acceptable normal range is as follows:

pH	(7.35-7.45)
PaO2	(75-100 mmHg)
PaCO2	(35-45 mmHg)
HCO3	(22-26 meq/L)
Base excess/deficit	(4 to +2)
SaO2	(95-100%)

It is preferable to approach arterial blood gas interpretation methodically. The interpretation process helps determine the degree or severity of abnormalities, whether acute or chronic, and whether the fundamental disease is metabolic or respiratory. Several studies have presented straightforward approaches to interpreting ABG data. However, for all levels of providers, the Romanski technique of analysis is the most simplified. In addition, this approach aids in determining the presence of an acid-base problem, its principal cause, and whether or not compensation exists [6-9].

The first procedure is to examine the pH and determine if acidemia (pH 7.35) or alkalemia (pH > 7.45) is present. If the pH falls within the usual range (7.35-7.45), use 7.40 as the threshold. In other words, a pH of 7.37 would indicate acidosis, but a pH of 7.42 would indicate alkalemia. Next, assess the respiratory and metabolic components of the ABG values, PaCO2 and HCO2, respectively. The PaCO2 reveals whether the acidosis or alkalemia is mainly respiratory or metabolic. For example, PaCO2 40 and pH 7.4 suggest respiratory acidosis, whereas PaCO2 40 and pH 7.4 imply respiratory alkalosis (but is often from hyperventilation from anxiety or compensation for metabolic acidosis). Next, check for indications of balance for the main acidosis or alkalosis by identifying the value (PaCO2 or HCO3) that is inconsistent with the pH. Finally, evaluate the PaO2 for any oxygenation anomalies.

An electrolyte imbalance or anion gap must be factored into assessing a patient's acid-base condition. To provide one illustration: In a patient with Diabetic Ketoacidosis, hyperchloremia causes the elimination of ketones and the closure of the anion gap, but also the persistence of metabolic acidosis. This occurs because of the robust ionic effect, which is outside the focus of this chapter.

5. Testing Methodology

Three electrodes measuring pH, PCO2, and PO2 at 371C are included in blood gas analyzers. They were launched in 1960, following the 1954 inventions of R. Stow (CO2) and L. Clark (PO2). Internal computers calculate

O2 saturation, base excess, bicarbonate, and other derived variables, such as the body's correction for acid–base imbalances, based on these outputs. Arterial PO2 and PCO2 can be estimated using heated "transcutaneous" skin surface electrodes, often employed in preterm newborns and nurseries. Also directly assessed by multiwavelength blood oximeters is the hemoglobin oxygen saturation (S02%). Pulse oximeters estimate arterial SO2 by detecting arterial pulsatile fluctuations in red and infrared light entering a finger, ear, or other tissue, a technique patented by T Aoyagi in Tokyo in 1973 and commercialized in 1983 [10].

Patient data must be input into the analyzer or laboratory information system (manually or with the aid of a bar-code reader, if one is available) and reviewed following the manufacturer's instructions to guarantee patient safety. In addition, recording the analysis's duration is necessary. Some analyzers allow for the entry of data on the patient's body temperature and oxygen supply, enabling the adjustment of BGA findings to the patient's current body temperature and for particular computations requiring data on oxygen supply. Only once the individuals working in BGA have received proper training should these extra elements be implemented. At 22 °C and 4 °C, respectively, the pH of freshly taken blood drops at a rate of 0.02 to 0.03 pH unit/hour and 0.01 pH unit/hour. Due to glycolysis and cell respiration, this pH fall is accompanied by a matching drop in glucose and a rise in lactate and pCO2 in the sample. Therefore, by evaluating the sample as soon as possible, ideally within 30 minutes of collection, undesirable effects on pH and blood gases can be avoided [11].

Laboratories must know the analyzer's limits, particularly concerning possible interferences. Polymeric pH sensors, generally utilized in cartridge-based analyzers and blood gas and related analytes sensors, are susceptible to interferences from lipophilic chemicals such as perfluorocarbon in emulsion-based quality control (QC) materials. Cationic surfactants, particularly benzalkonium compounds, may interfere with sodium, potassium, and ionized calcium tests. Ionized calcium and sodium can interfere with ionized magnesium sensors. Typical thiocyanate concentrations in smokers may lead to a reduction in ionized magnesium levels. Since chloride sensors rely on ion exchange membranes, they are prone to interference from more lipophilic anions (other than chloride) in the sample. Salicylates, thiocyanates, bromides, and iodides raise chloride concentrations artificially. Suppose the chloride electrode is frequently exposed to the anticoagulant heparin (for instance, when attempting

to wash clots off the analyzer). In that case, the heparin's negative charge might be extracted from the chloride membrane, resulting in a decrease in membrane sensitivity for chloride ions. Blood contains endogenous and external oxidizable chemicals that can interfere with glucose and lactate sensors (uric acid, ascorbic acid, acetaminophen, and dopamine). Fluoride and oxalate, commonly used as additives in blood collection tubes, may interfere with glucose and lactate biosensors by reducing the activity of oxidase enzymes employed in the measuring response. Conductivity measures of hematocrit may be affected by variations in electrolyte and protein contents. The presence of fetal and, to a lesser extent, sulfhaemoglobin (i.e., abnormal hemoglobins) interferes with co-oximetry readings. The medicinal administration of hydroxycobalamin and methylene blue (colored and interfering with spectrophotometric measures utilized in co-oximetry) may impact hemoglobin fraction measurement [11,12].

Due to the concentration gradient between cells (erythrocytes, leukocytes, and platelets) and plasma, hemolysis substantially impacts potassium concentrations. In addition, due to the dilution effect, hemolysis may result in erroneously low sodium and ionized calcium concentrations. Depending on the extent of hemolysis, additional analytes may also be impacted. Although the presence of hemolysis in whole blood samples cannot be seen visually, it has been demonstrated that it produces incorrect pO2 and pCO2 findings (i.e., significantly lower pO2 and higher pCO2, respectively). Moreover, hemolysis may result in a clinically significant reduction in sO2 and carboxyhemoglobin (COHb). Suppose a result does not accurately represent the patient's condition. In that case, hemolysis should be suspected and checked by transferring the whole blood sample to an additive-free tube and centrifuging it rapidly. The presence of lipemia in a sample impedes the determination of total hemoglobin and its components [11,12].

6. Clinical Considerations

Arterial blood gas monitoring is the gold standard for measuring a patient's oxygenation, ventilation, and acid-base status. Although non-invasive monitoring techniques have mostly supplanted ABG monitoring, it is still valuable for validating and calibrating non-invasive monitoring methods.

In the intensive care unit (ICU) and emergency department, oxygenation is commonly evaluated in the context of severe sepsis, abrupt respiratory failure, and acute respiratory distress syndrome (ARDS). Calculating an alveolar-arterial (A-a) oxygen gradient can help determine the etiology of hypoxemia.

For instance, the existence or absence of a gradient can assist in detecting if an aberration in oxygenation results from hypoventilation, a shunt, a V/Q mismatch, or hindered diffusion. The predicted A-a gradient equation assumes that the patient is breathing room air; consequently, the A-a gradient is less accurate with more significant percentages of inspired oxygen. The intrapulmonary shunt fraction, or the portion of cardiac output that flows via pulmonary units that do not contribute to gas exchange, provides the most accurate estimation of oxygenation status. Calculating the shunt fraction is generally conducted with a supplied FiO2 of 1.0, but if performed at a FiO2 below 1.0, the term venous admixture is more suitable. Calculating the ratio between PaO2 and the percentage of inspired oxygen (PaO2/FiO2 or P/F ratio) is the most commonly used to evaluate oxygenation. The difference between venous admixture and the P/F ratio for a particular shunt fraction depends on the supplied FiO2. The P/F ratio has also been used to define ARDS disease severity for research purposes [1,13].

The oxygenation index is another measure often used in ICUs to assess oxygenation (OI). Compared to the P/F ratio, this measure is regarded as a more accurate indication of lung damage, particularly in newborn and pediatric populations. It consists of the amount of invasive ventilatory assistance necessary to sustain oxygenation. The OI is the product of the ventilator-measured mean airway pressure (Paw) in cm H2O and the FiO2 as a percentage divided by the PaO2. Commonly, the OI is used to guide treatment decisions, such as beginning inhaled nitric oxide, providing surfactant, and identifying the possible need for extracorporeal membrane oxygenation [14,15].

A regular PaO2 measurement does not exclude respiratory failure, especially in the presence of supplementary oxygen. PaCO2 represents pulmonary ventilation and CO2 generation by cells. It is a more sensitive indicator of ventilatory failure than PaO2, especially in the presence of supplementary oxygen, due to its strong correlation with breathing depth and rate. The pulmonary dead space is an excellent predictor of lung function. Pulmonary dead space is the difference between PaCO2 and expired mixed PCO2 (physiological dead space) or end-tidal PCO2 divided by PaCO2. When pulmonary unit ventilation rises compared to their perfusion and shunting increases, pulmonary dead space increases. Consequently, pulmonary dead space is a good indication of lung function at the bedside and one of the most reliable prognostic indicators in ARDS patients. The pulmonary dead space fraction may also aid in diagnosing illnesses such as pulmonary embolism [1,16].

The aforementioned respiratory system anomalies can have an impact on acid-base balance. For example, acute respiratory acidosis and alkalemia cause acidemia and alkalemia, respectively. Furthermore, hypoxemic hypoxia promotes anaerobic metabolism, which results in metabolic acidosis and acidemia. Acute metabolic acidosis and alkalosis result in acidemia and alkalemia, respectively, as can metabolic system anomalies. Patients with diabetic ketoacidosis, septic shock, renal failure, medication or toxin ingestion, and gastrointestinal or renal HCO3 loss all exhibit metabolic acidosis. Kidney illness, electrolyte abnormalities, prolonged vomiting, hypovolemia, diuretic usage, and hypokalemia are all causes of metabolic alkalosis.

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CHAPTER XV

HEPATIC CLINICAL CHEMISTRY AND REDOX MARKERS IN DOMESTIC ANIMALS: DIAGNOSTIC SIGNIFICANCE AND RELATION

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1. Introduction

Clinical chemistry is a branch of laboratory medicine that involves the analysis of bodily fluids like blood and urine for the diagnosis and monitoring of diseases. It is a crucial aspect of clinical evaluation and is important for maintaining the health of domestic animals, ensuring productivity in agricultural industries, and improving the quality of life for animals and their owners (1).

The measurement of the reduction-oxidation (redox) relationship is a significant part of clinical chemistry as it helps determine the balance of antioxidants and oxidants in the body. This information can be used to evaluate biological processes like metabolism and cellular respiration and diagnose conditions like oxidative stress which can lead to various diseases. Understanding the redox relationship in biological systems is essential for developing effective therapeutic strategies and monitoring their efficacy (2,3).

Clinical chemistry markers such as reduced glutathione, oxidized glutathione, and redox potential can be used to determine the redox status of an animal. A decrease in reduced glutathione levels and an increase in oxidized glutathione levels indicate an imbalance in the redox system which could be a

sign of oxidative stress. Similarly, a shift towards more oxidizing conditions can be detected by a decrease in redox potential (4). This information is crucial for guiding treatment decisions, monitoring disease progression, and evaluating the effectiveness of antioxidant therapies.

In conclusion, the redox relationship plays a critical role in clinical chemistry and is a key tool in the diagnosis and management of various diseases in domestic animals. Clinical chemistry is an important component of veterinary laboratory assays, which are growing trends in prognostic and diagnostic medicine. Effective clinical evaluation and understanding of the redox relationship are essential for the well-being of domestic animals, the success of agricultural industries, and the improvement of animal health and quality of life (5).

Altered cellular oxidative balance can lead to changes in inflammation, immunity, and stress, making it important to assess these parameters in a relational manner. Clinical chemical parameters and other relevant measurements should be considered together to obtain a comprehensive understanding of the relationship between redox balance and hepatic function (2).

Oxidative injury and the generation of reactive oxygen species (ROS) play a key role in the development of various types of acquired liver damage. Many liver disorders related to inflammation are associated with oxidative damage as activated macrophages generate free radicals. The generation of ROS and oxidative injury is further exacerbated in cases of cholestatic liver disease (6).

Liver damage has been linked to oxidative stress, which is defined as a state where pro-oxidant forces outweigh antioxidant processes (7). The presence of ROS and their peroxidation by-products can trigger the activation of redox-sensitive transcription factors, leading to the release of pro-inflammatory factors that cause liver damage (8).

This section of the book focuses on domestic animals and investigates the relationship between redox balance and hepatic function tests through a review of current studies. It is important to note that a thorough veterinary examination, including liver function tests and a review of the animal's medical history and symptoms, is necessary for a proper diagnosis and treatment plan for any liver disease. Instead of discussing all the tools and methods, this section aims to interpret the relationship between hepatic laboratory tests and the redox system from an integrated perspective.

2. General Principles of Laboratory Testing and Diagnosis

The foundation of veterinary laboratory education is rooted in understanding the basic principles of laboratory instruments and equipment. Familiarity with these principles is crucial for a veterinary hospital and laboratory personnel. These principles can be divided into two main categories: hematology and chemistry techniques (1,9).

Hematology techniques rely heavily on microscopic examination and involve measuring the characteristics of blood samples. This includes the collection of blood samples, centrifugation, plasma protein concentration determination, cell counting, preparation of blood films, and counting of leukocytes and their subtypes (1,10).

Chemistry techniques involve the analysis of animal samples using physical and chemical principles. This includes photometry techniques such as absorbance, reflectance, and fluorometry, light-scatter techniques like turbidimetry and nephelometry, and electrochemical techniques like potentiometry, amperometry, conductometry, osmometry, and electrophoresis (11).

The accuracy and applicability of medical diagnoses can be influenced by various factors like proper sample collection, laboratory selection, location, and the expertise of the employees (12). Pathological effects of diseases can cause changes in laboratory data that exceed the reference values, which can be interpreted to provide important insights into the disease process (13).

3. Hepatic Clinical Chemistry and Its Diagnostic Significance

The liver plays a crucial role in vital functions such as metabolic processes, waste removal, and detoxification (14). When the liver is not functioning properly, abnormalities in laboratory analysis may occur, leading to the diagnosis of liver dysfunction. Evidence of liver disease, including hepatocellular damage and/or cholestasis or inadequate production of metabolic synthesis products and elimination of harmful substances in the blood, can be determined through serum enzyme assays and hepatic function tests (15).

Cells, tissues, and organs contain specific enzymes that can aid in diagnosing disorders and diseases. The diagnostic value of these enzymes depends on their specificity, with enzymes specific to the environment providing more direct answers than enzymes found in all living organisms (16). Leakage of enzymes due to degeneration and increases in enzyme synthesis can both be detected, allowing for inferences about the disorder or disease when clinical enzymology

is interpreted alongside other laboratory results such as hematology, cytology, and urinalysis (17).

Determining the cause of elevated enzyme levels in hepatic enzyme analysis, whether it be from enzyme leakage due to degeneration or increased enzyme synthesis, is crucial in interpretation. In most cases of hepatic diseases, both conditions are intertwined, making it difficult to make a clear distinction. However, it is more crucial to recognize the disorders or diseases causing the increased enzyme levels. For the diagnostic determination of hepatocyte damage, routine measurements of the alanine aminotransferase (ALT), aspartate aminotransferase (AST), sorbitol dehydrogenase (SDH), and glutamate dehydrogenase (GDH) enzymes are usually taken (1,15,16).

Cholestasis is a medical condition in which the flow of bile from the liver is obstructed, leading to a buildup of bile in the liver and a reduction in its release into the small intestine. The diagnosis of cholestasis is made through measurements of alkaline phosphatase (ALP) and gamma-glutamyltransferase (GGT) (18).

3.1. Alanine Aminotransferase

ALT is frequently used as a single test for detecting hepatocellular damage in dogs and cats and has a higher liver specificity than AST. It is an enzyme localized in the hepatocyte cytoplasm and is not just a liver-specific enzyme; it is also found in significant amounts in the kidney, skeletal muscle, and myocardium (19). Especially since skeletal muscles are heavier in mass than the liver, disorders and diseases associated with muscle damage can cause significant leakage of ALT. In the differential diagnosis, measurement of creatine kinase (CK) may be necessary due to its greater muscle specificity (11,15).

Many disorders and diseases, such as inflammation, metabolic disorders with lipidosis, bacterial toxins, toxic chemicals and drugs, and hepatic carcinomas, can cause hepatocellular degeneration and increase the amount of ALT in the blood (1).

However, the increase in ALT does not provide any distinguishing information about the degenerative or necrolytic process, or vice versa the regenerative recovery process of the injury. Acute liver damage can increase ALT activity, which may gradually decrease over several weeks but can be delayed due to liver regeneration. Chronic inflammatory liver damage can also cause periodic increases in ALT activity. In addition, if liver damage has caused the degradation of a large portion of liver mass or if certain toxins

have disrupted transaminase production, slight increases in ALT may be observed (1).

3.2. Aspartate Aminotransferase

AST is an enzyme found in various tissues including the liver, heart, muscle, and kidneys, with the highest levels found in the liver where it plays a role in cellular metabolism. The measurement of AST levels in the blood is a useful tool in diagnosing and managing liver disease in domestic animals (20).

Elevated AST levels can indicate conditions such as hepatitis, liver inflammation, or toxicity, as well as other factors such as muscle damage or medication. AST levels are often measured as part of a comprehensive blood profile or after a suspicious finding on physical examination or other diagnostic tests (21). The interpretation of results should consider other factors such as breed, age, and overall health of the animal and be made in conjunction with other findings and clinical information. The measurement of AST is useful for veterinarians in monitoring the progression of liver disease and the effectiveness of treatments (22).

3.3. Sorbitol Dehydrogenase

SDH is an enzyme found in the liver, heart, and other tissues that regulate glucose and other sugar metabolism. In the liver, it plays a critical role in regulating glucose levels (23). In domestic animals, measuring SDH levels in the blood is crucial for diagnosing and monitoring liver disease (24).

Elevated levels indicate liver injury or diseases such as hepatitis, inflammation, or toxicity and are often measured as part of a comprehensive blood profile or follow-up after suspicious findings. The results provide information about liver health and assist in making informed decisions on diagnosis and treatment. SDH testing is also useful for monitoring the progression of liver disease and the effectiveness of treatments and providing early warning signs of liver dysfunction (1,25).

3.4. Glutamate Dehydrogenase

GDH is an enzyme found in the liver, heart, and muscle that regulates the metabolism of glutamate, an important amino acid involved in energy production and neurotransmitter regulation. GDH plays a crucial role in glucose and energy metabolism, helping to maintain normal cell function (26).

In domestic animals, measuring GDH levels in the blood is crucial for diagnosing and monitoring liver and muscle disease. Elevated levels indicate liver or muscle damage or conditions like hepatitis, inflammation, injury, or toxicity (27). GDH testing is part of a comprehensive blood profile or performed after suspicious results from physical examination or other diagnostic tests. Results, along with other clinical findings, provide information on liver and muscle health and assist in diagnosis and treatment decisions. GDH measurement is useful for tracking disease progression and treatment effectiveness and serves as an early warning sign of liver or muscle dysfunction (11,27).

3.5. Other Enzymes and Parameters

ALP is an enzyme produced by the liver used as a marker for liver function and bile duct health. Elevated ALP levels in the blood indicate cholestasis and are used, along with other clinical findings and diagnostic tests, to diagnose and monitor the progression of the disease. Measuring ALP levels is crucial in the diagnosis and management of cholestasis (28).

GGT is an enzyme that catalyzes the transpeptidation and hydrolysis of glutathione, thus playing a very important role in cellular detoxification. Its enzyme association with glutathione is necessary for redox hemostasis and is a main reaction of intermediate metabolism (29). Oxidative stress exposure protects cells against oxidative damage by increasing GGT transcription. Additionally, cholestatic disorders cause bile acid solubility, resulting in high GGT release levels from the membrane. In dogs and cats, GGT is most concentrated in the kidney and pancreas, with a lower concentration found in hepatocytes and bile duct epithelium. However, the increase in serum GGT activity is largely attributed to acute liver injury (1,15).

In the evaluation of liver function, parameters such as bilirubin, serum bile acids (SBA), urine bile acids (UBA), plasma ammonia level, albumin, globulins, glucose, urea, cholesterol, and coagulation factors are measured. In addition, total protein (TP) and prothrombin time (PT) parameters are also frequently used to evaluate hepatopathies (17,30).

Bilirubin, SBA, UBA, plasma ammonia, albumin, globulins, TP, glucose, urea, cholesterol, coagulation factors, PT, and lactate dehydrogenase (LDH) are all indicators of various aspects of health and disease and can provide valuable information about liver function, liver disease, and the progression and effectiveness of treatment. Bilirubin is a waste product produced when red blood cells break down, while SBA and UBA are substances produced by the liver and

used to digest fats and excrete bile acids, respectively. Plasma ammonia level and glucose levels are indicators of liver function, as the liver is responsible for clearing ammonia and regulating glucose in the bloodstream. Albumin, globulins, TP, and cholesterol are all related to liver function and regulation, while coagulation factors and PT are measures of the liver's production of these substances and its ability to prevent bleeding. LDH, which is involved in energy production, is present in various tissues and can be used as a marker of tissue damage or stress, including liver disease (3,11).

Elevated levels of bilirubin can indicate liver dysfunction and diagnose liver diseases such as hepatitis, cirrhosis, and other conditions that affect the liver's ability to metabolize bilirubin. Elevated levels of SBA and UBA can also indicate liver disease and be used to monitor its progression and treatment effectiveness. Similarly, elevated plasma ammonia levels, as well as elevated glucose and urea levels, can indicate liver disease and be used to monitor its progression and treatment. The levels of TP in the blood can indicate liver function and diagnose liver disease, while elevated cholesterol levels can indicate liver disease. Measuring coagulation factors, such as PT, can also be used to diagnose liver disease and monitor its progression and treatment effectiveness. Elevated levels of LDH in the blood can indicate a range of conditions, including liver disease, and can be used to diagnose and monitor its progression and treatment (1,3).

4. The Importance of the Redox System in the Prognosis, Diagnosis, and Treatment of Diseases

The redox system is a vital component in overall health and disease prevention, playing a crucial role in energy generation, cell signaling, and protection against oxidative stress (31).

Measuring redox markers such as oxidative stress levels, antioxidant status, and redox potential provides important information for the diagnosis and prognosis of diseases. Imbalances in the redox system can indicate oxidative stress-related diseases, and changes in redox markers can be used to monitor treatment effectiveness and disease progression (4,32).

Antioxidant therapies can target the redox system directly to reduce oxidative stress and prevent cellular damage. The redox state of an organism is a significant aspect of its overall health, and a deep understanding of its workings is critical for effective, personalized medical strategies (33).

Some of the commonly used parameters in the evaluation of the redox system include:

Reduced glutathione (GSH) levels: The GSH antioxidant system is composed of various interrelated components, including GSH and oxidized glutathione (GSSG), glutathione peroxidase (GSH-PX), glutathione S-transferase (GSH-ST), and glutathione reductase (GR). The importance of GSH, as one of the primary non-protein sources of thiol in most cells, lies in its crucial role in preserving the redox state of the organism. A decrease in GSH levels is associated with mitochondrial oxidative damage, a characteristic of various liver diseases. The liver plays a crucial role in maintaining GSH homeostasis, with the levels of GSH in the liver and plasma commonly used to determine the redox state of the organism. However, liver failure can result in decreased availability of GSH, leading to elevated levels of ROS and subsequent hepatocyte death (34).

GSSG levels: GSSG is the oxidized form of GSH and is a key indicator of oxidative stress in the body. Increased levels of GSSG are associated with liver diseases (35).

Thiobarbituric acid-reactive substances (TBARS): TBARS are a measure of lipid peroxidation and are used to assess the level of oxidative stress in the body (36,37).

Superoxide dismutase (SOD) activity: SOD is an important antioxidant enzyme that converts superoxide into hydrogen peroxide, which is then converted into water by other antioxidant enzymes. SOD activity is often measured as a marker of antioxidant defense in the body (38).

Total antioxidant capacity (TAC): TAC is a measure of the overall antioxidant defense of the organism and is calculated by combining the levels and activities of various antioxidant enzymes and molecules (39).

In addition to these parameters, other markers of oxidative stress, such as malondialdehyde (MDA) and nitric oxide (NO) levels, are also used to evaluate the redox state of an organism. The results of these tests, in conjunction with other clinical information, can provide important insights into the health of the organism and help diagnose and treat various diseases related to oxidative stress (40).

5. Relation between Hepatic Clinical Chemistry and Redox Markers in Domestic Animals

5.1. Dogs

Some studies have investigated the correlation between hepatic clinical parameters and the redox system in dogs. ALT and ALP levels are known to increase in dogs with various diseases, such as inflammatory, metabolic, vascular,

and neoplastic diseases, according to liver histopathology. Meanwhile, GGT levels also increase except in vascular diseases and are often considered more specific but less sensitive than ALP for the detection of hepatobiliary disease (15). Corticosteroid administration or elevated endogenous corticosteroid levels may result in increased serum GGT activity in dogs, likely due to enzyme induction, and mild to modest increases in serum GGT can also occur with anticonvulsant therapy (17). However, there is a need for studies that directly investigate the relationship between GGT and the redox system in dogs.

Albumin increases in neoplastic diseases, while bile acids increase in inflammatory diseases. There is no change observed in bilirubin, cholesterol, glucose, PT, and ammonia levels (41).

Liver disease in dogs is associated with oxidative stress, as evidenced by an increase in urinary 8-isoprostanes/creatinine levels, which is a marker of lipid peroxidation. However, there is no correlation found between liver GSH concentration and systemic oxidative stress. Furthermore, there is no correlation between liver GSH levels and plasma and erythrocyte GSH levels (41).

Disease in dogs can cause disturbances in hepatic function and redox balance, not directly through hepatic injury, but through systemic effects, increased immune response, and complications (1). For example, dogs with babesiosis have been found to have increased levels of MDA, a marker of lipid peroxidation, as well as decreased TP and albumin levels and increased glucose and ALP. However, in complicated cases with additional conditions such as renal dysfunction, hepatic dysfunction, muscle damage, and acute respiratory distress syndrome, total bilirubin, ALT, ALP, and GGT levels also increase. An increase in MDA levels was also evident in these complicated cases (40).

Other diseases can also affect the hepatic function and redox balance, not just direct hepatic injury. For example, in dogs with acute diarrhea, elevated ALT and PT levels have been linked to impaired redox balance, but no change was observed in albumin and glucose levels (42). Similarly, type 1 diabetes in dogs has been linked to increased ALT, AST, ALP, glucose, and total cholesterol levels along with an impaired redox system (43).

Liver transplantation can cause alterations in hepatic function parameters and oxidative damage, depending on the transplantation conditions. In a case of canine partial liver autotransplantation, the liver tissue underwent oxidative stress during removal, manipulation, and storage, leading to increased levels of AST, ALP, and LDH (44).

It is well known that therapeutic agents can also cause liver damage. The liver function profile and related redox parameters can deviate from normal values during normal use doses or drug toxicities. In a case of acetaminophen toxicosis in a dog, GSH depletion and increased levels of AST, total bilirubin, direct bilirubin, and indirect bilirubin were reported (45). Lomustine, a frequently used alkylating agent in the treatment of neoplastic diseases in dogs, also causes liver damage and increases ALT, ALP, and SBA levels, while decreasing hepatic GSH levels (46).

Liver injury can occur as a result of essential trace element copper intake through diet. Copper toxicity can cause the potential creation of ROS and result in liver injury in dogs. Symptoms include elevated levels of ALT, ALP, SBA, and ammonia, as well as decreased levels of albumin, increased PT, and activated partial thromboplastin time (47).

In cases where the production of ROS cannot be buffered by the antioxidant system, systemic pathological changes can occur. One such scenario is hyperammonemia, which leads to increased production of ROS and results in various consequences. This includes changes in cellular and mitochondrial membrane permeability, causing cerebral edema. The release of ROS from hyperammonemia also leads to oxidative stress, systemic inflammation, and decreased immunity to infections by impairing neutrophil function (48).

This highlights the importance of evaluating oxidative parameters together with routine hepatic test parameters, especially when liver biopsy samples are taken. It is important to consider clinical chemical parameters and other relevant measurements together to obtain a comprehensive understanding of the relationship between the redox balance and hepatic function.

5.2. Cats

In cats, some diseases can cause alterations in the parameters evaluated in the hepatic panel. These cases can sometimes emerge complicatedly with other diseases. The liver's involvement can be investigated through liver function tests and oxidative parameters (11,17).

Some liver function test parameters may provide more specific results than others. For instance, GGT may be a more sensitive indicator of hepatobiliary disease than ALP in cats, due to ALP's short half-life in cats (17). However, studies directly investigating the relationship between GGT and redox are needed in cats.

In cats with feline hyperthyroidism, an increase in ALT and glucose levels, along with a decrease in albumin levels, has been linked to oxidative imbalance. This type of relationship, where changes in albumin levels are seen without changes in other parameters, has also been reported in cats with diseases such as infectious/inflammatory, chronic kidney disease, metabolic, and neoplastic diseases (49).

As an example of complicated cases, acute pancreatitis is a common condition in cats, often associated with inflammatory bowel disease and cholangitis, and can sometimes lead to conditions such as hepatic lipidosis and diabetes mellitus. In hepatic lipidosis, moderate to significant elevation of ALP and minimal elevation of GGT can be present (17). In cats with acute pancreatitis, increases in ALT, cholesterol, and glucose levels have been reported and this is associated with oxidative stress (50).

Feline hepatic lipidosis is a frequently seen and potentially fatal disease in cats. In this condition, significant increases in ALT, AST, and ALP levels, as well as bilirubin and ammonia levels, have been observed, along with decreases in albumin, TP, and cholesterol levels (51). Cats affected by feline hepatic lipidosis can develop very low hepatic GSH concentrations, which can lead to polysystemic disorders (52).

Therapeutic agents used for treatment can also cause alterations in liver damage and related redox parameters. For instance, the use of methimazole in the treatment of feline hyperthyroidism can contribute to oxidative stress without affecting ALT, AST, ALP, and albumin levels (49). Acetaminophen is known to cause oxidative stress and hepatotoxicity. In cats with acetaminophen toxicity, significant increases in the serum concentrations of ALT, AST, ALP, LDH, total bilirubin, and direct bilirubin have been observed and this is associated with oxidative liver damage (53).

5.3. Other Domestic Animals

Common diseases affecting livestock such as horses, cattle, and sheep can impact liver function and result in elevated levels of liver enzymes in the blood, indicating liver cell damage. Diseases such as hepatitis, liver abscesses, and liver failure from toxin exposure or metabolic diseases can cause this. However, ALT is not suitable for detecting liver cell damage in horses and ruminants as it is only found in low amounts in their liver cells (1). Oxidative stress can also play a role in the development and progression of liver diseases in these animals (54). Further research is needed to assess the

redox relationship and liver function parameters in these commonly farmed animals.

Liver function tests are routinely performed to improve the health and productivity of livestock. Both invasive and non-invasive methods can be used to obtain the necessary liver parameters. For example, ALT, AST, ALP, and LDH were measured from saliva samples taken every four hours during the day in pigs, with time-dependent differences detected but no differences in redox parameters except for a sex-dependent increase in SOD and time-dependent increase in UA (55).

In a study of the physiological changes in adult sows during pregnancy, calving, and lactation, alterations were found in the levels of liver enzymes such as AST, ALP, GGT, and LDH, and advanced oxidation protein products. The most significant alterations were seen during the farrowing period, with elevated levels of stress markers, inflammation indicators, and oxidative stress indicators. Changes were also observed in muscle and liver enzymes (CK, AST, ALP, GGT, and LDH) (56).

Heat stress can have lethal outcomes for living beings (57), including chickens where it can cause a rise in reactive oxygen species (ROS) levels and oxidative harm. Heat stress also causes significant increases in ALT, AST, and ALP levels and decreases in GSH-PX and SOD levels (38).

Rabbits are not only used as livestock but also as animal models in scientific research. In a rabbit liver ischemia model, a redox imbalance occurred due to cellular hypoxia and it was found that ALT activity increased (58).

The feeding of farm animals with dietary supplements and routine use of supplements is a commonly preferred choice. Supplementation of wild-ginseng adventitious root meal in broiler chickens' diet has been observed to have effects on lipid parameters. The supplementation resulted in a decrease in TBARS in breast muscle and serum cholesterol levels (36).

Supplementation of Bazhen powder in broiler chicken's diet has been found to support the synthesis of HDL cholesterol and inhibit the synthesis of VLDL and LDL cholesterol. A decrease in AST levels was reported while ALT levels remained unchanged. The results also showed an increase in the antioxidant system in the liver with the rise of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and catalase (CAT) and a decrease in TBARS and GSH (37).

Supplementation of a herbal mixture in the diet of ducks resulted in increased activities of SOD and CAT, while serum levels of ALT, AST, blood urea nitrogen (BUN), TP, and albumin remained unchanged. Total cholesterol

levels were reported to decrease during the 3-6 week feeding period. It has been suggested that the antioxidant effect may be caused by the inhibition of lipid oxidation and scavenging of ROS, which was revealed by the DPPH and alkyl-radical scavenging activity of the herbal mixture (59).

Endosulfan, a broad-spectrum organochlorine insecticide, has a negative impact on the aquatic environment and the liver function of Nile Tilapia fish. The exposure to endosulfan leads to oxidative stress by decreasing antioxidants (CAT, SOD, GSH-PX, and GSH) and increasing MDA, and altering normal liver histology and GSH-ST mRNA transcript levels. The exposure also results in a significant increase in liver enzymes (ALT, AST, and ALP) and a decrease in TP, albumin, and globulin levels (60).

Nile Tilapia fish exposed to the organophosphate pesticide diazinon showed oxidative damage to the liver, as indicated by elevated levels of liver transaminases and ALP in the blood. The exposure also resulted in decreased levels of TP, albumin, globulins, and the albumin/globulin ratio, as well as an increase in serum glucose and cholesterol levels. The activity of antioxidants SOD and CAT in the liver declined, along with the levels of GSH and GSH-PX. The concentration of MDA significantly increased due to diazinon exposure (61).

Retrospective human studies also often contribute to the literature. For example, the most common causes of hepatocellular injury, which manifests itself with an increase in ALT and AST serum concentrations in humans, are hypoxic hepatitis, congestive hepatopathy, septic shock, and drug-induced liver damage. In drug-induced liver injury, ROS that are produced during the metabolism of drugs can cause glutathione depletion in hepatocytes and lead to hepatocyte necrosis because they are highly cytotoxic (62).

Many studies in the literature that examine the relationship between hepatic clinical parameters and redox are based on experimental models using laboratory animals (63,64). Studies that examine the relationship between ALT, AST, and other hepatic parameters, as well as ROS, inflammatory cytokines, lipid oxidation, and oxidative stress, particularly in relation to nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, and cirrhosis (65), are noteworthy.

6. Conclusion

In conclusion, there is a need for further research on the relationship between hepatic function and redox, especially in the field of veterinary medicine. The limited number of studies available on different animal species, such as pets,

livestock, and farm animals, highlights the importance of expanding this research. However, in this chapter, only domestic animal species are covered within the scope of the chapter. A comprehensive understanding of this relationship would be crucial for the diagnosis, prognosis, and treatment of diseases in veterinary medicine. Thus, it is necessary to invest in this area to advance our knowledge and improve the health of animals.

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CHAPTER XVI

DIAGNOSIS AND TREATMENT OF MICROBIOLOGICAL DISEASES SPECIFIC IN DIABETES MELLITUS

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Diabetes mellitus is an important health problem due to its prevalence, and it has been reported in the literature that there are approximately 424.9 million patients worldwide and one third of these cases are over 65 years of age[1,2].

On the other hand, some studies have emphasized that patients with diabetes mellitus are less fortunate than the normal population in terms of catching and being affected by infection[3-6]. These infections can spread to a wide spectrum, such as soft tissue infections, vascular system infections, pneumonia and other pulmonary infections, fungal infections, wound infections such as diabetic foot, periodontitis infections, and intensive care or hospital-acquired infections[3,5,7,8].

1. Skin and Soft Tissue Infections in Patients with Diabetes Mellitus

Diabetic patients have a significantly higher risk of developing a wide range of skin and soft tissue infections (SSTI), including cellulitis, osteomyelitis, and postoperative wound infections[9]. Among the results shared in the literature of a very large retrospective study, which included more than 22 million SSTI cases, it was reported that it was seen in 10% of diabetic cases[10]. It was determined that the risk of developing SSTI complications in outpatients who were not hospitalized was more than 5 times higher in diabetic patients than in non-diabetic patients[10]. Similarly, the percentage of complications in patients

hospitalized and followed up with the indication of SSTI is approximately 5% in diabetics and 1% in patients without diabetes[10]. In addition, it has been reported in the literature that the incidence of bacteremia, endocarditis or septic complicating conditions is higher in patients with diabetes mellitus whose initial diagnosis is SSSI[10].

The main risk factors for skin and subcutaneous infections in patients with diabetes mellitus it can be summarized as the[3] ;

*High blood glycemia level

*Deterioration of the skin barrier by losing its effectiveness

*Sensory and autonomic neuropathies occurring as a complication of diabetes

*Exposure to trauma

*Formation of pressure ulcers due to continuous or regular pressure Insufficiency in the arteriovenous system

*Inability of the immune system to work effectively

The leading bacterial population responsible for diabetic soft tissue infections as a result of loss of the skin barrier Gram-positive skin that can normally colonize the skin are bacteria[11]. Here is an important point; In cases of superficial cellulitis or abscess formation, the pathogens are mostly monomicrobial, whereas infective ulcers of the lower extremity or wound infections are more likely to be polymicrobial[12]. In superficial skin ulcers or cellulitis cases; Streptococci and Staphylococcus aureus (methicillin-susceptible and non-methicillin-sensitive) bacteria make up the majority. Unfortunately, diabetes mellitus provides an almost open door for colonization, especially with methicillin-resistant Staphylococcus aureus (MRSA)[13]. In the literature, they emphasize that other gram-positive microorganisms such as Corynebacterium and its subspecies and Finegoldia species are responsible for biofilm formation[14]. Although gram negative bacteria are not dominant in deep wound infections; Although Pseudomonas aeruginosa and Enterobacteriace species are in this group, including diabetic foot, they are dominant and common[12].

2. Foot Infections in Diabetes Mellitus

In foot ulcerative lesions caused by diabetes; Diagnosis of infection is not always easy. The reason for this may be due to the unhealthy inflammatory response in diabetic cases after damage or infection, and the inability to feel pain and pain due to neuropathy[15].

Signs of infection in a granulated tissue wound[16];

*Delay in healing
*Massering and embrittlement of tissue
*Occurrence of a very offensive odor
*Abscess secretion
*Increase in lesion size
*Sharpening of pain or discomfort
*Prolonged state of exudate fluid formation

3. Diabetes Mellitus and Pneumonia

Scientific studies reported in the literature; They stated that pulmonary infections are significantly higher in type II diabetes patients than in those who do not have this disease. In addition, they reported that the risk of immune system failure and accordingly lung infections and complications would increase with increasing patient age, uncontrolled hyperglycemia, and increased vascular involvement [17-19]. Vague and occult pneumonia progresses without any obvious symptoms such as coughing, sputum, and chest pain while breathing. Unfortunately, this sepsis has the potential to be overlooked in the first examination. Pneumonia that progresses insidiously in this manner is often seen in advanced age, that is, elderly populations[20]. On the other hand, J.Hua et al. in the literature they shared their work, the main pathogens in all elderly patients with and without occult lung infection; They stated that Pseudomonas aeruginosa, Acinetobacter baumannii and Klebsiella pneumoniae are from Gram-negative bacteria family[20].

Since the course of the disease will vary depending on the physiological characteristics of this population after pulmonary infection in elderly patients, it is essential to use antibiotics to which the lepathogenic bacteria are sensitive, with continuous meticulous follow-up[20].

In addition, one should be vigilant against changes in the resistance of the pathogen, and antibiotics should be changed without losing time when necessary. From this point of view, it should be considered how dangerous a co-morbid factor such as diabetes mellitus can lead to, and blood glycemia should be kept under control[20]. If the pneumonic involvement coincides exactly with the sagittal position of the heart or spine, this may be overlooked in the diagnostic examination in the riegraphy. As a result, it may lead to delay in antibiotherapy, which may lead to life-threatening multi-organ dysfunction[18, 21-23]

It should always be kept in mind that the prognosis will not be good if the clinical follow-up is not well managed with a dynamic treatment and follow-up process, keeping in mind that lung infections can go away with indistinct symptoms, especially in the elderly population with diabetes, and Gram-negative bacteria are common pathogens[20].

4. Covid-19 and Diabetes Mellitus

Coronavirus (COVID-19) has affected 118 million people On the other hand, it has become a viral pathogen responsible for the death of more than 2.5 million people in the world[24,25]. Considering that diabetes is already causing problems in the functioning of the immune system, it poses a very serious clinical problem for this population[24,25].

In most scientific articles published in the literature in recent years, it has been clearly stated that diabetes increases mortality in cases with Covid-19 pneumonia, just as it did in previous viral diseases such as SARS-CoV and MERS-CoV[26,27].

A Elibol et al. stated in their study that the preference of DPP-4 inhibitors for the treatment of diabetes mellitus is an increased risk factor for pneumonia in cases diagnosed with Covid-19[28].

In summary, in cases close to Covid -19; Diabetes mellitus itself and the medical treatment chosen in the treatment of diabetes and the success of this treatment can lead to severe pneumonia and mortality due to respiratory failure.

5. Role of Diabetes Mellitus in Urinary Tract Infections

Another increased risk in diabetics is related to the urinary system microbiota. Urinary tract infection (UTI) is a serious health problem in diabetic cases. Many different types of UTIs can be seen in a wide range from bacteriuria to upper UTIs in cases with diabetes. Furthermore, it has been reported in the literature that patients with diabetes have a lower chance of receiving outpatient treatment for pyelonephritis or bilateral kidney infection compared to cases without diabetes[29]. Another misfortune of DM cases is the increased potential for severe urosepsis, such as intra or perirenal abscess and emphysematous infections of the urinary tract[29]. As reported in some studies in the literature; Type 1 or 2 DM significantly increases the risk of UTI and bacteriuria caused by Enterobacteriaceae in females. The reported relative risk (RR) of symptomatic UTI for DM cases has been reported to range from 1.5 to 2.2 compared to

patients without DM. This is especially true; It will increase in those who take oral antidiabetic or need insulin therapy and who have diabetes mellitus much earlier[30-34].

6. Dermatomycoses and Diabetes Mellitus

The risk of tinea pedis and onychomycosis is significantly higher in patients with diabetic cases. [35]. More importantly; The belief that onychomycosis is a marker in diabetic foot syndrome is well established [36]. It has been found that diabetic cases with onychomycosis are 1.6 times more likely to develop diabetic foot ulcer disease [37]. It is thought that being in advanced age, male gender, presence of metabolic syndrome, obesity values, detection of high triglyceride levels in serum or uncontrolled blood sugar level (high HbA1c) increase the risk of onychomycosis disease. In a study in the literature; It has been emphasized that diabetic patients undergoing hemodialysis have an almost 88% higher risk of onychomycosis compared to patients without diabetes mellitus[38].

7. Hospital Infections and Diabetes

In the literature, it has been emphasized that hospital-acquired infections (HAI) and mortality due to hyperglycemia are associated with each other, and it has been reported that the treatment of hyperglycemia can be further improved with the noncritical care[39,40] .

To date, there are many studies in the literature indicating that there is a significant link between diabetes mellitus and an increased of infection status.

In laboratory experiments; It has been proven that the secondary increased susceptibility to infection in diabetes is associated with disruptions in the neutrophil system (impaired functioning in chemotaxis, decreased phagocytosis ability, and severely reduced microbicidal activities) [41-44] or an incomplete adaptive immune response [45,46]. At another important point, the colonization of pathogenic microbial strains increases in cases due to hyperglycemia [47,48].

Because of all these factors, the possibility of developing nosocomial infections in diabetic patients should always be kept in mind and should not be overlooked.

8. Infections in Cases with Peripheral Vascular Disease and Diabetes

Unfortunately, comorbid peripheral vascular disease (PVD) occurs at a rate as high as 9.5% in patients with diabetes and 40 years of age [49], and the risk

of developing PVD in these patients is almost four times higher than in cases without diabetes mellitus [50] . Suaya JA et al. according to the results of their study, they stated that diabetic cases were more likely to have complications related to SSTI (scin soft tissue infection) compared to non-diabetic cases[51]**.** Esepcially foot infections are an important reason of morbidity and mortality in casas with diabetes, the important point here is that cellulitis and infected ulcers in other areas also contribute to the infection risk in such cases[52]. In particular, Staphylococcus aureus is the principal pathogenic agent responsible for soft tissue,skin infections[53].

In spite of diabetes is a well-known important risk factor for hospital-acquired MRSA cSSTIs [54], in a more recent study; It has been reported that the rate of admission to the US emergency department in patients with diabetes with the diagnosis of CA(community-acquired)-MRSA(Methicillin resistant S. aureus) cSSTI is two times higher than that of patients without diabetes[55]. In summary, it should always be kept in mind that the potential for soft tissue and skin infections will increase in cases with diabetes because of the risk of vascular disease and, consequently, circulatory problems.

9. Diabetes Mellitus and Sepsis

Sepsis is an important organ dysfunction that has the potential to lead to mortality as a result of a cascade of reactions that disrupt metabolic adaptation due to infection. There is a risk of developing a septic table due to the attack of microorganisms. In sepsis, especially intensive care units are of particular importance, as patients with the potential to result in cardiovascular failure, mortality and morbidity are frequently present[56].

In view of the significant potential of diabetes mellitus (DM) to cause sepsis, the risk of sepsis has also increased, as the incidence of diabetic patients has increased markedly worldwide[56].

10. Periodontitis and Diabetes Mellitus

The development of periodontitis increases inflammation. Increased inflammation impairs glycemic control mechanisms in cases with diabetes. Impairment of glycemic control increases the complications of diabetes[57]. As we mentioned above, these complications disrupt the circulatory system or the immune system, affecting the bacterial distribution (periodontal microbiota)[57] in the intraoral area with a widespread bacterial population. The most increased

bacteria is; Porphyromonas gingivalis. This pathogen is responsible for some of the periodantal diseases[58].

We see that diabetes mellitus is closely related to many diseases of microbial or viral origin, which we have explained in subgroups above.

Because the main underlying problem is that diabetes, which has an important place among chronic diseases, impairs the immune system, causes neural damage, deprives patients of the feeling of pain caused by skin injuries, or uncontrolled diabetes, which disrupts microvascular circulation over many years and undermines tissue healing. As a result, a reflection of diabetes mellitus complications is diabetic microbial or viral infections too.

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CHAPTER XVII

THE STATE OF DEPRESSION-LIKE BEHAVIORS IN ANIMALS WITH METABOLIC SYNDROME

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Metabolic syndrome (MetS), abdominal obesity starting with insulin resistance, glucose intolerance and It is a fatal endocrinopathy combined with systemic disorders such as diabetes mellitus, dyslipidemia, hypertension and coronary artery disease [1]. According to a systematic review and meta-analysis of epidemiological studies, report that there is a reciprocal relationship between depression and MetS. therefore, it supports the early diagnosis and treatment of depression in patients with MetS [2]. Some studies showed that depression was remarkably associated with the waist circumference, a component of MetS [3]. MetS and depression might share same dysfunctions such as the hypothalamus–pituitary–adrenal (HPA) axis dysregulation, chronic inflammation, oxidative stress[4]. In this study, depression-like behaviors were investigated in rats with metabolic syndrome. The general consensus is that depression-like behaviors are increased in metabolic rats and mice. (See Table 1). Some studies have not shown this result [4, 5] . In the other nutritional regimen (HFHF) used by Gancheva et al.2017, depression-like behaviors increased [5].

According to Ribeiro et al 2020, while latency was negatively shortened, the total immobility time did not change [6]. This situation can be partially interpreted as an increase in depression-like behavior. Swimming behavior related to serotonin was negatively affected in MetS rats [7]. Climbing behavior related to noradrenaline was not impacted on MetS rats [6, 7]. In this regard, the serotonergic system-related behaviors are more vulnerable to MetS. In mice treated with a carbohydrate-rich diet, depression-like behaviors were increased in another depression test, the tail suspension test [8]. Based on this brief evaluation, overall depression-like behavior was increased in metabolic animals. However, despite limited studies, serotonergic behaviors seem to be more sensitive. Further behavioral, molecular studies will can elucidate this comorbidity. The results of preclinical studies are consistent with the results of clinical studies.

1.Metabolic Syndrome Model

Mets is created by dietary, genetic, and pharmacological models. Different dietary regimes induce MetS in animals have been reported by experimetal rodent studies. This regime included single or multiple diets such as high-fructose, high-sucrose, high-fat, high-fructose/high-fat, or high-sucrose/high-fat diets [22].

Leptin- or leptin receptor-deficient rodent models are used as genetically obese and diabetic experimental models. This animal also showed that Mets clinical Picture. This animals also showed that Mets clinical PicturesFurther pharmacological models such as antipsychotic-induced MetS and glucocorticoid-induced MetS are in other rodent studies [22].

After inducing the model, hyperglycemia, hyperlipidemia, obesity and hypertension are examined as components of Mets [22].

2.Depression Tests

2.1.Forced Swimming Test

It was firstly described by Porsolt et al in 1977 [23]. It is the most commonly used animal depression test [24]. After a certain period of time, floating behavior (immobile) like a piece of wood is observed in rats or mice forced to swim. Forced swimming test have some advantages for researchers including timing, cheaping, recording so on. On the other hand, neurotransmitter-based behaviors such as serotonergic system related swimming behavior and noradrenergic

system related climbing behavior can be examined. Table 1 summarizes the behaviors analyzed in the forced swim test and their meanings [28].

2.2. Tail Suspension Test

It was firstly developed in rats by Seture et al. in 1985 [26]. Although tail suspension test performed generally in mice, it can also be done in gerbils and rats [27]. Some authors suggest that it should only be done in mice, as it will cause pain in heavy rodents such as rats [25]. Tail suspension test have similar advantages for researchers (by terms of cheap, time, analyze and record so on).

Unless Forced swimming test, behavior analysis on the basis of neurotransmitter cannot be done in the tail suspension test. Total immobility time is analyzed as depression like behavior [27].

In this book chapter, only the behavioral depression tests including forced swimming test and tail suspension test are examined by terms of Mets models.

Table 1: Behavior patterns in the forced swim test [28]

Behavior patern	Describtion	Mean
İmmobility (floating)	Total immobility time (The period when 3 or 4 limbs are immobile)	An increase in this period indicates an increase in depression-like behaviors.
Mobility time	Total mobility time (the period when at least 2 limbs are mobile)	An increase in this period indicates an increase in antidepression-like behaviors.
Latency	Time until the first inactivity time elapses	The shortening this period is considered depressive like behavior.
Swimming	Horizontal movements in water.	The increase in this behavior is considered as antidepressant activity. It is associated with the serotonergic system.
Climbing	Vertical movements in water.	The increase in this behavior is considered as antidepressant activity. It is associated with the noradrenergic system.
Head shake Dive	Head shaking behavior Diving behavior	adaptive behavior

Table 2: Mets and Forced Swimming Test's Behavior Pattern

Study	Metabolic Syndrome Model (Mets)	Animal	Behavior parameters	Result
Dinel et al, 2011 [4]	Genetic	*db/db* mice	Immobility time↔	DLB ↔
Lemos et al, 2015 [9]	sucrose	Male Wistar Rat	Immobility time ↑	DLB ↑
Ressler et al, 2015 [20]	High fat diet, Hight fat diet + dihydrotestosterone	female Long-Evans rat	Immobility time ↑	DLB ↑

Vargas et al, 2016 [15]	periodic maternal separation protocol	Male Sprague-Dawley rats	Immobility time ↑ Climbing time , Swimming↓ time↓, Latency time ↓	DLB ↑
Li et al, 2016 [10]	corticosterone	Male ICR mice	Immobility time ↑	DLB↑
Gancheva and Zhelyazkova-Savova, 2016 [13]	high-fat high-fructose diet	Male Wistar Rat	Immobility time ↑	DLB↑
Gancheva et al, 2017 5	HFHF (high fat+ high sucrose)	Male Wistar Rat	Immobility time ↑	DLB ↑

Table 3: Mets and Forced Swimming Test's Behavior Pattern

DLB: Depression-like behavior, ↑: increase, ↓: decrease, ↔: not change

Gancheva et al, 2017 [5]	HF (High fat+ sucrose)	Male Wistar Rat	Immobility time ↔	DLB ↔
Guo et al, 2018 [7]	D galactose/ D-galactose+sucrose	Male Sprague Dawley rats	Immobility time ↑, climbing time↔, swimming time ↓	DLB ↑
Oliveira et al, 2017 [11]	highly palatable diet	Male C57BL/6 mice	İmmobility time↑	DLB↑
Oliveira et al, 2018 [12]	highly palatable diet	Male wistar rat	İmmobility time↑	DLB↑

Table 4: Mets and Forced Swimming Test's Behavior Pattern

Pan et al, 2019 [14]	Chronic Immobilization-Stress	Male Sprague-Dawley rats	İmmobility time↑	DLB↑
Patterson et al, 2019 [19]	High Fat	Male C57BL/6J mice	İmmobility time↑, Fecal Pellets Number ↑	DLB↑
O'Flaherty et al 2019 [21]	High Fructose	Male Sprague Dawley rat	Immobility time↔ Climbing time ↔, swimming time ↔	DLB↔
Ribeiro et al, 2020 [6]	High Fructose	Male Wistar Rat	Immobility time↔ Latency↓ Climbing ↔	DLB↔, ↑
Zborowski et al, 2021 [18]	200 mg/kg, i.p single dose streptozotocin	Male swiss mice	İmmobility time↑	DLB ↑

Table 5: Mets and Tail Suspension Test's Behavior Pattern

Study	Metabolic Syndrome Model	Animal	Behavior parameters	Result
Bax et al, 2018 [16]	high fat diet	C57Bl6J female and male mice	İmmobility time↑	DLB ↑
Santos et al, 2018 [17]	High refined carbohydrate-containing diet	Male BALB/c mice	İmmobility time↑	DLB ↑
Zborowski et al, 2021 [18]	200 mg/kg, i.p single dose streptozotocin	Male swiss mice	İmmobility time↑	DLB ↑

DLB: Depression-like behavior, ↑: increase, ↓: decrease, ↔: not change

3.Conclusion

The general consensus is that depression like behaviors increase in animals with a metabolic syndrome. The association of this condition (depression like behavior) with hyperglycemia, hyperlipidemia, hypertension or obesity shown to confirm the metabolic syndrome has not been studied and displayed. This correlation relationship should also be reviewed in future studies. Performing the forced swimming test in both rats and mice and examining behaviors based on neurotransmitters seems to be more advantageous than the tail suspension

test. If there is a risk of infection as a result of swimming in the water, the tail suspension test can also be performed, provided that it is only in mice.

Acknowledgment

We would like to thank our friend Zeliha Yılmaz for encouraging in writing this chapter and scientific studies. Zeliha Yılmaz unfortunately lost her life in the earthquake that occurred in 2023. We attribute this book chapter to an important scientist, Zeliha Yılmaz.

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CHAPTER XVIII

THE USE OF GENETICS IN METABOLOMICS RESEARCH

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1. Introduction

An organism's metabolome provides a molecular phenotype that can be used to assess the external influences that shape its development and existence. As an addition to the metabolome, RNA sequencing techniques allow us to identify molecular phenotypes associated with differential gene expression (1). Metabolomics is a research approach that utilizes data to identify biomarkers for metabolic systems and metabolic confusion and to substantiate hypotheses. The process of metabolomics involves the detection and measurement of metabolites at a global level across different samples (2). The term "metabolomics" is often used to describe biochemical processes taking place within organisms as an analog of an "imaging approach." There are many diseases and genetic factors that affect a person's metabolism. When attempting to link genetic problems with human health, it is necessary to identify an individual genetically determined or genetically influenced metabotype (3).

Metabolomics has enabled the detection of genetically influenced metabotypes in large biological sample sets by measuring hundreds of metabolites simultaneously, using low-cost automated procedures. Metabolomics of single cells offers an excellent way to decode cell heterogeneity due to its ability to identify metabolic profiles of individual cells (4).

2. Metabolomics

A metabolomics study measures the metabolites in body fluids qualitatively and quantitatively. Genetic transcription and translation processes and interactions with environmental exposures produce metabolites at the downstream end of the process. When it comes to multifactorial diseases, these factors are thought to be closely connected to the phenotype (5). Metabolomics is the study of the pattern of metabolites in biological systems (cells, tissues, and organisms) under specific conditions. A metabolome is considered the complete set of metabolites within a given system. Biological processes result in the production of metabolites as their downstream products. As a result, metabolite profiles can change due to gene-gene interactions and gene-environment interactions. Analyzing chemical data and integrating biostatistics and bioinformatics into metabolomics is crucial for providing accurate answers to contemporary problems in human disease research. Besides identifying new biomarkers for endocrine-related diseases, this technology is now being used to explain the mechanisms of these diseases (6).

It has revolutionized many biomedical disciplines because of metabolomics, which emerged in the early 2000s as part of the omics movement. A hypothesis-driven approach to biology remains the golden rule (Figure 1a). Research on metabolomics using large data sets can show us how to alter the metabolome under basal and induced conditions. Biomarker images could be analyzed under favorable circumstances to devise hypotheses and test them. A metabolomics approach can sometimes produce unexpected results unrelated to the original problem (Figure 1b) (7).

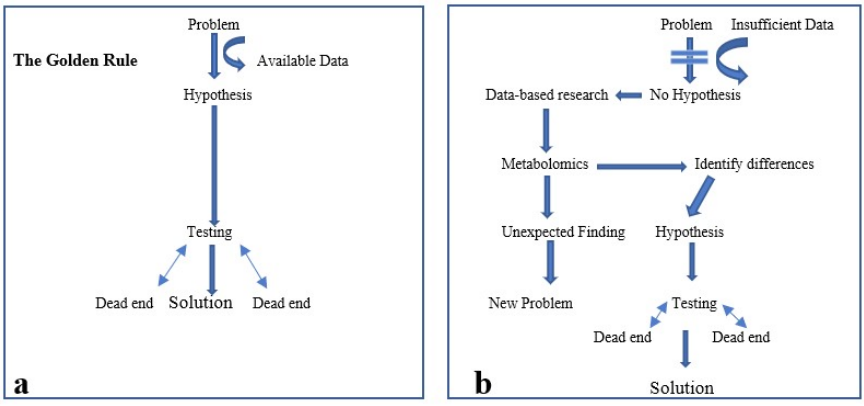

Figure 1: *a*, Golden Rule of Scientific Inquiry. *b*, Metabolomics Findings and Strategies.

Researchers are increasingly conducting large-scale metabolomics studies to study how genetically distinct individuals respond to environmental influences

over time. In general, the cost of applying all omics techniques to many samples varies, but they can all be widely applied. A metabolomics study provides 10x to 100x higher measurement rates than MS-based proteomics, which requires chromatography and peptide fragmentation. During the next few years, there will be three frontiers where high-throughput metabolomic measurements will be useful. They include large-scale profiling studies, high-resolution analyses of dynamic systems, and measurements of all cellular populations on an individual cell basis. These experiments have been enhanced by recent technological advancements, including sample sizes of 100,000, time resolution of seconds, and the ability to detect metabolism in single cells (Figure 2) (8).

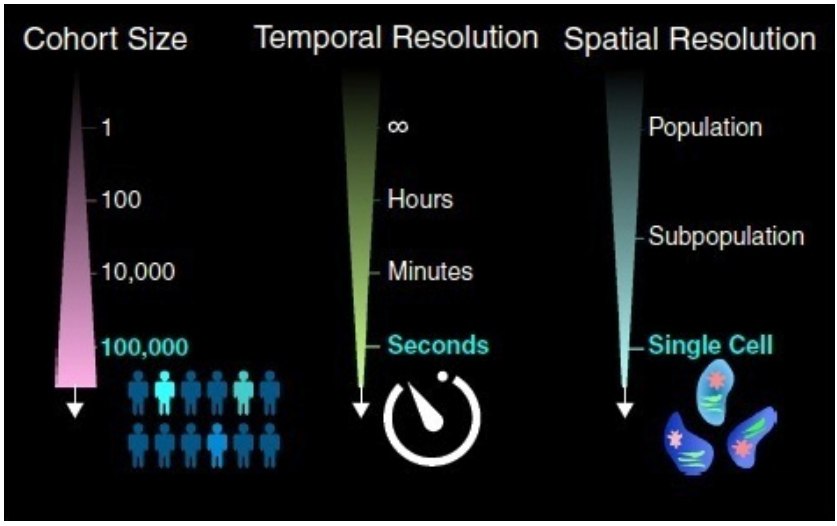

Figure 2: High-Throughput Metabolomics:Cohort Size, Spatial Resolution, and Temporal Resolution (8).

Metabolomics is discussed in this chapter as a tool for developing biomarkers of metabolic processes and genetics for metabolomics research. Information on metabolomics platform selection, study design, and data analysis in genetic studies is presented.

Molecular metabolomics is conducted primarily by nuclear magnetic resonance and mass spectrometry. Having both instruments allows high-throughput measurement of various metabolites in a reproducible manner (9).

2.1. Nuclear Magnetic Resonance (NMR) Spectroscopy

Because of its non-destructive nature and many organic compounds in biological samples, they frequently used NMR in metabolomics studies. As a

metabolomics technique, NMR has the significant limitation of its low sensitivity, which means it can only detect metabolites at very high concentrations. There are several techniques available in precision medicine, depending on the sample and the biological question (10).

NMR-based metabolomics workflow is demonstrated (Figure 3). Biological samples such as blood plasma, serum, urine, animal and human model tissues, and cells are used for metabolomic studies. The process involves the detection of metabolite signals, identification of metabolites using a combination of 1D and 2D NMR methods, database searches, and additional compounds, and then quantification of metabolites by comparing them with a single internal standard. Besides being increasingly important for diagnosing diseases in humans, metabolic profiling of intact tissue has gained growing interest. Tissue is one of the most effective methods for analyzing disease biomarkers since it highly concentrated them because of their close association with the pathological source, for instance, tumors (11).

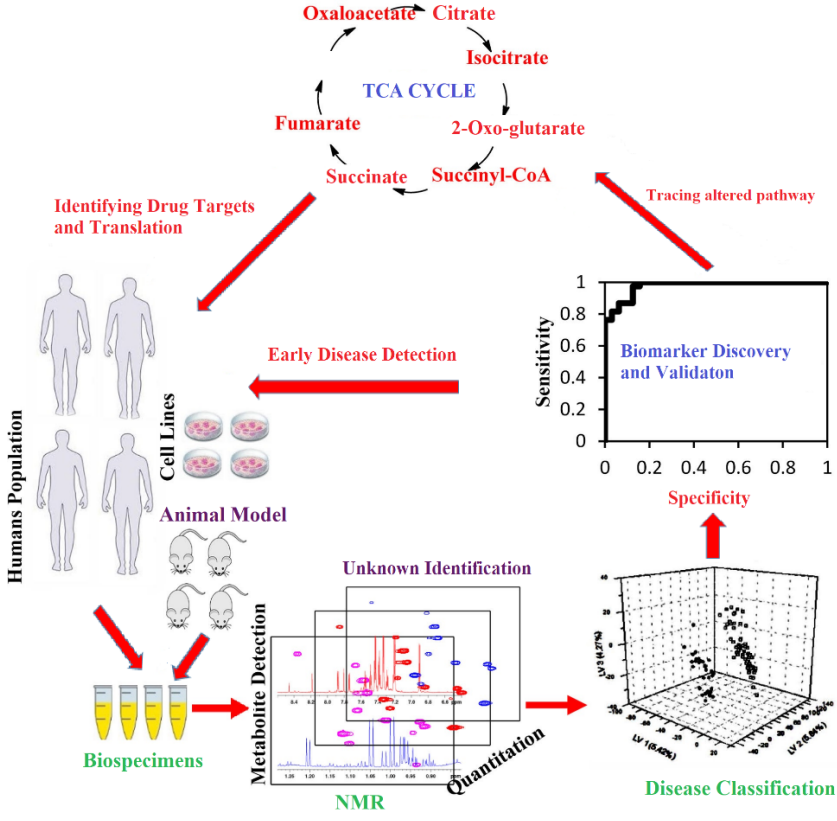

Figure 3: An Illustration of The Workflow in NMR-Based Metabolomics (11)

2.2. Mass Spectrometry (MS)

MS is commonly used to separate electrically charged species in the gas phase. Sources of ions produce charged species (ions). Solid-phase and liquid-phase analytes can be transferred to the gas phase using ion sources (12). A sample is separated using liquid chromatography (LC) or gas chromatography (GC) and ionized to identify the biomolecules. MS determines the mass-to-charge (m/z) ratios of fragmented parent compounds. The data acquisition software allows the acquisition of a full mass spectrum from target analytes (9).

There has been an increase in the use of mass spectrometry to study blood proteins and peptides, and many proteins not previously detected have been discovered. Researchers are now using mass spectrometry to identify diseases associated with polypeptides in the blood. Biomarkers can also be discovered through mass spectrometric measurements or protein discovery via mass spectrometry (13).

2.3. Gas Chromatography–Mass Spectrometry (GC-MS)

Its use in metabolomics is ideal for identifying and quantifying small molecular metabolites (for example, alcohols, hydroxyl acids, amino acids, sterols, catecholamines, fatty acids, and drugs). These compounds usually become volatile enough to be analyzed by gas chromatography through chemical derivatization. There are many types of GC-MS systems available, from classic detectors to target mass spectrometers to mass spectral instruments that are capable of providing accurate mass data (14).

Analyzing gaseous mixtures with GC is a very efficient analytical tool. Hydrogen (H2) can either be detected with a thermal conductivity detector or by a helium ionization detector when it is present in the sample, which is separated by specific columns. The new method is based on the detection and analysis of H2 isotopes by chemical ionization mass spectrometry with an electron ionization ion source and a quadrupole analyzer (15).

Metabolomic analysis can be enhanced by GC-MS due to certain features. These features can be listed as follows: (a) high sensitivity, enabling precise measurements using less raw biological material; (b) the gas phase allows better separation of compounds than the liquid phase; (c) a large number of compound databases and experimental protocols derived from the wide range of applications for which it has been used in the clinical, forensic, and biotechnology fields; (d) the least expensive metabolomic technology in terms

of purchase, operation, and repair; (e) its ease of use when compared to other technologies(16).

2.4. Liquid Chromatography–Mass Spectrometry (LC–MS)

Electrospray ionization provides a simple, robust interface to LC-MS, making it a routine technique. The assays can be developed with tandem MS and stable isotope internal standards for a wide range of biological molecules, although optimization is necessary to minimize ion suppression. Fast scanning speeds enable high degrees of multiplexing, allowing for simultaneous measurement of many compounds (17).

There has been an increase in the application of LC-MS research in clinical areas. A wide range of clinical tests can be conducted with LC-MS, including newborn screening, therapeutic drug monitoring, and identifying drugs of abuse, metabolites, and hormones (18). Pharmaceutical analysis has demonstrated great promise in the last decade using LC-MS-based techniques. This technology has been a key factor in studying drug metabolism, identifying and characterizing degradation products and impurities in drug products, and identifying and characterizing new drug candidates. LC is the gold standard for analytical strategies in pharmaceuticals thanks to recent innovations in LC instrumentation, chromatographic modes, and stationary phases. Nano-LC systems have the potential to become more important in pharmaceutical analysis in the future, especially microfluidic chip-based and miniaturized LCs (19).

3. Large Scale Metabolome Profiling

Metabolome data sets from human epidemiology and genome-wide association studies represent the largest collection, where genetic information has been combined with other markers related to disease, cellular organization, or gene-environment interactions (8). Cao and colleagues applied a targeted metabolomics approach to acquire MS and MS/MS data and investigate a variety of human samples, such as cells, tissues, and fluids, using a high-resolution Orbitrap mass spectrometer. Therefore, unbiased metabolite identification was achieved by searching mass spectral libraries. Then, using ultra-high-performance liquid chromatography coupled with triple quadrupole mass spectrometry (UHPLC-QqQ MS), a targeted metabolomics method was developed, allowing detection of over 400 biologically important metabolites and monitoring of 92 metabolic pathways (glycolysis, Krebs cycle, pentose

phosphate pathway, amino acid metabolism) in biological samples. By means of this method, isomeric metabolites can be separated, including glutamine and glutamic acid, as well as molecules of similar molecular masses (20).

Using non-targeted metabolomic profiling, a large part of a biological sample's metabolome is simultaneously analyzed. A large-scale metabolomic profiling of epidemiological studies has not been conducted because of the high costs and complexity of the data processing. Genetic and metabolomic data were combined in a study by Ganna et al. to identify four lipid-related metabolites with clinical potential and evidence of causal action in coronary heart disease. These types of studies provide an excellent platform to study metabolic variability across individuals and its impact on human health (21).

Metabolic profiling of cell lines has become a valuable tool for understanding the underlying causes of diseases, identifying drug mechanisms of action, and personalizing treatments for patients. The complex measurement procedures and time required for large-scale in vitro dynamic metabolic profiling are limitations. Through analyzing the metabolic responses, it became apparent that CoA biosynthesis plays a crucial role in dichloroacetate toxicity and that CoA homeostasis is important across a wide range of human cell types (22).

4. Time-Resolved Metabolomics

A dynamic optimization problem using time-resolved metabolomics data combined with metabolic network stoichiometry describes the dynamics following perturbations. An optimization problem is solved to obtain dynamic reaction rate profiles and upper and lower bounds on those rates(23). Metabolomics provides the major means to bridge the gap between genotype and phenotype, identifying and validating targets for improving health on an individual basis. A metabolomics biomarker is identified by assessing differences in metabolic profiles between different states. Whenever time-series studies are conducted, the datasets that are analyzed are extremely complex and may include variation due to constant differences in time among animals, time-dynamic variations, or combinations of these. There have been many methods used to analyze time-resolved metabolomics data, including methods borrowed from other disciplines. Multiple-level datasets can be studied using analysis of variance (ANOVA) and simultaneous component analysis (ASCA), an extension of ANOVA for multivariate data (24).

Metabolic profile correlation analysis can uncover silent phenotypes and genetic changes that aren't immediately apparent. Using time-series data, Kohonen self-organizing map (SOM) analysis can also classify and monitor metabolic change patterns. A time-resolved target analysis is a valuable tool for observing biochemical dynamics. Sato et al. examined hourly rice leaves for 56 essential metabolites over a 24-hour period. They developed capillary electrophoresis-mmass spectrometry (CE-MS) technology that enabled high-throughput analysis of Bacillus subtilis extracts, as well as the genetic and environmental deterioration of E. coli cells (25).

Nitrogen deprivation causes lipid hyperaccumulation by oleaginous microorganisms. Lu et al. collected metabolomics, lipidomics, and proteomics data to study how nitrogen deprivation caused metabolic changes that led to triacylglycerol (TAG) accumulation in M. alpina. The accumulation of TAG was primarily caused by the reallocation of carbon, nitrogen sources, and lipids under nitrogen deprivation rather than an increase in the biosynthesis of TAG. A global view of resource use by M. alpina under nutrient stress is provided in the study. Thus, some interesting relationships between resource reallocation and TAG accumulation are revealed. (26).

5. Single Cell Metabolomics

It is important to gain an understanding of biological processes from individual cells because their chemical activities can provide valuable information. Phenotypic dynamics and microenvironmental changes can cause cells with identical genotypes to have different chemical properties. Single-cell metabolomes have several scientific opportunities, but they also present several challenges because of the limited sample volume, minimal analyte amounts, and rapid turnover. Non-invasive, sensitive, rapid quenching and analysis of single cells, along with the ability to analyze large volumes of cells at once, are essential characteristics of the ideal single-cell metabolomics technique (27).

As high-throughput metabolism analysis becomes increasingly sophisticated, single-cell metabolomics (SCM) has become a powerful tool. An excellent method for decoding cellular heterogeneity is simple-cell metabolomics, which allows us to identify the metabolic profiles of individual cells. In recent years, there have been several advances in both equipment and technologies that have made SCM analysis possible for a variety of biological samples (28).

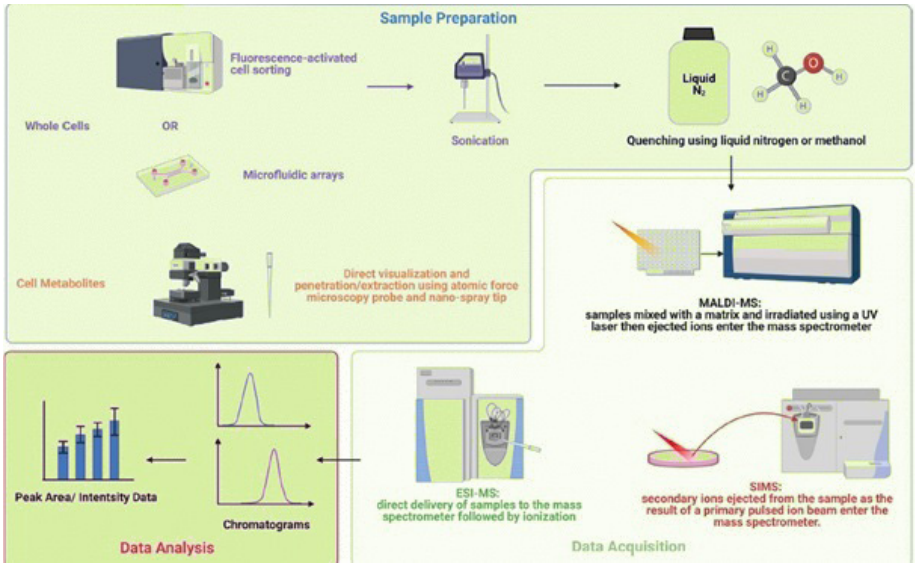

Figure 4: Single Cell Metabolomics Workflow (28)

As a typical workflow in SCM, samples are prepared for the isolation of individual cells or their components (Figure 4). There are other commonly used sample preparation methods such as fluorescence-activated cell separation (FACS), microfluidic arrays, and direct visualization and penetration by atomic force microscopes (AFM). There is evidence to suggest that sonication increases detection sensitivity for whole cell samples, while direct transmission from the probe to the mass spectrometer minimizes distortion. It is essential to quench isolated cells with an organic acid or primary solvent immediately after isolation in order to denature enzymes and prevent further metabolic transformations. Cellular metabolism is stopped by freezing, and freezing with liquid nitrogen is a common quenching method. Organic solvents are commonly used to quench cellular metabolism. The matrix-assisted laser desorption/ionization MS (MALDI-MS) utilizes acetonitrile both for quenching and extraction. Electrospray ionization MS (ESI-MS) involves delivering samples directly from a probe or capillary to the mass spectrometer with no pretreatment. Secondary ion MS (SIMS) is a new technique in ultrahigh resolution SCM; it requires the impact of an ion beam on a surface, followed by the measurements of the secondary ions removed by a mass spectrometer (28).

Typically, SCM analyses are hindered by the isolation and careful handling of single target cells and the transfer to downstream analyses, as this directly impacts data precision. The most common manual methods used for single-cell

analysis are limited dilutions, manual cell picking with a micromanipulator, and laser microdissection. Single-cell isolation is most commonly performed using fluorescence-based cell sorting, high-density microarrays, and microfluidics. An aptamer-based cell isolation method makes use of structural conformation to precisely bind aptamers to their target and facilitate the isolation of single cells. SCM can analyze whole cellular metabolites, but it is more often used to analyze a cluster of metabolites under specific conditions (29).

6. Conclusion

Metabolomics is a relatively new postgenomic field and one of the best technologies for developing new diagnostic tests in medicine for the future. The study of metabolomics examines the products of biochemical reactions in an organism and their interactions with the environment. Biomarkers and new diagnostic techniques for early disease detection are a key aspect of precision medicine and can be found in metabolomics. Oncological biomarkers have been discovered using mass spectrometry (MS)-based metabolomics, especially in the early stages of cancer, when it may be possible to prevent the development of tumors and the spread of metastatic diseases. In patients with lung cancer, metabolomics and metabolites from untargeted MS analysis have been identified as potential biomarkers to predict treatment effectiveness. A metabolism-based approach can help to improve glucose tolerance and type 2 diabetes diagnosis and prediction (30).

A wide spectrum of omics technologies utilizing MS are currently being applied to investigate the body's metabolic phenotypes by profiling small molecules in multiple matrices. Due to the complexity of organismal lipidomes, lipidomics has become an independent field of omics as opposed to molecular metabolism, which focuses on hydrophilic classes. The combination of metabolomics and lipidomics, an emerging approach for mechanistic examination, is evolving along with the development of high-coverage omics approaches. Through the integration of the metabolome and lipidome, a comprehensive map of the metabolic landscape is available, enabling the identification of the metabolic drivers that drive disease pathology as well as understanding the interconnections between lipids and other metabolites (31).

In this section, the types of metabolomics, how to interpret and analyze metabolomics data at the biological or clinical level, and recent developments in these areas are also discussed. The metabolome depends on the genotype, physiology, and environment of the organism. One or more different analytical

platforms can be used when considering many factors, depending on the type of metabolomics experiments, species-specific metabolome size, dynamic range, and application in different biological samples. Omix technologies are used to reveal metabolite dynamics unbiasedly, to provide fast, accurate, and precise analysis of metabolites, to define the patterns between biological samples, to identify biomarkers, and to evaluate the biochemical results of drug treatments.

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CHAPTER XIX

FORMATION OF PANCREATIC CANCER AND RISK FACTORS

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1. Introduction

The pancreas is a lobular mixed gland that produces exocrine and endocrine secretions. Pancreatic cancer is one of the most aggressive types of cancer. Despite its low incidence, it has a high mortality rate. Despite progress in cancer management, the survival rate has remained

virtually unchanged over the past decade. The one-year survival rate of pancreatic cancer patients is 20%, and the 5-year survival rate is approximately 7%. It is the seventh leading cause of cancer-related deaths all over the world. Poor prognosis; the insidious growth is due to nonspecific symptoms and nonspecific methods for early diagnosis. From a morpho-pathological perspective for pancreatic tumors, more than 95% of pancreatic tumors originate from the exocrine pancreas and less than 5% from the endocrine pancreas. Three leading lesions, pancreatic intraepithelial neoplasms, intraductal papillary mucinous neoplasms, and mucinous cystic neoplasms, play a role in developing pancreatic cancer. Most of the risk factors do not directly cause the disease, but the level of exposure to these factors generally affects cancer development. Limited treatment options and identifying controllable risk factors that may play a role in developing pancreatic cancer are significant for individuals with a family history of pancreatic cancer at high risk.

2. Pancreas

Herophilus defined the pancreas in 300 BC, and Rufus made it the first "pancreas" name. Pancreas means flat meat in ancient Greek and Latin (1). The pancreas is a retroperitoneal organ that emerges from the caudal part of the anterior intestine as posterior and anterior pancreatic buds in the fourth week of fetal life. It is located in the back of the stomach, from the duodenum to the spleen. It is yellowish, 15-25 cm long, and 70-150 g in weight in humans (2).

Pancreas; consists of 5 parts: head, uncinate process, neck, body, and tail. Head of the pancreas; It is the most significant part and is located in the arc made by the duodenum, and the last part of the common bile duct usually passes through the head of the pancreas. Processus Uncinatus is located behind the portal vein and superior mesenteric vessels, in front of the aorta and inferior vena cava. The uncinate process may not be in every person or surround the superior mesenteric vessels.

The neck is a relatively narrow part of the pancreas. Its body and tail are adjacent to the aorta, superior mesenteric vessels, left kidney, and suprarenal gland, and the tail part extends to the splenic hilum. The splenic vessels are located within the upper part of the pancreatic tail (2).

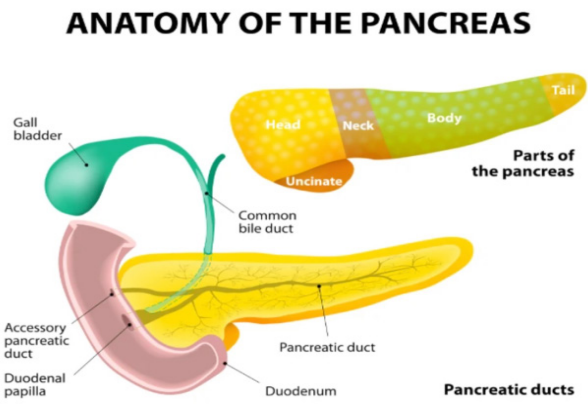

Figure 1: Anatomy of the Pancreas (3)

The pancreas is a lobular mixed gland that produces exocrine and endocrine secretions. The exocrine part, which makes up 98% of the pancreas, consists of acinar and duct cells. This part secretes the pancreatic fluid necessary for the digestion of carbohydrates, proteins, and fats. 2% of the endocrine part of the pancreas consists of Langerhans islet cells. The task of Langerhans islet cells is to regulate glucose metabolism with the hormones they secrete (Figure 2) (4).

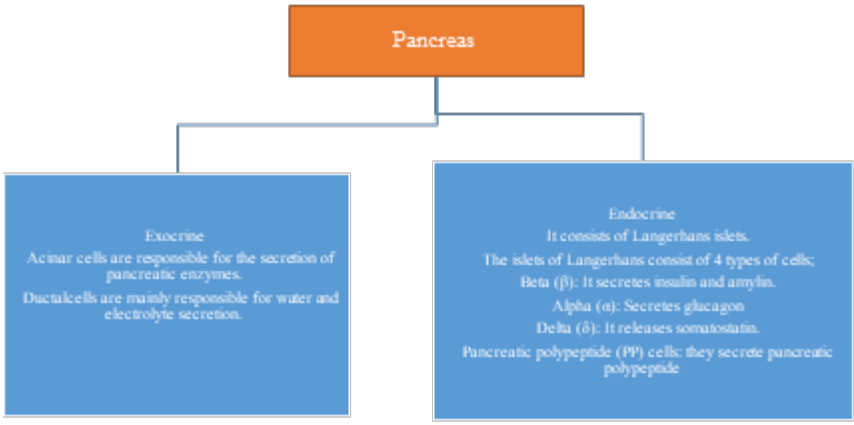

Figure 2: Description of the Exocrine and Endocrine Parts of the Pancreas (2,5)

From the perspective of human medicine, the pancreas suffers from two major related diseases. These diseases; Diabetes Mellitus and Pancreatic Cancer. Commonly, several broad categories of diseases affect the pancreas. These;

include pancreatitis, pancreatic insufficiency, cystic lesions of the pancreas, and pancreatic tumors (2).

3. Pancreatic Cancer (PC)

Pancreatic cancer (PC) is one of the most aggressive types of cancer (6). Although it has a low incidence, it has a high mortality rate. The reason for this situation is a late diagnosis. Despite the progress in cancer management, the survival rate has remained almost unchanged over the last decade (7). The one-year survival rate of pancreatic cancer patients is 20%, and the 5-year survival rate is approximately 7%. It is the seventh leading cause of cancer deaths worldwide (8). Poor prognosis; The insidious growth is due to nonspecific symptoms and the lack of sensitive and specific methods for early diagnosis (9,10).

Symptoms (e.g., jaundice, weight loss, and epigastric pain) do not appear until late in the disease. Surgical resection is the only therapeutic method that has the potential tot cure the disease. However, often it cannot be operated due to the spread of lesions to nearby structures. More than a third of tumors are in the fourth stage upon diagnosis, and less than 20% of these cancers are candidates for surgical resection (11,12). From a morpho-pathological perspective for pancreatic tumors, more than 95% of pancreatic tumors originate from the exocrine pancreas and less than 5% from the endocrine pancreas (13,14). The most common malignancy is ductal adenocarcinoma involving the exocrine glands; Most of these tumors are discovered in the head of the pancreas. Pancreatic neuroendocrine tumors (NETs) are malignancies in the pancreas' endocrine tissue. Also known as islet cell tumors, rare tumors occur in about 1 in every 100,000 people, and only 1% of all pancreatic tumors are NETs. NETs can cause specific clinical syndromes by overproduction and secretion of pancreatic hormones, including insulin, gastrin, glucagon, and vasoactive intestinal peptide (VIP) (13).

Generally, the term pancreatic cancer is used for ductal adenocarcinoma representing 85-90% of pancreatic tumors. Occur in many places; The anatomical location of 20% cannot be determined. Three leading lesions, pancreatic intraepithelial neoplasms (PanIN), intraductal papillary mucinous neoplasms (IPMN), and mucinous cystic neoplasms (MCN), play a role in the development of pancreatic cancer (15,16). Early detection of these lesions is complicated but provides patients with a chance to cure before contracting an invasive pancreatic carcinoma. Early detection and treatment of precursor lesions could save patients from progressing to invasive pancreatic cancer (Figure 3).

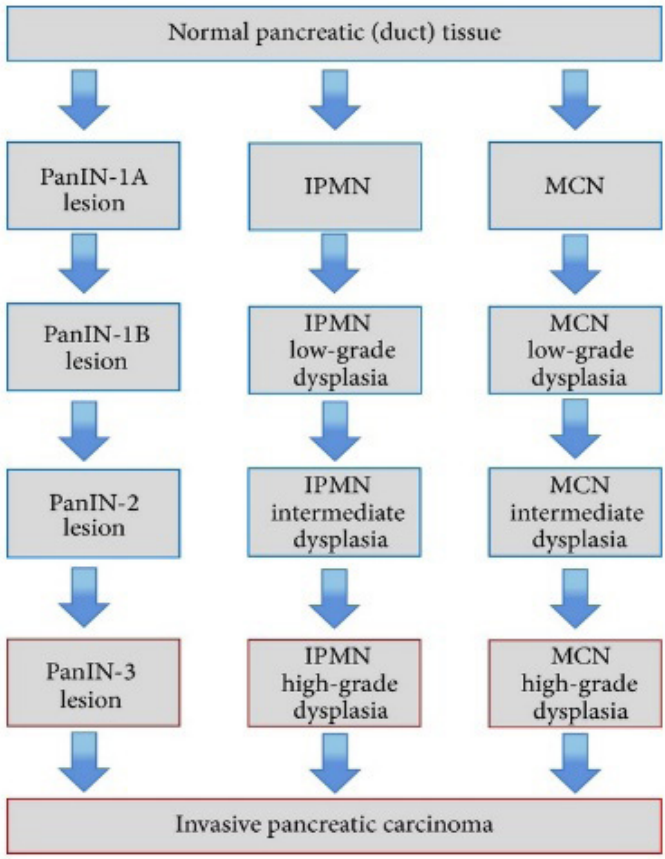

Figure 3: A Model of Three Morphological Pathways For Invasive Pancreatic Carcinoma (16).

3.1. Pancreatic Intraepithelial Neoplasia (PanIN)

The most important and best-known precursor of a PDAC is pancreatic intraepithelial neoplasia (PanIN). Hruban et al. first created the "PanIN scheme" in 2001. The most important and well-known precursor lesion of pancreatic cancer, PanIN, are noninvasive, smooth, and papillary microscopic lesions less than 5 mm in diameter that occurs in small intralobular pancreatic ducts. These lesions are characteristically asymptomatic (15).

The typical smooth cubic epithelial structure has altered in these lesions, commonly located in the head of the pancreas. Epithelial atypia divides into three subgroups PanIN-1A/B, PanIN-2 and PanIN-3 (17,18)

PanIN-1, with cylindrical epithelial cells, divides into two PanIN-1A (flat type) and PanIN-1B (papillary type). Epithelial atypia divides into three subgroups PanIN-1A/B, PanIN-2, and PanIN-3 (17,18).

PanIN-1, with cylindrical epithelial cells, divides into two PanIN-1A (flat type) and PanIN-1B (papillary type). In PanIN-2 lesions, the formation of pseudo-stratified epithelium, nuclear hyperchromasia, and loss of nuclear polarization compatible with moderate dysplasia started. PanIN-3 lesions with significant cytological atypia are characterized by complete loss of nuclear polarization, nuclear hyperchromasia, prominent nucleolus, and atypical mitotic shapes (19). (Figure 4)

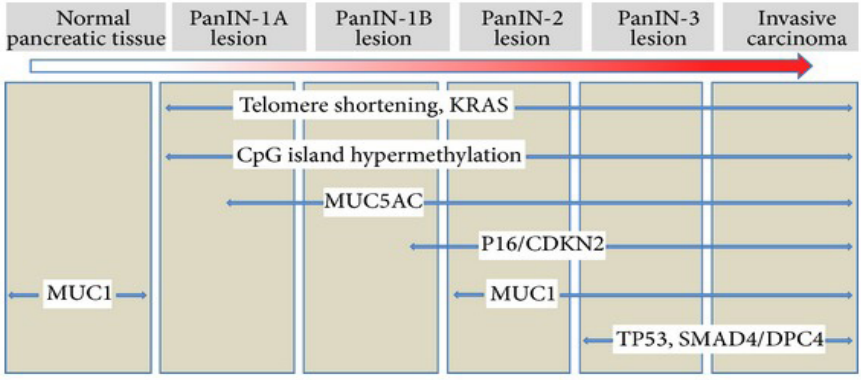

Figure 4: Summary of Molecular Changes During the PanIN Progression Model (16).

Although PanIN-1 and PanIN-2 have structures with 60% or more carcinomas, they are considered clinically insignificant since they can also be found in typical pancreatic structures. In addition, these lesion groups have different histopathological structures and differences in molecular pathologies according to their genetic changes (20).

Genetic abnormalities in PanIN lesions also reflect this histological progression observed in lesions. The K-ras mutation has an essential role in the development of PanIN lesions. The K-ras mutation was detected in 36% of PanIN-1 lesions, 44% of PanIN-1B lesions, and 87% of PanIN-2 / -3 lesions, showing that the increase in K-ras mutation was associated with the development of neoplasia. While mutations causing loss of CDK2NA/ p16 activation came to be seen in PanIN-2 lesions, loss of TP53, SMAD4 / DPC4, and BRCA2 activation is generally associated with PanIN-3, an advanced PanIN lesion (21,22,23).

3.2. Intraductal Papillary Mucinous Neoplasm (IPMN)

IPMNs belong to the heterogeneous group of cystic pancreatic lesions with an increasing incidence in recent years and are characterized by the cystic enlargement of the pancreatic ducts. IPMNs are macroscopic lesions that produce excessive mucin production due to the papillary proliferation of noninvasive mucin-producing epithelial cells. These cystic lesions were first reported in the 1990s (24). Although they occur at the beginning of the pancreas, their size is more significant than 1 cm (14). They constitute approximately 3% of exocrine pancreatic neoplasias and 20% of cystic pancreatic neoplasias (25). Most IPMN patients have a history of smoking. These lesions that develop from the principal or lateral channels of the pancreas are clinically separated into the main duct type IPMN (IPMN-MD) and the branch duct type IPMN (IPMN-BD). They are classified as high-grade dysplasia and invasive carcinoma dysplasia (26). Approximately 60% of IPMN-MDs show high-grade dysplasia, and approximately 45% are associated with invasive carcinoma (27). Approximately 50% of IPMNs with a low degree of dysplasia have point mutations that activate the K-ras oncogene, and the degree of dysplasia is related to the frequency of K-ras mutation. CDK2NA / p16 and TP53 activation loss were detected in IPMNs, showing a high degree of dysplasia(28). SMAD4 expression loss in approximately 30% of PanIN-3 lesions was observed in approximately 3% of IPMNs (29).

Morphologically, they are subdivided into the main ductal type (MD-IPMN) and the branch duct type (BD-IPMN) according to their origin regions also, cases where both the principal and branchial canal, called the combined ductal type, are observed. IPMNs divide into four subgroups gastric, intestinal, pancreatobiliary, and oncocytic. This classification is made according to their histological structures and differences in immunohistochemical staining with mucin antibody. The gastric type is of branchial ductal origin, while others are of primary ductal origin (30,31).

3.3. Mucinous Cystic Neoplasm (MCN)

MCNs of the pancreas are the rarest precursor lesions of pancreatic cancer. MCNs are mucinous cystic tumors with distinctive ovarian-type stroma of the pancreas that arise in the mucin-producing epithelial layers of the cells and form septated cysts mainly seen in women and do not communicate with the ductal system (17). These cystic lesions are almost solitary, and most MCNs

are asymptomatic and found incidentally (32). Cysts appear split on imaging and may contain calcification. It inhabits the body part and the tail part of the pancreas. It tends to grow in extensive areas, and their size is between 2-35 cm, but their average size is 6-10 cm (33). According to the histologically examined structures, MCNs are seen in the epithelial structure with various degrees of dysplasia consisting of cylindrical cells divided into three groups; low, moderate, and advanced MCN according to their dysplasia. The K-ras and the TP53 observed mutations are prevalent in MCN lesions; genetic abnormalities increase depending on the degree of dysplasia. In addition, loss of SMAD4 expression was observed in MCN lesions associated with invasive carcinoma (30). Approximately 1 in 3 MCNs can become invasive, usually to form ductal adenocarcinomas; however, these lesions are defined as noninvasive. Five-year survival for patients with noninvasive MCN is almost 100%. In addition, the five-year survival rate for patients undergoing resection for an invasive MCN is approximately 60% (15,34).

4. Pancreatic Cancer Epidemiology and Risk Factors

Although pancreatic cancer is the second most common gastrointestinal cancer globally, it is a highly aggressive tumor with epidemiology, survival, prognostic factors, and a high mortality rate. Pancreatic Cancer is expected to be the second cause of cancer death by 2030 (35). What makes pancreatic cancer prominent compared to other malignancies is the lack of clinical progress in the last decade regarding improved survival, and the 5-year survival rate is less than 5% (36). According to the World Health Organization report for 2012, pancreatic cancer is the 12th most common cancer in men worldwide and 11th in women. Pancreatic cancer is in seventh place as the cause of cancer-related deaths in the same year. The incidence of pancreatic cancer varies by geographic region and population. One-third of the cases in 2012 occurred in European countries, the highest incidence rates were in central and eastern Europe, North America, Argentina, and Uruguay, and the lowest were in African and East Asian countries (37). It stated that pancreatic cancer was the 10th most common cancer in the United States in 2013 and was indicated as the fourth cause of cancer-related deaths in men and women (38). According to the Ministry of Health of the Republic of Turkey Health Statistics Yearbook published by the Ministry of Health, pancreatic cancer is the 8th most common cancer in men, while it is not in the top 10

in women (39). According to the International Cancer Research Agency's GLOBOCAN 2018, 458,918 new cases of Pancreatic Cancer were registered in 2018, accounting for 2.5% of all new cancer diagnoses (40). Pancreatic ductal adenocarcinoma (PDAC) accounts for more than 90% of all diagnosed pancreatic cancer neoplasms (41). Pancreatic cancer ranks as the 12th most common cancer globally, with a global age-standardized incidence rate (ASIR) of 4.8 per 100,000 people. Pancreatic ductal adenocarcinoma (PDAC) is the third leading cause of cancer-related deaths in the United States, with an estimated 57,600 people diagnosed in 2020 (42,43). The overall 5-year survival rate for patients diagnosed with PDAC remains sluggish, between 7% and 9%. While surgical resection offers the only hope for recovery, this should be applied in multimodal therapy to include chemotherapy, chemoradiation, and potentially targeted therapies. Even in this best scenario where a patient completes all aspects of multimodality care, the median survival is 30 to 40 months compared to 10-13 months for patients with metastatic or unresectable disease receiving chemotherapy alone (44,45,46) The prognosis remains poor due to the high percentage of advanced disease at the time of diagnosis and the limited effectiveness of available drug and radiation therapy combinations. Despite decades of efforts, the reported 5-year survival was less than 5% in 2005, and mortality rates remained stable without significant changes. Surgery remains the only hope for long-term survival, but even for resected patients, the 5-year survival rate remains at approximately 20%, as almost 80% will recur within two years after surgical resection (47,48). The incidence of pancreatic cancer has been increasing in both genders in most countries in recent years and will likely increase worldwide(49). Contrary to the positive impact of the decline in smoking prevalence, the change in prevalence of other lifestyle risk factors, including obesity and an increase in the diabetic population, at least in men, an increase in intake of red or processed meat with inadequate fruit intake as well as improved diagnosis of vegetables and physical inactivity. Methods are likely to parallel the temporal trend in the incidence of Pancreatic Cancer worldwide (50,51). It is stated that many factors may be influential in the development of pancreatic cancer. Most of the risk factors do not directly cause the disease, but the level of exposure to these factors generally affects cancer development. Due to the limited treatment options, identifying controllable risk factors that may play a role in developing pancreatic cancer and avoiding them is particularly important for individuals with a family history of pancreatic cancer who are at high risk (52).

4.1. Cigarette

It is noted that the causes of cancer in the pancreatic tissue of smoking are carcinogenic compounds such as nitrosamine in cigarette smoke. Although it is known that it is responsible for approximately 25% of pancreatic cancers, there is approximately 2.2 times increased risk in smokers than non-smokers (53). It is known that metabolites formed by N-nitrosamines cause K-ras mutation by forming DNA breaks and increasing the activity of cyclooxygenase-2 (COX-2), which plays a role in carcinogenesis (54). Approximately 25% of pancreatic six cancer cases are due to smoking, and the cancer risk decreases to the level of non-smokers within 15-20 years in people who have stopped smoking. Passive smoking is not considered a risk factor for pancreatic cancer. The expression of stem cell markers significantly increases in PDAC cells exposed to Nicotine and Nicotine derived carcinogens. Studies have shown that cigarette smoke and its components can increase the stem cell properties of pancreatic cells. Stem cell properties enable cancer cells to self-renew and differentiate into other cell types (55).

4.2. Alcohol

In epidemiological studies conducted to reveal the relationship between alcohol consumption and the development of pancreatic cancer, any relationship between alcohol use and pancreatic cancer risk is unconfirmed (56). Alcohol consumption is a risk factor for pancreatitis and type II diabetes, which are associated with an increased risk of pancreatic cancer. Low and moderate alcohol consumption does not lead to an increased risk, while heavy alcohol consumption of 30 g or more per day accounts for approximately 22% of pancreatic cancer. It is noted that alcoholic metabolites, such as acetaldehyde, fatty acid ethyl esters, and ethanol, which are carcinogenic, can contribute to carcinogenesis indirectly or directly as a cause of pancreatic inflammation (57).

4.3. Age

Age is another risk factor for developing pancreatic cancer, and the incidence and mortality rate increase with age. The risk of developing pancreatic cancer in the first 30-40 years of life is low, and the incidence increases after age 30. Pancreatic cancer incidence peaked at the age of 70-80. A pancreatic cancer diagnosis is 10% before age 50, and the average age at diagnosis is 72 (58).

4.4. Chronic Pancreatitis

Chronic pancreatitis is a progressive inflammatory condition of the pancreas that causes irreversible histological changes, pancreatic fibrosis, and loss of islet cells. Frequent episodes of chronic pancreatitis can lead to disease progression and pancreatic exocrine and/or endocrine failure, eventually leading to abnormal pancreatic enzymes. SP can also disrupt the endoplasmic reticulum, mitochondria, and lysosomal autophagy systems of pancreatic cells, leading to cellular DNA damage, chromosomal mutations, and oncogene activation (59). Although acute pancreatitis is not considered a risk factor, it is stated that tropical and hereditary chronic pancreatitis increases susceptibility to pancreatic cancer (60). It reported that approximately 5% of patients with chronic pancreatitis develop pancreatic cancer over 20 years (61).

4.5. Obesity

For rapidly proliferating cancer cells, lipid oxidation, and biosynthesis are essential for cell survival. Epidemiological evidence suggests that obesity is a significant risk factor for Pancreatic cancer. KRAS mutation, a ring network between the YAP gene and obesity, contributes to the development of pancreatic cancer (62). Several prospective studies have shown a positive association between red meat and animal fat and the risk of pancreatic cancer and an inverse association between fruits, vegetables, and folate and Pancreatic cancer risk (63). It also reported that a diet high in animal protein and fat increases the risk of pancreatic cancer 2.5 times. Vegetable and fruit consumption and a diet rich in vitamins and fibers, especially vitamin C, have protective properties against pancreatic cancer (64). Obesity is a recognized risk factor for both women and men. Obese individuals have a 20% higher risk of pancreatic cancer than individuals of average body weight. Also, changes in Type 2 diabetes mellitus (T2DM), insulin resistance, inflammation, intestinal microflora, and gastrointestinal peptides can modulate downstream signals. There is evidence that fat distribution can also affect cancer risk. The waist-to-hip ratio or waist circumference is recommended in conjunction with body mass index (BMI) to measure the development of certain cancers (65,66,67).

When looking at body mass index (BMI), a BMI of 25 for both men and women is associated with an increased risk of pancreatic cancer, while this increased risk is much more pronounced in people with a BMI of 35 or more. Abdominal obesity is associated with the risk of developing pancreatic cancer at an early age. Physical activity can also reduce the risk of pancreatic cancer (61).

4.6. Ethnicity

According to the ethnic examined differences, the highest incidence of pancreatic cancer in African Americans, Northern European people, New Zealand and Hawaiian Polynesians. The mortality rate in the African-American population in America is 1.4 times higher than the Caucasian population (68).

As of 2018, the highest incidence of ASR among people with PC globally is in Europe (7.7 / 10 million) and North America (7.6 10 million). Conversely, the lowest incidence rate is in Africa. (2.2 per 100,000 people) (69).

4.7. Diabetes

Diabetes mellitus is an early finding and a significant risk factor for pancreatic cancer. In addition, long-term diabetes carries about two times the risk compared to non-diabetic conditions.In type 1 diabetes, the risk of PC increases 5-10 times in patients with a disease duration longer than ten years. People with diabetes who have had this disease for more than 20 years have a higher risk of PC (70). A study has shown that abnormal glucose metabolism is associated with the risk of pancreatic cancer. However, insulin resistance and secondary hyperinsulinism play a role in pancreatic carcinogenesis. New-onset diabetes is a potential early finding of pancreatic cancer; approximately 1% of new-onset diabetic adults are diagnosed with pancreatic cancer within three years after diagnosis of diabetes (71). When looking at the types of diabetes, it stated that type 1 diabetes mellitus and type 2 diabetes mellitus cause a 2 and 1.8 times increase in the risk of pancreatic cancer, respectively (72).

4.8. Blood Group

The ABO blood group antigen has on the entire surface of red blood cells. Recent studies emphasize that blood group antigens affect the risk of pancreatic cancer (73). When looking at people with diabetes, people with type A, AB, or B type blood are at higher risk than type O blood type (74). The gene encoding the ABO antigen involves cancer progression, progression of metastasis, and the inflammatory process by association with various plasma components such as soluble intercellular adhesion molecule-1 (sICAM-1) and tumor necrosis factor (TNF). These proteins are adhesion molecules required for immune cell recruitment and mediate systemic inflammation. These studies reveal that the gene encoding the ABO blood group plays a direct role in tumor formation and malignancy and plays a role in tumor cell immune surveillance, cell adhesion, tumor apoptosis, and angiogenesis (75).

4.9. Human Microflora

The human microbiota consists of various organisms, including bacteria, viruses, fungi, and protozoa. They play a vital role in human health and disease situations. Studies have shown that PC's occurrence, development, and prognosis are closely related to the human microbiota. Some hepatitis viruses and bile and specific oral, gastrointestinal, and pancreatic microbes may have potential etiological implications in pancreatic cancer development. The microbiota plays a role in cancer development mainly in the following ways. Immunomodulatory activity, microbial metabolites, Microbiota dysbiosis, microbial toxins and virulence relationship, oral microbes and oral diseases, gastrointestinal microbiota, intrapancreatic microbial system (76,77,78,79).

Immunomodulatory activity; intestinal microbiota triggers many natural and adaptive immune responses involved in tumor formation. The microbiota in the pancreas supports pancreatic tumors by inducing innate immune suppression and adaptive immunosuppressioN (80). Microbial metabolites: Microbial products secondary bile acids, lipoteichoic acid (LTA), and short-chain fatty acids (SCFAs) play essential roles in cancer cell growth. (Brown & et al. 2016) The metabolite produced by intestinal microbes travels and acts on pancreatic cells, causing PDAC. However, the specific mechanism has yet to be elucidated (81).

Microbiota dysbiosis: The disorder of the human microbial system causes a decrease in microbial diversity in the intestine and other organs in the body. After continuous antibiotic intake, the researchers caused microbial disorders in mice and found that various extraintestinal tumors, including PC, increased significantly. A growing body of evidence suggests that microbial dysbiosis is associated with the susceptibility, occurrence, and prognosis of PDAC. Microbial toxins and virulence relationship; some bacterial toxins can cause chronic inflammation, destroy cellular DNA, and cause carcinogenesis through ototoxins. Microbiologists have confirmed oral germs and diseases: How oral microbes spread through translocation or to the pancreas. Xiaozhou et al., in a case-control study, oral pathogens are associated with an increased risk of PC (82).

Gastrointestinal microbiota; the gut microbiota is a complex ecosystem made up of the largest microbial community in the human body. The microbes in the microbiota interact to protect the body from infection, ensuring the gastrointestinal tract's normal functioning. For example, Hydrolase secreted by the pancreas requires the breakdown of intestinal bacteria, and pancreatic juice

has antibacterial activity, protecting the pancreas from retrograde infection and helping the intestinal flora maintain normal function. In recent years, research has uncovered the potential pathogenic role of gut microbes in PC. Recent studies have also shown that H. Pylori for pancreatic cancer is indirectly related to inflammation and immunity avoidance (83).

Intrapancreatic microbial system; it is believed that most microorganisms will not survive in the pancreas because it contains large amounts of strong alkaline pancreatic juice and proteases (84). Some researchers have found that PDAC patients have 1000 times the number of microorganisms in their pancreas (85). A comparative study showed a significant increase in Bifidobacteria,- Proteus, H. pylori, and Clostridium bacteria in the pancreas of patients with PDAC. Proteus γ may be associated with drug resistance to the anticancer drug gemcitabine (86). Clinical studies have shown that H. pylori activate pathways that control the growth and progression of pancreatic PDAC associated with PC malignancy (87).

4.10. Hereditary Syndromes And Genetic Risk Factors

In addition to environmental factors, genetic factors also play an essential role in developing pancreatic cancer. 3-10% of pancreatic cancers are classified as familial pancreatic cancer. Depending on the number of affected individuals in the family, the risk increases by 4.46-32 times more (88). Research conducted in recent years has found that PC has a clear family basis and that a family history of Pancreatic Cancer dramatically increases the risk of disease (89,90). It mainly causes by genetic and acquired gene mutations. Although most pancreatic cancers are sporadic, 10% of them are familial (91,92). Most common in pancreatic cancer, K-RAS, CDKN2A (P16), TP53, SMAD4, BRCA2, BRCA1, STK11, PRSS1, and MMR are point mutations (93,94,95).

Mutations frequently identified in familial pancreatic cancer patients are BRCA1 and BRCA2 gene mutations. BRCA1 mutation increases the risk of pancreatic cancer 2.26 times. In the presence of BRCA2 mutation, the risk increases 3.5-8 times (96). There is also an increased risk of pancreatic cancer in some hereditary diseases and syndromes. For example, the most common hereditary nonpolyposis colorectal cancer, also known as Lynch syndrome, is an autosomal dominant inherited disease in which the incidence of pancreatic cancer is observed. This disease causes by mutations in the DNA repair genes (MMR) of MSH2, MLH1, MSH6, and PMS2. This syndrome creates 8.6 times the risk of pancreatic cancer compared to the average population (92). In cystic

fibrosis, an autosomal recessive disease caused by CFTR gene mutations, viscous mucus production obstructs the pancreatic duct and increases the risk of inflammation. Unfortunately, this situation also increases the risk of chronic pancreatitis and pancreatic tumor formation in patients with cystic fibrosis.

Peutz-Jeghers syndrome (JPS) is an autosomal dominant inherited disease characterized by hamartomatous polyps in the gastrointestinal tract and melanotic macules on the lips and mucous membranes. Approximately 80% of the patients carry a mutation in the STK11 / LKB1 tumor suppressor gene, regulating cellular energy metabolism (97). This syndrome creates 132 times more risk of pancreatic cancer than the average populatioN (98). The risk of developing pancreatic cancer in affected individuals is 36% (99). In addition to these diseases, it is also associated with pancreatic cancer, Li Fraumeni syndrome, ataxia-telangiectasia syndrome, multiple endocrine neoplasia type I syndrome, and von Hippel-Lindau syndromes (100). In familial orthopedic multiple mole-melanoma (FAMMM), approximately 38% of patients carry a mutation in the p16INK4A / CDKN2A tumor suppressor gene, which regulates the cell cycle and is inherited autosomal dominantly (92).This syndrome creates 38 times the risk of pancreatic cancer compared to the average populatioN (101). Familial adenomatous polyposis (FAP) syndrome progresses to malignancy in some, creating a definite risk factor for colorectal adenocarcinomas in their 40s. It is a syndrome characterized by the early development of colorectal adenomatous polyps (102). This syndrome is caused by an autosomal dominantly inherited germline mutation in the APC gene, functional in cell cycle control and microtubule stability, functioning in the Wnt /-catenin signaling pathway. As a result, there is 4.5-6 times increased risk compared to the average population (92).

Hereditary pancreatitis (HP), a rare form of chronic pancreatitis, a definite risk factor for pancreatic cancer, is inherited autosomal dominantly and has high penetrance. Although it depends on the ancestral origin in the family, it is a disease that usually manifests before the age of 30. In patients, there is autosomal dominant inheritance in the PRSS1 gene encoding cationic trypsinogen, and in the SPINK1 gene encoding a trypsin inhibitor, there are autosomal recessively inherited germline mutations (92). The incidence of pancreatic cancer in these families increases after 20–40 years of chronic pancreatitis, especially with an early onset of smoking and diabetes. There is a 53-times more increased risk compared to the average population (103). There are apparent epigenetic changes in pancreatic stem cells that occur mainly in mutation in chromatin

regulatory proteins and the control of epithelial-mesenchymal transition (EMT). However, these changes do not include changes in genetic sequence. Only DNA and chromatin structure/chemical changes are involved, so these changes ultimately affect the overall phenotypic state of the cell. Based on these ideas, some researchers have begun investigating whether blocking the epigenetic editing process contributes to developing new PC treatments (104).

5. Conclusion

Since pancreatic cancer usually shows no symptoms in its early stages, early diagnosis is highly complicated. In treating pancreatic cancer, chemotherapy, radiotherapy, and surgery are preferred. The surgical treatment of pancreatic cancer is "Whipple" surgery. This surgery can apply to a small number of patients who do not have metastases. As treatments for pancreatic cancer are inadequate, new therapeutic strategies for this disease are needed. New biomarker studies and genetic studies for early diagnosis are ongoing.

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CHAPTER XX

WALKING PATHOLOGIES IN CEREBRAL PALSY AND VISUAL WALKING ANALYSIS

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1. Introduction

Involuntary co-contraction accompanying muscle weakness, spasticity, balance problems and muscle control, soft tissue structural changes and compensatory mechanisms affect gait patterns in children with cerebral palsy (CP). (1) In later ages, functional impairments and ineffective energy utilization will both rise as a result of irregular gait patterns. (2) Depending on all these, various gait pathologies are seen. (3) Pathological gait is examined in three different planes as transverse, sagittal and coronal planes, and evaluations in the sagittal plane are frequently used in clinical decision making. (4)

2. Walking Problems in SP

2.1. Sagittal Plane

In the sagittal plane, children with diplegic and hemiplegicxCP have different abnormal gaitxpatterns.

2.1.1. Gait Patterns in Spastic Hemiparetic Children

The most popular categorization in spasticxhemiparetic patients is the one created by Winters et al. Based on sagittal plane kinematics, gait patterns were divided into 4 groups.

Children with hemiparetic type CP have slower walking speeds, step widths and step lengths than children with normal development. The fact that

these children have excessive muscle tension and poor balance and coordination causes their walking patterns to change and they develop compensation mechanisms. (5)

a. Type1

Due to tibialis anterior muscular weakness, this uncommon walking pattern in hemiplegic type CP is marked by foot drop during the swing phase. In the stance phase, the heels touch the ground depending on the presence of sufficient dorsiflexion angle. To compensate for drop foot, knee flexion rises in the first contact stage and hip flexion increases in the swing stage. In addition, an increase in pelvic lordosis is observed. (6, 7)

b. Type2

It is the most prevalent gaitxpattern in hemiplegic type CP. Drop foot is seen in both swing and stance phases of gait due to spasticity or contracture in the plantar flexors. As compensation, the knee is either in full extension or tends to go into the recurvatum.

Type 2 A: The hip is in extension, the knee is in neutral, and the ankle is in plantar flexion.

Type 2 B: The hip is in extension, the knee is in hyperextension, and the ankle is in plantar flexion. (7-9)

c. Type3

In addition to a lowered foot, it is distinguished by a "stiff-kneexgait" that results from the co-contraction of the hamstring and quadriceps muscles. Knee flexion is limited in the swing phase, and a stiff knee is seen in flexion. As a compensation mechanism, the same side walks by sickening the leg in the swing phase or by raising the fingertip of the opposite side in the stance phase. (3, 7)

d. Type4

Involvement is more in the proximal muscle groups of the lower extremities. The asymmetry due to the unilateral involvement has become quite evident. Pelvic retraction is evident due to asymmetry resulting from unilateral involvement. Dynamic hip flexion contracture is added to all disorders in type 4 gait. Plantarxflexion of the ankle, hip flexion, knee flexion, adduction and internal rotation are seen. (7, 8)

2.1.2. GaitxPatterns in Spastic Diparetic Children

Children having dipparatic CP can often ambulate independently, but pathology in their gait is noticed. Compared to normally developing

children, their energy expenditure increased and their walking speed decreased. (7)

a. Jump Knee Gait

In kids with diparetic CP, it is a frequent pathological gait. Due to spasticity in the muscles of the hip flexors, hamstrings, and gastrocnemius, it is characterized by increased ankle plantar flexion, knee and hip flexion, lumbar lordosis, and anterior pelvic tilt. During any phase of walking, the knee cannot reach full extension. (7)

b. Crouch Knee Gait

After equestrian posture, it is the most typical problematic gait in children having dipareticxCP. (10) The bent-knee gait exhibits significant hip and knee flexion together with foot dorsiflexion throughout the walking and swinging phase. It happens due to hip flexor and hamstring spasticity or hip extensor and triceps muscle weakness. In addition, an overextended Achilles tendon at an early age can cause bent-knee gait. (7)

c. True Equine Position

When walking begins in children with dipareticxCP, the ankle is in plantarxflexion in all phases of walking due to spasticity in the plantar flexors. The genurecurvatum can occasionally hide the real equine posture. In other words, children force their knees to recurvatum in order to have the capacitiy to press completely the soles of their feet, and they can walk in this way. The use of articulated AFO in these children provides more stable walking. (7)

d. Apparent equinus

In the stance phase of walking, the ankle dorsiflexion angle is close to normal. However, the child is in the equine position because the hip and knee are excessively flexed. Triceps surae weakness or lengthening surgery can cause this condition. Ground reaction AFO, articulated AFO or solid AFO can be recommended. (7)

2.2. Transverse Plane

Upper extremity position, patella angle and foot progression angle are evaluated in the transverse plane. Transverse plane deviations cause impaired gait in children with CP. Video analysis method is used based on the stance phase in the evaluations made in this plan. (4) In the transverse plane, deteriorations occur in the anatomical alignment of the body as a result of muscle weakness, balance, coordination and muscle control weakness. Lever arm dysfunction

develops due to these impairments. Pelvic rotation, torsion of the femur and tibia, pes varus or valgus are factors that cause lever arm dysfunction. For these reasons, the toes of children with CP may be turned inwards or outwards. Children with hemiplegic CP frequently have pes varus, which is known to be brought on by the tibialis posterior and anterior muscles. Hip internal rotation and tibial torsion are much more frequent in kids with diplegic CP. (11)

2.3. Coronal Plane

Gait disorders seen in this plane often develop depending on the transverse and sagittal plane. Evaluations are made on the basis of the stance phase. Increased hip internal and hip flexion rotation result in increased hip adduction. Similarly, valgum occurs in the knee due to hip internal rotation and knee flexion. (4) Pelvic oblique and hip abductor weakness is evaluated. (12)

3. Observational Gait Analysis in Children with CP

Determination of abnormal gait patterns in children with CP is important in determining the treatment process, using common language among clinicians, and predetermining gait pathologies that await the child in the future. (4, 13)

Gait analysis method of 3-dimensional is used as the gold standard in the assessment of pathological gait. However, since it is not available in every clinic, methods with practical use in the clinic can be preferred. (14)

Observational gait analysis (OGA) is widely and frequently used to detect gait problems in children with CP. In OGA, gait can be evaluated visually and gait abnormalities in different joints and planes can be determined through video recordings and various scales. Joint motions in the coronal, transverse, and sagittal planes are observed to make assessments. (15) While performing visual gait analysis; evaluation should be done every 6 months in patients under 3 years of age, every 12 months in patients aged 3-12 years, and every 2-3 years in patients over 12 years of age. These times should still be determined according to the patients and new records should be obtained in case of any change. (12)

Various scales are used to make the evaluation more objective with OGA. Commonly used OGA scales are : (16)

- Visual GaitxAssessment Scale
- PhysicianxRating Scale
- ObservationalxGait Analysis
- EdinburghxVisual Gait Scale

- SalfordxGait Tool
- VisualxGait Score
- ObservationalxGait Scale

3.1. EdinburghxVisual Gait Scale

The Edinburgh Visual Gait Scale (EVGS) was created to visually evaluate gait in ambulatory CP youngsters. EVGS is often utilized in CP patients to assess progress following orthopedic, selective dorsal rhizotomy, and botulinum toxin therapy. (17, 18)

Two cameras are placed to capture sagittal and coronal images. Markers are placed on the SIAS and the greater trochanter, and the height of the camera is adjusted so that it is flush with the greater trochanter. Six anatomical regions in each lower extremity, namely foot, ankle, knee, hip, pelvis, and trunk are evaluated separately in the stance and swing phases of gait. The images of the video recordings recorded while the patients were walking barefoot are taken and each sub-parameter in the 17 observation parameters is scored according to the observed condition or the measured joint angles. A three-point scale is used for each parameter. In scoring, 0 indicates normal, 1 indicates moderate deviation from normal, 2 indicates significant deviation from normal, and then all scores are added. The maximum total score per lower extremity is 34. Total score of zero is normal; scores above zero indicate abnormal gait. (19)

Intraobserver and interobserver reliability has been verified for experienced and inexperienced observers in gait analysis in children with CP. (20) Experienced observers generally have higher reliability than inexperienced observers, distal segments than proximal segments. (14, 20) A positive correlation has been shown between safety and patients with a better functional level. (20) At the same time, by contrasting it with 3D gait analysis techniques, its validity in children with CP has been investigated in several research. Validity was higher in experienced observers than inexperienced observers. (19, 21)

In a study, EGYS was confirmed to be a standard assessment tool with significant correlations with the Gilette Gait Index. (22) In another study, it was shown to correlate well with measurements from 3DGA and also detect postoperative changes after multilevel surgery. (19)

In a study that tested the reliability of the Physician Rating Scale and Edinburgh Visual Gait Analysis on children with CP, it was shown that both scales had excellent intra-observer reliability, but poor inter-observer reliability. (23)

3.2. Observational Gait Scale

It is a scale developed by Boyd and Graham by modifying the measurement sensitivity of the Physician Rating Scale. The Observational Gait Scale(OGS) consists of 8 parameters. These; knee position in the middle of the stance phase, first foot contact, foot contact in the middle of the stance phase, time of heel lift, rear foot position in the middle of the stance phase, support area width, assist device and change sections. Evaluation is made separately for both legs in the sagittal and frontal planes. The maximum score is 22, and low scores indicate poor walking. During the stance phase, the relationship between the knee and foot joints was emphasized. Validity and reliability have been determined to evaluate gait on children with CP. (15)

In a study conducted on 512 children with CP in 2022, OGS was shown to be a rapid tool for assessing gait and confirming the child's GMFCS level. (24)

In a study, three assessment scales, namely Visual Gait Assessment Scale, Edinburgh Visual Gait Scale and Observational Gait Scale, were used to evaluate walking in children with spastic diplegic CP. They found that Visual Gait Assessment Scale and Edinburgh Visual Gait Scale were more appropriate compared to Observational Gait Scale. (25)

3.3. Salford Gait Tool

The SF-GT is a tool consisting of 18 assessments to determine the position of the hip, knee and ankle in 6 distinct phases of gait. Users estimate the angular position (in degrees) of each joint at the time of each walking phase (normal, backward, forward) after watching the video of the walking subjects. A 5-point category system is used for the angular position of each joint (-2,- 1, 0, 1, 2). The sum of the 6 categories for each joint represents the joint's function over the entire gait cycle. Therefore, the output from using the tool is a numerical indicator for each of the 3 joints, and when the scores of the joints are summed, they provide an indication of the overall gait pathology. (26, 27)

3.4. Physician Rating Scale

It is a scale in which the structure and functions of the lower extremity joints are evaluated observationally during walking. (28) It was used for the first time by Koman et al. Observation is made in the sagittal plane. It is a clinically applicable assessment criterion with high applicability. It consists of three parts that enable to evaluate how foot contact is made in walking, whether or how far

the knee has recurved, and the degree of crouch position. Right and left sides are evaluated separately. Scoring ranges from 0 to. (29) During the evaluation, the child is asked to walk at the speed chosen by her, as in daily life, by removing the orthoses, if any.

3.5. Visual Gait Assessment Scale

The Visual Gait Assessment Scale consists of 7 items that are evaluated in the range of 3 points (1 to 3) or five points (1 to 5). This scale analyzes hip, knee, ankle and foot position in the sagittal plane during gait cycle events.

The reliability and validity of the Visual Gait Assessment Scale were investigated in children with hemiplegic CP. Experienced observers generally showed higher inter- and intra-observer reliability than inexperienced observers. In both groups, a higher fit was found in the evaluations of the ankle and foot compared to the knee and hip. It has been found that the scale can be used by inexperienced observers, but is limited to observations in the sagittal plane and has low reliability in the knee and hip for experienced and inexperienced observer. (30)

4. Conclusion

Due to the complex nature of SP, different types of patterns are seen in walking. Observational gait analysis methods, which are clinically proven effective, applicable and easy to access, can be used to determine gait pathologies in children with CP.

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CHAPTER XXI

NANOSTRUCTURED LIPID CARRIER AS A NOVEL INSIGHT FOR ORAL BIOAVAILABILITY ENHANCEMENT OF POORLY WATER SOLUBLE DRUGS

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1. Introduction of Lipid Based Formulations

These systems encompass a variety of formulations that are generally categorized into three types i.e., vesicular (**Figure 1**), emulsion based lipid particulate systems **(Figure 2)** (1).

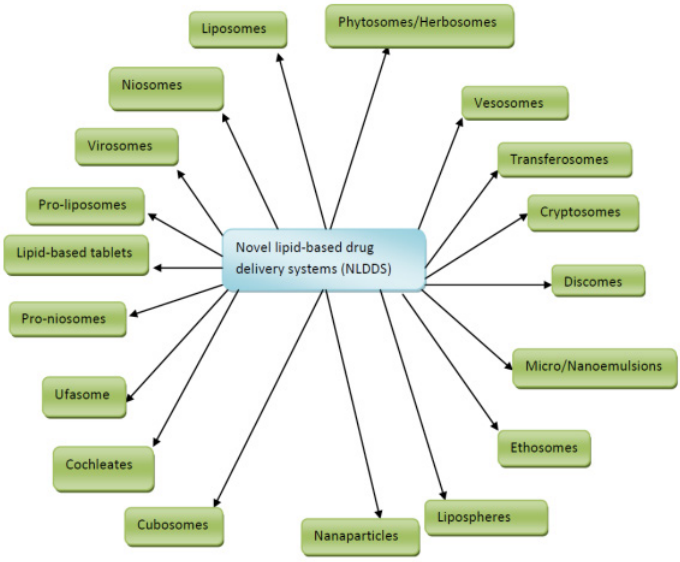

Figure 1. Novel vesicular lipid based drug delivery systems (4)

Lipid carriers consist of various structural and functional traits pliable to modification attained by adjusting lipid composition such as triglyceride oils, mixed glycerides, polar oils, and other additives (2). Lipid-based drug delivery systems have become increasingly prominent due to their potential to enhance the solubility and bioavailability of poorly soluble drugs (3). These systems are widely accepted, commercially viable, and can be customized to meet various product requirements. Lipid systems offer high stability, high carrier capacity, and can incorporate both hydrophilic and hydrophobic substances. These approaches can also be administered via different routes, including oral, topical, parenteral, and pulmonary, making them versatile drug carriers for challenging pharmaceutical molecules (4).

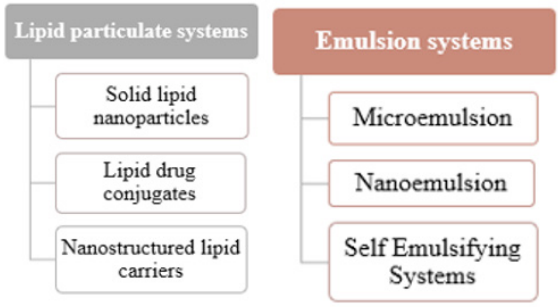

Figure 2. Lipid particular and Emulsion based lipid systems

2. Nanostructured Lipid Carriers

In recent years, there have been advancements in lipid-based drug delivery systems such as lipid drug conjugate nanoparticles, Solid lipid nanoparticles and nanostructured lipid carriers (5). Lipid drug conjugates were initially developed as a drug delivery system, but encountered several challenges including shelf life stability, uncertain gelation tendency, sterilization for a parenteral product, abrupt polymeric transitions, low drug loading capacity, and large scale production issues (6,7). In response, solid lipid nanoparticles were introduced in 1991 as a promising alternative. SLN is made up of solid lipids that exhibit biocompatibility and biodegradability and have been used for oral drug delivery of various drugs. Regardless of the small size and large surface area of SLNs, limitations such as limited drug loading capacity due to internal structure of crystal lattice and hence drug expulsion tendencies persisted (8). Consequently, nanostructured lipid carriers (NLC) sometimes referred to as the new unstructured-matrix SLN was developed in the early 2000s to address these challenges. NLC offers greater flexibility in controlling drug release profiles, and improved drug payload (9) attributed to the imperfection internal arrangements. In addition to that, NLC can accommodate higher lipid content that facilitates formulation of the final product (10). Moreover, NLCs have shown to offer protection of active substances from chemical degradation, and less chance of expulsion of drug molecules from the carrier (11). NLCs are also less susceptible to unexpected gelation due to their lower water content.

Nanostructured lipid carrier is a second-generation lipid nanocarrier ranging from 10 to 1000 nm in size and are made up of liquid and solid lipids in different ratios from 70:30 up to a ratio of 99.9:0.1 (12). Despite the high proportion of liquid lipids, NLCs are solid at room temperature, and their blended matrix of solid and liquid lipids creates an unstructured matrix with more imperfections that can result in high entrapment efficiency and hence offer a promising alternative to SLNs for oral route applications (13).

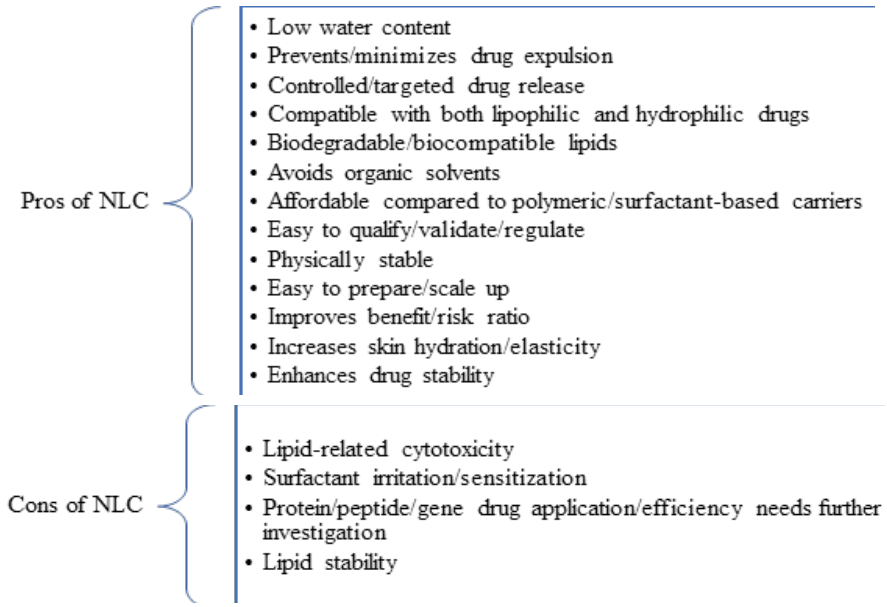

Figure 3. Pros and Cons of Nanostructured Lipid Carriers

3. Nanostructured Lipid Carrier Classification

As described in (Table **1**), nanostructured lipid carriers (NLC) are classified into three types based on preparation methods and formulation composition.

Table 1. Types of NLC (14)

Type	Classification	Composition	Description
Type I	Imperfect type	Solid lipid +low liquid lipid	Highly disordered lipid matrix structure that allows space for bioactive molecules to be accommodated.
Type II	Multiple type	Solid lipid + High liquid lipid	Multiple carriers of oil, fat, and water, with a miscibility gap that can cause phase separation.
Type III	Amorphous type	Solid lipid + Amorphous liquid lipid	Amorphous lipid matrix that hinders crystallization and drug expulsion.

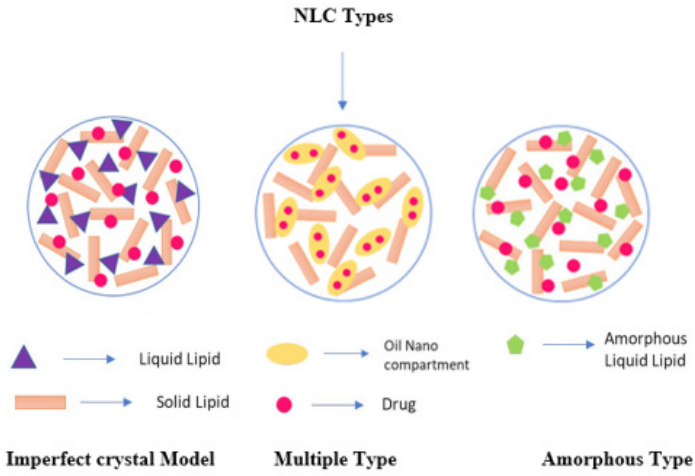

Figure 4. Schematic representation of NLC types (13)

4. Formulation Excipients of NLC

The composition and process parameters are crucial in regulating the size, drug capacity, and release profile of nanostructured lipid carriers. Researchers have successfully exploited these factors, such as using small-sized NLCs to improve drug efficacy during oral administration in rats. Lipids are essential for the stability and behavior of NLCs, and a range of biocompatible, well-tolerated, and biodegradable lipids are used in their development. Moreover, the exact composition of NLCs can vary depending on the specific application and desired properties, but the common components of NLC formulations are shown in figure 5.

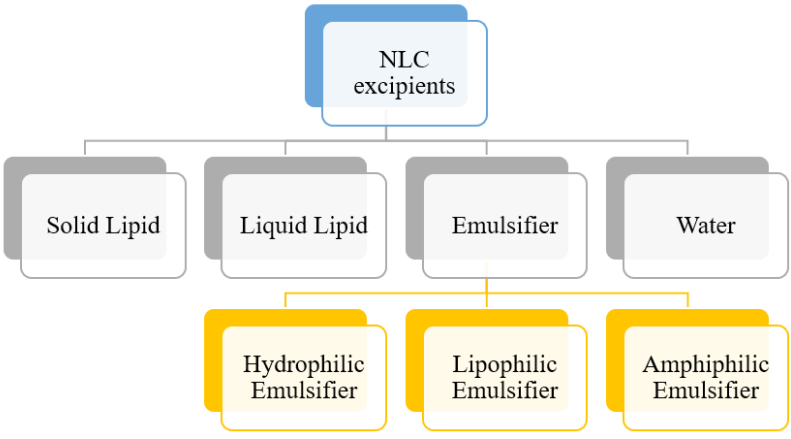

Figure 5. Formulation components of NLC

❖ Solid lipids such as hard fat, triglycerides, partial triglycerides, and waxes are the primary component of NLCs and form a matrix structure that stabilizes the particle and prevents drug expulsion (15).

❖ Liquid lipids are incorporated into the NLC formulation to improve the flexibility of the particle structure, allowing for easier drug incorporation and release (16).

❖ Emulsifiers or surfactants are added to the NLC formulation to stabilize the particle structure and prevent particle aggregation. They also facilitate the interaction between the lipid particles and the aqueous environment, which is essential for drug release (17).

Table 2. Various examples of Excipients used in the preparation of NLC.

Excipient Type	Example
Solid lipids	Stearic acid, Behenic acid, Palmitic acid, Theobroma oil, Goat fat, Beeswax, Carnauba wax 2442, Cholesterol, Apifil, Geleol, Trilaurin (Dynasan 112), Trimyristin (Dynasan 114), Tripalmitin (Dynasan 116), Tristearin (Dynasan 118), Tribehenate (Dynasan 122), Hydrogenated Palm Oil (Dynasan P 60), Hydrogenated Palm Oil (Softisan 154), Cetyl palmitate (Precifac ATO), Glyceryl Monostearate (Imwitor 900), Glyceryl Monostearate (Imwitor 491), Hydroxyoctacosanyl hydroxystearate
Liquid lipids	Castor oil, Oleic acid, Davana oil, Palm oil, Olive oil, Sweet almond oil, Caraway essential oil, Soy bean oil, Linoleic acid, Decanoic acid, Argan oil, Coconut oil, 2-octyl dodecanol, Isodecyloleate, Parafn oil, Black cumin oil, Propylene glycol dicaprylocaprate, Miglyol 812, Transcutol HP, Labrafac Lipophile WL 1349, Labrafac PG, Decyl Oleate
Hydrophilic emulsifier	Tween (20, 40 and 80), Polyvinyl alcohol, Poloxamer 188 and 407, Sodium oleate, Polyglycerol methyl glucose distearate, Sodium deoxycholate, Sodium glycocholate
Lipophilic emulsifier	Span (20, 40 and 60), Myverol 18-04 K
Amphiphilic emulsifier	Egg lecithin, Soya lecithin, Phosphatidylcholines, Phosphatidylethanolamines

5. Preparation Methods of NLC

The main preparation techniques are summarized below (18).

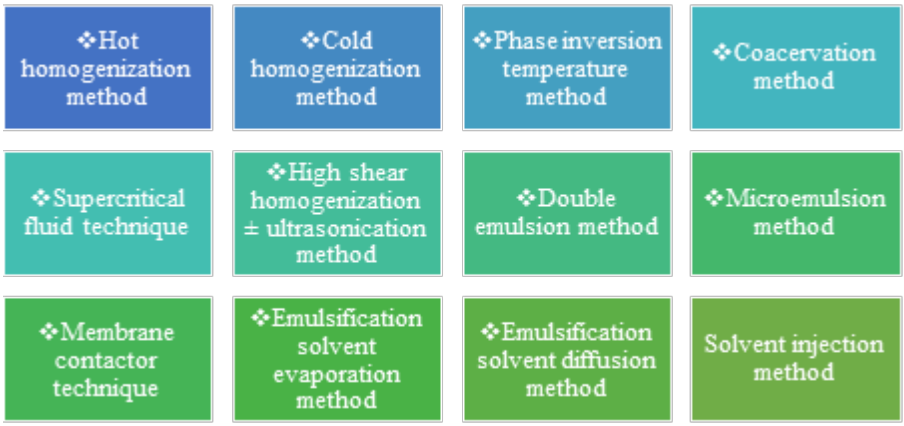

Figure 6. Preparation Methods of NLC

Lyophilization is a useful method for stabilizing NLCs and prolonging their storage duration by creating a solid-state material that can be easily re-dispersed when required. Cryoprotectants or lyoprotectants, such as mannitol, trehalose, fructose, sorbitol, lactose, glucose, sucrose, and aerosil, are used to protect the formulation from freezing and drying stresses during the lyophilization process (19).

NLCs can be easily scaled up for large-batch production in the pharmaceutical industry through organic solvent-free processes such as high-pressure homogenization method and that makes them an ideal candidate for drug delivery due to their GRAS components and improved drug safety (18).

6. Characterization of NLC

Various NLC evaluation parameters will be briefly mentioned (20,21).

6.1. Particle Size Distribution analysis is an important parameter that affects their stability, bioavailability, and drug release kinetics. The size distribution can be measured using techniques such as:

- ✓ Photon correlation spectroscopy (PCS)
- ✓ Dynamic Light Scattering (DLS)
- ✓ Laser diffractometry (LD)
- ✓ Nanoparticle Tracking Analysis (NTA)

6.2. Zeta Potential Analysis of NLC is a measure of its surface charge, which can affect their stability and interactions with biological membranes. It can be measured using techniques such as electrophoretic mobility or laser Doppler velocimetry.

6.3. Crystallization and Lipid Modification Analysis

6.3.1. Thermal Analysis Techniques

- ✓ Differential Scanning Calorimetry (DSC)
- ✓ Thermogravimetry Analysis (TGA)

6.3.2. Surface Morphology and Lipid Modification Analysis

- ✓ Light microscopy
- ✓ Scanning Electron Microscopy (SEM)
- ✓ Transmission Electron Microscopy (TEM)
- ✓ FTIR-Infrared and Raman spectroscopy
- ✓ Atomic force microscopy (AFM)
- ✓ X-Ray Scattering

6.4. Encapsulation Efficiency (EE%) and loaded drug (L%): by using HPLC or UV-Visible spectroscopy.

$$EE\% = \frac{\text{Actual amount of drug in the filtered formulation} - \text{Soluble unencapsulated drug}}{\text{Amount of drug added in the formulation}} * 100$$

$$L\% = \frac{Actual\ amount\ of\ \ encapsulated\ drug}{Amount\ of\ \ lipid\ added\ in\ \ the\ formulation} * 100$$

Figure 7. Calculation of Entrapment Efficiency % and Loaded drug %

6.5. In Vitro Drug Release Kinetics: will be characterized using techniques such as dialysis or Franz diffusion cells. These techniques can provide information on the rate and extent of drug release from the particles.

6.6. Stability Studies

6.7. Evaluation of Intestinal Permeation

- ✓ In vitro studies: by using intestinal epithelial cell monolayers to evaluate the effects of NLCs on drug transport and absorption.

✓ In vivo studies: to be conducted in rats to evaluate the pharmacokinetics and biodistribution of the developed NLCs.

7. Optimization of NLC through Quality by Design (QbD)

QbD adoption has led to statistically designed experiments (DOEs) for determining the impact of multiple parameters and their interactions on product profile. Optimization of NLC formulations requires setting QTTP to improve their biopharmaceutical performance. To ensure a successful Quality by Design (QbD) approach, several steps need to be followed (22). These include:

✓ Identifying critical material attributes (CMAs) such as API composition, solid lipid concentration, liquid lipid concentration, excipient ratio, surfactant concentration, drug–lipid ratio etc.

✓ Screening critical process parameters (CPPs**)** as independent variables such as preparation method, temperature, sonication time, lipid type, stirring time, homogenization time, etc.

✓ Identifying working design space, and

✓ Analyzing critical quality attributes (CQAs) such as Particle size distribution, polydispersity index, Zeta Potential, phase separation, Entrapment Efficiency, In vitro release etc.

8. Oral Bioavailability of Poorly Water-Soluble Agents

Oral route is the most preferred means of drug administration due to its high patient compliance, non-invasiveness, and therapeutic efficacy. However, many drugs often have limited oral bioavailability due to various physiological and physicochemical/formulation-related factors (23). These include first-pass metabolism, efflux transporters in enterocytes, gastric fluid instability, fast gastric emptying, and intestinal barrier restrictions, as well as poor solubility, improper drug partition coefficient, and high molecular weight of the drug. This necessitates the development of strategies to enhance solubility of drugs. In recent years, lipid-based delivery systems have emerged as a promising approach to address this challenge, offering several advantages in terms of drug delivery and absorption. Remarkably, many researchers developed NLCs to explore its potential to enhance solubility and permeability via oral application. While the exact mechanisms underlying the absorption-enhancing effects of NLCs are not fully understood, some researchers have suggested the possible hypotheses (10).

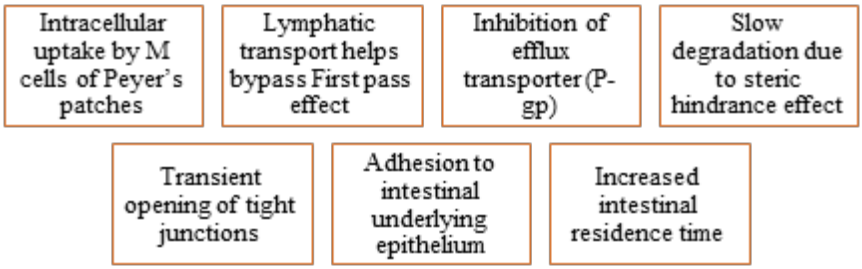

Figure 8. Mechanism for NLC bioavailability Enhancement

9. Critical Analysis of Previous NLC Research for Oral Application

Zaky et al. explored oral bioavailability and effectiveness of apixaban, an oral anticoagulant drug, by formulating it in a nanostructured lipid carrier (NLC). NLC was optimized using a Box-Behnken design and showed improved pharmacokinetic and pharmacodynamic properties in male Wistar rats. The optimized NLC formulation demonstrated increased AUC and Cmax, longer half-life, and reduced clearance compared to oral suspension. It has been concluded that incorporating apixaban in an NLC formula significantly enhances its oral bioavailability and pharmacodynamic activity (24).

In another study, optimized nanostructured lipid carrier formulation has been developed by incorporating Atorvastatin and olive oil and it demonstrated sustained drug release, high stability, and significant reduction in the evaluated biochemical parameters. This emphasizes the potential of the formulation for enhancing drug solubility and pharmacological activity (25).

Ezetimibe is a BCS class II drug with poor bioavailability due to its low aqueous solubility and extensive hepatic metabolism, which calls for the development of a novel carrier system to improve its therapeutic efficacy. Agrawal et al. encapsulated Ezetimibe into nanostructured lipid carriers using a high-pressure homogenization technique. The optimized formulation showed a particle size of 134.5 nm, and high entrapment efficiency of 91.32%. The in-vivo studies demonstrated the superior efficacy of Ezetimibe loaded NLCs in ameliorating hyperlipidemia in a high-fat diet-induced rat model, with reduced dose-dependent side effects and improved bioavailability (26).

Shahzadi et al. developed and characterized nanostructured lipid carriers (NLCs) incorporating insulin and sodium dodecyl sulfate to improve insulin lipophilicity and provide enzymatic protection. NLCs containing PEG-ester, PEG-ether, and PG-ester surfactants were prepared and evaluated for particle size, biocompatibility, lipolysis, and proteolysis. Results showed that PEG-ether NLCs provided the highest protection for incorporated insulin complex against gastrointestinal proteases, with PG-ester and PEG-ester NLCs following closely behind. These findings suggest that NLCs with less susceptible surface substructures can provide higher protection for incorporated peptides (27).

Pyo et al. studied solubility and bioavailability of fenofibrate, a poorly soluble compound used to lower cholesterol levels. Fenofibrate-loaded nanostructured lipid carriers (FFB-NLCs) were formulated and coated with chitosan, a biodegradable polymer, to allow controlled drug release. The CF-NLCs demonstrated increased solubility and encapsulation efficiency of fenofibrate and improved pharmacokinetic and pharmacodynamic parameters after oral administration, suggesting their potential use in improving the bioavailability and stability of fenofibrate (28).

Table 3. Recent studies of NLC based formulations for oral bioavailability enhancement

Drug	Composition of NLC	Research Outcome	Ref.
Pioglitazone	Solid and liquid lipid mixture, surfactants (Tween 20, Span 40)	Enhancement in permeability and bioavailability of pioglitazone was reported	(29)
Raloxifene	Solid lipid (Glyceryl Behenate), liquid lipid (Oleic Acid), surfactant (Poloxamer 407)	3.19-fold enhancement in oral bioavailability compared to raloxifene suspension	(30)
Exemestane	Solid lipid (Precirol ATO 5), Liquid lipid (flaxseed oil), Surfactant (Poloxamer 188, Tween 80, and Tween 20)	Exemestane shown sustained release pattern with 3.9-fold enhancement in bioavailability	(31)
Amisulpride	Solid lipid (Gelucire43/01), liquid lipid (CapryolTM90), surfactant (Tween-80)	NLC-based capsules exhibited enhancement in oral bioavailability of Amisulpride, as evidenced by a relative bioavailability of 252.78%	(32)
Olanzapine	Solid lipid (glyceryl tripalmitate), liquid lipid (castor oil), surfactants (Pluronic F-68 and soy lecithin)	Oral bioavailability increased more than 5 times compared to olanzapine suspension	(33)
Telmisartan	Solid lipid (glyceryl monostearate), liquid lipid (oleic acid), surfactant (Tween 20)	Increase in bioavailability compared to marketed formulation (2.17-fold) and pure drug suspension (3.46-fold)	(34)
Simvastatin	Solid lipid (stearic acid), liquid lipid (oleic acid), surfactant (Pluronic F-68)	Significant improvement in bioavailability compared to simvastatin suspension by 4-fold	(35)

It's important to note that the use of NLCs as a drug delivery system is still a relatively new and evolving field, and many products are still in the clinical trial phase. Because lipid-based formulations like liposomes are already in the market, there may be more NLC-based oral products will be available in the near future.

10. Conclusion

Nanostructured Lipid Carriers (NLC) have emerged as a promising alternative to Solid Lipid Nanoparticles (SLNs), microparticles, polymeric nanoparticles, and liposomes in recent years, garnering the attention of researchers. NLC is a versatile carrier system with potential applications in oral, parenteral, ocular, pulmonary, topical, and transdermal drug delivery, as well as site specific targeting possibility. After the development, optimization, and characterization of the NLC formulation in this study, we anticipate that it will yield improved outcomes, based on previous findings that have demonstrated the efficacy of NLCs as a delivery system for enhancing drug bioavailability and reducing side effects. Specifically, we expect that our optimized NLC formulation will exhibit a high encapsulation efficiency, amorphous state of the drug in lipid matrix, and desirable particle size and zeta potential values, and will result in increased bioavailability of the model drug. In addition, development of standardized characterization methods and regulatory guidelines will be crucial for successful translation of NLC-based drug formulations to clinical practice.

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CHAPTER XXII

ASSESSMENT AND EVALUATION IN NURSING EDUCATION: THE USE OF PORTFOLIO

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1. Introduction

Nursing education consists of theoretical knowledge and practices. This is why the nursing curriculum is important for theoretical information and practices. Many methods are used in the measurement and assessment process of theoretical knowledge and practices in nursing education. Portfolio is one of the assessment methods that has become widespread in recent years as an assessment tool. The portfolio has advantages such as guiding progress towards individual and professional goals, supporting professional development, providing data collection and assessment (1).

1.1. Portfolio

The portfolio (student development file) is a tool in which all studies reflecting the ways and achievements followed in order to achieve these goals are systematically collected in accordance with predetermined learning goals. The portfolio has been created in order to show the student program, student studies in many fields, student performance, success and progress in these studies (2).

Portfolios intended use;

- Monitoring the student's progress throughout the teaching process, step by step.

- Encouraging the student to take responsibility for learning and develop self-directed learning skills.
- Students' realization of learning by establishing a relationship with real life
- Collaboration of students with their peers
- Ability to combine formative and appraisal assessments and make a holistic assessment
- Discovering the interests and abilities of students
- Assisting the student's development process
- Increasing the student's self-awareness and competences
- Creating records that show the student's progress
- Follow the student's development in the theoretical and practical process of the training program
- Demonstrating individual and professional development within the professional structure of nursing with documents
- Preparing the students to pursue professional development
- Ensuring quality in education and practice
- Assessment
- It is aimed to create a connection between theoretical knowledge and practice (1).

Portfolios are separated from traditional measuring tools due to some of its features. Here are some of the highlights of the portfolio:

- *Individual:* Portfolio is structured according to individual choices.
- *Developmental:* The portfolio allows students to see their own progress.
- *Original:* It is a unique assessment method used to show and demonstrate the development of the student's own concrete products.
- *Performance is based:* The portfolio is based on the target.
- *Selective:* The students decide for themselves how to prepare a portfolio.
- *Interactionist*: Increases interaction between teacher-student and student-student
- *Reflective:* Reflects the evidence of what the student has learned (3).

The use of the portfolio is prepared for a specific purpose and goal. However, some situations require the use of a portfolio, while some situations do not require the use of a portfolio.

Situations where the use of a portfolio is appropriate;

- Used in the training program to assess the results of individualized gains and each portfolio must be configured for the student's requirements and objectives.
- It is used to monitor changes that individuals have shown in the process
- It may be requested to prepare a portfolio in order for students to better recognize and interact with the environment and society in which they live.
- The portfolio can be used to monitor changes in attitudes and behaviors that need to be achieved by monitoring the student during the process
- The portfolio is an easy-to-use tool for measuring and evaluating complex and high-level behaviors. However, if the target behaviors are in the top level steps, the portfolio is both learning and assessment (2).

Situations where portfolio use is not appropriate;

- The use of portfolio as a time assessment tool is not appropriate when target behavior should be gained to all students in the curriculum.
- The use of portfolio as an assessment tool in measuring low-level behaviors will not be efficient.
- The scoring and assessment of the portfolio may not be completely objective, so the portfolio should not be used in performance-based evaluations.
- It is not appropriate to use a portfolio to determine the students' readiness levels.
- It is not efficient to use portfolio as it will be difficult for the educator to plan the portfolio, follow the process and guide the file preparation process in crowded classrooms (2).

1.1.1. Advantages and Limitations of Using a Portfolio

There are many advantages of using a portfolio in the education process. Advantages of using a portfolio;

- Allows the student to be monitored in detail
- Allows the assessment of the student's performance individually and in group work.
- Guides the trainer to prepare a training plan in line with student requirements.
- Allows the student to take responsibility for learning
- Provides students with learning experience by doing and living
- Monitoring students throughout their education process ensures that their strengths and weaknesses are identified.

- Allows the student to make self-assessment
- Helps the student develop their skills
- Promotes active learning
- Improvement of clinical qualifications
- Ensures students' satisfaction with assessment
- Demonstrates students' competence for critical thinking and lifelong learning.
- Encourages clinical and professional development (4,5,7,9).

The limitations of portfolio use are; lack of clarity and portfolio use is time consuming (4,5).

1.2. Types of Portfolios

There are different types of portfolio available. The most commonly used portfolio types are showcase portfolio, developing portfolio, selected works portfolio and transition portfolio.

1.2.1. Showcase portfolio

The showcase portfolio consists of the best reflecting work of the student and is the work that is intended to motivate the student to develop. Therefore, only completed studies are included in the file, drafts and unfinished studies are not included in the file. The showcase portfolio does not reflect the daily performance and does not give information about the teaching process because it is made up of the files that most reflect the student's abilities. The showcase is used for portfolio level determination assessment (2).

1.2.2. Developing Portfolio

The devoloping portfolio shows personal developments and growth over time. Personal development may focus on academic or intellectual skills, content knowledge, or any other area. A development portfolio that reflects development may be selected to reveal one of the best working examples. This situation helps students to see their processes by setting goals and evaluating themselves (6).

1.2.3. Selected Works Portfolio

It is the type of portfolio created by the selection of a limited number of studies prepared for a specific purpose and to demonstrate the student's development in the process (7).

1.2.4. Transition Portfolio

A transition portfolio is prepared to show student achievement realized in accordance with the special purposes of the educational program, to accumulate selected studies made for acceptance by other educational institutions or programs, or to certify success for job purposes. These types of portfolios are suitable for training or use of individuals planning to take a job (7).

1.3. Sections of Portfolio

Pre-Part / Introduction Part

Introduction section; It consists of a cover page, a table of contents and a student introduction page.

Cover page: This page contains the name and surname of the student, class, number, name of the course and the name and surname of the educator.

Contents page: Specifies the page on which the data in the file is located.

Students introduction page: This is the page where the students introduce themselves. The student's resume are on this page (3).

Main section

In the main section, there are self-selection form, student studies, self-assessment form, peer assessment form, group assessment form and grading rubric.

Self-selection form: In this form, it states the studies chosen by the students and why they chose these studies.

Student studies: The activities that the students want to be in their file are included in the students studies.

Self-assessment form: It is the form in which the students evaluate their strengths and weaknesses after completing their studies.

Peer Assessment Form: It is the form in which students assessment each other's performance after completing their studies. This form can also be used to assessment both the product and the process.

Group assessment form: It is the form in which the opinions of the groupmates about the performance of the students in the studies carried out with their groupmates are included.

Rubric: It is a measurement tool used by the educator to score student work. A good rubric also indicates the level of qualifications that each criterion should have (3,8,9).

1.4. Preparation Stages of the Portfolio

The process of preparing a portfolio consists of three consecutive main steps.

Organizing and Planning: Organize and plan for monitoring and evaluation of student and educator progress (material and time selection, organizing and presenting process, retention process) (2).

Collection: This is the stage of collecting the materials. Material selection; It is carried out by considering factors such as specific subject area, learning process and special projects (2).

Reflection: The reflections of the educators and their peers on the thoughts, processes and products in the development file are included in the students' work and results. Thus, students can see the level of success in their studies and the aspects they need to develop (2).

1.5. Assessment of the Portfolio

The evaluation of the portfolio begins with the determination of the purpose. The purpose of the portfolio is decided and the purpose is to shed light on the selection of materials. However, criteria and standards are determined to achieve the goals, and whether these criteria and standards are reached in achieving the goals is included in the portfolio (2).

The accuracy of the assessments in the portfolio is possible with the validity and reliability of the measurement results. Scoring in the portfolio is realized by self-assessment and peer evaluation. Checklists and scoring guidelines are prepared to ensure that the scoring is objective. These checklists, guidelines, and many independent raters evaluate the portfolio. The average of the scores given by the raters is taken. To evaluate the accuracy of the scorers' scores; reliability, simple correlation techniques, kappa statistics, generalizability or multi-facet Rasch model are used according to the situation (2, 9).

In order to increase the reliability and standardization of the portfolio while evaluating it, David et al. (2001) offered recommendations. These recommendations;

- Defining the success criteria for each component of the portfolio
- Defining and being clear about the tasks and evaluation criteria

-Providing clear/explicit instructions to students.

-Preparation of written instructions for evaluators, organization of trainings

- Using standard guides for monitoring and questioning the portfolio with students (9,10).

1.6. The Use of Portfolios in Nursing Education

The use of the portfolio in education guides reflection on learning, assessment and practice (11).

Davis (2015) emphasized that the portfolio in nursing should be prepared for basic professional competencies. In the portfolio; name and surname, health records (including vaccinations and current physical condition), educational background, specialty certifications and renewal dates, professional organization memberships, anadal performance review (school transcripts), professional awards, academic publications and presentations, supervisor, educator, patient or letter of thanks from family members, letters of recommendation for career advancement, and future career goals (12).

Molahadi et al. (2018) nursing students benefited from the portfolio in their mentoring practices and reported that the portfolio was effective in the mentoring process (8). Alradini (2022) used a portfolio in medical education in his study and the researcher reported that the use of a portfolio is a valuable part of the process that assesses student achievement, in-depth learning, trust and self-confidence (11). Hoveyzian et al. (2021) examined the clinical competencies of nursing students using portfolios. Consequently; With the clinical portfolio, nursing students can see their strengths and weaknesses, and their self-directed learning skills develop (13). According to Salem and Abd Elrasoul (2021), portfolio use is time-consuming, so strategies and guidelines should be created for the use of the portfolio in clinical education, which are clear and easy to use for students and educators (4).

Nowadays, e-portfolios are used instead of paper-based portfolios. Madden et al. (2019) emphasized that nursing students prefer e-portfolio in their study. On the other hand, nursing students stated that the e-portfolio provides pleasure to use and supports lifelong learning (14). According to another study, the e-portfolio; It is an assessment method that encourages nursing students to think for themselves, is accepted by the educator/students, and increases interaction (15). In the study of Kutlu et al. (2014), students prepared a web-based portfolio and they showed a positive attitude towards the portfolio preparation process, but it was determined that the students had difficulties throughout the study (16).

2. Conclusion

As a result; Portfolio is one of the assessment and evaluation tools used in nursing education. The students with the portfolio; they can observe their own development process, discover their strengths and weaknesses, and develop self-directed learning skills. Therefore, the use of portfolio in the assessment and evaluation process is important for the student's development.

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CHAPTER XXIII

THE IMPORTANCE OF BREAST MILK AND FACTORS AFFECTING EXCLUSİVELY BREAST MILK

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1. Introduction

Breast milk is the most suitable nutrient for the healthy growth and development of the newborn. Breastfeeding has many short- and long-term benefits for maternal and infant health in terms of immunological, developmental, social and economic aspects.(1) Therefore, early cessation of breastfeeding negatively affects maternal, child and community health and increases the financial burden of the health system. (25) While there has been an increase in early breastfeeding rates with breastfeeding programs carried out worldwide, the increase in breastfeeding rates alone is not at the desired level. (11) In addition, due to the development and commercialization of infant formulas from the 18th century to the present, there has been a significant decline in breastfeeding rates worldwide. (38)

Today, the necessity of supporting and promoting breast milk for the health and growth of babies is felt all over the World. (41) Although many studies have been carried out in the international arena to support breast milk in the last 40 years, only breast milk intake for the first 6 months is not at the expected level all over the world. In developing countries, the rate of exclusive breastfeeding of infants under 6 months is 39%. It was observed that 58% of infants who

received exclusive breast milk between 18-24 months continued to be fed with breast milk. (7,18) Although progress has been made with efforts to promote and support breastfeeding, global breastfeeding indicators are still below target levels. According to UNICEF's breastfeeding report, 44% of babies start breastfeeding within the first hour. Again, in the same report, 42% of infants younger than 6 months receive exclusive breast milk. (37) The rate of mothers in Turkey to breastfeed their babies in the first 6 months with exclusive breast milk is 41.6% in the 2008 report of the Turkey Population Health Survey (TNSA), 30.1% in the 2013 report, and 41% according to the 2018 report. (34,35) The "Global Breastfeeding Scorecard" of UNICEF and the World Health Organization, which covers 194 countries and evaluates breastfeeding rates, reports that the rate of babies fed exclusively with breast milk in the first six months is 40%. As a result of these data, the rate of infants receiving exclusive breast milk in exclusively 23 countries out of 194 is over 60%. (37)

2. Importance and Benefits of Exclusive Breast Milk

Breast milk has many benefits. It has been stated that feeding exclusive breast milk reduces the risk of gastrointestinal infection (GIS) by 64%, while feeding with formula increases the risk of diarrhea in infants by 80%. One in 10 babies who are exclusively breastfed prevent a case of Necrotizing Enterocolitis (NEC) and one in every eight infants prevent a case of complicated NEC that leads to death. (9) In addition, a Cochrane review showed that exclusive breastfeeding for six months is highly beneficial in reducing the risk of gastrointestinal disease compared to exclusive breastfeeding or breastfeeding and formula feeding for three to four months. (20) Regarding respiratory tract infections, it has been reported that the mortality rate due to pneumonia is approximately fifteen times higher in infants who are not breastfed when compared to infants who are exclusively breastfed in the first six months of their lives, compared to infants who are not breastfed. (21). It has also been reported that continuation of exclusive breastfeeding for more than 3 months significantly reduces the risk of otitis media (77%), atopic dermatitis (42%), asthma (40%) and respiratory tract infections (75%).(4). In addition, it has been stated that breastfeeding for more than 6 months reduces the risk of leukemia by 19% and the risk of sudden death by 36%. (2,10) The World Health Organization (WHO) recommends that babies should exclusive be breastfed until they are at least 6 months old, and that breastmilk should be continued with complementary foods until they are over 2 years old. (27)

The concept of exclusive breast milk is defined as taking breast milk alone without consuming solids or other liquids other than vitamins, minerals and other drugs necessary for babies in the first 6 months of life. (29) Studies have reported that exclusive breastfeeding for the first six months is an effective method in reducing infant mortality, as well as reducing infant mortality by 13%. (9,44) However, a growing body of research shows that breastfeeding has important long-term health consequences for babies and mothers. It has been shown that there is a two-thirds reduction in the incidence of childhood diseases (obesity and diabetes) and mortality in children under 5 years of age in breastfed infants. It has also been observed that it has positive effects on children's mental and motor development scores (16). Another study on the intelligence scores of babies determined that the IQ of babies who were breastfed for less than one month was 6.6 points lower than those who were breastfed for 7 to 9 months. (3) In addition, it has been determined that there is a 15% to 30% decrease in the risk of obesity in adolescence and adulthood in breastfed babies compared to non-breastfed babies (9). It has been reported that increased use of formula in infancy causes an increase in insulin-like growth factor 1 (IGF-1) secretion and, consequently, an increase in early weight gain and obesity. (28) In a study conducted in developed countries, it was determined that breastfeeding for at least three months reduced the risk of asthma by 27% in children without a family history of asthma, and by 40% in children younger than 10 years of age with a family history of asthma. (17) A 2016 meta-analysis of the health consequences of breastfeeding for mothers and children concluded that babies who were breastfed for longer had a lower rate of infectious disease and mortality than infants who were breastfed for a shorter period of time or not. (39)

2.1. Reasons to Stop Breastfeeding in the First 6 Months

A study conducted in the USA shows that many mothers want to breastfeed for a longer period of time and approximately 60% of mothers stop breastfeeding sooner than they would like. When the reasons for early termination of breastfeeding by mothers are examined; difficulties with breastfeeding, concerns about the baby's weight gain, illness or the need to take medication, and the thought of insufficient milk production play an important role. (22) The duration of breastfeeding in high-income countries is shorter than in low-income and middle-income countries. (39) As a result of a community-based, cross-sectional study conducted in 26 poor rural districts, 98.3% of children were breastfed following birth. However, the rate of infants receiving exclusively breast milk

for the first 6 months was 28,7%. (12) In the United States in 2019, it was stated that approximately 83.2% of babies were never breastfed, 55,8% of breastfed babies were breastfed until 6 months and 35,9% up to 1 year. Considering the rates of exclusively breastfeeding in the USA; It has been reported that 45,3% of babies younger than 3 months and 24,9% of babies up to 6 months are fed exclusively with breast milk. In other words, only 1 out of every 4 babies is fed exclusively with breast milk for the first 6 months. In the United States, the rate of births in baby-friendly hospitals that support breastfeeding was increased from 3,8% in 2010 to 28,9% in 2021, aiming to increase breastfeeding rates. (5)

When the problems experienced in taking exclusive breast milk in the first 6 months are examined, the low education level of the mother, the working status of the mother, the late start of breastfeeding, the lack of adequate information and support by health professionals about breastfeeding in the early period of pregnancy and postpartum, insufficient family support, especially spousal support. Many important factors were stated, such as the time to start complementary foods, the first postnatal food, water, sugary water or formula, and the use of pacifiers and bottles. (6) In addition, many factors such as insufficient weight gain and insufficient milk perception, mastitis, breast tenderness and swelling, pain and trauma in the nipples, difficulty in grasping the breast, and low self-efficacy in mothers negatively affect the success of breastfeeding alone. (8,19) Postpartum maternal anxiety is also an important risk factor in reducing adequate breastfeeding and exclusive breastfeeding in the first 6 months. (1) Although breastfeeding rates are gradually increasing in the Netherlands and 80% of mothers start breastfeeding, the proportion of babies who are exclusively breastfed for the first 6 months is 39%. In the Netherlands, mothers with higher education started breastfeeding more frequently (90%) than mothers with medium and low education. While 57% of one-month-old babies are fed exclusively with breast milk in the Netherlands, this rate is 47% at 3 months and 39% of those who are fed only with breast milk at 6 months. (23) However, despite substantial evidence and many policies showing that breastfeeding is a healthy behavior for infants, mothers, and society, the rate of exclusive breastfeeding at 6 months in China was 29,5%. (42) Exclusive breastfeeding rates in infants younger than six months; It has been reported as 16% in Afghanistan, 51% in China, 32% in East Asia, and 30% in South Africa. (18) According to the results of another study conducted by Çiftçili et al. the rate of starting solid food before 6 months is 44,6%. The most common time interval to start complementary foods is 0-3 months. (18) The decrease in exclusively

breast milk intake in the first 6 months; It was found that the history of preterm birth, use of pacifier and bottle, and initiation of formula in the hospital after birth were found to be related. During the breastfeeding period, the duration of breastfeeding and the total duration of breastfeeding are adversely affected by the problems for the mother and the baby. Problems for the mother usually occur in the first 1-2 weeks of breastfeeding. At the beginning of these problems are breast-related conditions that sometimes prevent breastfeeding to a great extent. (33) These breast-related problems adversely affect the breastfeeding of the mother and cause the baby not to benefit from breast milk sufficiently. (13) When the problems related to the postpartum breast were investigated, it was stated that the most common problems were breast abscess, mastitis (33%), nipple pain and trauma (34-96%), fullness of the breasts, and sunken or flat nipples. It has also been reported that one out of every three mothers experienced nipple problems during breastfeeding. (33) Incorrect placement of the baby to the breast, excessive breast fullness and inadequate emptying, and candida infection cause sore and cracked nipples. (11,29) In another study, it was stated that 83,8% of the mothers after birth were not sufficient in breastfeeding and that breastfeeding was not done in accordance with the technique, and at the same time, breastfeeding the baby with the right technique was important in terms of preventing possible nipple pain and trauma, as well as increasing breast milk and ensuring the continuity of breastfeeding. (13)

In studies conducted, one of the most common reasons for the decrease in mothers' exclusively breastfeeding in the first 6 months is the perception of insufficient milk in the mother. (26) Perception of insufficient milk is the situation in which mothers perceive that they cannot produce enough milk to meet their infants' needs or that their infants do not get enough milk. (25) The frequency of perception of insufficient milk varies between 29% and 76,2%. (27) The idea that breast milk is insufficient prevents mothers from feeding exclusively with breast milk. Studies have shown that mothers with high breastfeeding self-efficacy have a higher rate of feeding their babies with exclusively breast milk, while mothers with low breastfeeding self-efficacy are concerned that their milk is not enough and exclusively breast-feeding rates are lower. (25,26,32) Mothers with high breastfeeding self-efficacy believe that they can produce enough breast milk to meet the needs of their babies, but mothers with low breastfeeding self-efficacy feel inadequate to meet their babies' breast milk needs. This situation adversely affects the infants' ability to receive exclusively breast milk. (40) In order to increase mothers' breastfeeding self-efficacy,

breastfeeding education should be given to mothers in the prenatal and postnatal period, such as the importance of breastfeeding, breastfeeding techniques, the benefits of breastfeeding for the mother and the baby, the physiology of breast milk production, and breastfeeding problems. (14,25) For this reason, it is stated by midwives and other health professionals that it is important to inform and support mothers and their relatives about breastfeeding from the beginning of pregnancy, and to encourage breastfeeding, both in terms of increasing the mother's confidence and increasing exclusively breast milk intake. (6)

3. Conclusion

Individualized prenatal breastfeeding education and postnatal support can significantly increase exclusive breastfeeding rates up to 4 months postpartum compared to routine care. (14) Su et al. study showed that prenatal breastfeeding education or postnatal lactation support can significantly increase rates of exclusive breastfeeding up to 6 months postpartum. (31) Systematic reviews have shown that prenatal education and postnatal support programs improve exclusive breastfeeding rates. In addition, prenatal individual counseling and postnatal education and support were found to be more effective in increasing the rate of exclusive breastfeeding. (14) In the study of Huang et al. 70,9% of the women in the intervention group who received breastfeeding education and 46,2% of the women who did not receive breastfeeding education in the control group fed their baby with breast milk at the 4th month. In the study, it was thought that regular phone calls made by the intervention group about breastfeeding could be effective in both improving breastfeeding practice and reducing economic costs. Similarly, in the study of Van Dellen et al. when mothers who received a breastfeeding support program were compared with those who did not receive a breastfeeding support program, it was seen that the risk of stopping breastfeeding was 66% less in mothers who received a breastfeeding support program. (38)

Skin-to-skin contact at birth, early initiation of breastfeeding, breastfeeding support, Baby-Friendly Hospital Initiative (BFHI), breastfeeding education and following professional advice are important for initiating and maintaining breastfeeding. A systematic review has shown that professional support, such as doctors, nurses, midwives, and lactation consultants trained in breastfeeding support, is beneficial in prolonging breastfeeding. (30) It has been stated that improving factors affecting breastfeeding such as interventions for exclusive breastfeeding after birth, prenatal and postnatal counseling, support and

breastfeeding management, community-based practices, better workplace conditions (availability of breastfeeding rooms and breastfeeding breaks) will positively affect exclusive breastfeeding. In order to achieve breastfeeding, a behavioral pattern should be developed within six weeks after birth that will enable mothers to improve their breastfeeding knowledge and skills and to cope with breastfeeding difficulties and negative emotions.(42) This behavior pattern positively affects the mother's cognitive status, decision-making mechanism, or breastfeeding action for six months after birth and even during the next pregnancy period. (43) However, due to the fact that the mother's physical and/or psychological support needs cannot be fully met in the postpartum period, mothers tend to develop behavioral disorders and develop an ineffective breastfeeding behavior model, and this causes the baby's breastfeeding status to be adversely affected. (42).

Socioeconomic, cultural and individual factors have an important role in the feeding process of mothers' infants. It can be a guide for mothers on how to follow a path during the breastfeeding process. Mothers need information and support to start breastfeeding and to continue breastfeeding successfully. The information and support to be provided to mothers by midwives about breastfeeding is important in terms of increasing the duration of feeding the baby with "exclusive breast milk".(18)

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CHAPTER XXIV

DIABETES MELLITUS AND CONSIDERATIONS FOR DENTISTRY

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1. Introduction

Diabetes mellitus (DM, diabetes) is a chronic metabolic condition characterized by hyperglycemia caused by an absolute or relative deficiency of insulin, a hormone secreted by the β- cells of the pancreas, as well as disorders in protein, fat and carbohydrate metabolism, capillary membrane changes and accelerated atherosclerosis. (1-10) In other words, diabetes is defined as an endocrine disorder that can occur in the absence of insulin and is characterized by hypofunction of the islets of Langerhans β-cells, resulting in excessive increase of blood glucose levels and excretion of glucose from the urine. With this metabolic disorder, the pancreas may produce insufficient insulin, or peripheral tissues may become insulin resistant. (1,4,6,7)

2. Diagnosis and Classification

The history of Diabetes Mellitus goes back to ancient times. Before 1500 BC, it was described in the Egyptian Ebers Papyrus as a condition in which excess urine is produced and sugar is lost through urine. Areateus of Cappodocia named

the illness Diabetes 200 years after Christ. Langerhans discovered pancreatic islets in 1860, Claud Bernard identified the neuro-hormonal mechanism of diabetes in 1875, V. Mering and Minkowski revealed the central organ of diabetes through pancreaotectomy in 1889 and Best and Banting discovered pancreatic extract in 1922. (11)

Diabetes may be found in all age groups, affecting roughly 3-4% of the general population, but with only 75% of these people recognizing it, it remains a primary cause of mortality and disability. The number of diabetics in the world, which was 171 million in 2000, reached 347 million in 2013. According to the International Diabetes Federation (IDF) 2019 Diabetes Atlas, there are approximately 463 million patients diagnosed with diabetes in 2019. (1,3,8,10) The IDF Diabetes Atlas (9th edition) predicts that by 2045 there will be 629 million people with diabetes, of whom 438 million are aged 20-64 and 191 million are aged 65-79. (3) This increase corresponds to an increase of 48%. In the 2045 projections for our country, it is stated that there will be more than 11 million diabetics. (12) The reasons for this increase in the frequency of diabetes are the prolongation of life expectancy in societies, the rapid change in lifestyle, especially nutrition and exercise habits, and the increase in obesity accordingly. (12,13)

Diabetes risk factors include family history (if a close relative, such as a parent or sibling, has the illness), obesity, inactivity, age (the chance of getting type 2 diabetes increases with age, especially beyond age 45), and race. Genetics play a part as well. Diabetes type 1 is more prevalent in the Caucasus and in European nations such as Finland and Sweden. Type 2 diabetes is more frequent among Black Americans, Asians, and Hispanics, and it has a stronger hereditary basis. Diabetes affects one-third of the Asian elderly population. Around half of all adults among Pima Indians in the United States and Nauru in the Pacific Area have type 2 diabetes, which is among the world's highest rates of the disease. (3)

Significant progress has been made in the diagnosis and categorization of diabetes and other diseases of glucose metabolism. The American Diabetes Association (ADA) first presented new diagnostic and classification criteria in 1997 and the World Health Organization (WHO) approved these criteria with minor adjustments in 1999. (14, 15) Later in 2003, a minor revision was made by the ADA for the diagnosis of impaired fasting glucose (IFG). On the other side, in the 2006 report produced by WHO and IDF, it was decided to keep the 1999 criteria (Table 1, Table 2). (16)

Table 1: Criteria for the Diagnosis of Diabetes Mellitus (8)

FPG ≥126 mg/ dL (7.0 mmol/L). *Hunger is defined as no calorie intake for at least 8 hours.**
OR
2-hour PG ≥200 mg/ dL (11.1 mmol/L) during OGTT. *The test should be performed in accordance with WHO standards using 75 g of anhydrous glucose dissolved in water. **
OR
A1C ≥ 6.5% (48 mmol / mol). *The test should be performed in the laboratory using a method standardized according to the DCCT test. **
OR
Random plasma glucose ≥200 mg/dL (11.1 mmol/L)

*DCCT, Diabetes Control and Complications Study; FPG, fasting plasma glucose; OGTT, oral glucose tolerance test; WHO, World Health Organization; 2-hour PG, 2-hour plasma glucose. * In the absence of definitive hyperglycemia, diagnosis requires two abnormal test results from the same sample or from two separate test samples.*

Table 2: Etiological Classification of Diabetes Mellitus (10)

I. Type 1 diabetes mellitus (usually there is β-cell destruction leading to absolute insulin deficiency)
II. Type 2 diabetes mellitus (characterized by progressive insulin secretion defect on the background of insulin resistance)
III. Gestational diabetes mellitus (GDM: It is a form of diabetes that occurs during pregnancy and usually resolves with birth)
IV. Other specific types of diabetes
β - genetic defect of cell functions (monogenic forms of diabetes) Genetic defects in the action of insulin Exocrine tissue diseases of the pancreas Endocrinopathies Pharmaceuticals or chemical agents Immune rare forms of mediated diabetes Genetic syndromes associated with diabetes Infections

2.1. Type 1 Diabetes Mellitus

Type 1 Diabetes Mellitus is commonly diagnosed at the age of 12 and occurs before the third decade, but it can occur at any age. It is characterized by antibodies against insulin and pancreatic islets of Langerhans. It is associated with other organ-specific autoimmune disorders.(1-6,8-10) It is a type of diabetes characterized by absolute insulin insufficiency as a result of pancreatic β-cell damage. It is usually seen under the age of 35, but it is most common between the ages of 10-15. It constitutes approximately 5-10 % of all diabetics and the number of patients diagnosed each year is thought to be 70,000. Since there is absolute insulin deficiency, insulin is absolutely necessary for treatment. (1,3,4,8-10,17)

2.2. Type 2 Diabetes Mellitus

85-90% of diabetics have type 2 diabetes. It mainly affects overweight people over the age of 40 who are genetically susceptible. Insulin resistance and reduced β- cell function are seen in the patients. It is a kind of diabetes caused by hereditary and environmental causes, and it can result from insulin resistance and glucose tolerance problems. Rather than insulin insufficiency, this kind of diabetes has insulin excess and insulin resistance. Type 2 diabetes is the most prevalent kind of diabetes in the world and among adults, with 5-10% of the population in developed nations suffering from it. (1-6)

It mostly occurs after the age of 30, but because of the increase in obesity in the last 10-15 years, type 2 diabetes cases are also encountered in childhood or adolescence. Initially, it is not prone to diabetic ketoacidosis (DKA), but DKA may be seen in the case of prolonged hyperglycemia or in later periods when β-cell reserve is reduced. (8-13) The disease develops over time and most people have no symptoms at first. Symptoms such as impaired vision, numbness and tingling in the hands and feet, repeated fungal infections or delayed wound healing may occur in certain patients. (1,3,8,18)

2.3. Gestational Diabetes

Gestational diabetes is an insulin-resistant condition that increases the chance of developing type 2 diabetes later in life. (3,8) It develops during pregnancy and terminates with delivery. The children of these mothers are at risk of obesity and these children may develop diabetes at a young age. The mother is also at risk for type 2 development. (3,8,10,18,19)

2.4. Other Specific Types of Diabetes

This form of diabetes is caused by conditions that damage the insulin-producing cells in the pancreas, as well as medication usage. Pancreatic diseases that induce secondary diabetes include pancreatitis, trauma/pancreatectomy, neoplasia, cystic fibrosis and fibro calculous pancreas. Secondary diabetes is caused by endocrinopathies such as acromegaly, Cushing's disease, glucagonoma, pheochromocytoma, hyperthyroidism, and somatostatinoma. (1,3) Among the substances that cause secondary diabetes, nicotinic acid, glucocorticoids, thyroid hormone, diazoxide, thiazides and dilantin can be counted. (1,3,4,8,10).

It is important for clinicians to be aware of this classification to avoid misdiagnosis. Type 1 and type 2 diabetes are discrete diseases with widely varying clinical manifestations and disease development. Although classification is critical for establishing treatment, some people cannot be categorized as having type 1 or type 2 diabetes at the time of their diagnosis. (1,8-10)

Traditional conceptions of type 2 diabetes appearing only in adults and type 1 diabetes occurring just in children are no longer valid, as both age groups are affected. Children with type 1 diabetes are generally followed with polydipsia/polyuria and approximately one-third develop diabetic ketoacidosis (DKA). (8,10-12,16,17) In adults, the onset of type 1 diabetes may be more variable. They may not exhibit the usual symptoms found in children and may have a temporary relapse from the requirement for insulin. DKA occurs in patients with type 2 diabetes on occasion, especially in ethnic minorities. It is critical for clinicians to recognize that diabetes type classification is not always clear, and misinterpretation is common. (1,3,4,8-10) Although it is difficult to determine the type of diabetes in all age groups at first, the diagnosis becomes apparent with time.

A range of genetic and environmental variables can induce progressive loss of β-cell mass and/or function in both type 1 and type 2 diabetes, resulting in hyperglycemia. Patients with all forms of diabetes are at risk of developing the same chronic problems when hyperglycemia occurs, although the rates of progression may vary. (1,3-6)

Improving personalized diabetic treatments in the future will necessitate a deeper understanding of the numerous processes leading to β-cell death or dysfunction. Analysis of the underlying pathophysiology in type 1 diabetes is more advanced than in type 2 diabetes. According to studies of first-degree relatives of type 1 diabetes patients, the detection of two or more islet autoantibodies is an almost reliable predictor of clinical hyperglycemia and diabetes. (8)

The rate of progression is determined by the age at which autoantibodies are first detected, the number of autoantibodies, the specificity of the autoantibody, and the autoantibody titer. Glucose and A1C values rise long before the clinical onset of diabetes, enabling early detection. (3,8,9,14-16,20)

3. Diagnostic Tests and Monitoring

3.1. Diagnostic Tests

Diabetes can be diagnosed using plasma glucose parameters such as fasting plasma glucose (FPG) values, 2-hour plasma glucose (2-hour PG) values, or A1C values obtained after a 75 g oral glucose tolerance test (OGTT). (1,3,8) FPG, 2-hour PG, and A1C during the 75 g OGTT are all equally appropriate for diagnostic screening. Diabetes may be diagnosed using fasting and 2-hour plasma glucose levels. The agreement between the FPG and the 2-hour PG tests, as well as the A1C and both glucose-based tests, may be debatable. The 2-hour PG value identifies more patients with prediabetes and diabetes than the FPG and A1C endpoints. (1,3,4,6,8,14)

The efficacy of interventions for the primary prevention of type 2 diabetes has been demonstrated in individuals with impaired glucose tolerance (IGT), whether they have elevated fasting glucose, rather than patients with isolated impaired fasting glucose (IFG) or prediabetes defined by A1C criteria. The same tests can be used to screen and diagnose diabetes and identify individuals with prediabetes. Diabetes can be detected anywhere within the spectrum of clinical scenarios in ostensibly low-risk individuals getting glucose tested, individuals tested based on diabetes risk assessment, and symptomatic patients. (3,4,8,10,14-16,18-20)

3.2. Monitoring Criteria

3.2.1. Hemoglobin A1C (HbA1c)

HbA1C measures the average glycemia during a three-month period. The test is the most significant tool for evaluating glycemic control and it has a high predictive value for diabetes complications. (1,4,8,10,14,20) As a result, HbA1C testing should be conducted frequently in all diabetic patients, both during the initial evaluation and as part of ongoing therapy. Patients' glycemic targets are reached based on measurements obtained generally every three months. (8, 20, 21) HbA1C testing frequency should be determined by the clinical circumstances, treatment plan and medical decision. On-target glycemia

may perform well with HbA1C testing just twice a year in people with stable type 2 diabetes. Patients who are unstable or intensively managed or who are not on track with therapy modifications, may require more frequent testing. (20-24)

The HbA1C test serves as an indirect indicator of average glycemia and consequently has limitations. (8) The measurement of HbA1C is subject to variation, just like any other laboratory test. While such variability is lower than with blood glucose testing, clinicians should proceed cautiously when using HbA1C as the only basis for assessing glycemic control, especially if the result is close to the threshold for medication therapy changes. (4,8,20) For patients prone to glycemic variability, particularly those with type 1 diabetes or type 2 diabetes with severe insulin deficiency, glycemic control is best assessed by a combination of Self-Monitoring of Blood Glucose (SMBG) or Continuous Glucose Monitoring (CGM) and HbA1C results. (8, 22-24)

3.2.2. Self-monitoring of Blood g (SMBG) or Continuous Glucose Monitoring (CGM) at Home

SMBG is an essential component of the efficient management of insulin-treated patients. CGM has evolved as a tool for measuring glucose levels in recent years. (4,8,21) Glucose monitoring helps patients to assess their individual response to medication and whether glycemic objectives are being fulfilled safely. SMBG is essential for monitoring and preventing hypoglycemia and hyperglycemia, particularly in insulin-treated individuals. Most patients on rigorous insulin regimens (multiple daily injections or insulin pump treatment) should be encouraged to use SMBG (and/or CGM) to monitor their glucose levels before meals and snacks, at sleep and occasionally afterwards when low measurements are suspected. (4,8,20,21) Many patients who use SMBG may need to be checked up to 6-10 times per day, however requirements may vary. CGM measures interstitial glucose using two types of sensors and correlates well with plasma levels. (4,8,20-26)

4. Clinical Profiles and Risk Groups

4.1. Type 1 Diabetes Mellitus

4.1.1. Immune-mediated Type 1 Diabetes:

This type of diabetes, formerly known as “insulin-dependent diabetes” or “young-onset diabetes,” accounts for 5-10% of all diabetes cases and is caused by cellular-mediated autoimmune destruction of pancreatic β-cells. (1,6,8) Islet cell

autoantibodies, GAD autoantibodies (GAD65), insulin, tyrosine phosphatases IA-2 and IA-2b, and zinc transporter 8(ZnT8) are examples of autoimmune indicators. The presence of two or more of these autoimmune markers defines stage 1 of type 1 diabetes. The disease exhibits considerable human leukocyte antigen (HLA) correlations with the DQA and DQB genes. These HLA-DR/DQ alleles might be protective or predisposing. Type 1 diabetes refers to any kind of diabetes caused by autoimmune β-cell damage.

There are crucial genetic implications since many of the mutations that end up causing diabetes are inherited. Certain mutations are linked to other disorders, which may necessitate extra testing. (8) The rate of β-cell death varies greatly, being rapid in some individuals (mostly newborns and children) and slow in others (mainly adults). DKA may be the initial symptom of the disease in adolescents and children. Some patients have moderate fasting hyperglycemia, which can quickly progress to severe hyperglycemia and/or DKA. (8,16,18,21,22)

Adults can acquire adequate cell function to prevent DKA for many years. Some patients may have remission or reduced insulin needs for months or years before becoming insulin-dependent and at risk of DKA. (8,21) At this stage, there is little or no insulin production, as demonstrated by low or detectable plasma C-peptide levels. The immune-mediated type of diabetes is most frequent in childhood and adolescence, although it can occur at any age, including the eighth and ninth decades of life.(8,21,22) The progressive degeneration of its cells has numerous hereditary predispositions and is also linked to environmental variables that are still unknown. Although people with type 1 diabetes are not normally obese, obesity is becoming more widespread in the overall population, and there is information that it may be a contributing factor for type 1 diabetes. Type 1 diabetes patients are also prone to other autoimmune disorders such as Hashimoto's thyroiditis, Addison's disease, Graves' disease, celiac disease, vitiligo, autoimmune hepatitis and pernicious anemia. (8)

4.1.2. Idiopathic Type 1 Diabetes

Certain types of type 1 diabetes have no apparent etiology. These individuals exhibit chronic insulinopenia and are predisposed to DKA, but no indication of cell autoimmunity has been found. (1,4,8) Unfortunately, only a small percentage of type 1 diabetes patients fall into this group. People with autoantibody-negative type 1 diabetes of African or Asian ancestry who suffer

from episodic DKA may experience various degrees of insulin deficiency between episodes. This type of diabetes is highly hereditary and is not linked to HLA. In affected patients, insulin replacement treatment may become absolutely necessary. (8)

4.2. Prediabetes and Type 2 Diabetes

4.2.1. Prediabetes

Prediabetes is a term used to describe patients whose glucose levels are too high to be considered normal but do not fulfill the requirements for diabetes. Patients with prediabetes have IFG and/or IGT, as well as a HbA1C of 5.7% -6.4 (39-47 mmol/mol) (Table 3). (4,8,10,14) Prediabetes should be seen as a risk factor for diabetes and cardiovascular disease (CVD). Tables 4 describe the diabetes or prediabetes testing criteria in asymptomatic adults, children, and adolescents. Obesity (particularly abdominal or visceral obesity), high triglyceride and/or low HDL cholesterol, dyslipidemia, and hypertension are all risk factors for prediabetes. (4,8,10,14,16-20)

Table 3: Criteria defining prediabetes * (8)

FPG 100 mg/ dL (5.6 mmol/L) to 125 mg/ dL (6.9 mmol/L) (IFG)
OR
75 g OGTT 140 mg/ dL (7.8 mmol/L) to 199 mg/ dL (11.0 mmol/L) (IGT)
OR
A1C 5.7 –6.4% (39–47 mmol/mol)

FPG, fasting plasma glucose; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; OGTT,

Oral glucose tolerance test; 2 -h PG, 2 hour plasma glucose.

** The risk for all three tests is continuous, extending below the lower end of the range and increasing disproportionately at the upper end of the range.*

Table 4. Diabetes or Prediabetes Testing Criteria in Asymptomatic Adults (8)

1. Testing should be considered in adults who are overweight or obese (BMI ≥ 25 kg/m2 or 23 kg/m2 in Asian Americans) who have one or more of the following risk factors: - First degree relative with diabetes -High-risk race/ethnicity (eg. African American, Hispanic, Native American, Asian American, Pacific Islander) -CVD history -Hypertension (≥140 / 90 mmHg or for the treatment of hypertension) -HDL cholesterol level < 35 mg/ dL (0.90 mmol/L) and/or a triglyceride level >250 mg/ dL (2.82 mmol/L) -Women with polycystic ovary syndrome -Physical inactivity -Other clinical conditions associated with insulin resistance (eg, severe obesity, acanthosis nigricans) 2. Patients with prediabetes (A1C≥ 5.7% [39 mmol/mol], IGT or IFG) should be tested annually. 3. Women diagnosed with GDM should have a lifetime test at least every 3 years. 4. For all other patients, testing should begin at age 45. 5. If results are normal, testing should be repeated at intervals of at least 3 years and more frequent testing should be considered depending on initial results and risk status

CVD, cardiovascular disease; GDM, gestational diabetes Mellitus; BMI,Body Mass Index

4.2.2. Type 2 Diabetes

Type 2 diabetes, previously called “non-insulin dependent diabetes” or “adult-onset diabetes”, is responsible for 90-95% of all diabetes cases. (1,4,5,8,10) Those with relative (rather than absolute) insulin insufficiency and peripheral insulin resistance are included in this category. Some people may not require insulin therapy to survive at first, and quite often throughout their lifetimes. (5,8,10,13) Type 2 diabetes has several causes. Although the underlying etiologies are unclear, autoimmune destruction of β -cells does not exist, and individuals do not have other recognized causes of diabetes. The majority of people with type 2 diabetes are overweight or obese. High weight increases insulin resistance. (4,5,8,10,17))Patients who are not obese or overweight, according to typical weight definitions, have an elevated amount of body fat distributed largely in the abdomen. DKA seldom happens on its

own, and when it does occur in type 2 diabetes, it is frequently associated with the stress of another condition, such as infection or the use of certain drugs. (8,10, 13,17,18,22) Since hyperglycemia develops gradually and is frequently not severe enough for the patient to identify the classic symptoms of diabetes in the early stages, type 2 diabetes is routinely misdiagnosed for years. (8,18,22,27-29) Yet undiagnosed patients are at significant risk of developing macrovascular and microvascular complications. Age, obesity, and a lack of physical exercise all raise the probability of developing type 2 diabetes. (8,27-31)

Screening and testing for prediabetes and Type 2 diabetes in asymptomatic adults

Prediabetes and type 2 diabetes meet the requirements for circumstances where early identification is recommended. Such disorders are widespread and have major clinical and public health implications. (4,8) Before being diagnosed with type 2 diabetes, there is frequently a considerable presymptomatic period. Basic tests for detecting preclinical disease are commonly available. Other considerations for screening of type 2 diabetes and prediabetes in asymptomatic patients include the following: (8)

Age:

Age is an important risk factor for diabetes. For all patients, testing should begin no later than the age of 45. Screening should be considered in individuals of any age who are overweight or have one or more risk factors for diabetes and obesity.

BMI and ethnicity:

In general, BMI≥25 kg/m2 is a risk factor for diabetes.

Drugs:

Some drugs, such as glucocorticoids, thiazide diuretics, certain HIV medications, and atypical antipsychotics, have been linked to an increased risk of diabetes and should be considered.

Test intervals:

The ideal time between screening tests is uncertain. The three-year gap is justified by the fact that it reduces the amount of false-positive tests that require confirmation. (8)

1.1. Diabetes Symptoms

When the effect of insulin is blocked, glucose cannot enter the cells, resulting in exhaustion due to a lack of energy production. Glucose then builds up in the blood (hyperglycemia) and in the urine (glucosuria), osmotically absorbing enormous amounts of water (polyuria). This causes dehydration, which creates thirst and an excessive desire to drink water (polydipsia). (1,3-5,8,10,13,17,18,21) In type 1 diabetes, because glucose is no longer a viable source of energy, fat and protein reserves are metabolized through weight loss, peripheral muscle atrophy, and the generation of ketone bodies (acetoacetate, hydroxybutyrate, and acetone). In severe cases, ketone bodies (especially acetone) can be detected in the breath and can accumulate in the blood (ketonemia) and may also be excreted in the urine (ketonuria). (1,4,10,18,20)

Polyuria, polydipsia, and polyphagia are the most prevalent symptoms of uncontrolled diabetes. While type 1 diabetes symptoms appear quickly (weeks or months), type 2 diabetes symptoms appear considerably more slowly and may be unclear or absent. (3-5,8,10) Others include impaired vision, headaches, fatigue, slow wound healing, and itchy skin. Glucose uptake in the lens of the eye causes visual alterations and changes in the structure of the eye, which can be induced by long-term high blood pressure in diabetes individuals. Diabetic dermadromes are a group of skin rashes that can emerge because of diabetes. Table 5 summarizes the acute and chronic symptoms of diabetes mellitus. (8,10,32)

Table 5: Acute and Chronic Symptoms of Diabetes Mellitus (1)

Acute	**Chronic**
Increased thirst Polyuria and polydipsia Weight loss and fatigue Lethargy Confusion/Behavioral changes Stomach ache Nausea and vomiting Ketoacidosis Dehydration Kidney failure Coma	Increased thirst Polyuria and polydipsia Weight loss and fatigue Lethargy Irritability Recurrent skin, oral and genital infections Visual impairment Paresthesia (hands and feet)

2. Complications

People with well-controlled blood glucose levels show much less common and serious complications of diabetes (Table 6**)**. Broader health problems exacerbate the negative effects of diabetes.(1,4)

Table 6: Complications of Diabetes Mellitus (1)

Central and peripheral nervous systems	• Cerebral palsy • Autonomic neuropathy • Peripheral neuropathy (Motor and sensory dysfunctions)
Ocular	• Retinopathy • Cataract • Blindness
Cardiovascular system	• Cardiomyopathy • Myocardial infarction • Atherosclerosis • Hypertension • Endothelial cell dysfunction
Oral cavity	• Caries, gingivitis, periodontal infections)
Renal system	• Nephropathy • Proteinuria • Glucosuria • Kidney failure
Gastrointestinal system	• Diarrhea • Constipation • Dyspepsia • Exocrine gland insufficiency
Genital system	• Sexual dysfunction • Urogenital dysfunction
Skin and soft tissues	• Impaired wound healing • Skin infection
Bones	• Osteopenia, fractures
Foot	• Foot ulcer • Foot amputation

2.1. Acute Complications

2.1.1. Hyperosmolar Hyperglycemic Syndrome (HHS)

Although HHS has many of the symptoms in common with DKA, it is an acute complication of a completely different origin and a different treatment. In this case, water will osmotically move from the cells into the blood and the kidneys will begin to excrete glucose into the urine [(usually considered >300 mg/dl (16 mmol/L)] in a person with very high blood glucose levels. (1,4,8,10,20,29,33) Water loss and an increase in blood osmolarity are the end results. The osmotic effect of high glucose levels, coupled with water loss, will eventually lead to dehydration if fluid is not replaced orally or intravenously. (33,34) Cells gradually dehydrate as they lose water, and electrolyte imbalances are also common. As with DKA patients, emergency medical treatment is required, usually beginning with fluid volume replacement. Although it is more common in type 2 diabetes than in type 1 diabetes, lethargy may progress to coma. (33-36)

2.1.2. Hypoglycemia

Hypoglycemia, or an unusually low blood glucose level, is caused by a few diabetic treatments that might result in an emergency consequence. Otherwise, it is uncommon in either diabetic or non-diabetic people. (1,8) The patient may become agitated, sweaty, and weak, as well as exhibit several indications of sympathetic autonomic nervous system activation, leading in emotions of anxiety and immobilized panic. In severe circumstances, patients may lose consciousness, resulting in coma, seizures, brain injury, and death. (1,4,8) In patients with diabetes, this may be due to several factors, such as too much insulin, too much or incorrectly timed exercise (exercise reduces insulin requirements), or insufficient food (especially carbohydrates containing glucose). (1,8,21,37) Hypoglycemia is usually treated with sugary drinks or meals. In most situations of severe hypoglycemia, a glucagon injection or an intravenous infusion of dextrose is administered, but only if the person is unconscious. Intravenous dextrose is most commonly used in hospitals. (1,8,21)

2.1.3. Diabetic Coma

The medical emergency that occurs in an unconscious person with DM is known as diabetic coma. Acute complications of diabetes such as severe diabetic

hypoglycemia, advanced DKA and extreme hyperglycemia may contribute to diabetic coma. (8,35,36)

2.2. Chronic Complications

Damage to blood vessels in people with diabetes is caused by chronically elevated blood glucose levels. Endothelial cells lining blood vessels take up more glucose than normal because they are not bound to insulin. (1,4,6,8,13,17,18) Later, as these endothelial cells make more surface glycoprotein than normal, the basement membrane becomes thicker and begins to weaken. The problems that occur in diabetes are grouped under "microvascular disease" (due to damage to small blood vessels) and "macrovascular disease" (due to damage to the arteries). (8,21)

Recent research suggests that autoimmune disease that initially destroys pancreatic β-cells may also cause retinopathy, neuropathy, and nephropathy in type 1 diabetics. The familial clustering of the degree and type of diabetic complications suggests that genetics may also play a role in causing complications such as diabetic retinopathy and nephropathy. (1,4,8,10,17,18,20,21,29) It is generally thought that complications will improve over time by maintaining normal blood glucose levels. In terms of pathophysiology, studies show that the two main types of DM (type 1 and type 2) cause alterations in the stabilization of metabolites such as carbohydrates, lipids and blood coagulation factors, and subsequently lead to complications such as microvascular and cardiovascular complications.(8,21,29)

Types of Chronic Complications:

Microvascular:

One or more of the following are caused by damage to the small blood vessels that cause microangiopathy:

- Diabetic cardiomyopathy
- Diabetic nephropathy
- Diabetic neuropathy
- Diabetic retinopathy
- Diabetic encephalopathy.

Macrovascular:

One or more of the following are caused by macrovascular disease:

• Contributes to cardiovascular disease accelerating atherosclerosis. Coronary artery disease leading to angina or myocardial infarction ("heart attack")

• Diabetic myonecrosis ("muscle wasting")

• Exercise-related leg and foot pain and intermittent claudication with diabetic foot due to peripheral vascular disease

• Paralysis (mainly ischemic type). (8)

Diabetic foot often occurs due to a combination of sensory neuropathy (numbness or insensitivity) and vascular damage, increasing rates of skin ulcers (diabetic foot ulcers) and infection, and in severe cases, rates of necrosis and gangreneIn developed countries, it is frequently the leading reason for adult toe and/or foot amputation. (38,39)

3. Medical Therapy

Individuals with diabetes should take an active role in their own treatment. Diabetes treatment aims to prevent or delay complications while maintaining quality of life. (8) At the initial patient assessment, a complete medical evaluation should be performed, taking the following factors into account:

• Diagnosis should be confirmed and diabetes should be classified.

• Diabetes complications and potential for comorbid conditions should be evaluated.

• Previous treatment and risk factor control should be reviewed in patients with established diabetes.

• Patient participation should be initiated in the formulation of a care management plan.

• A plan for continued treatment and care should be developed.

The cornerstones for attaining diabetes treatment goals are effective behavioral control and psychological wellness. To attain these objectives, diabetes self-management education, medical nutrition treatment, regular physical exercise, smoking cessation counseling where appropriate and psychological counseling are required. (8,20,37,40,41)

3.1. Pharmacological Therapy for Type 1 Diabetes

3.1.1. Insulin Therapy

Insulin therapy is very important for individuals with type 1 diabetes, as type 1 diabetes has little or no β-cell function. (1-4,8,10) In addition to

hyperglycemia, it can contribute to other metabolic disturbances and life-threatening tissue catabolism, such as insulinopenia, hypertriglyceridemia and ketoacidosis. Severe metabolic decompensation was mostly prevented by once or twice daily injections for sixty or seventy years after the discovery of insulin. (8,10,21-23,40,41,43) However, over the past three decades, evidence supporting more intensive insulin replacement suggests using multiple daily injections of insulin or continuous subcutaneous administration via an insulin pump, providing the best combination of efficacy and safety for people with type 1 diabetes. Based on the Diabetes Control and Complications Trial (DCCT), intensive treatment with multiple daily injections or continuous subcutaneous insulin infusion (CSII) decreases HbA1C and is associated with improved long-term outcomes. (44) Comprehensive treatment, on the other hand, has been related to a higher risk of severe hypoglycemia than standard therapy.

In the last 25 years, rapid-acting and long-acting insulin analogs with different pharmacokinetics have been developed compared to recombinant human insulins. (18,21,43) In general sense,basal insulin analogs have a longer time period of action, flatter, more stable plasma concentrations, and activity profiles than Neutral Protamine Hagedorn (NPH) insulin. Rapid-acting analogues (RAA) have a faster onset, peak, and duration of action than standard human insulin. In people with type 1 diabetes, treatment with analogous insulins is associated with less hypoglycemia and weight gain and lower A1C compared to human insulins. (1,8,18, 21,43) More recently, two new insulin formulations with improved rapid-acting profiles have been introduced. Insulin analogues in type 1 diabetic patients, the expense and/or intensity of therapy necessary for their usage is prohibitive for some. (43)

There are many approaches to insulin therapy. The basic principle in the management of type 1 diabetes is to administer some form of insulin to achieve the patient's glycemic goals and in a tailored regimen designed to keep patients safe, avoid diabetic ketoacidosis, and avoid severe hypoglycemia. (18,21,43)

The patients with type 1 diabetes usually receive 50% of their daily insulin basal and 50% prandial. Total daily insulin requirements can be estimated based on weight, with typical doses ranging from 0.4 to 1.0 units/kg/day.(43) The American Diabetes Association/JDRF Type 1 Diabetes Sourcebook states that a typical starting dose in patients with metabolically stable type 1 diabetes is 0.5 units/kg/day, half given as prandial insulin to control blood sugar after meals and the other half. states that it is as basal insulin to control glycemia in the periods between meals and absorption.(43-47)

3.2. Pharmacological Therapy for Type 2 Diabetes

A patient-centered approach should be used in the selection of pharmacological agents. (43) Considerations include cardiovascular comorbidities, risk of hypoglycemia, cost, risk of side effects, and patient preferences. Intensification of treatment should not be delayed in patients with type 2 diabetes who do not meet treatment goals. The drug regimen and drug-taking behavior should be reassessed at regular intervals (every 3-6 months) and adjusted as necessary to include specific factors influencing treatment choice. (10,21,43)

3.2.1. Monotherapy

Metformin should be begun as soon as type 2 diabetes is identified, unless there are contraindications, and for many individuals, this will be monotherapy in conjunction with lifestyle adjustments.(10,21) Metformin is an effective, safe, and economical diabetes treatment that may lower the risk of cardiovascular disease and mortality. Metformin is offered in two forms: immediate-release for twice-daily administration and extended-release for once-daily administration. Compared with sulfonylureas, metformin as first-line treatment had positive benefits on HbA1C, weight and cardiovascular mortality. (4,8,10,14,21,41-43). The main side effects of metformin are bloating, abdominal discomfort and gastrointestinal intolerance from diarrhea these can be alleviated by gradual dose titration. The agent is removed via renal filtration, and extremely high circulating levels (due to overdose or acute renal failure, for example) have been linked to lactic acidosis. This consequence, however, is now recognized to be extremely uncommon, and metformin can be safely supplied to individuals with lower estimated glomerular filtration. (21,43-45)

In patients with contraindications or metformin intolerance, initial therapy should be based on patient factors and many patients will require dual combination therapy to achieve their target A1C level. (21,43,48,49) When hyperglycemia is severe and catabolic characteristics (weight loss, hypertriglyceridemia, ketosis) are present, insulin has the benefit of being efficacious in the absence of other drugs and should be considered as part of any combination treatment. It is common practice to initiate insulin therapy in patients with blood glucose levels of 300 mg/dL (16.7 mmol/L) or A1C >10% (86 mmol/mol) or if the patient has symptoms of hyperglycemia (polyuria, polydipsia, or weight loss). (51) As glucose toxicity improves, it is often possible to simplify the regimen and/or switch to oral agents. However, there is evidence that patients with uncontrolled hyperglycemia associated with type 2 diabetes can also be treated with a sulfonylurea. (52)

3.2.2. Combination Therapy

Because type 2 diabetes is a progressive disease in many patients, maintenance of glycemic targets with monotherapy is usually possible after a few years. Current recommendations are to use the gradual addition of metformin drugs to keep HbA1C on target. This provides a clearer assessment of the positive and negative effects of new drugs and reduces patient risk and expense. (8,10,21,43,53-55) Depending on these factors, the sequence of oral agents It has become the standard of metformin therapy.

The choice of drug with metformin added depends on the clinical characteristics and preferences of the patient. Important clinical features include the presence of established atherosclerotic cardiovascular disease (ASCVD) or high ASCVD risk indicators, other comorbidities, and risk of specific adverse drug effects, as well as safety, tolerability, and cost. (21,43)

If the HbA1C target is not reached after approximately 3 months, metformin may be combined with any of six preferred treatment options: sulfonylurea, thiazolidinedione, DPP-4 inhibitor, SGLT2 inhibitor, GLP-1 RA, or basal insulin. The choice of agent to add is based on drug-specific effects and patient factors (Table 7). (43)

Table 7: Drug-Specific and Patient Factors to Consider When Choosing Antihyperglycemic Therapy in Adults with Type 2 Diabetes (40)

		Activity	**Hypoglycemia**	**Weight change**	**Oral/SC**
Metformin		High	-	There is some weight loss potential	Oral
SGLT-2 inhibitors		Middle	-	Weight loss	Oral
GLP-1RA		High	-	Weight loss	Subcutaneous, oral
DPP-4 inhibitors		Middle	-	Neutral	Oral
Thiazolidinediones		High	-	Weight gain	Oral
Sulfonylureas (2nd Generation)		High	-	Weight gain	Oral
Insulin	Human insulin	Very high	Present	Weight gain	Subcutaneous, inhaled
	Analogue				Subcutaneous

DPP-4, dipeptidyl peptidase 4; GLP-1 RAs, glucagon-like peptide 1 receptor agonists; SGLT2, sodium-glucose transporter 2; SC, subcutaneous

There is no known prevention method for type 1 diabetes. A person who maintains a normal body weight, does physical exercise, and follows a healthy diet can prevent type 2 diabetes. A diet high in whole grains and fiber, as well as choosing beneficial fats such as nuts, vegetable oils and polyunsaturated fats found in fish, are all proven to help prevent diabetes. (40-43) Diabetes can be prevented by consuming less red meat and other sources of saturated fat in their daily lives, as well as limiting sugary drinks. Active smoking is also associated with an increased risk of diabetes, so quitting smoking can also be an important preventative measure. (42,43,56)

4. Diabetes and Oral Health

Chronic hyperglycemia causes a variety of problems throughout the body, including the oral cavity, therefore blood glucose management is critical. Impairment of neutrophil function, increased collagenase activity and reduced collagen synthesis, microangiopathy, and neuropathy are all possible mechanisms related with diabetic oral complications. (7,57-59) The severity of diabetic complications is generally related to the degree and duration of hyperglycemia. Oral manifestations and complications of DM include dry mouth, dental caries, periapical lesions, gingivitis, periodontal diseases, oral candidiasis, burning sensation, change in taste, geographic tongue, oral lichen planus (OLP), recurrent aphthous stomatitis, increased susceptibility to infections, and impaired wound healing. (57,58)

4.1. Gingivitis

Plaque is a thin layer of bacteria that constantly builds up on teeth. Gingivitis develops when plaque builds up and is called gingivitis in its early stages. The gingiva looks erythematous and edematous and may bleed while brushing teeth. (7,57,60)

4.2. Periodontal Diseases/Periodontitis

Periodontitis is advanced gum disease. Periodontitis, if left untreated, can result in tooth loss. Poor glycemic control may be associated with progression of gingivitis, periodontitis, and alveolar bone loss. (57,59,60)

Effect of diabetes on periodontal tissue:

Periodontitis is preceded by various stages of gingivitis. Symptoms of periodontitis include erythematous and edematous gums that bleed easily, periodontal pockets and pus formation, halitosis or a bad taste in mouth, loose teeth that have lost their supporting bone tissue (Figure 1-3). It is commonly related to bacterial infection by organisms such as *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans.* (57,60-63)

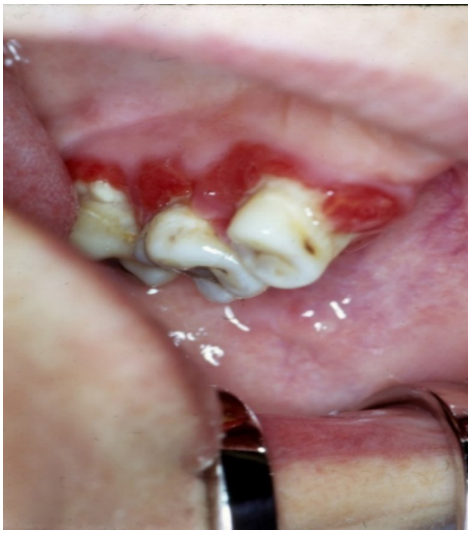

Figure 1: Erythematous Appearance of the Marginal Gingiva

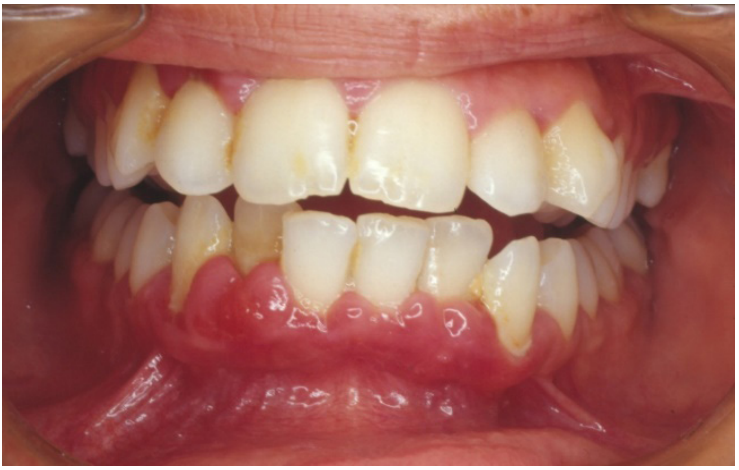

Figure 2: Erythematous, Edematous Appearance of the Anterior Gingiva of the Mandible

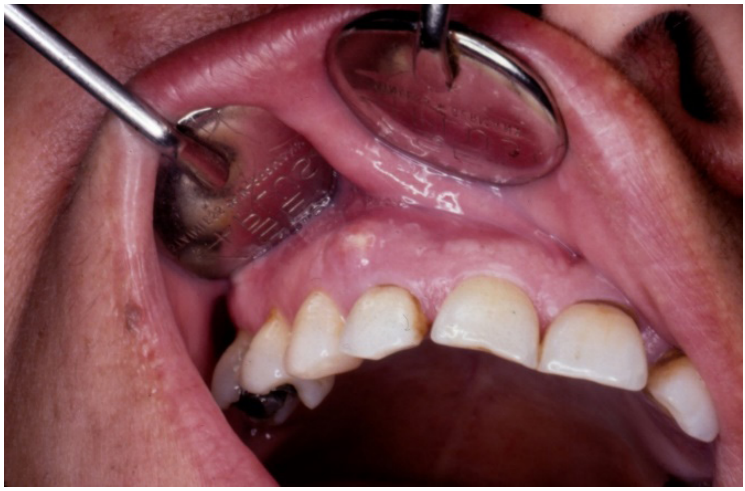

Figure 3: Periodontal Pocket Formation in a Diabetic Patient

Periodontitis is about twice as common in children and adolescents with type 1 diabetes than it is in children and adolescents without diabetes. (57,58,62,63) Moreover, adults with type 2 diabetes have higher rates than adults without diabetes. Uncontrolled glycemia is a predisposing cause for periodontal disease, but the resulting infection also contributes to the continuation of hyperglycemia. Fungi, spirochetes and anaerobes are the agents of periodontitis, which is seen two to six times more frequently in young adults and children. The risk of bacterial infection is increased in poorly controlled diabetes because chronic microvascular complications caused by prolonged hyperglycemia reduce vascular lumen width. As a result, tissue perfusion is impaired, oxygen consumption decreases and anaerobic microorganisms increase. In a situation where the chemotactic functions of leukocytes are also reduced, that is, diabetes, these microorganisms initiate supra or subgingival invasion and secrete endotoxin when host resistance is reduced. (4) These bacterial products react with phagocytic cells and fibroblasts, causing the release of inflammatory mediators such as IL-1, TNF-α, IL-6, and activating the excessive collagenolytic mechanism. Thus, catabolism in connective tissue and bone increases and periodontal ligament destruction accelerates. Another issue caused by prolonged hyperglycemia is the glycosylation of tissue proteins. When the advanced glycation end products (AGEs) formed meet the receptors in the gingiva, the receptor for advanced glycation endproducts (RAGE) complex formed causes the release of inflammatory mediators (TNF-α, IL-1) that initiate oxidative stress. Thus, the collagenolytic mechanism initiated by pathogenic

bacteria is further accelerated. The patient's age and duration of diabetes are among the factors that determine the picture. (4,57)

Periodontal ligament loss:

Periodontitis is the loss of dental supporting tissues such as connective tissue attachment and bone (Figure 4,5). Diabetes and attachment loss have a statistically significant association, and this association is strongly affected by the level of glycemic control. Severe periodontitis resulting in alveolar bone loss is believed to include the action of inflammation on both osteoclasts and osteoblasts. Diabetes has a significant influence on osteoclastogenesis and osteoblast apoptosis (Figure 6). (57,61-65)

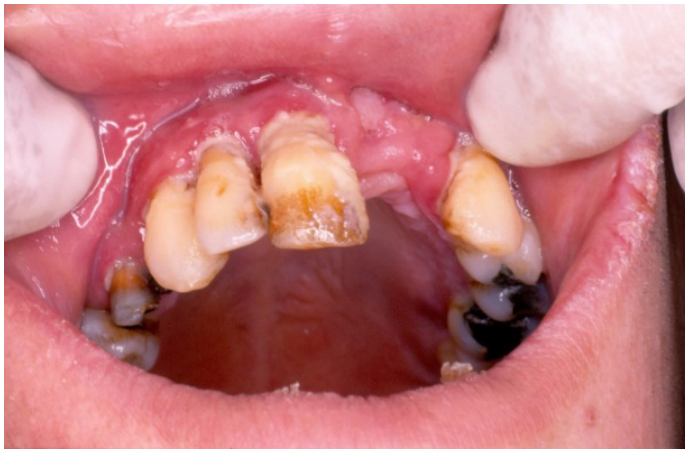

Figure 4: Periodontitis and Severe Bone Destruction in a Patient Diagnosed with Diabetes

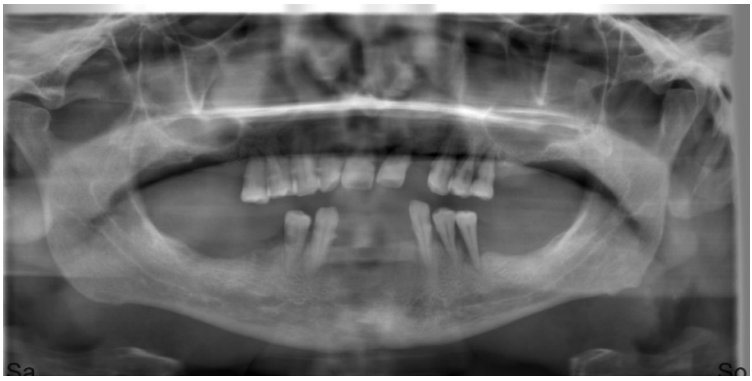

Figure 5: Generalized Alveolar Bone Destruction on Panoramic X-ray in Another Case Diagnosed with Diabetes

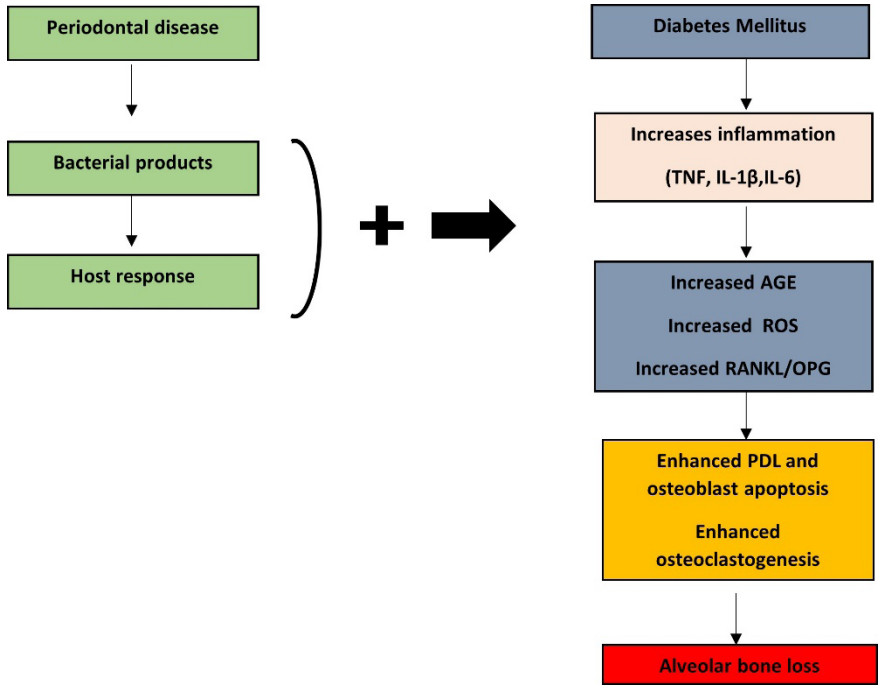

Figure 6: Pathogenesis of Periodontal Destruction (65)

AGE, advanced glycation end product; IL, interleukin; OPG, osteoprotegerin; PDL, periodontal ligament; RANKL, receptor activator of nuclear factor kappa-B ligand; ROS, reactive oxygen species; TNF, tumor necrosis factor

Changes in host defense response, subgingival microbiota, collagen structure and metabolism, vascularity, and gingival crevicular fluid, as well as patterns of heredity, might all explain the increased susceptibility to periodontal diseases. Moreover, various risk factors have been identified that predispose these individuals to periodontal disease development, including poor dental hygiene, poor metabolic management, diabetes for a long period of time and smoking. (57,61,62)

4.3. Oral Candidiasis

People with diabetes are also more likely to develop candidiasis, an oral fungal infection caused by *Candida albicans.* (57,66) A variety of factors can contribute to the onset of oral candidiasis. The salivary dysfunction in these patients may contribute to further carriage of fungi. Candida -associated lesions include prosthetic stomatitis, angular cheilits and median rhomboid glossitis.

Diabetes patients who smoke, wear dentures, have poor glycemic control, and take steroids and broad-spectrum antibiotics are more likely to get Candida infection. While those who have type 1 diabetes show greater rates of candida colonization than those with type 2 (84% vs. 68%), it is only about 27% in non-diabetics. (66-68)

4.4. Dry Mouth

Diabetics suffer from salivary dysfunction, which can lead to reduced saliva flow and changes in saliva composition. Dry mouth can cause several problems, including difficulty in speaking, eating and swallowing. In fact, it can have a negative impact on patients' quality of life. Although the etiology is unknown, it may be associated with polyuria, autonomic neuropathies and changes in the basal membranes of the salivary glands. (57,69,70) The rate and amount of saliva secreted from the parotid and other salivary glands do not change in diabetics without glycemic control, but the glucose and calcium ratio in saliva is higher. The salivary glucose level, which is 0.2-3.3 mg/dl in normal people, may increase twofold in diabetic patients and reach 0.45-6.3 levels. As a result, dry mouth can be observed. (4)

4.5. Dental Caries

Diabetic people are more prone to developing new and recurring dental caries.(57,62) Higher carbohydrate levels in saliva, as well as increased levels of oral candida, mutans streptococci, and lactobacilli, may all contribute to an increased prevalence of tooth decay. Furthermore, persistent hyperglycemia might result in permanent pulpitis and pulp necrosis. According to several research, apical periodontitis and radiolucent periapical lesions are more prevalent in diabetics than in non-diabetics. (57,71,72)

4.6. Burning Sensation

Burning sensation in the oral cavity of diabetic individuals is associated with poor glycemic control, metabolic changes in the oral mucosa, angiopathy, candida infection, and neuropathy. Neuropathic pain in these patients may manifest as a burning, tingling, or even electric shock sensation. These pain sensations have a significant impact on physical and psychological functions and are associated with sleep disturbance, anxiety, and depression levels. (57,69)

4.7. Taste Disturbance

Taste dysfunction can arise in patients with poorly managed diabetes. Saliva dysfunction can cause altered sense of taste or elevated perception thresholds. Neuropathy also increases the taste threshold. This sensory impairment might make it difficult to maintain a healthy diet and result in impaired glucose management. (57,61,69,70)

4.8. Oral Mucosal Changes

Some oral mucosal changes such as fissure and rusty tongue, geographic tongue, recurrent aphthous stomatitis, and some autoimmune conditions including OLP can be associated with diabetes. (57,61,69,70,72) The sensitivity of these patients to alterations in the oral cavity is still debatable, but poor control of diabetes, immunological alteration, microcirculation changes with reduced blood flow, xerostomia and changes in saliva flow and composition, and smoking have been mentioned.

OLP is more common in patients with type 1 diabetes compared to type 2 because type 1 diabetes is considered an autoimmune disease and there is an autoimmune mechanism underlying OLP. (61,70) Acute hyperglycemia causes changes in immune response in diabetic patients. (57,70)

4.9. Delayed Wound Healing

A significant complication of dental procedures is delayed recovery of soft and hard tissues in diabetic individuals. (70) Prolonged wound healing of these patients includes delayed vascularization, decreased blood flow and hypoxia, decreased innate immunity, decreased growth factor production, and psychological stress. Oral complications in DM patients are considered to be the main complications of the disease and may affect the patients' quality of life. Chronic and persistent oral complications in these patients adversely affect blood glucose control. Therefore, prevention and management of diabetes-related oral complications are important. (57, 61,70)

5. Dentist's Approach to Patients with Diabetes Mellitus

Type 1 diabetes usually occurs in young individuals. For treatment, subcutaneous insulin preparations and insulin pumps are used. Diabetic ketoacidosis will occur if these patients remain without insulin for longer than 48 hours due to continuous exogenous insulin intake. (1,8,10,14) Cortisol,

catecholamines and glucagon, which are released in the body in physiological stress situations, cause a stress-induced glucose intolerance by eliminating the effects of insulin.(8,10,23,41) Therefore, in some cases, insulin dose adjustment may be required before the procedures to be performed on the patient. However, since wound healing is impaired in cases where blood glucose is high, no action should be taken until the blood glucose level is regulated in patients whose blood glucose is not under control or within normal limits.(1,4, 64,68,73)

Before starting the treatment of a diabetic patient, several key points should be considered to help minimize the risk of an intraoperative diabetic emergency and reduce the likelihood of oral complications of the disease:

- The patient's doctor should be consulted to evaluate diabetes control.
- Medical history and medications should be updated at each appointment.
- Before starting the treatment,it should be confirmed that the patient is taking the drug.
- Hypoglycemia should be anticipated and prepared to manage.
-Infections should be prevented and treated immediately.
-Compounds containing aspirin should not be used.
- Oral hygiene and comprehensive preventive treatment should be provided.
-Regular diet and medication regimen should be reinforced before and after dental appointments.
- If the patient is in the high-risk group and uses insulin or will be operated, measurement should be made with a glucometer.(73-75)

At the start of each appointment, the dentist should make sure that the diabetic eats and takes their medication as usual. Otherwise, the patient may be at risk of hypoglycemics. In some cases, it is necessary to measure the blood glucose level and before the dentist begins treatment. If the level is low, the patient should consume an oral carbohydrate source before starting treatment. If the level is high, treatment should be delayed and the dentist should refer the patient to their physician to reassess glycemic control. Target values for blood glucose in diabetic patients are shown in Table 8. (1,4,64,73-75)

Table 8: Target Values for Most Patients with Diabetes Mellitus (4)

Glycemic control **	
HbA1c	< 7%
Preprandial glycemia	90-130 mg/dl(5-7.2mmol/l)
Postprandial glycemia	<180 mg/dl (<10 mml/l)
Arterial pressure	<130/80 mmHg
Lipids	
LDL	< 100 mg/dl (<2.6 mmol/l)
Triglyceride	< 150 mg/dl (< 1.7 mmol/l)
HDL	>40 mg/dl (>1.1 mmol/l)
Criteria for setting glycemia targets:	
-Target values should be individual.	
-Special populations (child, pregnant, diabetic, elderly) require a special approach.	
-In patients with severe hypoglycemia, strict glycemic control with intense treatment should be prioritized.	
-It should be known that providing tighter glycemic control (HbA1c <6%) reduces costs by preventing chronic complications despite the risk of hypoglycemia.	
- If preprandial glycemia is normal but HbA1c is high, the target should be PPG in treatment.	

LDL; low-density lipoprotein, HDL; high-density lipoprotein

*** The peripheral blood values of the sample measured in the venous plasma by the glucose oxidase method are 10-15% lower.*

Diabetes patients also have an increased risk of other systemic diseases such as coronary artery diseases, cerebrovascular accident, and kidney failure. Therefore, these risk factors should be considered when evaluating diabetic patients. When planning the procedure for the patient, the type and weight of diabetes, whether the disease is under control, the time of the procedure, the duration of the procedure to be performed, and when the patient can start feeding after the procedure are the factors that affect the anesthesia and surgical procedure and should be considered. During the procedure, the hypo /hyperglycemic state of the patient should be constantly monitored. (1,4,62-68)

Initial signs and symptoms of hypoglycemia include hunger, fatigue, sweating, nausea, tremor, irritability, and tachycardia. If a hypoglycemic episode is suspected, the dentist should immediately stop the dental treatment and give 15 g of oral carbohydrates via sugar, juice, or glucose tablet. Studies have shown that 15 g of glucose will cause a blood sugar increase of about 2.1 mmol/L in 20 minutes. (1,67,68)

Following emergency treatment, the dentist should monitor the patient's blood glucose level to determine if repeated carbohydrate dosing is necessary. If the patient is unconscious or unable to swallow, the dentist should seek medical attention. In these cases, the patient is given intravenous 20–50 mL. A 50% dextrose solution or 1 mg of glucagon should be given by intravenous, intramuscular, or subcutaneous injection. (4, 58,60,61,68)

In general, morning appointments are recommended for patients with diabetes because endogenous cortisol levels are typically higher at this time. Since cortisol raises blood sugar levels, the risk of hypoglycemia is less. For patients using short and/or long-acting insulin therapy, appointments should be scheduled so that they do not coincide with peak insulin activity, which increases the risk of hypoglycemia. It is important to confirm that the patient is eating normally and taking all scheduled medications prior to the appointment. (4,62-64,72-75) If a treatment is scheduled with the assumption that the patient may change their typical eating patterns prematurely (e.g., sedation), the dose of diabetes medication may need to be changed in consultation with the patient's physician. Patients with well-controlled diabetes can usually be managed conventionally for most surgical procedures. If the patient's food consumption will be affected after oral or dental surgery, the patient's diabetes medications should be balanced and food intake should be provided in advance. Dentists should exercise caution when treating patients with marginal or poorly controlled diabetes. (60,67,68,74-76) Good clinical judgment is important because in some cases it may be necessary to delay elective dental treatment until the patient's diabetes is stable or better controlled. Dental implants can be applied to patients with well-controlled diabetes, and implant therapy can be applied to patients with moderately controlled diabetes. However, implant placement in patients with poorly controlled disease has an unpredictable prognosis and should be avoided if possible. Coordination with the patient's physician may be necessary to determine the patient's state of health and whether the planned dental treatment can be performed safely and effectively. Physicians should provide laboratory test results to the dentist upon request and inform the dentist of any related diabetic complications. (4, 67,68,73-80)

6. Conclusion

With the present growth in diabetes cases, practitioners must have a basic awareness of the disease. The consequences for dental care supply should be clearly grasped. The informed diabetic patient may be a great source

of information about how to effectively manage therapy. Encouraging this "teamwork" attitude will assist to make treatment as trouble-free as feasible.

**** We would like to thank Prof. Semih Özbayrak for image support.***

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CHAPTER XXV

REGENERATIVE ENDODONTICS

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1. Introduction

Regenerative endodontics is defined as biologically based procedures designed to replace damaged tooth structures, including dentin-root structures and cells of the pulp-dentin complex (1,2). Root canal treatment is difficult due to the weak root walls of necrotic teeth with incomplete root development (3). Root apex occluded using calcium hydroxide in open apex teeth, and the teeth are prepared for a traditional root canal treatment (4). Apexification treatment with calcium hydroxide may require several visits. Alternatively, Mineral Trioxide Apexification treatment, which can be completed in a single visit with obturation using Mineral Trioxide Aggregate (MTA), is also frequently preferred for teeth with open apex (5). Although the apexification treatment performed with this method is successful, the tooth roots do not thicken, root development does not continue, and the teeth cannot be expected to be vital again (6,7). MTA has a short shelf life, is expensive, and is difficult to manipulate as a material. In the treatment of immature necrotic permanent teeth, ensuring the continuity of root development and thickening the dentin walls, encouraging the increase in root length, and restoring the vitality of the tooth will be the ideal treatment (8).

Nygaard-Ostby is one of the pioneers in regenerative endodontic treatments. In his study in the early 1960s, he demonstrated that new vascularized tissue formation could be induced in the root apical region of endodontically treated mature teeth with necrotic pulp and apical lesions (9). Nygaard-Ostby and Hjortdal attempted to revascularize the pulp in necrotic teeth with apical

periodontitis, but mostly unsuccessfully. It is thought that these results are related to the inadequacy and effectiveness of the materials used 40-50 years ago (10). There are various developments in intracanal drugs and application methods used in regenerative endodontic studies. The placement of artificial scaffolds and access to the cavity sealed sealing allows the infected pulp to be disinfected. By obtaining a sterile tissue matrix, the development of new cells is encouraged, and it is possible to regain the vitality of the pulp (11). Iwaya et al. coined the term revascularization, which has ensured positive vitality tests and root development in immature teeth in contrast to apexification for the first time (12). In the following years, it recommended using revitalization instead of revascularization, as the results of this treatment may cause regeneration in soft and hard tissues (13). The European Endodontic Society (ESE) has also used the term revitalization (14). The American Society of Endodontists (AAE) also calls these procedures regenerative endodontics treatment (RET) (15). Later, Banchs & Trope published a study supporting this treatment. In the treatment follow-up, the symptoms disappeared, the dentin thickened, and the open root apex was closed (16). So, RET has suggested the first choice in teeth with an open apex. (17).

2. Biological Basis of Regenerative Endodontic Treatment

2.1. Stem Cells and Growth Factors

To transfer regenerative principles into practice in endodontics, tissue engineering applications are needed. The tissue engineering triad can be defined as stem cells, scaffolding, and induction of morphogenetic signals (18). The most important cell of regenerative therapy is stem cells. Stem cells originating from the apical papilla were described in 2006 (19). The apical papilla is rich in undifferentiated mesenchymal stem cells with high proliferation ability and odontogenic differentiation capacity (20). Apical papilla stem cells (SCAP) are regulated by hertwig epithelial root sheath cells (HERS). HERS is involved in root development and formation (21). In a study conducted in 2011, the apical region was stimulated, and bleeding was achieved, a step of the regenerative procedure. The presence of mesenchymal stem cells (MSC) in this region was evaluated, and reported that during the regenerative procedures, a significant amount of MSC migrated into the root canal space, resulting in a more than 700-fold increase in the expression of MSC markers. In addition, these cells can be cultured from clinical specimens and examined under a confocal

microscope. With this study, it was shown for the first time that regenerative endodontic treatments are based on stem cells (22). Even in the presence of apical periodontitis and apical abscess, significant concentrations of MSC have been reported to migrate into the root canal. RET receives help from living cells in apical tissues such as SCAP, periodontal ligament stem cells, and dental pulp stem cells (23). In addition, SCAP is self-renewing cells that can differentiate into odontoblast-like cells that are very similar to the dentin microstructure by promoting regeneration. At the same time, the apical papilla is a structure rich in MSC. Therefore, SCAP should remain healthy and alive, especially in teeth with open apex, which is essential for RET (20).

MSCs are transported into root canals by apical bleeding in teeth with an open apex. Stem cells entering the root canals through bleeding form the basis for starting the regenerative process (22). It was emphasized that the critical point, in this case, is the survival potential of these cells (23).

The second component of RET is growth factors. Growth factors are proteins that attach to receptors on the cell surface and act as signals that enable cell proliferation and differentiation (24). Recent research showed that dentin leads to the release of a series of bioactive proteins thought to lead to regeneration disinfection materials can affect release growth factors (25). For example, growth factors embedded in dentin can be released from dissolving dentin tissue by EDTA chelation (26). Moreover, growth factors regulate stem cell functions and support regeneration by placing them in scaffolds (27). Increases angiogenesis and matrix synthesis. Thus, they help the healing and regeneration of tissues (28).

2.2. Blood Clots and Scaffolds

The third component needed is a physical scaffold. Tissues are three-dimensional structures that need a three-dimensional physical scaffold to grow and differentiate. This scaffold structure lays the groundwork for cell organization, proliferation, differentiation, and vascularization. In current RET clots, platelet-rich plasma (PRP) or platelet-rich fibrin (PRF) is used for scaffolding (15). In addition to traditional PRP and PRF scaffolds, concentrated growth factor has started to be used as a new-generation scaffold in regenerative endodontics (29). Thanks to its concentrated growth factor-rich cytokine content, it contributes to regeneration by directing the stem cells in the apical papilla into the canal (30). It has been reported in a study that collagen solutions are also used as artificial scaffolds in the root canal (31).

Empty pulp chamber, from the periapical region to the canal, unable to stimulate tissue growth. A suitable scaffold in the root canal promotes cell proliferation and differentiation. The scaffold must be able to selectively bind to cells, localize them, contain growth factors, and be resorbable (32). The simplest and easiest method to create a scaffold in RET is clot formation in the canal by providing apical bleeding. MSC and growth factors will create regeneration with bleeding from the apical into the canal entering the root canals (22). There are also defense cells such as phagocytes, immunoglobulins, antibacterial polypeptides, and proinflammatory cytokines in the blood. Although it has not been discussed until now, a blood clot can antibacterially remove bacteria that may live in root canals (5). It was emphasized that the bleeding created in the root canal in RET should reach 3 mm below the enamel-cementum line and wait 15 minutes for clot formation to occur (33). In this method, it may not always be possible to bleed from the apical in cases where the periapical tissues are severely damaged. If bleeding is not achieved, treatment may be delayed until the periapical tissues have healed (34). Ding et al. reported the absence of clot formation due to insufficient bleeding in the canal as one of the causes of unsuccessful regenerative procedures (33). They considered using PRP in patients who could not bleed with a file inside the canal (33). Hargreaves et al. reported that platelet-rich plasma (PRP) meets many of the ideal scaffolding properties for RET (35). In studies conducted to date, the superiority of PRP and PRF scaffolds over blood coagulation has not been proven (5).

3. Regenerative Endodontic Treatment Related to The Mechanism of The Procedure Theories

There are 5 fundamental theories about the continuation of root development and the formation of new tissue in the root canal with the RET.

1. Even in the presence of pathology in the periradicular region of permanent teeth that have not completed root development, pulp cells that have preserved their vitality may be found in the apical root canal. It has been argued that new matrix formation is induced by differentiation into odontoblast cells by the organization of hertwig epithelial root sheath cells, which are more resistant to inflammation and destruction (36).

2. It has been thought that it may be related to multipotent dental pulp stem cells (DPSC). DPSC can be found in high concentrations in permanent immature teeth in the apical region of the dentinal walls. It has been suggested

that the continuity of root development can be ensured by depositing tertiary or atubular dentin by differentiating multipotent dental pulp stem cells into odontoblasts (37).

3. It is thought to be related to the presence of periodontal ligament stem cells. These cells can proliferate into the apical region and the root canal and provide hard tissue deposition in both regions (38).

4. It is based on the belief that it is related to apical papilla root cells or bone marrow. It is aimed to transplant the MSC in the bone into the root canal lumen with the bleeding created in the apical region. The proliferation capacity of these cells is relatively high (39).

5. It is thought that the clot formed in the root canal plays an important role in regeneration because it contains growth factors. The resulting clot contains platelet-rich growth factor, vascular endothelial growth factor, platelet-rich epithelial growth factor, and tissue growth factor. All these factors stimulate the transformation of immature and undifferentiated MSC into cells such as fibroblast, odontoblast, and cementoblast in the newly formed tissue matrix (40).

4. Root Canal Disinfection

Minimal or no mechanical debridement is suggested in RET. Therefore, chemical debridement and intracanal medicament are very intensive to eliminate the infection in the root canal. However, during the selection of chemical agents used during regenerative procedures, attention should not be paid to whether they are bactericidal or bacteriostatic but also that they can increase the proliferative and survival capacity of stem cells (23). The first step in root canal debridement is eliminating infected necrotic organic material from the canal cavity with copious irrigation. Sodium hypochlorite agents have been used in regenerative endodontics at rates ranging from 1-6% (41). While ESE recommends using sodium hypochlorite at rates ranging from 1.5% to 3%, AAE recommends using 1.5% (14,15) due to its cytotoxic effect on SCAP (14,15,42).

Another recommended irrigation agent in regenerative endodontics is EDTA (14,15). EDTA is mainly used in RET by chelating, allowing growth fibers to be released from the dentin matrix (43). The use of EDTA at a concentration of 17% in irrigation reverses the cytotoxic effect of sodium hypochlorite on stem cells and reduces its harmful effect (42). Since minimal mechanical debridement is performed in regenerative endodontics, the smear layer is removed using EDTA, which may facilitate the regenerative tissue's adhesion to the canal walls

(44). Growth factors released from the dentin matrix with EDTA have been shown to signal stem cells in the apical papilla to transform into odontoblast-like cells (45).

There are also reported cases of the use of chlorhexidine in RET (46,47). However, it has been reported that chlorhexidine has harmful effects on stem cells (48).

5. Intracanal Medicament Use in Regenerative Endodontic Treatment

It has been reported that using intracanal medicaments in addition to irrigation in RET is effective in reducing root canal decontamination (49). For this purpose, triple antibiotic paste (TAP), calcium hydroxide, or chlorhexidine gel were used for 1-4 weeks (47,50,51). Infections in the root canal system are considered a polymicrobial pathological condition caused by both aerobic and anaerobic bacterial species. Therefore, using more than one antibiotic in combination was deemed appropriate. It has been reported that the most promising combination in this regard is metronidazole, ciprofloxacin, and minocycline (52). The TAP contains bactericidal (ciprofloxacin, metronidazole) and bacteriostatic (minocycline) properties. Metronidazole, a broad-spectrum bactericide, is highly effective against anaerobes (12). In selecting an appropriate irrigant and medicament, regenerative properties should be considered, as well as antimicrobial effect. Tetracycline causes the growth of host cells in dentin by causing the exposure of hidden collagen fibrils or growth factors (53).

Antibiotic concentration is essential for the survival of stem cells. Therefore, it is recommended to use low concentrations to reduce the cytotoxic effect of TAP on stem cells in the apical papilla (5). 1-5 mg/ml for TAP was recommended by AAE (15). Despite the successful results of TAP in RET, there are also some disadvantages. Coronal discoloration, bacterial resistance, and allergic reactions are some of them (54, 55). Nosrat et al. reported that using amoxicillin + clavulanate instead of TAP as an intracanal drug is effective in periradicular tissue healing (56). Shin et al. developed a single-session regenerative endodontic treatment technique and excluded intracanal medicaments (57). Due to its antimicrobial properties, calcium hydroxide has been used as a root canal treatment intracanal medicament since ancient times (58). An in vitro study showed that apical cells' attachment to dentin was more successful when calcium hydroxide was used than with antibiotic paste (59). Cehreli et al. used calcium hydroxide in the coronal part of the canal after

disinfection with sodium hypochlorite in a tooth with an open apex that lost vitality due to trauma. They reported that the apex was closed three months later, and root development continued (60). It has been reported that more successful results are obtained when calcium hydroxide placement is limited to half of the coronal third (61). Ruperal et al. evaluated the effects of TAP, dual antibiotic paste (ciprofloxacin, metronidazole), and calcium hydroxide on SCAPs. They demonstrated that using other medicaments, except calcium hydroxide, during regenerative procedures has detrimental effects on SCAPs (62). For this reason, the intracanal medicament should be used at a concentration that provides antibacterial activity but is not toxic to stem cells (49).

However, some studies reported that neither irrigation solutions nor in-canal medicaments had wholly eliminated the biofilms in infected root canals (63,64).

6. Coating Material

One of the ideal coating materials to be used for hermetic coronary sealing is MTA. Because it has been reported that the physical properties of MTA in terms of covering and sealing are quite good (65). The MTA should be 1-2 mm thick and 3-4 mm from the cementoenamel junction to allow for more root development. After the MTA has hardened, the cotton pellet should be removed, and the tooth should be restored with glass ionomer cement or resin cement (16). Jung et al. suggested that resorbable barriers could serve as a matrix for MTA and recommended using Collatape (Zimmer Dental, Warsaw, IN, USA) for this purpose. A study also shows the successful use of glass ionomer cement (CIS) as a coating material instead of MTA (67).

7. Is Apical Foramen Diameter Important in Regenerative Endodontic Treatment?

The apical foramen width is not the only success factor in revascularization in the canal and the formation of new tissue after transplantation (49). While it was stated that the previous studies' critical diameter is 1 mm, successful cases have been reported in further studies, even with 0.6 mm and 0.32 mm diameters (68-70).

8. Protocol of Regenerative Endodontic Treatment

Although teeth with necrotic pulp and open apex are indicated for RET, they are contraindicated if support from the pulp chamber is required for post

or core construction. The systemic health status of the patient should be ASA 1 or ASA 2, and the patient should not have drug allergies. An informed consent form must be taken from the patient before the treatment. The RET procedure is based on the guidelines updated by AAE in 2021 (15).

8.1. First Visit

For RET, local anesthesia is performed in the first visit, and isolation is provided with rubber dam application. After opening the access cavity, abundant irrigation with 20 ml of NaOCl (irrigation needles and EndoVac systems can be used) should be done to minimize the overflow to the periapical tissues. Irrigating the canal in 20 ml/canal for 5 minutes with a minimum concentration of NaOCl (1.5%) is recommended. Then, the irrigation needle should be positioned 1mm above the apex and irrigated with saline or EDTA 20 ml/canal for 5 minutes. This procedure prevents damage to stem cells in apical tissues and reduces cytotoxicity. Afterward, the canals should be dried with paper points, and calcium hydroxide or low-concentration TAP should be placed. If a triple antibiotic paste is to be placed, the pulp chamber should be covered with a dentin bonding agent to minimize discoloration, and ciprofloxacin: metronidazole: minocycline should be mixed in a 1:1:1 ratio. Minocycline's final concentration should be 0.1-1.0 mg/ml. However, since minocycline causes discoloration, double antibiotic pastes without minocycline or triple antibiotic pastes prepared with cefaclor, amoxicillin, and clindamycin can be used instead of minocycline. The medicament should be placed in the canal with a syringe. If the TAP is used, care should be taken to keep the medicament below the cementoenamel junction. The first visit is completed by restoring the access cavity with a temporary restorative material of 3-4 mm thick (15).

8.2. Second Visit

In the second visit, 1-4 weeks after the first visit, the controls of the initial treatment should be done. Additional treatment time with alternative antimicrobials is needed for persistent infection symptoms.

If there is no symptom, anesthesia should be performed with 3% mepivacaine without vasoconstrictor, and isolation with a rubber dam should be provided. Gently irrigate with 20 ml of 17% EDTA. Afterward, the canal should be dried with a paper point. Bleeding into the canal should be created with over-instrumentation. Filling the entire canal space with blood up to the cementoenamel junction by rotating the pre-curved K-file that passes the apical

foramen 2 mm is provided. PRP, PRF, and autological fibrin matrix (AFM) can be used to form blood clots. A resorbable matrix such as CollaPlug™, Collacote™, or CollaTape™ should be placed on the clot to shape the white MTA placement as coating material. The coating material should be covered with 3-4 mm glass ionomer cement and cured for 40 seconds. Bioceramics or tricalcium silicate cement can be used in cases where there is a possibility of discoloration and aesthetic concerns. For anterior teeth and premolars, it is recommended to use Collatape/Collaplug, and cover the clot with a 3 mm thick non-coloring material, followed by restoration with composite resin. For molars and crowned teeth, it is recommended to use Collatape/Collaplug, cover the clot with 3 mm thick MTA, then restore with resin-modified glass-ionomer or composite resin.

8.3. Follow-up

It has been reported that healing of the periapical lesion after RET is usually expected within 6-12 months, and closure of the apex with thickening of the root walls is expected in approximately 12-24 months (15). In cases where clinical symptoms were not observed, follow-up was recommended every 3 months in the first year and annually after that (71). Cone-beam computed tomography is recommended to be used in follow-ups (15).

9. Clinical, Histological, and Radiological Evaluation

RET in immature necrotic teeth has three goals (15). The first goal is to eliminate symptoms and to see radiographic healing. It has been reported that a high success rate is seen in periapical healing and the disappearance of clinical symptoms (72).

The second goal is an increase in dentin thickness and root length. The newly formed hard tissue in the dentinal walls of the root canal cavity is markedly separated from the dentin, bone, or bone-like tissues. It has been reported that it resembles cement, but collagen matrix organization and maturation are significantly different (44). Although it is believed that dentin thickening and root length increase will strengthen the tooth structure, there are not enough studies to prove this (5). However, a study on dogs showed that cement deposition after RET increases fracture resistance (73).

The third goal is for the teeth to respond positively to vitality tests. It has been reported that an average of 50% of RET cases have reached vitality in studies (23). The lack of pulpal response may not be associated with the

presence or absence of regenerating a nerve tissue. According to Torabinejad and Turman, the response to the electric pulp test and cold test can be affected according to the coronal level of the regenerated tissue and the thickness of the filling material covering this tissue (74). According to clinical and histological studies, regenerating tissue in the root canal is classified into 4 types:

Type 1: Increased dentin formation results in pulp canal obliteration,
Type 2: Periodontal ligament (PDL) and cementum formation,
Type 3: PDL-cement-bone formation,
Type 4: Defined as bone-bone marrow formation. (75)

It has been reported that the formation of hard tissue after RET in root canals may be because the stem cells coming into the canal have the potential to produce hard tissue such as periodontal ligament and bone marrow (5). It is believed that immature teeth treated with RET will have the best prognosis if they heal with type 1 tissue. Human teeth with immature apex have effective stem cell sources for hard tissue regeneration (76). Many factors, such as root development of teeth, elongation of their apex, or canal calcifications, can be examined radiographically after RET (77-79). Chen et al. classified the root response types that emerged (78):

Type 1: Elongation and thickening of the root apex,
Type 2: Blind closure of the apex,
Type 3: The apex remains open,
Type 4: Severe canal obliteration,
Type 5: Dentin barrier under MTA (78).

In addition, another common finding after RET is calcifications in root canals (79). These radiographic results are expected for RET. Re-planning of endodontic treatment is not recommended unless symptoms occur (80). According to the results of a study in which the soft tissue formed after RET was examined histologically, the tissue formed shows the characteristics of pulp-like connective tissue but does not contain odontoblast cells (81). Regeneration is the restoration of the biological functioning of the tissues in the past with the tissue formed similarly to the original tissue. Repair is the repair of worn tissues with tissues different from the original tissue (82). As a result of the research, bone and cement-like reparative tissues are formed rather than regenerative pulp tissue after RET. Therefore, it is more accurate to say that the pulp dentinal

complex formed because of this treatment is healed with repair tissue instead of regeneration (5).

10. Evaluation of Success after Regenerative Endodontic Treatment

The success rate of regenerative endodontic treatments can be evaluated based on clinical feedback. According to the results of a previous study, the survival rate of teeth after RET was reported as 100%, healed cases 80%, and ongoing healing cases 20% (83).

Post-treatment symptoms (pain, percussion tenderness, fistula, mobility, swelling) are a sure indicator of failure (84). In addition, previous results in the literature, such as discoloration, no increase in root length, no blood entering the canal, coronal leakage, and tooth fracture, were discussed as failures (34,56,85-87).

The etiology of the RET cases may also affect success (23). Traumatic injuries can cause root resorption and damage the apical papilla and hertwig epithelial root sheath (88). Therefore, RET may fail in traumatic dental injuries (89). It has been reported that persistent infection results are highly effective in the failure of RET cases (89). In addition, it has been reported that residual bacterial biofilms and their by-products can cause failure by significantly changing the osteogenic differentiation of stem cells (90). It has been reported that the blood clot will be weak physically in short-rooted and open apex teeth. Therefore, it cannot fully provide pulpal regeneration (71). However, this is not considered a failure (15). Because it has been shown that dentin thickness and root length increase even if adequate bleeding is not achieved (91). Causes such as antibiotic paste containing minocycline, bleeding in root canals, and use of MTA may cause coronal discoloration after RET. Although it is not considered a failure, it is an aesthetically undesirable result (92,93).

MTA apexification is the most preferred treatment option in treatment failure (89).

11. Conclusion

With RET, a new biological and clinical era has begun in endodontics. In cases where success has been reported in the literature, removal of inflammation, stimulating regeneration of dental stem cells, and release of growth factors from the dentin walls provide repair instead of regeneration. As a result, prospective studies evaluating the long-term clinical success of these treatment techniques,

which are offered as an alternative to traditional apexification treatment, are required.

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CHAPTER XVI

PREOPERATIVE RADIONUCLIDE LABELİNG BEFORE AXILLARY SENTINEL LYMPHADENECTOMY WITH INTRAOPERATIVE GAMMA PROBE

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1. Introduction

Deaths from breast cancer have been decreasing in recent years, thanks to early diagnosis and improved treatments. Removal of breast cancer without metastatic spread increases survival. The main examination findings in breast cancer; are a lump in the breast or armpit, dimpling of the breast skin, nipple retraction, nipple discoloration, and bloody nipple discharge. Mammography is the most commonly used imaging method in breast cancer screening. 3D tomosynthesis, a type of enhanced digital mammogram, allows the examination of multiple images of the breast. 3D tomosynthesis; It allows the benign and malignant features of the lesion to be evaluated in finer detail by the radiologist(4-6). Breast ultrasonography; helps to evaluate whether an existing lesion in the breast is cystic or solid. However, ultrasonography examines a limited area of the breast and cannot be used as a screening test for the whole breast instead of mammography. Breast MRI can help diagnose breast cancer in some clinical situations. Breast MRI can be used in cases where cancer is detected in the armpit lymph nodes but there is no evidence of breast cancer on mammography. In order to diagnose breast cancer, it is necessary to take a sample from the abnormal area. A

breast biopsy can be done with the help of imaging. The sample obtained by fine-needle aspiration biopsy is not sufficient to characterize some features of cancer. Therefore, it is preferable to obtain a larger sample with a larger diameter core needle biopsy. Core needle biopsy is performed under local anesthesia and the lesion is marked with a clip to locate the lesion. The earliest breast cancers are called "in situ" cancers. If cancer originates in the ducts of the breast and does not grow outside the ducts, the tumor is called ductal carcinoma in situ. Ductal carcinoma in situ can progress to invasive cancers if left untreated. Treatment for ductal carcinoma in situ is usually the removal of the cancerous area followed by radiation therapy. Radiation therapy is not always necessary. Surgery may be sufficient, especially in the presence of a tumor in a very small area, in the presence of hormone receptor-positive and low-grade disease, and in elderly women whose tumor has been completely removed.

When ductal carcinoma in situ is widespread, occult invasive cancer may accompany it. A mastectomy may be required. A sentinel lymph node marking is a special technique to identify and remove the most important lymph nodes in the armpit. If cancer has affected the lymph nodes, this will affect treatment decisions. Hormonal therapy may be recommended to prevent a recurrence. In invasive breast cancer, the tumor has affected the surrounding breast tissue beyond the ducts or lobules of the breast. The presence of hormone receptors and HER2 protein is important in the selection of treatment. The pathologist evaluates these proteins when grading cancer. Higher-grade tumors are more likely to need chemotherapy. Estrogen-dependent breast cancer cells produce proteins called hormone receptors, which can be estrogen receptors (ER), progesterone receptors (PR), or both. If a woman has hormone receptors in her breast cancer, she is likely to benefit from treatments that lower her estrogen levels or block the effects of estrogen. These treatments are called endocrine or hormone therapies, and such tumors are called hormone-sensitive or hormone-receptor-positive. Women who do not have an ER or PR do not benefit from endocrine therapy and are not recommended. HER2 is a protein found in approximately 15 to 20 percent of invasive breast cancers (1-3). The presence of HER2 in breast cancer identifies women who may benefit from treatments for the HER2 protein. In cancer, if the hormone receptors and HER2 are negative, it is called "triple negative" disease. The cells of cancer that start in the breast can go to other places through the bloodstream or lymph channels and form metastases.

One of the first places where breast cancer spreads is the axillary lymph nodes. When these nodes enlarge, they can be felt during the examination. Enlarged axillary lymph nodes can also be seen on mammography, MRI, or ultrasound. However, even if the lymph nodes are enlarged, the only way to determine if they truly contain cancer cells is to examine the tissue sample under a microscope. The presence or absence of lymph node involvement is one of the most important factors in determining the long-term outcome of cancer and often guides treatment decisions. If the armpit lymph nodes contain cancer (positive nodes), the cancer cells are more likely to spread elsewhere, and most of these women are advised to receive adjuvant systemic therapy. Underarm lymph nodes should be examined for tumor spread. If a suspicious lymph node is found, a needle biopsy is done to obtain a tissue sample. If there is cancerous involvement in the axillary lymph nodes, a surgical procedure called axillary lymph node dissection is performed to remove all of the axillary lymph nodes during breast surgery (4). A surgical procedure called a sentinel lymph node biopsy is often performed in patients with early-stage breast cancer who do not have significant involvement of the axillary lymph nodes. In this procedure, the lymph nodes where cancer will go first are marked. Two viewers are used for It is then removed for pathological examination. Problems such as upper extremity lymphedema, which are at risk of developing secondary to axillary lymph node dissection, are less common in sentinel lymphadenectomy. It also provides important information for staging. The relapse score is evaluated with genetic tests performed on tumor tissue and it is used to decide on chemotherapy. In general, patients with low recurrence scores and other low-risk features of their cancer may not need chemotherapy, while those with high scores will benefit more from chemotherapy. Antiestrogen therapy is typically administered to patients with hormone receptor-positive disease regardless of relapse score. Tumor stage and grade are different concepts. In a standard system of abbreviations called the TNM staging system, “T” stands for the primary tumor, “N” stands for regional lymph nodes status, and “M” stands for the presence or absence of metastases to other organs. Bone scintigraphy, thorax CT, abdomen and pelvic CT, and PET-CT may be done to help determine if cancer has spread beyond the breast and axillary lymph nodes. Women with stage I or II breast cancer are said to have early-stage localized breast cancer. Generally, stage I breast cancer refers to a tumor less than 2 cm (0.8 inches) in size that is node-negative. In general, stage II tumors are tumors that have spread to

the axillary lymph nodes and/or have a tumor size greater than 2 cm but less than 5 cm (approximately 2 inches)(1-3). Women with stage III tumors are referred to as having locally advanced breast cancer. These include large breast tumors (greater than 5 cm or about 2 inches in diameter), with extensive axillary nodal involvement (more than 10 lymph nodes with cancer), and nodal involvement of both axillary and internal mammary nodes (behind the ribs). Supraclavicular and infraclavicular lymph nodes may be involved. A tumor is also called stage III if it extends into the muscles under the chest wall or the overlying skin. Inflammatory breast cancer, a fast-growing type of cancer that makes the breast appear red and swollen, is at least stage III, even if it's small and doesn't involve lymph nodes. Stage IV breast cancer refers to tumors that have metastasized to areas outside the breast and lymph nodes to the bones, lungs, liver, or other organs. The primary tumor can be any size and can have any number of affected lymph nodes. This is called metastatic breast cancer.

2. Sentinel Lymph Node Marking Methods

Sentinel lymph node biopsy (SLNB) is a method used for axillary staging in lymph node-negative breast cancer. A properly constructed SLNB identifies patients who need further axillary therapy, while potentially saving others from morbid axillary lymph node dissection (ALND) (5,6). Appropriate surgical technique in SLNB minimizes the risk of understaging and undertreating patients, which in turn affects outcomes. SLNB typically begins with the injection of a tracer intradermally or subcutaneously around the tumor or areola. They enter the follower lymphatic channels and the lymph nodes where they first accumulate are called sentinel lymph nodes. Although sentinel nodes can be variably located, they are usually located in the lower axilla (6).

2.1. Blue Dye Method

In the blue dye method, 3 to 5 mL of blue dye (1% isosulfan blue or diluted methylene blue) is injected. It is important not to inject the dye into the tumor itself or into the seroma space following breast biopsy. These errors in technique are likely to cause the mapping to fail. The use of isosulfan blue dye for SLNB is associated with serious anaphylactic reactions requiring resuscitation in 0.16 to 1.1 percent of cases (7-9). However, routine prophylaxis is not standard practice due to the low reported rate of anaphylaxis (7). In high-risk cases, prophylactic treatment with hydrocortisone 100 mg (or 20 mg

methylprednisolone or 4 mg dexamethasone), 50 mg diphenhydramine and 20 mg famotidine intravenously just before or during anesthesia induction appears to reduce the severity. Methylene blue is an alternative to isosulfan blue dye with a lower rate of an anaphylactic reaction (0.0006%) (10-11). Methylene blue also has side effects. Intradermal injection of methylene blue may cause skin necrosis. The intraparenchymal injection may cause hardening and erythema along with pain (12). Pulmonary edema and serotonin syndrome have also been reported in patients taking serotonergic drugs. By diluting methylene blue with normal saline, side effects can potentially be minimized (13). Following the follower injection, the breast should be massaged for five minutes to expand the mammary lymphatics (14). The armpit fascia is then entered through the armpit incision. If blue dye is used as the sole tracer, the surgeon cannot use an intraoperative gamma probe to locate the protective node. A careful and systematic search is made for blue lymphatic channels. The sentinel nodes at the end of the blue lymphatic channel are identified. Care should be taken to identify the bluest node and the closest blue node. Dye transition is rapid in the axilla and distal, nonsentinel axillary lymph nodes are also seen as blue. Suspicious palpable nodes should also be removed for evaluation, as a tumor-replaced lymph node will likely not be able to receive tracer dye.

2.2. Radionuclide Labeling

Tecnetium-99m-nanocolloid can be used as a radioactive marker. The radioactive material can be injected into the peritumoral, intradermal, or subareolar plexus. Intradermal injection of radiocolloid seems to be superior to subcutaneous injection (15,16). The half-life of technetium 99m nanocolloid or sulfur colloid is six hours. Typically, 0.5-1 mCi is injected on the day of surgery or 2.5-4 mCi is injected the day before surgery. After chest massage, a portable gamma probe is used to determine the maximum radioactivity in the axilla. If a hot spot cannot be found with the gamma probe prior to the incision, 10 to 40 mL of saline or local anesthetic may be injected at the radioactive colloid injection site. Breast massage can be done again to increase the interstitial pressure and force more viewers to enter the lymphatic channels. In patients who have had previous breast or armpit surgery, normal lymphatic channels may be blocked, causing alternative drainage pathways. In such patients, the risk of not localizing sentinel lymph nodes with the gamma probe is higher than normal. Preoperative lymphoscintigraphy and the use of dual tracers may help better localize sentinel nodes in these patients. The

lymph node with the most radioactivity as determined by the gamma probe is removed first and an ex vivo count is obtained. Removal of subsequent lymph nodes follows the "10 percent rule." All lymph nodes with more than 10% of the ex vivo count of the most radioactive nodes are removed (17). Usually, two to three sentinel lymph nodes are removed. After removing four or five guard lymph nodes, the value of additional nodes is extremely low (18). If the count from other lymph nodes is still greater than 10 percent of the radioactive node but does not appear suspicious, some surgeons may choose not to remove any additional nodes. It is suggested that the marginal efficiency of removing more than four or five guard nodes is extremely low. Any suspicious palpable nodes should be removed, regardless of whether they are radioactive or not, as tumor-laden nodes may not receive much tracer. In patients with tumors in the upper outer quadrant of the breast or axillary tail, signals from the gamma probe, injection site, and axilla overlap and there may be a failure to localize sentinel lymph nodes. Subareolar injection or a smaller volume of radioactive colloid can be injected instead of peritumoral. Or the axilla can be reevaluated after tumor resection. Thus, the count from the injection site is eliminated.

2.3. Other Methods

Other techniques for localizing sentinel lymph nodes have been reported using novel tracers such as indocyanine green (ICG), superparamagnetic iron oxide (SPIO), and microbubble contrast agent. However, these new techniques have wide variability in results between studies, small number of patients, and short patient follow-up. Currently, these techniques should be considered for research purposes until there is conclusive evidence that they can identify sentinel lymph nodes reliably and with a low false-negative rate (19–21).

2.4. Combined Methods

In patients with clinically node-negative breast cancer, both blue dye and radiocolloid injection can be used to detect sentinel nodes intraoperatively in SLNB (6). In cases where a high false-negative rate is expected, the use of dual viewers may be preferred (7). If the patient received neoadjuvant therapy before SLNB, if the patient has a history of previous breast or armpit surgery, if the patient is obese, and if the surgeon has little experience, the

false-negative rate may be high (22-25). Lymphoscintigraphy is performed using a gamma camera to identify areas of increased radioactivity and mark the skin in such areas (26). The efficacy is higher when a radionuclide marker is used in combination with a blue dye than with a blue dye alone. In this case, an intraoperative gamma probe can also be used (27). Preoperative lymphoscintigraphy is recommended before re-SLNB in patients with a previous operation history in this region. The probability of having an abnormal drainage pattern is high in these patients (28). About a quarter of patients with positive sentinel nodes have residual disease in the axilla, and in some cases, a complementary ALND may be required (29). Although several intraoperative techniques can be used to identify a positive protective node, due to sampling limitations, none of these methods can identify all patients with positive nodes intraoperatively. Evaluation is more accurate than permanent (paraffin) sections.

The mean false-negative rate of intraoperative sentinel lymph node evaluation is about 25% (30). Additional improvements of the intraoperative evaluation may improve the identification rate of nodal metastases. The combination of frozen section and rapid cytokeratin immunostaining has been tried, and this method has reduced the false-negative rate of intraoperative analysis (31). Most studies have found a false positive rate of 0%. Patients should be informed that a second surgery may be required to complete ALND. Potential adverse outcomes from missing node metastases include understaging of the patient and increased risk of cancer recurrence (32). Thus, every effort is made to reduce the false negative rate. A systematic review of 69 SLNB studies, including 8059 patients, showed that sentinel lymph nodes were detectable in 95 percent of patients, with a 7.3 percent false-negative rate (range 0 to 29 percent) (33). When performed by experienced surgeons, SLNB is a safe procedure with few complications. The success of sentinel node biopsy depends on lymphatic drainage. A previous excisional biopsy has the potential to increase the false negative rate compared to a previous percutaneous biopsy. For this reason, open surgical biopsies should be avoided if diagnosis by core biopsy is possible. If open surgical biopsy has already been performed, care should be taken to avoid injecting the blue dye or radioactive tracer directly into the seroma space. Patients who have had previous breast or armpit surgery may benefit from preoperative lymphoscintigraphy and/or the dual tracer technique. The false-negative rate is directly related to the number of sentinel nodes excised (34). Also, gentle palpation of the axilla along the

incision should be made to identify any definitively suspicious nodes. Not only blue and warm lymph nodes but also palpable suspicious lymph nodes should be considered sentry nodes (35).

The condition of the axillary lymph nodes remains one of the most important prognostic factors in women with early-stage breast cancer. Histological examination of the excised lymph nodes is the most accurate method to evaluate the spread of the disease to these nodes. Sentinel lymph node biopsy (SLNB) is important for axillary staging in clinically node-negative breast cancer. A standard SLNB can be made with blue dye and/or radioactive colloid. Routine lymphoscintigraphy is not required before SLNB. Preoperative lymphoscintigraphy should be performed before repeating SLNB, as the probability of an abnormal drainage pattern is high in patients with previous SLNB or ipsilateral axillary dissection. Successful sentinel lymph node identification with blue dye is defined as the identification of any blue node or non-blue node containing blue afferent lymphatics. For radioactive SLNB, the "10 percent rule", which refers to the removal of all nodes with more than 10 percent of the most radioactive node, has been proposed as a guideline. On average, two or three sentinel nodes are identified. Some authors stop the procedure after removing three guard lymph nodes, while others continue until all lymph nodes meet the criteria and are removed. Intraoperative evaluation of sentinel lymph nodes may not identify all patients with positive nodes due to sampling limitations. The permanent section is the most accurate evaluation method. The false negative rate is an important measure of the procedural accuracy of the SLNB.

Breast-conserving surgery; can also be referred to as wide excision, quadrantectomy, or partial mastectomy. Radiation therapy may be needed after breast-conserving surgery to reduce the chance of cancer recurring in the same breast. Radiation therapy may not be needed in small, hormone-receptor-positive elderly patients and lymph-node-negative patients. The combination of surgery and radiation often results in cosmetically acceptable preservation of the breast without compromising breast cancer outcomes. Radiation therapy to the chest wall and surrounding lymph node may also be recommended for patients who have had a mastectomy. Factors such as positive lymph nodes, large tumors, and positive margins play a role in the decision. Systemic anticancer therapy given before or after surgery is called "adjuvant systemic therapy." The term "neoadjuvant" is used when treatment is given before surgery. Many patients with early disease who are triple negative or HER2 positive will receive

neoadjuvant therapy. Then, depending on the results of the surgery, they may also receive additional treatment. The goal of systemic therapy is to eliminate or prevent the growth of metastatic cancer cells that may have escaped from the breast and grown in other organs. The first place where breast cancer spreads is the axillary lymph nodes. When breast cancer metastasizes to the axillary lymph nodes, the cure rate is lower than when it is only in the breast. Patients with metastases or cancer cells in other organs, such as the liver, lung, or bone, rarely recover. However, systemic therapy can prevent metastases in the majority of patients and thus cure many women who would otherwise not recover. Therefore, systemic therapy has become an important component of breast cancer treatment. Systemic treatment is recommended for the vast majority of women with stage II breast cancer and for many women with stage 1 disease. There are three types of systemic therapy. Some women may receive more than one type of these treatments, depending on the characteristics of the tumor. Endocrine therapy is only recommended for women with estrogen receptor (ER) positive breast cancer. It is recommended for almost all women with ER-positive disease, regardless of stage, as it has few life-threatening side effects and is very effective. Endocrine therapy reduces the chance of breast cancer recurrence by about 50 percent. There are many types of chemotherapy used in the adjuvant setting, and they are usually given in combination or sequentially. Some women with "triple negative" cancer will benefit from immunotherapy, which uses the body's own immune system to fight cancer. For women with BRCA1 or BRCA2 genetic mutations and whose cancer is HER2-negative but with high-risk traits, it may be helpful to use a drug called a poly-ADP ribose polymerase (PARP) inhibitor following adjuvant therapy. Locally advanced and inflammatory breast cancer is less likely to cure than smaller cancers. Treatment usually includes a combination of systemic therapy, surgery, and radiation therapy. Depending on the cancer receptors, additional treatments may include endocrine therapy (if the tumor is hormone receptor-positive), anti-HER2 therapy (if the tumor is HER2 positive), and immunotherapy (if the tumor is triple-negative). In a minority of patients with metastatic breast cancer, the disease goes away completely. However, significant progress has been made in improving the length of time patients live with metastatic breast cancer and their quality of life during that time. Not all patients do well with the treatment of metastatic disease, but overall for most patients, treatment can prolong life, delay cancer progression, and reduce cancer-related symptoms. can alleviate and improve quality of life.

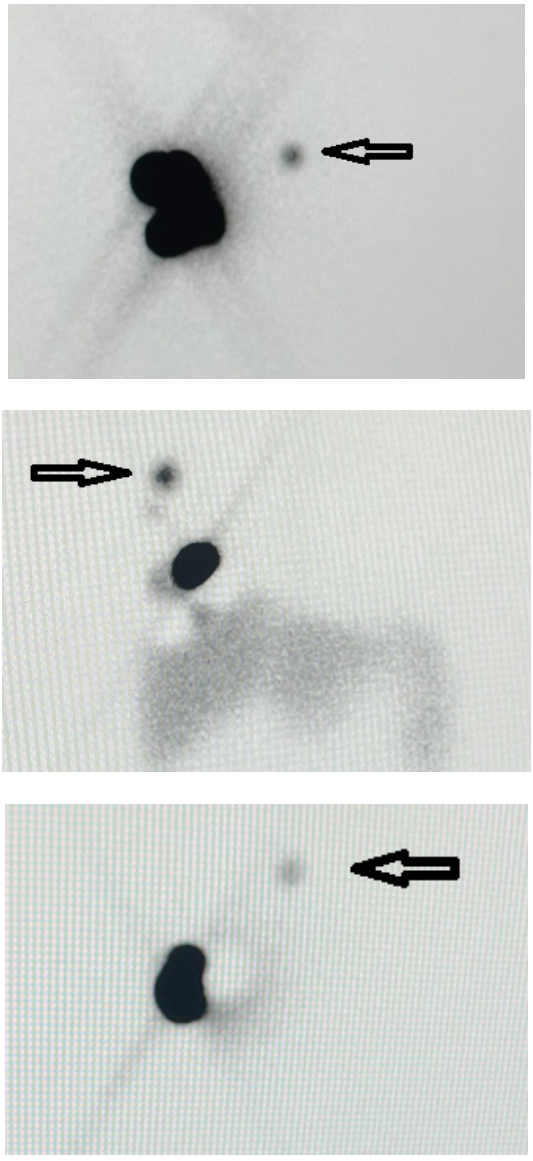

Figure 1: Preoperative Scintigraphic Imaging Before Sentinel Lymphadenectomy With Intraoperative Gamma-Probe In 3 Different patients With A Diagnosis Of Breast Cancer (arrow: radioactive material accumulation in the axillary sentinel lymph node)

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