ADVANCES IN HEALTH SCIENCES

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DUVAR

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Advances in Health Sciences
Editors: Assist Prof. Dilek ATIK Ph.D
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Editor in chief: Berkan Balpetek
Cover and Page Design: Duvar Design
Printing : First Edition-March 2022
Publisher Certificate No: 49837
ISBN: 978-625-8109-18-4

© Duvar Publishing
853 Sokak No:13 P.10 Kemeraltı-Konak/Izmir/ Turkey
Phone: 0 232 484 88 68
www.duvaryayinlari.com
duvarkitabevi@gmail.com

Printing and Binding: REPRO BİR
Repro Bir Mat Kağ. Rek. Tas. Tic. Ltd. Şti.
İvogsan 1518. Sokak 2/30 Mat-Sit iş Merkezi Ostim
Yenimahalle/Ankara
Certificate No: 47381

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Chapter 1

Current approach to diabetes and its diagnosis and treatment

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Diabetes Mellitus (DM)

Diabetes mellitus (DM) is a disease caused by absolute or relative insulin deficiency or insulin resistance, manifested as hyperglycemia, and characterized by impaired carbohydrate, fat, and protein metabolism (1). Rapid population growth in societies, unhealthy and irregular nutrition, increased prevalence of obesity and sedentary lifestyle, aging, and urbanization have been causing a rapid increase in the number of patients with diabetes. Owing to this increase, the International Diabetes Federation estimates that globally, the number of patients with type 2 DM in 2025 will be 334 million (2).

It is the most common endocrine disease worldwide. Chronic hyperglycemia in diabetes is particularly responsible for the damage and dysfunction of the eyes, kidneys, nerves, heart, blood vessels, and other organs. In addition to acute metabolic complications, chronic micro- and macrovascular complications are the most important causes of disease-related morbidity and early mortality associated with this disease (3,4).

History: The earliest information regarding DM dates back to Before the Common Era (BCE). DM was defined as a disease characterized with excessive urination (thamuria) in Egyptian papyrus scrolls in 1500 BCE. In the 5th century BCE, the Indian physician Sushruta described a disease characterized with excessive thirst, severe halitosis, fatigue, and honey-sweet urine; further, he described DM as a disease with increased urinary volume, excessive thirst, and weight loss in his 5-volume work titled "Sushruta Samhita." Diabetic urine has been known to be sweet for centuries; however, in 1674, Willis coined the term "Diabetes mellitus" because of the sweet taste of urine similar to that of honey. The words "diabetes" and "mellitus"—used in current medical literature—are derived from the Greek words "diabetes," which means "to go through," and "mellitus," which means "sweet as honey" (5).

DM Classification: The first comprehensive classification for DM was published by the World Health Organization (WHO) in 1980, which was later revised in 1985 and re-published. In the classification published in 1980, the WHO Diabetes Mellitus Expert Committee classifies DM into two categories:

1) Insulin-dependent DM (IDDM) or type I DM
2) Non-insulin-dependent DM (NIDDM) or type II DM

However, the current expert committee has proposed some changes to this classification, considering the latest researches and developments (3).

The guidelines have recommended the continuation of the use of terms type 1

and type 2 DM and discontinuation of the use of the terms IDDM and NIDDM. Type 1 DM primarily includes patients prone to ketoacidosis associated with pancreatic islet cell damage, whereas type 2 DM is the most common form of diabetes, caused by impaired insulin secretion along with concurrent insulin resistance (6).

Classification: The first widely accepted classification of diabetes was established by the American National Diabetes Data Group in 1979, which laid the foundation for the WHO classification (7). The diagnosis and classification of this disease was revised by the American Diabetes Association (ADA) in 1997. ADA classified it into three main groups based on etiology rather than treatment requirements and pathogenesis; Moreover, the terms Type I and Type II diabetes were proposed instead of insulin-dependent and noninsulin-dependent diabetes.

1. Type 1 diabetes (autoimmune or idiopathic)
2. Type 2 diabetes
3. Other specific types
   - Genetic defects of pancreatic β cells
   - Genetic disorders caused owing to insulin
   - Endocrine pancreatic diseases
   - Endocrinopathies
   - Disorders caused by drugs or chemical agents
   - Rare forms of autoimmune diabetes caused by infections
   - Other genetic syndromes that may occasionally be associated with diabetes

4. Gestational diabetes mellitus (GDM)

Type 1 diabetes mellitus (IDDM): Although this autoimmune-induced disease is common in childhood and adolescence, it can occur at any age. This diabetes type involves progressive β-cell destruction in the pancreas and requires insulin-dependent treatment. Type 1 diabetes is more common in winter; this seasonal relationship is observed in most age groups, and the disease is reported in all seasons in younger age groups (8). The prevalence of type 1 DM in the general population is between 0.5% and 1%. Reportedly, the incidence of Type 1 DM has been increasing in recent years (9).

Antibodies active in type 1 diabetes that results from the autoimmune β-cell destruction are islet cell autoantibodies (ICA), insulin autoantibodies (IAA), glutamic acid decarboxylase autoantibodies (GADA), and tyrosine phosphate IA-2 and IA-2b. The initial symptom in some patients is ketoacidosis, whereas it

is infection and rapidly developing hyperglycemia in others. In the advanced stage of the disease, insulin secretion is extremely low or absent, consequently resulting in low or undetectable C-peptide levels. Moreover, comorbidities with other autoimmune diseases, such as Graves' disease, Hashimato thyroiditis, Addison's disease, and Pernicious anemia, were observed in these patients (1,10).

Latent Autoimmune Diabetes Adult (LADA): LADA is a form of type 1 autoimmune diabetes that demonstrates gradual progression. Most patients are not obese, and they are determined to be GADA- and ICA-positive. Type 2 diabetes is associated with insulin resistance and insulin secretion disorder (11). It typically develops after 30 years of age; however, the number of cases with type 2 diabetes, particularly in childhood or adolescence, has begun increasing owing to the increasing prevalence of obesity in the last 10–15 years (12). It is more common in individuals with advanced age, obesity, sedentary lifestyle, previous gestational diabetes, hypertension, and dyslipidemia. It constitutes 85%–90% of all patients with diabetes. Most patients are obese, which leads to the emergence of diabetes as well as worsens existing diabetes (11,13).

Type 2 DM pathogenesis: With regard to glucose balance, type 2 DM is typically characterized by three pathophysiologic mechanisms in clinical settings (14).

1. Decrease in insulin sensitivity
2. Functional pancreatic β-cell impairment with insulin deficiency
3. Increased glucose production in the liver (which occurs owing to a change in the ratio of glucagon and hepatic insulin, thereby increasing gluconeogenesis in the liver)

When stimulated by glucose, pancreatic β-cells secrete insulin in two phases: a fast phase and a slow phase wherein insulin is steadily secreted. The first symptom in type 2 DM development is β-cell dysfunction, which can typically be detected before the appearance of clinical symptoms (15). Decreased insulin-induced glucose uptake or insulin resistance is the earliest detectable dysfunctions in type 2 DM development.

Several factors, including age, sex, ethnicity, obesity, high blood pressure, familial DM, smoking, and ischemic heart disease, affect insulin sensitivity (17). Decreased insulin effect and insulin secretion disorders may be present even before the diagnosis of type 2 DM. Therefore, in patients with severe insulin resistance, if the levels of insulin secretion can be sufficiently increased, glucose levels can remain normal (18). The insulin-dependent glucose transport is performed by

glucose transport proteins. The glucose transporter family (GLUT) comprises 11 different proteins that facilitate D-glucose transport into the cell regardless of the concentration difference, with GLUT-4 being mainly responsible for this process. GLUT-4 is the glucose transporter in the adipose tissue and skeletal muscle. Several GLUT-4 glucose transporters have been identified within the intracellular stores in unstimulated fat cells (13,16). Low GLUT-4 levels lead to insulin resistance and low GLUT-2 levels are responsible for the absence of an early insulin response to glucose (14).

In type 2 DM, the insulin efficacy decreases due to low insulin receptor kinase and glycogen synthase activity in the muscle tissue. Insulin efficacy is associated with glucose uptake as well as exerts an effect on several processes such as insulin secretion and resistance, energy consumption within the mitochondria, changes in metabolically active adipocytes, changes in gene pathways regulating cell growth (15,18).

Type 2 DM Risk Factors:

1. Age
2. Sex
3. Genetic factors
4. Genetic interference (mixing)
5. Familial clustering
6. Genetic markers
7. Obesity and body fat distribution
8. Physical inactivity
9. Diet
10. Sex hormones
11. Alcohol consumption and smoking

Individuals should undergo screening for diabetes using the FBS test every 3 years after the age of 45 years, particularly those with central obesity, those who are overweight [body mass index (BMI) $\geq$ 25 kg/m^2], and those with a high waist circumference (>88 cm in women, >102 cm in men). In addition, individuals with a BMI of $\geq$25 kg/m^2 should undergo screening from a younger age, with more frequent screening if they belong to one of the following risk groups:

1. Individuals who have first degree relatives with DM
2. Individuals from ethnic groups that have a high prevalence of diabetes
3. Women who gave birth to an overweight baby or who have previously

been diagnosed with gestational DM
4. Individuals with hypertension (blood pressure ≥ 140/90 mmHg)
5. Individuals with dyslipidemia (requiring HDL-cholesterol vaccine)
6. Individuals with IFG or IGF
7. Women with polycystic ovary syndrome (PCOS)
8. Individuals with clinical findings related to insulin resistance (acanthosis nigricans)
9. Individuals with coronary, peripheral, or cerebrovascular disease
10. Individuals born with a low birth weight
11. Individuals with sedentary lifestyle
12. Individuals with dietary habits rich in saturated fats

Gestational diabetes mellitus: GDM is defined as various degrees of glucose tolerance that begin during pregnancy and is initially diagnosed during pregnancy (19). Moreover, GDM is defined as transient hyperglycemia that continues throughout pregnancy or glucose intolerance that develops during pregnancy affecting perinatal outcomes (20). Changes in insulin sensitivity and secretion can be detected before pregnancy in women with GDM, and these disorders typically persist after pregnancy (20-22).

GDM is one of the most common complications that occur during pregnancy. The prevalence of GDM is 1%–14% worldwide and varies according to geographical distribution; it is observed in approximately 4% of all pregnant women in the United States, >200,000 women per year (1). Studies conducted in Turkey have reported the prevalence of GDM between 3% and 8% (42). With an increasing prevalence of obesity in women of childbearing age, there is an increase in the incidence of both GDM and subsequent type 2 diabetes diagnosed after birth (25-27).

The prevalence of GDM may vary according to race and ethnicity, with it being most common in women of Asian origin, followed by Hispanic, African-American, and Caucasian women. GDM is 5–10 times more common in Asian pregnant women compared with Caucasian pregnant women (28).

Etiopathogenesis:

Although the etiology of GDM is not completely elucidated, it is a heterogeneous disorder comprising a combination of factors responsible for decreased insulin sensitivity and inadequate insulin secretion (29). With the progress of pregnancy, a higher amount of insulin is required owing to the increased insulin resistance observed in tissues. In most pregnant women, this requirement is easily met and the balance between insulin resistance and insulin supply is maintained. However,

the deterioration of this balance toward insulin resistance causes hyperglycemia in pregnant women (30). It is considered that β-cell dysfunction underlying GDM development is caused by obesity and chronic insulin resistance, with most cases exhibiting these factors; such cases demonstrate that GDM may be a type of developing type 2 diabetes (31).

Risk Factors for GDM: GDM is a critical public health issue affecting 1%–14% of all pregnant women and is associated with maternal, fetal, and neonatal short- and long-term adverse health outcomes. Environmental or genetic risk factors may be associated with GDM, and these risk factors may exist before pregnancy or develop during pregnancy (32).

Environmental Risk Factors

1. High maternal age
2. High parity
3. Diet rich in saturated fats
4. Vitamin D deficiency
5. Previous pregnancy complications
   a. Congenital malformations
   b. Stillbirth
   c. Macrosomia
   d. Cesarean section
6. Multiple pregnancy
7. Lifestyle habits
8. Short stature
9. Maternal weight
   e. Excess weight before pregnancy
   f. High BMI before pregnancy
   g. Gestational weight gain
   h. Weight gain during adulthood
10. Being the child of a diabetic mother
11. Fetal growth retardation

Genetic Risk Factors:

1. Age
2. Birth order of the mother
3. Ethnicity
4. Genetic history

Screening Tests and Diagnostic Criteria: Since the first diagnostic criterion was established in 1964, the diagnosis of GDM has been updated by various institutions and different diagnostic criteria have been adopted. These criteria facilitate the categorization of individuals with certain characteristics for establishing values that allow treatment, prognosis, or outcome prediction (33,34).

GDM diagnostic criteria were developed to accurately predict perinatal mortality and morbidity and likelihood of developing maternal diabetes in the postnatal period based on standard deviation in mean values after oral glucose tolerance test (OGTT) (35). For GDM diagnosis, two different applications containing different glucose levels are used for performing OGTT (33).

Diagnostic criteria were established based on the risk of developing postpartum diabetes by neglecting the perinatal risk. To this end, O'Sullivan and Mahan (36) evaluated the mean and standard deviation values of blood glucose levels in 752 women who were administered 50 and 100 g OGTT for 3 h (fasting blood glucose and blood glucose at 1, 2, and 3 h). According to this method, if two of the four blood glucose values were higher than the average, the diagnosis of GDM was confirmed.

Further, the diagnosis of GDM was confirmed if value obtained after performing 75 g OGTT for 2 h (fasting blood glucose, blood glucose at 1 and 2 h) is higher than the average (37,38).

Treatment:

Objectives of DM Treatment:

1. Amending symptoms associated with hyperglycemia
2. Reducing long-term micro- and macrovascular complications
3. Ensuring that the patient is, for the most part, able lead a normal lifestyle

The "Global Guidelines for Type 2 Diabetes Treatment," which was created by IDF in 2005 for increasing treatment compliance in patients with type 2 DM and decreasing disease morbidity and mortality by reducing HbA_{1c} levels, was updated in 2012. In this guideline, an earlier and more aggressive treatment of type 2 DM is recommended. According to the guidelines, the targeted HbA_{1c} value is ≤7.0% (≤53 mmol/mol), which should be achieved within the first 6 months of treatment (lower HbA_{1c} targets should be determined in eligible patients who can tolerate these targets).

Healthy lifestyle changes should be applied at all stages of treatment from onset of type 2 DM. Pharmacological treatment can be initiated from the disease onset

in cases where lifestyle changes are considered insufficient. The patient should undergo follow-up visits 3 months after each treatment change. With regard to glycemic control targets, in patients with type 2 DM, these targets should be determined on an individual basis considering the patient's characteristics and clinical status.

In general, in the absence of a specific condition that increases the risk of hypoglycemia in patients with type 2 DM, if the patient has a sufficiently long life expectancy, the HbA_{1c} target should be set to ≤6.5% (48 mmol/mol) for reducing microvascular complications. However, HbA_{1c} target can be determined as 6% (42 mmol/mol) in conscious patients and in some special cases (e.g., risky pregnancy), provided that no hypoglycemic attacks occur. The benefit of maintaining HbA_{1c} low should not increase the risk of hypoglycemia and mortality, particularly in patients with a high risk of cardiovascular disease (CVD). If the patient has a low life expectancy and a high risk of hypoglycemia, glycemic control targets should be maintained higher. Patient preferences should be considered with regard to the treatment choice, provided that they are adequately informed about the subject. Typically, if HbA_{1c} was 6.5%–7% (48–53 mmol/mol) or if the individual's glycemic targets could not be achieved, lifestyle should be primarily examined. If HbA_{1c} is >7% (> 53 mmol/mol) despite lifestyle changes, treatment should be modified. Patients should undergo follow-up visits 3 months until the HbA_{1c} target value is reached.

1) Lifestyle Change Training: After providing the patient general information regarding DM, treatment steps, such as exercise and oral antidiabetic and insulin use, should be introduced. Incidents that can be encountered during the general course of diabetes and its treatment (hypoglycemia and hyperglycemia), their causes, consequences, and strategies to manage these incidents should be explained. Patients should be instructed regarding the prevention of acute and chronic complications as well as the follow-up required in case the complications are already present. In addition, personal care training (skin, teeth, and foot care) should be provided (39).

2) Diet: Because most patients with type 2 DM are overweight, the primary goal of dietary therapy should be weight loss. Calorie restriction helps to significantly improve hyperglycemia (40). BMI—calculated as weight / height (m^2)—is used for determining the ideal weight of patients. This value should be between 20–25 kg/m^2. Calorie intake should be adjusted with an aim to reach the patient's ideal weight. Carbohydrates with low glycemic index should be preferred (41).

3) Exercise: For patients with DM, achieving the expected benefit from exercise depends on normal metabolic balance; if not, the metabolic balance may be completely impaired. Regular exercise reduces hyperglycemia because it reduces liver glucose secretion and increases glucose utilization. Besides glycemic regulation, the benefits of exercise include increased fibrinolytic activity, decreased triglyceride levels, increased HDL, decreased blood pressure, and weight loss. The patient should be cautioned with regard to hypoglycemia that may occur hours after exercise (41, 42, 43).

Oral Antidiabetics

1. Biguanides: Metformin is the major member of this group. It reduces blood glucose levels by reducing glucose production and insulin resistance in the liver. It can be used as monotherapy or in combination with other drugs in patients with type 2 DM. Because metformin is not an insulin-releasing agent, it does not cause hypoglycemia (44). An important difference of metformin from other oral antidiabetics is the preventive effect exerted by this agent on weight gain. Metformin alone provides a reduction of 60–70 mg/dL in blood sugar and 1.5%–2% reduction in HbA_{1c} level.

Side effects: Gastrointestinal irritation (including flatulence and bloating, which are typically temporary), cramps, diarrhea, metallic taste in the mouth, vitamin B1 deficiency, and lactic acidosis, which rarely occurs, particularly in cases with impaired tissue perfusion. The risk of lactic acidosis is 0.03/1000. The drug is ingested on a full stomach, and the maximum daily dose is 2 g (45).

Contraindications: Renal dysfunction is contraindicated in cases with an eGFR of <30 mL/min, half dose of 30–45 mL/min as well as in cases of chronic alcoholism, liver disease, congestive heart failure, acute myocardial infarction (can be administered after the acute phase), chronic lung disease, major surgical intervention, sepsis or decreased tissue perfusion, and intravenous radiographic contrast examination (should be discontinued at least 2 days prior, can be administered 1 day after the procedure).

2. Glitazones: Rosiglitazone and pioglitazone are members of this group of drugs. They primarily exert their effect by acting on the peroxisome proliferator-activated receptor gamma (PPAR-γ) in the adipose tissue and affect the fat metabolism and distribution in the tissue (46). They provide 30–60 mg/dL decrease in FBS, 1%–1.5% decrease in HbA_{1c}, and do not cause hypoglycemia. The most important side effects are weight gain and peripheral edema (45).

Clinical benefits: Its benefits include reduction in cardiovascular events

(secondary prevention) and stroke risk (primary prevention), regression in NASH progression, low frequency of hypoglycemia, and sustainable efficacy. It reduces insulin resistance by exerting an effect on enzyme and carrier proteins in insulin and lipid metabolism via PPAR-γ stimulation.

Side Effects: Weight gain, fluid retention, increased osteoporotic fracture risk, and macular edema progression (requires close monitoring).

Contraindications: Heart failure, bladder cancer, pregnancy

3. Alpha Glucosidase Inhibitors: With a competitive inhibitory effect for alpha-glucosidases on the intestinal brush border membrane, these drugs lead to a delay in the absorption of complex carbohydrates. Moreover, they do not result in weight gain.

Side Effects: Gastrointestinal symptoms, excessive flatulence

Contraindications: Liver and kidney failure, pregnancy, anemia, inflammatory bowel diseases

The chewable drug is ingested along with first bite at all three mealtimes: (1) 25 mg during dinner on the first day, followed by 50 mg during all three meals for 2–3 days and continued as 3 × 1 100 mg thereafter.

4. Insulin Secretagogues

Glinides (Short-acting insulin secretagogues): This group of drugs includes repaglinide and nateglinide. Their mechanisms of action and side effects are similar to sulfonylureas. However, unlike sulfonylureas, the effect begins and ceases faster. Therefore, they are ingested with meals (47). They provide 60 mg/dL reduction in postprandial glucose (PPG) and 1.7%–1.9% decrease in HbA_{1c} levels (48). Nateglinide, which is not a meglitinide derivative but an insulin secretagogue, has a rapid activity, and its effect disappears faster than repaglinide (49). They provide increased insulin secretion from β-cells via postprandial hyperglycemic effect associated with type 2 DM.

Side Effects: Hypoglycemia, weight gain Clinical Benefits: Suitable for flexible meal timing

Contraindications: T1DM, liver failure,

Sulfonylureas (medium and long-acting insulin secretagogues): They are essential drugs for the treatment of type 2 DM. These drugs bind to their receptors in the pancreatic islet cell and increase insulin secretion. They provide 60–70 mg/dL reduction in FBS and 1.5%–2% decrease in HbA_{1c} levels. The most important side effects are hypoglycemia and weight gain (7, 50, 51). Clinical benefits include strong and rapid efficacy and reduction in microvascular risk (UKPDS

and ADVANCE trials).

Side effects include hypoglycemia, weight gain, allergic reactions (which rarely occur), and hepatotoxicity

Contraindications: Type 1 DM, liver and kidney failure, pregnancy, stress, severe infection, trauma, surgical interventions, ketoacidosis, and hyperglycemic hyperosmolar syndrome

GLP-1 Analogs (Incretin mimetics)

The most important incretin secreted from the gastrointestinal tract is glucagon-like peptide-1 (GLP-1). This peptide is synthesized by L-cells located in the jejunum and distal ileum of the gastrointestinal tract. GLP-1, GLP-2, and glycentin are synthesized from L-cells. GLP-1, the most potent incretin, is similar to glucagon with 50% homology. It slows gastric motility and prevents sudden glucose peaks after meals. It regulates insulin secretion via a protective effect on β-cells and reduces glucagon levels. The drug decreases body weight (mean, 2–5 kg). GLP analogs include exenatide (Byetta, consumed twice daily) and liraglutide (Victoza, consumed once daily).

DPP4 Inhibitors

DPP4 inhibitors constitute another novel group of antidiabetic drugs; these drugs inhibit the enzyme DPP-4, which provides rapid degradation of the GLP-1 molecule during the postprandial period, and increase GLP-1 and GIP levels, which are endogenous, natural incretins. The main drugs of this group include sitagliptin (Januvia), vildagliptin (Galvus), saxagliptin (Onglyza), and linagliptin (Tradjenta).

After DPP4-inhibition, antidiabetic effects are observed via the increase in GLP-1 levels. The increase in GLP-1 levels is postprandial and it remains continuous throughout the day; further, it improves fasting glycemia. Dose should be reduced if renal function is impaired (creatinine clearance <50 mL/min) and dose reduction should be continued as clearance decreases. A three-fold increase in GLP-1 levels and marked suppression of glucagon levels were demonstrated following DPP-4 inhibitor treatment. It decreases HbA_{1c} level by 0.5%–1% and can be used with other oral diabetes medications and insulin. It has no known effects on blood pressure and ECG and may cause slight increases in insulin secretion. The drug has a neutral effect on weight loss and no effect on appetite. like symptoms and rarely the development of pancreatitis. The hypoglycemic side effects are relatively uncommon. It contraindicated in pregnant and lactating women.

5. SGLT-2 Inhibitors

Indications for Use: The drugs reduce glucose reabsorption from the kidney by inhibiting SGLT2, which is the carrier responsible for glucose reabsorption, in the proximal nephron. Further, they result in an increase in glycosuria.

Clinical Benefits: Low risk of hypoglycemia, mild weight loss, decreased blood pressure, reduced likelihood of a new attack, and reduced mortality risk in patients with cardiovascular disease (particularly empagliflozin, as reported in the EMPA REG study)

Side Effects: Genitourinary infections, polyuria, volume depletion, and increased risk of diabetic ketoacidosis (normoglycemic ketoacidosis)

Type 1 Diabetes Treatment

Principles of Insulin Treatment in Type 1 Diabetes

Basal insulin doses control fasting blood glucose, and bolus insulin doses control postprandial blood glucose. Therefore, basal insulin dose should be adjusted if fasting blood glucose is high, and bolus insulin dose should be adjusted if postprandial blood glucose is high. Bolus insulin change in a meal should typically not exceed 2–4 U. In special cases, such as acute hyperglycemia, necessary modifications can be made. According to blood glucose values, insulin titration should be performed after at least 3 days of follow-up. Before each hyperglycemic measurement, a hypoglycemic attack should be investigated. The bolus insulin dose should be reduced in patients with a fasting blood glucose level of <80 mg/dL.

In new-onset type 1 DM, dynamic monitoring should not be discontinued after achieving glycemic regulation. Insulin doses may decrease due to an increase in insulin receptor sensitivity within 2–4 weeks after achieving glucose regulation. It should be considered that insulin needs may decrease in patients with newly diagnosed type 1 DM during the remission period. However, regardless of this decreased need, insulin therapy should not be discontinued.

Glucagon should be prescribed to every patient undergoing intense insulin therapy, and glucagon training should be provided to individuals who reside with the patient.

Insulin Types

Basal Insulins

Basal insulins control fasting plasma glucose and account for approximately 50% of the daily requirement. The following basal insulins are available in Turkey.

- ✓ **Moderate-acting (NPH) insulin:** NPH Insulin
- ✓ **Long-acting (analog) insulins:**

I. Insulin Glargine U-100
II. Insulin Glargine U-300
III. Insulin Detemir
IV. Insulin Degludec

Bolus Insulins

Bolus insulins control postprandial glycemia and constitute 10%–20% of daily insulin requirement at each meal. The following bolus insulins are available in Turkey.

1. Short-acting insulins,

I. Regular insulin,

II. Fast-acting (analog) insulins: Insulin Aspart, Insulin Lispro, Insulin Glulisine

2. Biphasic insulins

- ✓ Regular + mixture insulin; Regular insulin 30%, NPH insulin 70%
- ✓ Analog + Analog protamine insulin; Insulin Aspart 30%, Protamine Insulin Aspart 70% Insulin Aspart 50%, Protamine Insulin Aspart 50% Insulin Lispro 25%, Protamine Insulin Lispro 75% Insulin Lispro 50%, Protamine Insulin Lispro 50%
- ✓ Analog + Degludec mixed insulin: Insulin Aspart 30%, Insulin Degludec 70%

Measures taken during the early period of DM exert a positive effect on the quality of life. Studies have shown that the risk of type 2 DM can be prevented or delayed by up to 58% with healthy lifestyle changes. TURDEP study has shown that the prevalence of diabetes in Turkey is 2-fold higher than the prevalence worldwide and 3-fold higher the European prevalence. There are 11 million patients with DM in Turkey; moreover, the incidence of diabetes in Turkey has increased by 100 percent every decade. Most costs associated with diabetes-related healthcare are owing to the treatment of life-long complications caused by inadequate blood glucose control. Maintaining the blood glucose levels, blood pressure, and cholesterol levels close to normal can help prevent or delay DM-related complications. To avoid DM, a balanced and adequate diet should be followed. Further, 6 meals should be consumed as main and intermediate meals at 2-3-h intervals during the day. Healthy foods should be included in appropriate proportions. The Diabetes Food Pyramid can be used for this purpose. Pulp that

is contained in dried legumes, vegetables, fruits, and grains should be consumed as required. To prevent blood sugar from becoming uncontrollable, carbohydrate consumption should be limited. Excessive carbohydrate consumption is considered a factor that disrupts blood sugar balance. Red meat consumption should be restricted, and fish and white meat consumption should be preferred; these should be grilled and steamed instead of fried. Solid fat consumption should be avoided. In particular, patients who are overweight should consume less fat. Breastfeeding of babies for the first 6 months reduces the risk of diabetes. Weight should be maintained at an ideal range. Patients with DM are typically overweight. Therefore, excess weight should be reduced with a healthy diet. A regular exercise plan should be followed. Sports activities should be evaluated according to personal characteristics.

Regular exercise, which is as important as nutrition in preventing DM, is effective in maintaining health. Unhealthy habits, such as smoking and alcohol consumption, should be avoided. Stress should be avoided because it is one of the factors that adversely affect general well-being of individual. The balance of the body should not be disturbed and a regular sleep pattern should be maintained. If patients care about their health, they can successfully follow a healthy lifestyle. Individuals should attempt to devote time to himself/herself, acquire various hobbies, and maintain his/her mental health at a high level for a healthy life. Following these measures will protect the individual against diabetes as well as other diseases.

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Chapter 2

A Pediatric Analysis of Carol Gilligan's Theory of Moral Development

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A Pediatric Analysis of Carol Gilligan's Theory of Moral Development İntroduction

Definition of Morality

Morality; They are the forms of behavior and rules that people adopt and have to comply with in the society. *Morality* is about the distinction between "good" and "bad". Morality generally refers to rules about what individuals "do" and what "not to do" (Güngör, 2003).

The subject of morality and values, which is expressed as a set of principles or rules that people live and guide according to themselves, is a phenomenon that accompanies people's existence on earth. Ethics and values play an important role in a person's individual and social life, whether they manifest as certain religious beliefs, or the tradition or understanding of life is seen as a necessary result of collective living. For this reason, the subject of morality has been a subject of interest and research since the first periods of the history of human thought, many ideas have been expressed and works have been given in this field. In addition to the religious and philosophical explanations regarding the origin of morality, the subject of moral development has also been a separate area of interest and study with the development of human sciences and especially social sciences. The 20th century is a period in which important developments were experienced in this field (Cevizci, 1999).

Subject of Morality

The subject of morality is human behavior. Willful, conscious, good or bad which can be described as behavior is the subject of morality (Aydın, 2003). Man is under the influence of the world he feels in. This feeling of the world affects their mental state. Human behaviors shaped in this way Since it is directed towards the members of the society, it also affects their relations with them. Thus, in response to his moral behavior in the society, a person is good or bad from the society. gets reactions. These existing emotions in man, the forces that belong to man himself and their the behaviors that he/she performs and the behaviors that these behaviors shape with other people. Relationships form the subject of moral science (Can and Gürhan, 2003).

On the other hand, the science of ethics is a science of rules; religious, personal, familial and determines the rules and laws that they must abide by in their social lives. It is a science and therefore the nature of the values ascribed to the behavior of the moral agent, searches for its size and source; commands to do good deeds and abandon bad deeds (Çağdaş et.al., 2002).

The Purpose and Necessity of Morality

The aim of moral science is to prevent human beings from acts contrary to their creation, ıt is to awaken the beauties that exist in your nature. For this, virtue and he will get himself into trouble in life, as he informs and advises what the values are explains all kinds of disgrace and makes suggestions to stay away. The purpose of moral science is to enable them to act in accordance with their creation. For this reason, moral science is what is right for people. It informs and recommends what is considered well, that is, what is considered to be a virtue, that is, the beautiful (Çağlayan, 2005).

It shows the ways to ensure that individuals are happy one by one, spiritually and physically. Balanced by showing the excesses of feelings and thoughts, behavior and inclinations tries to help gain life (Durmuş, 2005).

Moral Development Principles

Principle of Love: The most important factor that keeps people together is mutual benefit and solidarity. However, human relations in which love does not interfere are more than interests relations. Love is the unifying power of social life as well as family. Therefore, we define love in the broadest sense as

> "positive things that bring people closer together.
> and all of the beautiful feelings" (Ergün, 2006).

Authority Principle: A perfect balance is required in providing love and discipline in education. What one must not give up love in order to establish discipline, nor should love break it. Mom a harmonious, understanding, and loving nature that stems from the personality of the father and the teacher authority is necessary for education and training (Erden ve Akman, 2006).

Freedom Principle: Childhood years have an important place in the development of personality. "When you were born, the child, who is completely dependent on the adult, naturally moves away from this situation as he grows up and he wants to be more self-sufficient. Freedom, the ability to dominate one's own development, in a sense. Some have defined it as shaping capacity. Freedom is the self in this sense. It is the other side of consciousness. As the individual moves away from self-awareness, considered to be far from its independence. Therefore, a free self-structure. It can reach a complete and consistent wholeness with the choices that will be made freely belonging to its essence (Fidan ve Erden, 2006).

Sociality Principle: Getting the cultural elements of the society in which people live through education, adopting it, making it his own personality and in this way a member who participates in that society. It is called socialization. The basis that

distinguishes it from other assets. One of the features is social life. The social life of the human mind and thinking is the product of his talent and is in a state of constant change. This is the personality of the person. It means that it is formed in the process of socialization (Kızıltepe, 2004).

A Pediatric Analysis of Carol Gilligan's Theory of Moral Development İntroduction

Moral Development Theorist

Carol Gilligan, She received her clinical psychology specialization from Radcliffe College. She completed her doctorate in social psychology from Harvard University. Carol Gilligan is a psychologist working on the development of morality. She argued that Kohlberg's studies of moral development are not universally valid. She is also Kohlberg's graduate student. Taking into account the criticisms of feminist philosophers to Kohlberg's theory, Carol Gilligan proposed an alternative theory of moral development to Kohlberg's theory in 1982. Gilligan takes a new approach to moral development in response to Kohlberg's work especially with men. Gilligan found in her studies that men and women differ significantly from each other regarding moral development (Yeşilkayalı, 2014).

He defined the focus of developmental theories on men as a problem, starting with Freud, and showed that men and women differ significantly in moral development through his studies (Austrian, 2008).

Carol Gilligan's Moral Development

According to Gilligan's theory, the important thing in moral development is not to reach the next level of development, as Kohlberg states, or to instill certain virtues in children, as character educators put it, but to establish a "caring"-centered moral education in the school and classroom environment. is to be done (Noddings and Slote, 2003).

According to Gilligan's definition, the ethics of care is to respond to the needs of the other, to care for the other, to take care of him, to prevent him from being harmed. In terms of ethics of care, moral education focuses on the care-centered construction of the moral atmosphere, which is related to the implicit part of moral education, rather than the direct teaching of certain virtues in education (Noddings and Slote, 2003).

Moral Development in the Information

Gilligan, the origins of the ethics of care and justice; She states that it lies in the experiences related to the attachment figure and relationships in childhood,

and in different education and practices for girls and boys in societies. According to her, in many societies, when girls are raised, their closeness and commitment to their caregivers, that is, to their attachment figures, usually their mothers, is supported. (Gillian, 2003).

Unlike girls, boys are allowed to grow up separately and independently from their mothers. In other words, while attachment experience and commitment are more important in the education of girls in many societies; In the education of men, separation, that is, independence, is in a more prominent position. Based on this, it correctly establishes the link between gender and moral orientation in childhood and adolescence (Autrian, 2008; Ekşi, 2006).

Gilligan Moral Development: The Three Stages

- Selfishness (Pre-conventional Stage): The individual is selfish. He rejects those who do not meet his own interests.
- It carries the Social Formation in an unexpected way.
- Principled Morality (Post-Traditional Phase): Individually received and assumed. He speaks as an individual and will live (Berzonsky, 2008).

Moral Development of In the Acquisition

According to Carol Gilligan, the important thing in moral development is not reaching the next level of development, as Kohlberg states (Adolescent, when young adult reaches maturity; both girls and boys fulfill their duties as mature beyond the post-conventional level). "Morality is to win love (Autrian, 2008).

It states that everyone will behave fairly and honestly towards others, according to their own inner stimuli. Therefore, he argued that Kohlberg's stages of moral development are not definitive and universal. Gilligan argues that there is a much more mature operating phase "above morality"; it is "morality love" that enables them to understand their mutual needs from a psychological point of view (Smetana and Turiel, 2003). According to Gilligan, there are gender differences in moral development and people need to be recognized in order to be understood adequately. Ideally, both responsibility and fairness should be combined in moral decision making (Özen, 2011).

Conclusion and Recommendations

Moral development is very important at every stage of childhood. In the literature on moral development, it is claimed that there are some important variables that can affect moral development steps. Age, gender and educational status and economic status are the leading variables among these variables. These variables are according to Gilligan moral development theory. Affect the moral development of children. In the field of moral development, theoretical and applied comparative research examining socially different variables should be encouraged. There is a need to increase and develop the lessons that can contribute to the moral development of children.

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Chapter 3

Where we are and what should we do about Glaucoma?

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Glaucoma (high eye pressure)

Glaucoma causes loss of vision by damaging the optic nerve due to degeneration of axons in macula and optic nerve and high intraocular pressure. Worldwide, the prevalence of glaucoma is reported to be over 65%. Its prevalence is estimated to increase by 20-30% in the next year. It has been suggested that glaucoma may be responsible for mild to moderate vision loss in populations. There is also a relationship between the prevalence of glaucoma and ethnicity. It is useful to reveal the role of aqueous humor in order to understand the etiopathology of glaucoma. Therefore, in this part, the production of aqueous humor will be mentioned.

Production of aqueous humor

The aqueous humor (AH) fluid is synthesized from a triangular-shaped structure called ciliary body. The main components of this liquid are ions are amino acid, oxygen, carbon dioxide and water. It plays an important role in the hemostasis of ocular tissues such as nutrition of avascular ocular structures (lens, cornea etc.) and removal of toxic substances.AH fluid is produced from ciliary epithelial cells by diffusion, active secretion and ultrafiltration. Active secretion plays an important role in hemodynamics by forming the main part of aqueous production. The trabecular and uveascleral pathway passes through the pupillary area into the anterior chamber and participates in the systemic circulation (figure1). An increase in intraocular pressure (IOP) occurs due to increased production or resistance to drainage of this fluid. The clinical condition associated with this increased IOP is known as glaucoma (1).

Overview of anatomical parts responsible for aqueous humor production(2,3).

Corpus Ciliare: Responsible for the production of AH. It consists of epithelium, stroma, ciliary muscle and vascular structure located in the middle of choroidal structure and iris.

Ciliary Muscle: Anatomically it consists of three different layers. The outermost longitudinal fibers are the only parts with a scleral connection that contract and expand the trabecular and schlemm's canal to help the drainage of AH. Radial fibersare in the middle and circular fibers are at the bottom.

Ciliary Epithelium: It consists of two pigmented and non-pigmented (pigment-free) epithelial layers located on the posterior chamber side of the corpus ciliare.

Pigmented Epithelium: It consists of cuboidal cells. There are many melanin cells in the cytoplasm that provide pigment production.

Pigment-free Epithelium: As it is responsible for the majority of aqueous humor production and metabolite events; more endoplasmic reticuloma, mitochondria

and golgi apparatus are present. There are important connections between the pigmented and pigment-free epithelium that control the passage of ion, water and certain molecules. These connections, called Tight Junction (zonula occludens), form the aqueous – blood barrier.

Stroma: Stroma, consisting of vascular structures and collagen fibrils, is fed from the anterior and posterior ciliary artery which have anastamoses at the root of the iris. The anterior ciliary artery passes through the sclera near the limbus and contributes to the nutrition of the ciliary muscle. Since the permeability of the arterioles from the anastamosis is high, their contribution to the aqueous humor is quite high.

Anatomical Structures that have a role in the drainage of aqueous humor

Schwalbe's Line: It is a gray and fluffy transition zone at the line where the apical anterior part of the trabecular meshwork and the corneal descement membrane terminate.

Trabecular meshwork: It consists of endothelial cells that cover the anterior part of the Schlemm's canal and are located in the lower part of the schwalbe's line. These endothelial cells are surrounded by collagen fibers and have phagocytosis property. It is divided into three main subgroups.

Uveal Network: It is the closest part of the anterior chamber to the aqueous and consists of duct-shaped bands extending from the iris to the schwalbe's line.

Corneascleral Network: It is the middle part of the trabecular meshwork that extends from the scleral spur to the anterior part of the scleral sulcus.

Juxtacanalicular Network: It forms the inner wall of the Schlemm's canal and is the part with the greatest resistance to drainage of the aqueous humor.

Schlemm's canal: It is located behind the trabecular meshwork and its inner wall is formed by endothelial cells. Unlike the blood-aqueous barrier, there are weak-pass connections between these endothelial cells. There are pores which provide aqueous output towards the channel lumen. They do not have these pores on the posterior wall and they are connected directly to the collector channel openings. From here, they are connected to the episcleral and conjunctival vessels through the intrascleral aqueous vessels, which allow them to enter the systemic circulation.

Outflow pathogenesis of aqueous humor (2,4).

Trabecular meshwork: The aqueous humor is synthesized from the corpus ciliare and then drains through the pupillary space into the anterior chamber. From here it passes through the trabecular meshwork to the schlemm canal. From here it drains into the cavernous sinus through the episcleral veins and

ophthalmic vein. The evaluation of the outflow of aqueous is done by tonography. The measured value is in mmHg.

Uveoscleral Pathway: In this way, 20% of the aqueous contributes to the systemic circulation. The aqueous humor fluid passes through the iris and ciliary muscle to the sprachoroidal area and then into the systemic circulation. Drugs that increase the drainage of this pathway are the prostoglandin group. While mydriasis creates a positive contribution to flow, myosis causes resistance and reduces drainage.

Episcleral Vein: The increase in pressure in the episcleral vein, which plays a major role in the transition of aqueous to systemic circulation, also results in a significant increase in IOP. Conditions such as cavernous sinus thrombosis, orbital pathologies etc. increase pressure.

Evaluation of Optic Nerve Head (5).

Anatomically, the optic nerve head is evaluated as neuroretinal rim, optic disc diameter and cup-to-disc ratio.

Neuroretinal rim (NR): This area, which is the retinal nerve fiber extension, is the part between the optical cup and the outer edge of the optic disc. In healthy people, this area is seen as a pinkish color. This area is evaluated as 360 degrees horizontal diameter; ISNT (Inferior-Superior-Nasal-Temporal) rule applies. That is, the widest part of NR is in inferior area and the narrowest part is in the temporal area.

Optic disc diameter (ODD): In determining the ratio of cup-disk (C / D) which is guiding in fundus examination in glaucoma; ODD is also important. Correction factors are important here depending on the type of lens used during the examination.

Cup-to-disc ratio (CDR):Since the optical disc is vertical and the cup is horizontal oval, the optical disc is horizontally wide. Physiologically, the cup / disc ratio (C / D) is 0.3 and a value of 0.2 for this ratio between two eyes should not be ignored in terms of glaucoma. Additionally, neurological and systemic diseases that may cause optic neuropathy should be ruled out instead of only glaucoma. (Figure 2)

Optic Nerve Changes in Glaucoma (6).

Changes in optic nerve head: Pathologically, localized tissue loss in the optic disc, reduction in NR thickness (ISNT rule), color change from pink to gray-white, and asymmetry between the two eyes are important.

Peripapillary Changes: The perimeter of the optic disc is surrounded by a

scleral ring and the peripapillary area is formed around it(alpha and beta zone). This peripapillary atrophy in glaucoma is significant. The internal beta zone is characterized by chorioretinal atrophy and is more common in eyes with glaucoma. The alpha zone is characterized by thinning in the retinal pigment epithelium. Beta zone is more important in determining the severity of glaucomatous damage.

Retinal Nerve Fiber Layer Thickness (RNFL): They are caused by the axons of the ganglion cells in the retina. Their thickness increases as they approach the optical disc. The close ones of these fibers that form the optical disc are on the surface and in the center, while the distal fibers are located in the periphery and deep. The importance of this is important in determining the origin of the anatomical defect when evaluating the visual field in glaucomatous injury. Losses in RNFL can be localized or diffuse. OCT is used for early detection and follow-up of these pathological changes.

Pathogenesis of OD Damage in Glaucoma (7,8).

Although many factors are effective in the formation of optic neuropathy in glaucoma, the most important of these is high IOP values. In this way, two theories on optic nerve injury are emphasized.

Ischemic Theory: The persistence of glaucomatous optic neuropathy, even though IOP is not high or decreased, indicates that different factors are effective.

a. Low Blood Pressure: It is seen as a result of hypoperfusion of optic nerve as a result of systemic hypotension. This situation is more important especially in elderly patients.

b. Hypoperfusion of the Optic Nerve Head: Perfusion in the retina is controlled and regulated by locally acting mediators such as Endothelin, Nitric Oxide, Prostoglandin, etc. in vascular structures. Ocular blood flow increases when perfusion pressure is high and vascular resistance is low. Conversely, glaucomatous optic neuropathy may develop secondary to insufficient feeding of the optic nerve.

c. Apoptosis: Glutamate binds to its receptors in the cell wall and provides a lot of Ca ++ input into the cell which is resulted in programmed cell death (apoptosis) because of increased free radicals. Apoptosis-like retinal ganglion damage was detected in glaucomatous optic neuropathy.

d. Increased Vascular Resistance: Endothelin-1, an important vasoconstrictor mediator, can increase systemic vascular resistance and reduce ocular blood flow and cause optic nerve damage.

Mechanical Theory: In this theory defined by Müller, the increased IOP pits the lamina cribrosa backward and the perfusion of the nuclei in the lateral geniculate

is prevented axoplasmically. As a result, the release of neurotrophins is prevented and glaucomatous atrophy occurs in the optic disc due to excitoxic effect.

Diagnosis in Glaucoma

In glaucoma, the pathological appearance of the optic disc distinguishes it from other optic neuropathies. The vertical cup disc ratio (C / D) in OD forms an important clinical image for glaucoma. This ratio in OD should be evaluated comparatively in both eyes of the patient. Because the physiological or familial C / D ratio can be detected even higher than the normal limit. This can be accidentally diagnosed as glaucoma. However, you should keep in mind that there is not always the right ratio between the C / D ratio and the visual field. In other words, although this ratio is normal, visual field defects may be seen. OD, IOP and visual levels are evaluated together to confirm the diagnosis of glaucoma (9).

Diagnostic methods in glaucoma

1. Ophthalmoscopic Examination

2. Angle Inspection: In glaucoma, iridocorneal angle, which is one of the first step examination methods, is evaluated. Evaluation using gonioscopic lenses is important in the classification, treatment and follow-up of glaucoma. Gonioscopically, the ciliary body, scleral spur, schwalbe's line and vascular structures are seen at the angle. The most commonly used and still valid schaffer classification is used for angle evaluation.

Schaffer Classification and Scheie (10).

Grade 4 (35-45): This is the angle at which the ciliary body at the bottom can be easily seen. Since it is the widest angle, there is no possibility of closing. It is characteristically seen in myopia and aphakia.

Grade 3 (20-35): It is an angle in which scleral spur can easily be seen and there is no possibility for closing of this angle.

Grade 2 (20): It is a very narrow angle where only the trabecular mesh is seen and tends to close.

Grade 1 (10): Although only the Schwalbe line is seen, only the upper part of the trabeculum can be seen. It is a very narrow angle and the risk of closing is very high.

Narrow angle: No irregular element is seen even though iridocorneal contact

is not apparent.

Grade 0 (0): It is a closed angle type with iridocorneal contact. Diagnosis is made when the top of the corneal wedge cannot be selected. No angle elements can be seen here.

Lenses Used in Gonioscopy (11).

a. Direct gonioscopic lenses

Koeppe Lens: It is often applied to infantile patients with congenital glaucoma under general anesthesia. It is dome-shaped and forms the diagnostic lens group. The patient lies on his back, after the methylcellulose viscoelastic liquid applied to the eye, the lens is attached to the eye and the angle is evaluated with a certain light source. Biomicroscope is not used in this method.

Barkan, swan and Workshop Lens: Unlike Koeppe lens, they are used in angle surgery in glaucoma.

b. Indirect Gonioscopy lens

Goldman Lens: Since the faces of these lenses, which may be one or three mirrors, are more perpendicular to the cornea, the gap between them must be filled with a viscoelastic liquid such as methylcellulose. Thus, corneal epithelial damage is minimized by obtaining a clearer image.

Zeiss 4 Mirror Lens: The advantage of this is that an optimal inspection is achieved without the need to rotate the lens. Trabecular lens: It has four mirrors and is used for diagnostic purposes. However, it can also be used during the procedure in laser trabeculoplasty (figure 3).

3.Visual Field (VF)

The visual field refers to the total area in which objects can be seen in the side (peripheral) vision as you focus your eyes on a central point. GA has an important place in glaucoma. Although many methods have been used historically for evaluating visual field, Humphrey perimetry is the most important and one of the most valid tests. Perimetry classically refers to peripheral vision. Perimetry can be kinetic and static. In kinetics, a moving target is detected, and in static, the eye focuses on a certain point and is a test of detection of light or object-like stimuli from the periphery. The width of the VF obtained in this test is expressed in degrees and the depth in sensitivity. The results should be consistent with C / D. It should be evaluated in terms of false positivity or negativity. Otherwise you need to repeat the test (12). The easiest and most practical way to test VF is by using the method of confrontation. In this test, the patient and the physician close their opposing eyes with their palms, 1 meter apart. The physician advances an

object in his hand until the patient sees this object through the periphery. Here, the physician has an idea about the visual field.

Another test for VF is done with the Amsler grid card. Here, the 10-degree field of central vision is evaluated qualitatively. The patient holds the Amsler card at a distance and looks at it with one eye. If blurred vision and shifts in lines are seen, it should be evaluated for macular pathology and modified shapes improved (13).

Optical Coherence Tomography (OCT) has been used in the last two decades due to patient discordance, non-coperation and false positivity during visual field evaluation. OCT is a method used in early detection and follow-up of glaucoma and has been accepted for analysis of RNFL, ganglion nerve layer and optic nerve head. The axons of the ganglion cells form RNFL bundles in the peripapilar area to form the optic nerve. OCT plays an important role in the early detection of structural changes in RNFL. This shows the importance of OCT for early detection of glaucoma. Because despite the pathological changes in RNFL, the optical disc may have a normal appearance (14).

OCT is more sensitive than perimetric methods in detecting physiopathological changes in ganglion cells in glaucoma and detect the changes in these cells earlier in the pre-glaucoma stage. Based on this result, structural progression in glaucoma occurs before functional progression (15).

4. Optical Coherence Tomography (OCT)

OCT has an important role in the evaluation of retinal pathologies because of its advantages such as being invasive without contacting the eye and recording of the shots used in the follow-up of the disease. It is similar to Ultrasonography B mode. However, faster waves of light are used instead of sound waves. In OCT, images are obtained from partial coherent light. Partial coherent light is infrared laser light with a wavelength of ~ 800 nm. Depending on the characteristics of the device, the wavelengths can vary is around 900 nm imaging the retina(16). The images obtained here are projected onto the RNFL yellow-red screen which is seen as white thanks to the software used. The rapid RNFL protocol can be used for analysis relative to the obtained RNFL dials.

In this protocol, starting from the optic nerve center, 256 A scans are obtained, covering four 3.4-mm quadrants (superior-inferior, nasal-temporal) and three images are obtained. These images are averaged and presented as a single image in microns with the help of software. Here both average and four quadrants (superior-inferior, nasal-temporal) measurements are obtained and amalysis is made in micron (μm) (figure 4) (17).

5. Tonometer

Intraocular Pressure (IOP) is measured by tonometry. IOP is the tension caused by the aqueous humor in the chambers to the cornea and sclera. Different methods have been used to evaluate IOP throughout history. Applanation tonometry, developed by Goldman in 1954, is still a valid method today. This method works according to the Imbert-Fick rule. Applanation tonometry indicates the IOP value in mmHg after the reaction force obtained by smoothing an area of 3.06 mm in diameter of the cornea is multiplied by 10 in grams (18). When tonometry applies pressure to the cornea and makes IOP measurements, the structural state of the cornea may cause false positivity or negativity at the measured value. That is, it is measured higher in thick corneas and lower in thin corneas (19). Daytime changes in IOP values are expressed as diurnal, and changes in 24 hours are expressed as circadian. This change should be less than 10 mmHg. Since the measured values are different at certain times during the day, it is concluded that the diagnosis of Glaucoma is not correct with a single measurement. In healthy people, normal IOP values are 10-21 mmHg.

Central corneal thickness should be measured simultaneously with pachymetry when IOP measurement is performed by applanation tonometry. IOP values should be revised with correction formulas created according to the results (20).

6.Electrophysiological Tests(21, 22, 23).

Electroretinography (ERG): It is the electrical response to light illumination caused by the stimulation of the retina. The Ganzfeld dish is used here for illumination. It records the outer layers of the retina up to the ganlion cells. ERG is significant in patients with glaucoma with focal loss. As the response in ERG is full-thickness, retinal damage in focal areas will be detected as normal. For this focal, pattern ERG models are developed.

Pattern Electroretinography (PERG): It contains two basic components, P50 and N95. The P50 component is more specific to the macula. N95 is affected by the damage of ganglion cells in optic nerve disease. PERG has an important role in the differentiation of macular and optic nerve injury.

Visual Evoked Potential (VEP): It is the electrical response of the visual cortex in the occipital lobe against visual stimuli. In patients with glaucoma who develop optic neuropathy due to hypoperfusion, the latency of VEP is prolonged, amplitude decreases, and the duration of positive wave VEP wave formation is prolonged. However, it should not be forgotten that there is prolonged VEP also in macular diseases. PERG is required to differentiate the origin of this prolongation in VEP.

Electro-Oculography (EOG): There is resting potential due to interaction in the retinal pigment epithelium. Here, the potential is measured by illuminating the retina. Ganzfeld dish is used for lighting. The decrease in light amplitude in the EOG indicates a dysfunction at the level of the photoreceptor complex of the retinal pigment epithelium.

7.Dark Adaptation: Here, the sensitivity of the retina due to self-regeneration of rhodopsin is measured in a dark-adapted eye.

8.Color Vision: The perception of colors is greatest at the center of the retina and is not detected when reaching the peripheral retina. According to the Kollner rule, changes in red-green axes can be seen in optic nerve degenerations. As an exception to this rule, however, the blue-yellow axis is affected in glaucoma.

9.Ultrasonography: It has an important role in the evaluation of intraocular anatomy especially in cases of malignant glaucoma, retinal detachment, tumor etc. which are considered among the causes of glaucoma.

10.Magnetic Resonance Imaging (MRI): It is a non-ionizing imaging method that has an important role in the detection of orbital and intracranial optic nerve injury pathologies.

11.Computed Tomography (CT): This imaging system is superior to MRI in imaging of orbital bone structures but there is exposition to ionizing radiation.

12.Contrast Sensitivity Test: Contrast sensitivity decreases and visual performance decreases when illumination is diminished although visual level is good (Example, Optical Neuritis).

Classification of Glaucoma

Due to physiopathology of glaucoma, clinical manifestations, anatomic involvement area and different treatment options; different classifications were made in the form of subgroups. The common feature of these classifications is to reduce irreversible visual loss and optic atrophy by reducing the increase in IOP to a certain level.

In this study, classification was made based on the grouping in the European Glaucoma Society Terminology and Guidelines for Glaucoma (24).

1. Primary Congenital Glaucoma (PCG)

An idiopathic disease resulting in loss of visual function at birth or in the first two years of life. It is known to be secondary to dysgenesis in trabecular meshwork in physiopathology. It shows autosomal recessive inheritance. Although it is stated that it is genetically related to the mutation in cytochrome P4501B1 gene, its genetic map is not fully solved. Epiphora, photophobia and blepharospasm are known as classic triple in these patients. In addition, wrinkles of the descement membrane and agenesis of the schlemm's canal may be seen. Early diagnosis is very important in these patients. Treatments performed before optic disc atrophy may be sufficient for the patient to survive. The aim should be to control IOP levels and to preserve vision and prevent amblyopia. Among the treatment options, medical agents (beta adrenergics, prostoglandin analogs) are often used to control IOP fluctuations before and after surgery. The surgical option is almost always necessary in all patients, with the priority is for goniotomy followed by trabeculutomy and / or only trabeculectomy (25,26).

1.2 Conditions associated with childhood glaucoma (ESC)

1.2.1 Ocular Anomalies: Aniridia, microftalmus, microcornea, peters anomaly etc.

1.2.2 Chromosomal Anomalies: Down syndrome

1.2.3 Connective Tissue Disorders: Marfan syndrome, Stickler syndrome etc.

1.2.4 Metabolic Diseases: Homocystinuria, Mucopolysaccharidoses

1.2.5 Phakomotoses: Sturge Weber syndrome, Norofibromatosis 1-2 etc.

1.2.6 Others: Retinopathy of prematurity, trauma, drug use, tumors, uveitic diseases

2. Primary Open Angle Glaucoma (POAG)

Human life is prolonged in the developing world. The degree of vision becomes an important public health problem in the aging population. Optic neuropathy, which significantly affects visual acuity in glaucoma; can cause blindness if no action is taken (27). POAG is an important part of glaucoma in adult age group. Kreft et al. Found that the incidence of POAG was 3.22% in a study conducted on 250 thousand healthy people older than 50 years. The study showed that there are some risk factors for POAG. The systemic ones are Hypertension, Diabetus Mellitus, Ischemic Heart Disease, Obstructive Pulmonary Disease and Myopia, Retinal Vascular Diseases and Ocular Traumas in order of frequency. The incidence is higher in women (table 1) (28). Clinical symptoms may not be seen until neuronal damage progresses. Since the quality of life is significantly affected when symptoms occur, it is very important to diagnose the disease at the

early stage and to regulate treatment before symptoms appear. In other words, neuronal damage occurs before visual loss (15). As with other types of glaucomas, in POAG, the aim is to reduce the IOP to the target level to prevent neuronal damage. The target value should be adjusted according to the patient's quality of life, damage to the optic disc, severity of the disease, and predisposing risk factors for glaucoma (age, family history, high IOP values, topical or systemic corticosteroid use, black race).However, the range of target reduction in the initial IOP usually ranges from 20-50%. The primary goal of treatment should be to use medication that will reduce IOP to the target level and has minimal side effects and is not cost effective. Prostaglandin analogs are the first choice for medical treatment in POAG. These drugs are topically administered to the eye once a day. Following the use of these drugs, some patients may experience hyperemia in the conjunctiva, elongation and thickening of the eyelashes and increased pigmentation around the eyelids. Alternatively, alpha adrenergic agonists and / or beta adrenergic blockers may be topically administered to the eye for patients with insufficient treatment or disturbed by these side effects. If target IOP values are not achieved despite all these medical treatments, laser or surgical options are applied. In spite of laser trabeculoplasty, if the desired target is not reached, incisional surgery is performed. Trabeculotomy is one of the commonly used surgical methods. The aim here is to drain the aqueous fluid under the conjunctiva by incision from the corneal-scleral area. As a result, it is necessary to apply appropriate treatment options to the patient in order not to decrease the visual acuity of the patient (29).

3. Secondary Open Angle Glaucoma (SOAG)

SOAG forms one of the main classes of glaucoma including a heterogeneous group that is capable of inducing optic neuropathy due to ocular or non-ocular causes. These will be considered as individual titles.

3.1 Lens-Induced Glaucoma (LIG): This type of glaucoma is usually seen in the elder patients. LIG causes glaucoma by increasing IOP in two different ways. First; IOP increases due to lens particles preventing the passage of aqueous liquid into the anterior chamber. Second, Increases in IOP occur due to obstruction of trabecular meshwork due to proteins secreted from the lens or because of an anaphylactic reaction. Different subgroups of LIG are available(30).

a. Pupillary blockage: The iris root was placed in the trabecular meshwork, causing blockage and drainage of the aqueous fluid was prevented. Nd: YAG laser is used for treatment.

b. Ectopia lens: It occurs due to congenital diseases such as Marfan syndrome

and Homocystunuria. Lens malposition should be corrected by surgical treatment.

c. Phakolitic: Anterior chamber angle is occluded secondary to mature or Morgagni cataract, thus preventing aqueous drainage. Surgical treatment should remove these lens fragments.

d. Phacoanalytic: There is an immune response to lens particles and antiinflammatory and topical antiglaucomatous agents are used for treatment

3.2 Pseudoexfoliation Glaucoma (PXG): PXG, one of the causes of SOAG, is characterized by high IOP. It occurs in individuals with pseudoexfoliation syndrome. A typical feature of this syndrome is a systemic disease characterized by the accumulation of fibrillary matrix of ocular and extraocular tissues (31). Ocular involvement is often diagnosed by the presence of fibrillary material deposited in the anterior capsule, zonules and anterior chamber of the lens. It is usually characterized by involvement in one eye. In addition, this fibrillar material may accumulate in the trabecular meshwork and iris and may develop pupillary block due to posterior synechiae. As a result, it also causes closed angle glaucoma. In PXG, the clinical condition generally progresses slowly and quietly (32). Since IOP values are higher than POAG, follow-up frequency of these patients by physicians is important. Initial treatment is generally topical antiglaucamtosis agents in combination with Prostoglandin analogs and Beta blockers. Selective laser trabeculoplasty is performed in cases where medical treatment is inadequate. However, peripheral laser iridotomy is the first choice in cases with pupillary block. Despite all these options, tarbeculotomy is still used as a surgical option when the target IOP is not reached. Mitomycin C, which is an antineoplastic agent, is applied locally to prevent scar formation that prevents drainage which becomes possible with trabeculotomy. In these patients, the incidence of complications due to cataract surgery increases due to the accumulation of fibrogranular materials (31,33).

3.3 Pigmentary Glaucoma (PG)

In PG, the drainage of the aqueous fluid due to the accumulation of iris pigments in the tarbekular network is impaired, resulting in an increase in IOP. The clinical progression of these patients is generally asymptomatic. However, since men with young myopia are at risk for glaucoma, it is important to follow up this group of patients. In treatment, pilocarpine is the first primary option that causes myosis by acting on cholinergic muscorinic receptors. Beta antagonists, alpha-adrenergic agents and prostoglandin agents are also used as adjuncts. In case of insufficient medical treatment, Laser Trabeculoplasty, Laser iridotomy

and trabeculotomy as surgical options are applied(34).

3. 4 Uveitic Glaucoma (UG)

UG is one of the secondary glaucomas that develops secondary to infection of intraocular tissues and can be prevented by proper diagnosis and treatment. Uveitis patients are at risk for the incidence of glaucoma. This risk varies according to the type of infection and response to treatment. Both open angle and closed angle glaucoma can be seen in UG. The mechanism of glaucoma formation in uveitis patients has not been clearly resolved, but inflammation cells, debris and proteins can accumulate in the trabecular meshwork and may increase IOP by preventing aqueous output. In uveitis, elevated IOP may be acute or chronic. Viral infections and Posner schlossman syndrome cause acute IOP increases. Herpetic, Possner schlossmnan syndrome, Fuchs heterochromatic iridocyclitis, sarcoidosis, Toxoplasmosis, Juvenile rheumatoid arthritis, etc. are all involved in the etiology of UG. Antiinflammatory drugs are used for the causative agent and topical antiglaucomatosis is used to reduce intraocular pressure. It should be noted that topical or systemic corticosteroids used in treatment may also increase the IOP. Surgical treatment options for glaucoma are applied when medical treatment is inadequate (35).

3.5 Neovascular Glaucoma (NVG)

It is one of the causes of secondary glaucoma characterized by periocular pain and high IOP which is generally resistant to medical treatment. NVG may develop due to many infections and systemic diseases that cause retinal hypoxia / ischemiaAs a result of ischemia due to retinal involvement such as retinal vein occlusion, diabetic retinopathy, ocular ischemic syndrome, ocular tumors; mediators are released from the retina, such as Vascular Endothelial Growth Factor (VEGF), Tumor Growth Factor beta (TGF-B), Fibroblast Growth Factor (FGF), which trigger the formation of new vessels (angiogenesis) in the retina. These mediators first pass through the aqueous fluid and then from the pupilla to the anterior chamber (Rubeosis iridis) and initiate the process of angionesis in the anterior chamber. These new vessels formed in the iris reach the trabecular meshwork and block the angle causing closed angle glaucoma. The classic sign of NVG is rubeosis iridis and a fibrovascular-like membrane at the iridocorneal angle. IOP elevation may not be seen or open angle glaucoma may occur before these new vessels are occlude the angle. Visual acuity decreased in these patients due to the underlying disease. The primary problem in NVG is severe pain because of the increased IOP due to complete closure of the angle by the fibrovascular

membrane. Therefore, the primary goal is to reduce the elevated IOP so that both the patient's vision levels do not fall further and the pain that occurs is reduced. The main treatment here is to treat the underlying disease. Panretinal photocoagulation (PRP) is applied in order to prevent the increase of released mediators due to ischemia in the retina. In addition, intravitreal administration of anti-VEGF agents should reduce the levels of mediators that trigger angionesis. If angle closure has occurred, peripheral iridotomy is performed with Nd: YAG laser. If the angle is opened, topical antiglaucomatous drugs are administered. If the patient's pain persists and IOP values are still high despite these treatments, drainage of aqueous fluid is provided by ocular surgery. Sometimes all these treatments may be inadequate. After this stage, aqueous humor production is reduced by cyclodestructive method. The most recent solution is enucleation in patients without visual acuity (36).

3.6 Glaucoma Due to Retinal Detachment (RDG)

In vitrectomy, secondary glaucoma occurs due to increased IOP perioperatively or postoperatively as a result of intravitreal silicon and gas administration. During the scleral buckling, due to the pressure applied to the vortex veins, one of the ways in the drainage of the aqueous hormor, the passage of the aqueous liquid to the systemic circulation is prevented and swelling occurs in the backward ciliary body and an increase in IOP occurs. This increase can last from several days to several weeks. Topical antiinflammatory agents and cycloplegin are used to reduce congestion in the ciliary body. In addition, topical antiglaucomatosis is added to the treatment. YAG laser iridoplasty can be used in addition to medical treatment. In case of IOP increase due to intravitreal silicone or gas administration, topical antiglaucomatosis and corticosteroid agents should be added to the treatment after removal of these materials with aspiration (37).

3.7 Ocular trauma-induced Glaucoma (TG)

Due to ocular trauma, either the trabecular meshwork or the lens is damaged, or the IOP may increase due to closure of the angle, resulting in secondary glaucoma. High IOP values up to one month due to injury are usually secondary to hyphema, inflammation or lens damage. If this increase lasts more than 6 months, it is more likely to be related to posterior synechia or endophthalmitis in the iris. Trauma can also cause lens-induced glaucoma (see LIG). In TG, treatment should be tailored to the trauma-related complications. If hyphema develops, topical cycloplegic, corticosteroid and anti-glaucomatous agents should be used. In such cases such as lens dislocation or drop to the vitreous; surgical option should be at

the forefront(38).

3.8 Iatrogenic Glaucoma (IG)

Steroids are indispensable drugs in various autoimmune diseases and inflammatory conditions. They have beneficial effects as well as side effects such as glaucoma and cataracts in ocular tissues. Other than ocular use of steroids, nasal, systemic, and so on. use of the drug may also cause these side effects. Steroids cause extracellular matrix accumulation in the trabecular meshwork and increase the IOP by affecting the aqueous drainage. In patients, IOP change usually occurs a few weeks after taking the drug. People with family history, Diabetes, Rheumatoid Arthritis, Myopia are at risk. The first step in the treatment is to discontinue this drug and start topical antiglaucomatous drug. If there is no improvement, the next steps are laser trabeculoplasty and surgical options (39). Iatrogenic glaucoma may also be seen after vitroretinal surgeries(37).

3.9 Glaucoma Due to Intraocular Tumors (ITG)

In patients with a history of unilateral glaucoma, intraocular tumors should always be considered in the differential diagnosis. Melanoma, one of the anterior segment tumors, invades the anterior chamber and decreases aqueous humor drainage and increases IOP level. Metastastic and infiltrative (Leukemia, Multiple Myeloma, etc.) tumors block the angle and trabecular meshwork and prevent aqueous circulation. Retinoblastoma, which is an intraocular tumor seen in childhood, obstructs the drainage by obstructing the angle. Radiologic (MRI, CT) imaging is necessary to rule out tumor group in treatment-resistant glaucoma cases. Medical treatment, chemotherapy, radiotherapy and surgical options are used for the underlying cause (40).

3.10 Glaucoma Due to Extra Ocular Diseases (EOG)

It is seen in several different ways. Structural anomalies of congenital or hereditary orbit, vascular diseases etc. conditions preventing aqueous drainage. The treatment is cause-oriented and the aim is to keep the IOP at the target point and to preserve the visual acuity of the patient (41).

4. Angle Closure Glaucoma(ACG)

4.1 Primary angle-closure glaucoma (PACG)

PACG, is a type of glaucoma characterized by acute elevation of IOP due to the inability of aqueous fluid to reach trabecul between the chambers by blocking the iridocorneal angle of the iris root.

Pathogenesis: The anterior and posterior chamber are separated by iris tissue. The aqueous fluid cannot pass from the pupil to the anterior chamber due to the pupillary block formed by the iris due to factors that push the iris forward or attract to the trabecular meshwork. Clinically, the volume of the anterior chamber decreased, the volume of the iris increased, the lens moved towards the anterior and its thickness was increased. Genetically; plateau iris syndrome, small cornea and shallow anterior chamber are important risk factors for PACG (42,43).

Clinically divided into 5 different groups:

A. Acute Form: The iridocorneal angle closes acutely and the aqueous humor cannot pass into the anterior chamber. Patients present with sudden onset of periocular pain, loss of vision or even nausea and vomiting. In the examination, enlargement of episcleral and conjunctival vessels, corneal edema, non-reactive pupil light reaction, flattened iris and shallow anterior chamber can be seen. The IOP value is generally 3-4 times higher than the normal value. Topical (beta blockers) and systemic (intravenous mannitol, carbonic anhydrase inhibitors) anti-glaucomatous drugs are initiated in the treatment. If pupillary block develops, Nd: YAG laser iridotomy allows the passage of aqueous fluid into the anterior chamber.

B. Subacute Form: In this form, iridocorneal angle closes due to a triggering factor and IOP rises in the presence of predisposing factors (shallow anterior chamber, small cornea and family history). In general, the angle improves spontaneously and the increased pressure returns to normal.

C. Chronic Form: In general, optic nerve damage becomes apparent due to a quiet, gradually increasing IOP. The clinical course progresses similarly to open-angle glaucoma. Here the angle is partially closed. Filtration surgery is performed in patients whose general condition does not improve despite of using topical antiglaucomatosis.

D.Latent Form: Acute glaucoma attacks develop in one eye and no clinical symptoms are present in the other eye. However, the intact eye is always at risk. Therefore, prophylactic laser iridotomy is required in the intact eye.

E. Absolute Form: There is periocular pain due to increased IOP without visual acuity. Treatment should be aimed at reducing pain. In addition to topical and systemic antiglaucomatosis, anti-inflammatory drugs should be added to the treatment. If pain persists, ocular filtration surgery is performed

4.2 Secondary Angle Closure Glaucoma (SACG)

Although the clinical symptoms vary depending on the underlying cause, they all have an elevated IOP in common.

4.2.1Conditions causing glaucoma by blocking the pupil

- Cataract due to ocular trauma
-Lens dislocation due to ocular trauma
- Irido-pupillary synechiae due to infections

4.2.2Causes of glaucoma without pupillary block

a. Neovascular Glaucoma (see NVG)

b.Malignant Glaucoma: Aqueous humor fluid is normally synthesized from the ciliary body and then passes from the posterior chamber to the anterior chamber and is introduced into the systemic circulation through the trabecular and uveascleral routes through the episcleral veins. In malignant glaucoma, the aqueous humor flows into the vitreous rather than the anterior chamber, or accumulates behind the lens, causing an increase in IOP. Clinically it may be confused with pupillary block glaucoma. However, the central anterior chamber is shallow while the peripheral anterior chamber is shallow in the pupillary block. In addition, iris cambering is available in the pupillary block. Choroidal detachment is another condition that should be considered in the differential diagnosis. Choroidal detachment occurs more frequently after filtration surgery, where hypotonia is present. Suprachoroidal hemorrhage usually occurs a few days after intraocular surgery. Although the eye has hypotonia similar to choroidal detachment, the ocular structure has the appearance of inflammation. In malignant glaucoma; painful eye, blurred vision and congestion due to dilatation of scleral and conjunctival vessels are present. Ocular ultrasonography is used to differentiate the conditions considered in the differential diagnosis. In malignant glaucoma, medical treatment (antiglaucomatosis and cycloplegic agents) is the first choice. If there is no response to medical treatment, YAG laser iridotomy and incisional surgery are applied. Since the other eyes of these patients are at risk, prophylactic laser iridotomy can be performed and myotic agents should be avoided (44).

Treatment In Glaucoma

Treatment in glaucoma is divided into three main groups: medical, laser and surgical.

1. Medical Treatment

The aim of treatment in glaucoma is to avoid the development of optic neuropathy or to minimize the progression of neuropathy. Here, it is necessary to increase ocular blood flow and aim to reduce GiB to the target level. Medications prescribed for glaucoma should be regulated according to the patient's anamnesis and risk status. The drug with the minimum side effects that will provide maximum benefit should be selected and the patient should be informed about this. There are 4 main groups of drugs used in antiglaucomatosis in medical treatment. They are used topically or systemically.

1.1 Parasympathomimetics(45).

Mechanism of Action: They act by making myosis over muscarinic receptors in the longititinal muscles in the ciliary body and in the sphincters of the pupilla. They increase the flow of aqueous fluid through the trabecular meshwork and expand the angle by pulling the peripheral iris on the trabeculum.

Preparations: Pilocarpine; It is one of the first drugs used in the treatment of glaucoma. It can be used as monotherapy or in combination with beta-blockers. Carbachol; It is preferred in resistant cases because it is more effective than pilocarpine. However, it has more side effects.

Side effects: Because of myosis, narrowing of the peripheral visual field and shift to myopia due to accommodation spasm may occur.

1.2 Prostaglandin analogues(46).

Mechanism of action: They generally lower the IOP by increasing the flow of aqueous fluid through the uveascleral and trabecular routes. This group of drugs is usually used once a day topically at night.

Preparations: Latanoprost; is a F2-alpha analogue and is the first drug in this group to increase aqueous humor drainage via the uveascleral route. Travoprost: It has a similar effect to latanoprost. Bimatoprost: They act by increasing the flow of aqueous fluid both from the uveoscleral and trabecular routes.

Side effects: Conjunctival hyperemia, pigmentation increase at iris (irreversible) and periocular area (reversible). They may increase the incidence of cystoid macular edema and viral keratitis (herpetic) after cataract operation. Systemically; side effects such as respiratory problems and skin rashes can be seen. They can also trigger chronic headaches.

1.3 Sympathomimetics and Sympatholytics (47,48, 49).

Mechanism of action: Beta-blockers can generally be used in all types of glaucoma as they reduce the production of aqueous fluid. Alpha 2 agonists lower IOP in two ways: by reducing aqueous secretion and increasing uveascleral flow.

They show their effects on four different receptors including alpha 1-2 and Beta 1-2;

Alpha 1:It is found in the arterioles, pupil (dilatator muscle) and eyelid (müller muscle). Stimulation of this receptor causes mydriasis, hypertension and eyelid retraction.

Alpha 2:It is found in the ciliary body. As a result of stimulation of this receptor, the drainage of the aqueous liquid becomes more effective.

Beta 1:Only found in cardiac tissue. Heart rate increases due to stimulation.

Beta 2:It is found in the bronchi of respiratory system and ciliary bodies. As a result of stimulation, the production of aqueous liquid increases and dilatation of these bronchi occurs.

Beta Blocker Preparations: timolol; There are different forms used topically. Used once or twice a day. Betaxolol: It may be a primary option in hypotensive patients because it is less hypotensive than timolol. In addition, it can be effective in optic neuropathies due to its neuron protective effect as it increases ocular blood flow. Carteolol: It shows less cardiac effects than timolol.

Side effects: may cause allergic reactions and corneal keratopathies in ocular structures. Systemically, they may cause hypotension and cardiac rhythm disturbance due to their cardiovascular effects. They can trigger an asthma attack by narrowing the bronchi in the respiratory tract. They also impair blood lipid levels, can cause dizziness and psychiatric disorders.

Alpha 2 agonist Preparations: Brimonidine; This can be first choice in addition to beta-blockers in glaucoma patients with optic neuropathy due to its neuroprotective effect as it increases ocular blood flow. They act by reducing aqueous humor production and increasing uveascleral flow. Apraclonidine: It is often used prophylactically to avoid an increase in IOP after YAG Laser iridotomy and laser trabeculoplasty. Long-term use is not recommended. Because topical side effects are more.

Side effects: May cause conjunctivitis and fatigue.

1.4 Carbonic anhydrase inhibitors(50,51).

Mechanism of action: They inhibit carbonic anhydrase enzyme and reduce IOP and intracranial pressure. They also increase ocular and cerebral blood flow due to these effects. Topical and systemic use is available.

Preparations: Dorzolamide; Used topically. They act by reducing aqueous humor release. It can be used topically three times a day. Brinzolamide: Similar to Dorzolamade.

Acetozolamide: Available in oral and intravenous form. Intravenous form is very effective especially in acute glaucoma crisis. Oral tablets such as methazolamide and dichlorphenamide are also available. They act in a similar way to suppress aqueous humor production.

Side effects: May cause conjunctivitis and corneal endothelial damage in ocular structures. Systemically, they may cause paresthesias by affecting the central nervous system, irritations by affecting the gastro intestinal system, blood dyscrasias by affecting the hematological system, and stones in the kidney by affecting the urogenital system. In addition, stevens-johnson syndrome and hypokalemia (due to long-term use) may be seen very rarely.

1.5 Osmotic treatment(52,53).

Mechanism of Action: Osmotic agents increase the oncotic pressure of the blood, creating osmotic pressure in the blood-retinal barrier and with the difference of gradient, fluid absorption from the vitreous to the blood vessels occurs. In this way, intraocular fluid passes into the systemic circulation and the IOP drops. It is important not to use osmotic agents that cross the blood retinal barrier. They should also be used with caution in glaucoma associated with inflammatory disease in which the permeability of the blood retinal barrier is increased.

Preparations: Mannitol; It is the most commonly used intravenous antiglaucomatous agent. Glycerol and isosorbide nitrate are also used for this purpose.

Side effects: As the oncotic pressure increases in the systemic circulation, fluid retention may occur. Careful use of these drugs is necessary in patients with heart and kidney disease.

2. Laser Treatment (54, 55, 56).

2.1 Laser Iridotomy (LI)

Two different laser methods are available: Nd: YAG and Argon laser.

Indications: Narrow angle glaucoma, Primary angle-closure glaucoma, Pupillary block glaucoma

Technique: After application of topical anesthetic agent to the eye, iridotomy is opened through Abraham Iridotomy lens 4-10 millijoules (mJ) and 50-70 micron diameter for Nd: YAGand 200-600 milliwolt (mW) power and 200-500 micron diameter for Argon.

Complications: Bleeding in the form of leakage and inflammatory reactions in the iris, stinging and burning sensation in the eye, corneal damage and an increase in IOP may occur. After this procedure antiglaucomatous drugs and steroid drops to be used for an average week is prescribed.

2.2 Argon Laser Trabeculoplasty (ALT)

By applying laser shots to the trabeculoma, it is aimed to increase the trabecular intervals due to the burns and increase the aqueous outflow.

Indications: PACG, Pigmentary Glaucoma, PXG.

Technique: Prior to the procedure, topical anesthetic agent and apraclonidine are administered to prevent IOP increase. Shoots are applied to the trabecular area with a spot diameter of 50 microns for 0.1 second at 300-700 mW. The presence of air bubbles indicates that the shot is effective.

Complications: Eye irritation, small spot bleeding, peripheral anterior synechiae, acute increase in IOP may occur. After the procedure, apraclonidine and steroid are given with prophylactic purposes.

2.3 Selective Laser Trabeculoplasty

In the trabecular meshwork with 532 nm frequency-doubling, the Nd: YAG laser is used in this method, which targets only melanin pigments. It is more advantageous and reliable than ALT because it has indications similar to ALT and does not cause tissue damage.

2.4 Laser Iridoplasty (LIP)

It is a method for enlarging the anterior chamber angle by applying laser to the peripheral iris and increasing the drainage of the aqueous humor.

Indications: Narrow angle glaucoma, angle-related glaucoma crisis

Technique: Routine procedures are performed before the procedure similar to other laser procedures.

Complications: Micro hemorrhages in the iris, iritis and IOP increase may be seen. After the procedure, apraclonidine and steroid are given with prophylactic purposes.

2.5 Diode Laser Cycloablation (DLS)

Objective: To reduce the aqueous production by destroying a certain part of the ciliary body where aqueous humor production takes place.

Indications: It is a method used for the relief of periocular pain in patients with almost no visual acuity.

Technique: Following local anesthesia in the peribulbar region, it is applied to the back of the imbus at 1200-1500 mW power for 1-2 seconds at 360 degrees at 1 mm intervals (without touching the vascular and neural bundles at 3 and 9 o'clock level). Following the procedure, topical steroid, cycloplegine and antibiotics are prescribed and frequent follow-up is recommended.

Complication: Ocular pain may occur during and after the procedure. Rarely, corneal damage, retinal detachment, suprachoroid hemorrhage and phytisis bulbi secondary to hypotonia can be seen.

3. Surgical Methods

Surgical options are applied in cases where IOP is not decreased despite medical and laser treatments, non-compliance in medical treatment, rapid progression of visual loss, etc. The aim of surgery is to provide the flow of aqueous fluid to the subscleral or subconjunctival area.

3.1 Penetrating Trabeculectomy: The drainage of the aqueous fluid from the anterior chamber to the subtenon cavity is achieved with the help of a fistula. The fistula formed here is covered with scleral tissue or conjunctiva. The bubble formed by the aqueous at the outlet of the fistula is called the blep.

Indications: It is applied in glaucoma cases which do not improve despite medical and laser treatments.

Technique: After retrobulbar or peribulbar local anesthesia; a suture fixation was placed to the upper part of the rectus muscle. Following the conjunctival incision, bleeding control is performed with cautery and then scleral flap is prepared and paracentesis is performed to the anterior chamber. Following sclerostomy and peripheral iridectomy, the conjunctival sclera is closed with adjustable sutures and the procedure is terminated.

Complications: Drainage problems due to scleral or conjunctival flap rupture, excessive drainage of aqueous fluid (streaks in the corneal descement membrane, iris is in contact with the corneal endothelium, hypotonia and choroidal detachment, etc.), shallow anterior chamber, Pupillary block, Malignant glaucoma, Endophthalmitis due to infection in the blep site may be seen (57).

3.2 Non-penetrating Trabeculectomy (58,59).

Indications: PACG, PXG, PG and Congenital Glaucomas

Technique: The anterior trabecular meshwork is preserved and a deep scleral flap is removed. It is a more sensitive and difficult method than penetrating trabeculectomy. However, since the anterior chamber and trabecular meshwork

are preserved, the risk of over-filtration and associated complications is less.

3.3 Trabectome surgery: It is a microsurgical method performed by removing a small portion of the trabecular network and the inner wall of the schlemm canal with the help of a Trabectome device. It is a more reliable method than other surgical methods.

3.4 Drainage implants: The common feature of implants with valve and non-valve models of different lengths and sizes is that they have external connection with the anterior chamber and thus provide aqueous humor drainage.

Indications: In cases where trabeculotomy is inadequate, Traumatic and Neovascular glaucoma, presence of conjunctival scar or insufficiency, glaucoma due to infections, etc.

Complications: Inadequate or excessive drainage, endophthalmitis, corneal damage, hypotonia, shallow anterior chamber, fant glaucoma, etc.

Anti-metabolites in Filtration Surgery(60).

They increase the success rate of penetrating surgeries by suppressing cystic formations and similar conditions in blebs that decrease the chance of surgical success. It should be used according to the package insert in the areas where topically flaps are formed, otherwise ocular complications which may be difficult to treat may occur.

Preparations: Mitomycin C, 5-fluorouracil

Complications: Corneal ulcers, keratitis, conjunctivitis, hypotonia, etc.

Conclusion

Glaucoma is a major public health problem affecting seventy million people worldwide. As in all diseases, early diagnosis is important in glaucoma. In the present study, the etiopathology of glaucoma was discussed and the diagnosis and treatment of the disease were also presented systematically. Therefore, we think that the current study will provide ophthalmogists with a road map of what can be done in glaucoma in the light of current information.

Table 1:Risk Factors for Glaucoma

Female gender
Ocular injuries
Myopia
Retinal Vascular Occlusion
Hypertension
Diabetes Mellitus

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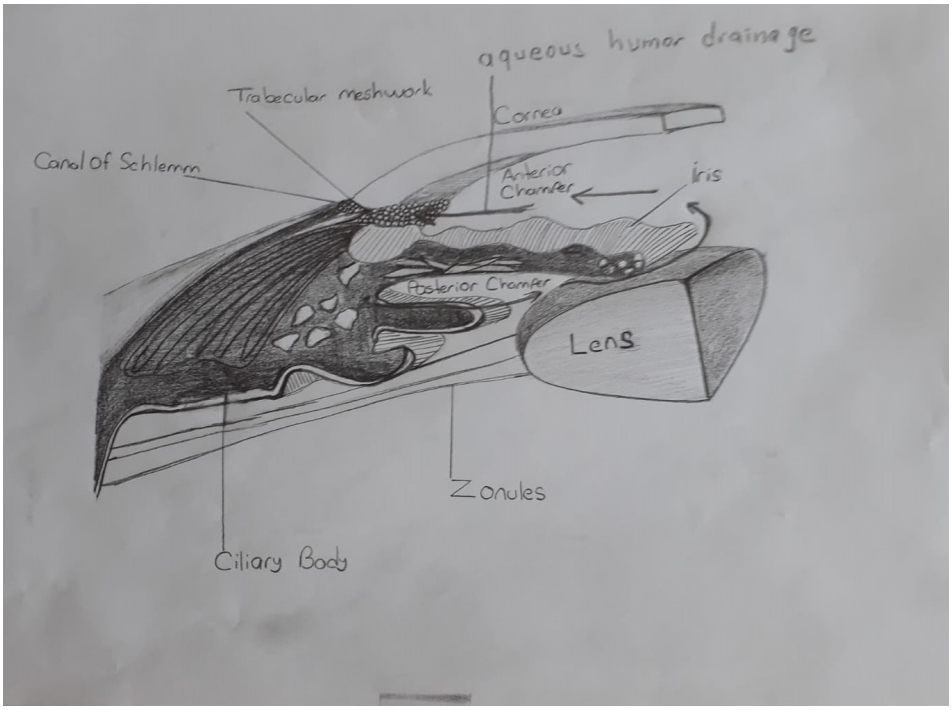

Figure 1: Anatomical and physiological structures involved in aqueous humor drainage(Hand drawing was done on Fig 13.14 from the reference number 43.)

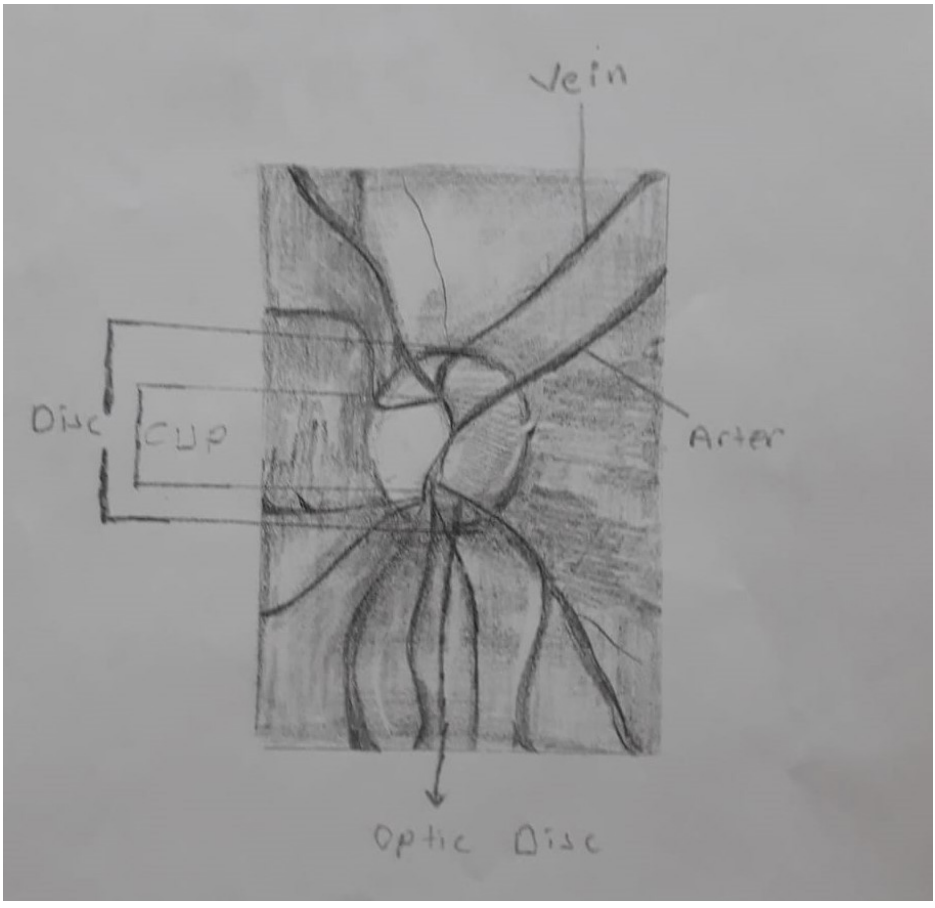

Figure 2: Cup-Disc ratio(C/D)(Hand drawing was done on Fig 13.16 from the reference number 43.)

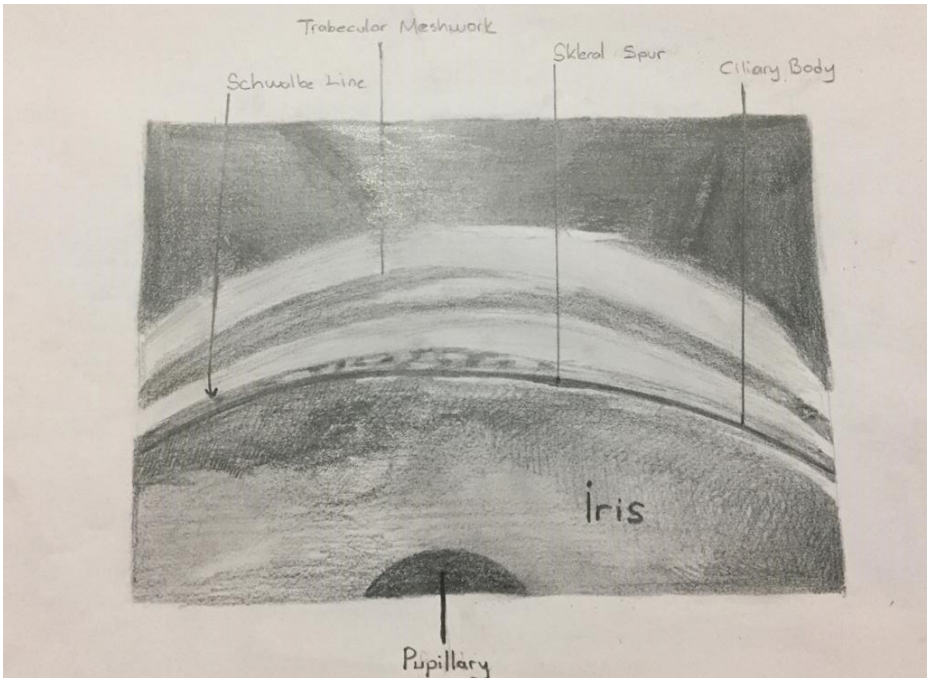

Figure 3: Gonioscopically, the angle elements are observed from front to back; Schwalbe line, Trabecular meshwork, Scleral spur and Ciliary body (Hand drawing was done on fig 7.13 from the reference number 10.)

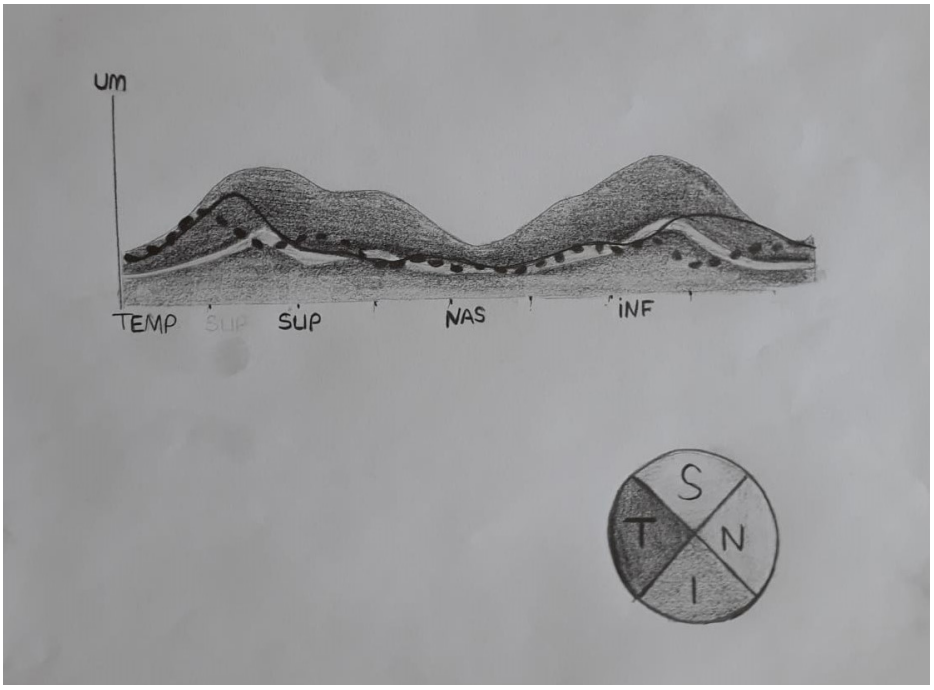

Figure 4: Retinal Nerve Fiber Layer Thicknesswith measurement Optical Coherence Tomography (hand drawing of the result we measured with optical coherence tomography)

Chapter 4

Overview of Epidemiology, Diagnosis, and Treatment of Neuroendocrine Tumors

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1. Introduction

Neuroendocrine tumors (NET) are epithelial tumors which can develop in almost every body part as a result of malignant transformation of various endocrine cells; they originate from the neuroendocrine system (1). The neuroendocrine tumor concept used currently has historically undergone some developments. For example, the origin of the term NET is based on the studies of the scientists Langhans and Lubarsch. Following the description of Langhans and Lubarsch, Dr Siegfried Obendorfer (1876 Munich-1944 Istanbul) named them as carcinoid (similar to carcinoma) structures. In 1995, neuroendocrine tumor term began to be used. However, in the years 2000 and 2004, World Health Organization (WHO) recommended the use of the term endocrine tumor. Since 2010, the name neuroendocrine tumor began to be used again (2,3). In addition to these terms, there is also the term mixed endocrine–exocrine tumor. These are tumors which include various ratios of exocrine and endocrine components. Development of two separate tumors is called composite. If there are two separate components within themselves, they are called combined. If the same cell includes two components, it is called amphicrine along with the goblet cell carcinoid definition (4). However, throughout this study, neuroendocrine tumor term will be used unless otherwise stated.

2. Epidemiology

In the United States of America (USA), according to the Surveillance, Epidemiology End Results (SEER) database, 64,971 NET cases registered between 1973 and 2012 were retrospectively analyzed. It was detected that 34,233 (52.7%) of these were female; its incidence increased by 6.4 fold from 1973 until 2012. This increase was observed in all regions and stages involving the tumor, and the highest incidence rate between the years of 2000 and 2012 was 1.49/100,000 for lungs, 3.56/100,000 for the gastrointestinal pancreatic region, and 0.84/100,000 for the ones without any determined primary according to SEER. Survival increased for all NETs between the years 2000 and 2012. This increase was more distinct particularly in advanced stage gastrointestinal NET (GEP-NET) and pancreatic NET (pNET) as a result of positive advancements in treatment (5). In the US, among the gastrointestinal tumors, colorectal cancers are the most common type, followed by GI-NETs. In a study conducted in Switzerland, it was reported that out of 478 patients with GEP-NET, 80.1% had small intestinal involvement, 10.3% had pancreatic involvement, and 9.6% had other regional (stomach, colon, and rectum) involvement (6). In Middle East and Asia Pacific regions, NET is most prevalent in the pancreas (49%), followed by

colon (13%), small intestine (11%), liver (10%), and others (4%) (7). Colon NETs account for 8% of all NETs, whereas rectal NETs account for 18% (8). Their prevalence in Europe is less compared with that in USA and more compared with that in Japan. The prevalence of gastrointestinal NETs in England increased in both sexes from 1970s until the year 2006. In the same time period, its prevalence was lower than that in USA. Although the differences in the incidence rates are explained by regional genetic factors or environmental conditions, this also raises concerns regarding the accurate identification and registration of patients by institutions (9). In Turkey, GEP-NETs are not specifically recorded in the cancer data system. Thus, sufficient information is not available regarding their incidence and prevalence. It is believed that future multi-center data entries will shed light on retrospective and prospective epidemiologic studies (10).

NETs are still considered rare diseases, whereas the increase in nonfunctional tumors is more distinctive. However, silent but functional new tumors (silent gastrinoma) may also occasionally occur. Although its prevalence is 35/100.000 people/year, its incidence rate is 5/100.000 people/year. Approximately 50% of these comprise small intestine NETs, and 30% comprise pancreatic NETs (11,12). Although NETs may develop at any age, they are usually more prevalent after the 5th decade of life. Conversely, although appendix carcinoid is more prevalent in the 4th decade of life, it is reported that GEP-NET with syndromes (multiple endocrine neoplasia-1 Von Hippel–Lindau) develops in early ages (11,12).

Worldwide, the incidence of NET of pancreas is 1–4/1,000,000, and it accounts for 1.5% of all pancreas tumors. Its clinical characteristics vary depending on the hormone it secretes. Overall, 60%–80% of these tumors are functional tumors, whereas the remaining 15%–40% are non-functional and metastatic tumors with a large diameter (13,14). In total, 90%–95% of tumors have a solid structure, and 10%–16% have a cystic structure. Because pNETs are vascular tumors and as a result of bleeding/necrosis into the lesion, cystic structure is formed (15).

3. Classification of Neuroendocrine Tumors

The current classification system 2017 WHO and AJCC 7 and 8 are used for the classification of NETs. Details and comparison of this classification are presented in Table 1. Moreover, TNM staging is performed according to the American Joint Committee on Cancer/Union for International Cancer Control (AJCC/UICC) 2014 guideline (Table 1).

Table 1. Comparison of "T" category criteria AJCC/UICC (2010) and ENETS classifications pancreas NET

	ENETS TNM	AJCC/UICC TNM 7	AJCC 8 2017
T1	Limited in pancreas, < 2 cm	Limited in pancreas, < 2 cm	Limited in pancreas, < 2 cm
T2	Limited in pancreas, 2–4 cm	Limited in pancreas, > 2 cm	Limited in pancreas, 2–4 cm
T3	Limited in pancreas, 4 cm or duodenum or bile duct invasion	Peripancreatic involvement without major vein invasion	Limited in pancreas, 4 cm or duodenum or bile duct invasion
T4	Neighbor organ or major vein	Celiac axis or SMA	Adjacent organ or major vein

Stages of neuroendocrine tumors are classified as G1: mitosis < 2, Ki67 < 2, G2: mitosis 2–20, Ki67 3–20, G3: mitosis > 20, Ki67 > 20 as per the WHO 2010 guidelines (Table 2). European neuroendocrine tumor society (ENETS), recommend a separate TNM classification for each region in their 2012 and latest 2016 guidelines.

Table 2. Stages of Neuroendocrine tumors according to ENET and WHO

GRADE	Mitosis Count / 10 BBA	Ki–67 Index (%)
NET–G1	<2	≤2
NET–G2	2–20	3–20
NET–G3 (NEC)	>20	>20

Mitosis - at least 50 large growth regions in the H&E-stained slides; Ki-67 - 500–2000 tumor cells in the regions with the most intense staining ("hot spots")

In the immunohistochemical (IHC) analysis of the samples collected with endoscopic ultrasound-guided fine needle aspiration (EUS-FNA), chromogranin A (CgA), synaptophysin, neuron-specific enolase (NSE) protein gene product (PGP 9.5), CD56, and pancreatic hormones are evaluated in well differentiated tumors. NSE, PGP 9.5, CD 56, and synaptophysin are evaluated in poorly differentiated tumors.

Histopathologic diagnosis is the golden standard for NETs. Joint evaluations, including all systems, must be a part of pathology reports. Organ location, tumor size, differentiation, mitosis, proliferative index, and invasion depth/area should be indicated. Result of the pan-neuroendocrine indicators (chromogranin and synaptophysin) should be provided. NET grade should be presented. Mitosis and Ki67 index are the most important biological indicators. Organ-specific staging

systems should be used, and the system used should be stated in the report. Moreover, the list of immunohistochemical antibodies used in the assignment of the primary focus in NETs is presented in Table 3 (16), and Table 4.

Table 3. List of immunohistochemical antibodies

Antibody	**"Ideal" specificity**
TTF-1	Lung, thyroid
CDX-2	Intestine
SAXB-2	Colorectum
PAX-8	Pancreas
Islet-1	Pancreas
Pax-6	Pancreas
DK7, CK20	-- (Merkel cell)

Table 4. IHC use to determine primary site in NET

	ENET Stomach	**TNM (AJCC) 8 (2017)**
Tx	Primary tumor not found	-
T0	No primary tumor	Limited in lamina propria or submucosa or ≤1 cm
Tis	Tumor in situ/dysplasia (<0.5 mm)	
T1	<1 cm and L. propria or submucosa invasion	
T2	>1 cm or M. Propria or subserosa invasion	>1 cm or muscularis propria invasion
T3	Serosa invasion	Subserosa
T4	Adjacent organ invasion	Serosa or adjacent organ invasion

As outlined above, the recognized classification of NETs is developed by WHO. This study will attempt to systematically explain neuroendocrine tumors by evaluating them based on their locations in the organism.

4. Gastrointestinal Neuroendocrine Tumors

There is no complete consensus on the classification of this disease group which is usually rare and highly heterogeneous. Currently, although there are different classification criteria based on prognostic factors, we can separate into

three main groups considering the structural characteristics and clinical behavior: tumors originating from foregut (bronchi, lungs, thymus, stomach liver, biliary system, pancreas, and first part of duodenum), those originating from the midgut (second part of duodenum, jejunum, ileum, appendix, right colon, proximal transverse colon, and ovaries), and those originating from the hindgut (distal transverse colon, descending colon, and rectum). Clinical results are divided into two groups: nonfunctional and functional. Those which do not secrete bioactive material cause symptoms owing to mass effect in their region of origin. In tumors with secretions, symptoms of the materials they secrete occur. As they are usually asymptomatic, they are diagnosed either incidentally or at a late stage.

4.1. Esophagus

They are extremely rare and account for 0.04%–4.6% of all NETs. They are prevalent in the 6th and 7th decades of life, M>F, and they are mostly located in the middle and lower esophagus. There is no treatment algorithm for NETs yet, and guidelines for lung and other GIS NETs are used. Most are detected incidentally; the patients are admitted with dysphasia, abdominal pain, melena, weight loss, chest pain, and rarely, carcinoid syndrome symptoms. During diagnosis, metastatic lymph node is found in 50% of the patients. Esophagus NETs are generally accepted as neuroendocrine cancer (aggressive course). Overall, 90% of these are also known as small cell carcinoma of the esophagus. Treatment is performed according to location, type, grade, and stage. <2 cm, mucosa–submucosa endoplasmic treatment + adjuvant chemotherapy (CT), >2 cm, open or laparoscopic surgery, and lymph node dissection are performed in lymphovascular and perineural invasion (17).

4.2. Stomach

In total, 7%–8% of all NETs originate from enterochromaffin-like cells. Endoscopic excision (<2 cm mucosa/submucosa, LN−) and surgery (>2 cm, >submucosa, LN+, and distant metastasis) are performed for the treatment of type 1. Treatment of type 2 is similar to that of type 1, i.e., gastrinoma resection. Type 3 is treated similarly as gastric adenocarcinoma, and LN dissection is performed. If the tumor measures <2 cm, is well-differentiated, with submucosa involvement and no lymphovascular invasion, endoscopic treatment is adequate (17).

4.3. Duodenum

It is rare and accounts for 1%–3% of all duodenal tumors. In total, 5%–8% of all GI NETs and 20% of duodenal NETs are located in the periampullary

region. It is accompanied by 90% non-functional, 10% ZES symptoms, and 3% carcinoid syndrome. In the presence of incidental, solitary, small (75% <2 cm), 9%–13% multiple, MEN-1 ZES, 60%–90% are multiple, and most are located in the mucosa and submucosa. Overall, 40%–60% are accompanied by LN metastasis, and <10% are accompanied by liver metastasis. G1: 50%–75%, G2: 25%–50%, and G3: 3%. Duodenal gastrinoma accounts for 50%–60%; somatostatin-excreting tumors account for 15%; non-functional serotonin-containing tumors account for 19%–27%; poorly differentiated neuroendocrine carcinoma accounts for < 3%; and gangliocytic paraganglioma accounts for <2%. Duodenal gastrinoma can be sporadic or MEN1-ZES related and solitary/multiple. It is usually small. LN metastasis is common and is present in 5%–10% of cases at the time of diagnosis. The 10-year survival rate is 60%–80%. Somatostatinoma is ampullary or periampullary located. Approximately 20%–30% cases are accompanied by neurofibromatosis. Its clinical picture includes hepatitis and pancreatitis. Gangliocytic paraganglioma is located in the periampullary region, with >2-cm diameter, muscularis propria involvement, and benign behavior. Poorly differentiated NEC is located in ampullary/periampullary region and is nonfunctional; LN and distant metastasis are present at the time of diagnosis. Ampullary duodenal cancers are aggressive, advanced stage, and poorly differentiated tumors with high mitotic activity, distant metastasis, and poor survival. There is no consensus regarding its treatment. Follow-up is not recommended for even small tumors. Surgical resection is performed (17).

4.4. Small intestine

It is the most prevalent small intestine tumor. Its early stage diagnosis is difficult. It is generally metastatic at the time of diagnosis. Long survival is possible. There are uncertainties regarding optimal treatment. A carcinoid crisis may occur during the operation or anesthesia. It is most prevalently located in the ileum. Prophylaxis with octreotide may be required. The 5-year survival rate is 70%–100% in localized disease. Proliferation index is low. In the presence of distant metastasis, the 5-year survival rate is 35%–60%. Overall, 80% of the deaths are owing to liver failure or intestinal obstruction. Its treatment includes complete oncologic resection of the primary tumor, excision of regional lymph nodes, and excision of mesenteric fibrosis as much as possible. Duration of operation, quality of life, experience of the surgeon, and cost must be considered in its treatment (18).

4.5. Appendix

It is the most prevalent tumor of the appendix. It accounts for 0.2%–0.7% of all appendectomies. It is more prevalent in the 2nd and 3rd decades of life. It has the best prognosis among GI NETs. It is detected incidentally and usually in the G1 and G2 stages. G3 (with goblet cells), are mixed with aggressive, 20% metastatic adenocarcinoma. Its clinical course, treatment, and prognosis are different from those of other NETs. Its treatment is appendectomy for G1 < 1 cm, right hemicolectomy for G2 and G3 >2 cm (19) .

4.6. Colon

The mean age of patients with this cancer is 65 years. NETs are generally localized in cecum and are diagnosed at a late stage. Lesion diameter is >2 cm in 90% cases. There is metastasis in >50% cases at the time of diagnosis. In <5% patients, carcinoid syndrome is also observed. It has a poor prognosis. In its treatment, EUS is performed if the tumor measures 1–2 cm. Endoscopic treatment is usually not appropriate if >2 cm and over the submucosa; operation in compliance with oncologic principles (colectomy + LND) and high grade colectomy independent of size + LND are performed. Palliative resection can be performed in patients with metastatic disease (18).

4.7. Rectum

It accounts for 2% of all rectal neoplasia cases. It is usually located within the first 5–10 cm. The mean age of patients is 57 years. It is generally asymptomatic. It is the group with the best prognosis after appendix NETs. Overall, 80% tumors are <1 cm, 152% are 1–2 cm, and 5% are >2 cm. They are usually located in the submucosa. If it is >1 cm, over the muscular layer, and lymphovascular invasion is present, metastasis risk increases. Tumors sized <1 cm are treated with endoscopic excision; those sized 1–2 cm are treated with endoscopy/ surgery, and those >2 cm are treated similar to rectal adenocarcinoma. Surgery/chemotherapy is performed in G3 advanced disease (18).

4.8. Insulinoma

It is the most common functional pNET (35%–40%). Hypoglycemia and excess catecholamine excretion due to hypoglycemia are its most prevalent symptoms. Incidence rate is 1–3 in one million. Less than 10% are malignant. pNET are smaller than 2 cm in two-third of the patients. Its incidence peaks in the 5th decade. It is slightly more common among women. Approximately 10% cases are multiple, and 5% are with MEN-1. In surgical treatment, if the tumor

is present at >2–3 mm from the pancreatic duct, enucleation is performed; if enucleation is not possible, formal resection is performed. There is no need for additional lymph node dissection. Laparoscopy is beneficial; however, blindly performing distal pancreatectomy is not recommended. The role of surgery in those with MEN-1 is to release the tumor load (20).

4.9. Gastrinoma

Its prevalence is 16%–30%. It is localized in the duodenum and pancreas. It causes Zollinger–Ellison syndrome. Approximately 25% cases are related to MEN-1. A total of 60%–90% cases are of duodenal gastrinomas. Duodenal gastrinomas are usually small, may not be detected in preoperative examinations, and can only be detected with duodenotomy. Lymph node metastasis can be found in 40%-60% cases. Its treatment requires an experienced surgeon. Laparoscopy is not recommended; lymph node dissection is required. 30% of sporadic gastrinoma cannot be completely localized in preoperative examinations. Patients with pancreatic gastrinoma along with <2 cm MEN-1 have long-term life expectancy. Thus, surgery is limited in those with >2 cm lesions (21).

Other functioning tumors such as vipoma, somatostatinoma, growth hormone producing adenomas (GRHoma), and corticotropin secreting pituitary adenomas (ACTHoma) are usually large, and their metastasis is prevalent. Curative resection is rarely possible. Enucleation and laparoscopic surgery are not recommended (20,22).

In 54%–93% cases, gastrinoma is accompanied by MEN-1 owing to hereditary diseases (hyperparathyroidism - 100%; pNET - up to 75%; and pituitary tumor - <50%). Pituitary microadenomas are commonly <5 mm, and 50% patients develop Zollinger–Ellison syndrome, 20% develop insulinoma, and 3%–5% develop vipoma and glucagonoma owing to gastrinoma. In approximately 10%–17% patients, it is accompanied by Von Hippel–Lindau syndrome (VHL). It is rarely accompanied by nonfunctional (NF)-pNET and functional (F)-pNET. In 0%–10% cases, it is accompanied by neurofibromatosis (NF)-1). Duodenal somatostatinoma, NF-pNET, and F-pNET are rare; in <1% patients, gastrinoma is accompanied by tuberous sclerosis (23).

4.10. Pheochromocytoma–paraganglioma

It accounts for 0.2%–0.6% of HT cases. Its incidence is 2–8 cases/1,000,000 individuals. F=M, and it is more prevalent in the 4th and 5th decades of life. It can be hereditary in up to 40% cases. There is continuous/paroxysmal HT in 95% of the patients. Its symptoms include headache, palpitation, anxiety, and

sweating. Approximately 80%–85% tumors originate from the adrenal gland (pheochromocytoma), and 15%–20% do not originate from adrenal gland [paraganglioma (PGL)]. Overall, 10% of pheochromocytoma and 25% of PGL are malignant. In total, 90%–95% tumors are located in the intra-abdominal region and 85%–90% in the intra-adrenal area. Pheochromocytoma should be suspected in the presence of the classical triad of headache, sweating, and tachycardia; individuals with hyperadrenergic seizures; presence of HT at an early age (<20 years); resistant HT, accompanying early onset or atypical diabetes; in the presence of familial syndromes (MEN2, NF1, VHK, and family history of pheochromocytoma); adrenal incidentaloma; individuals who were hypertensive during anesthesia, surgery, and angiography; individuals with idiopathic dilated cardiomyopathy; and individuals with a history of gastric stromal tumor or pulmonary chondroma (Carney triad). Tests performed for pheochromocytoma are as follows: in 1–24-h urine, assay of total metanephrines and catecholamines and plasma catecholamines and metanephrines. Moreover, in 24-h urine, VMA assay, clonidine suppression, regitine and glucagon stimulation, chromogranin A, and neuropeptide Y are also tested. In diagnosis, for 24-h urine fractionated metanephrine and catecholamines; Normetanephrine > 900 mcg or Metanephrine > 400 mcg, Norepinephrine > 170 mcg, Epinephrine > 35 mcg, Dopamine < 700 mcg are significant for diagnosis. For plasma fractionated metanephrines, in previously catheterized fasting patients, metanephrine < 0.3nmol/L and/or normetanephrine < 0.66 nmol/L, diagnoses are excluded. In fed patients who are sitting down from whom direct sample is collected, if metanephrine is <0.5 nmol/L and/or normetanephrine is <0.9 nmol/L, then the diagnosis is excluded. Functional monitoring in pheochromocytoma are adrenal/extraadrenal pheochromocytoma and PGL diagnosis and localization in patients with high Catecholamine, evaluation of nonfunctional adrenal lesions, evaluation of presurgical metastasis and multiple foci, evaluation of retroperitoneal extrarenal masses and suspected lesions in the head-neck region, patient selection for goal directed radionuclide treatments, response evaluation in CT and goal directed treatments, Active monitoring of some hereditary mutations such as SDx, VHL and guidance for radiotherapy indications (24). **Metaiodobenzylguanidine (MIBG-I131, I123, I124):** it imitates norepinephrine, a guanethidine analog. It enters the cell with MIBG active transport and is stored in the neurosecretory vesicles. It was first used in 1970 in Michigan University Medical Center in the imaging of adrenal medulla. In 1981, it was introduced for clinical use and began to be used in the diagnosis and localization of pheochromocytoma. **Indium-111 Octreotide**: it is the analog of somatostatin; its sensitivity in head-neck PGL is higher than

that of MIBG. Its use is recommended in patients with MIBG(−) patients with high clinical suspicion. Today, the most sensitive Ga-68 DOTA peptides are used. **F18-DOPAMINE PET**: Catecholamine precursor enters the cell and is stored in the intracellular vesicles with volumetric arch treatment (VMAT). Ii is recommended that it should be preferred in sympathetic paragangliomas. It is superior to MIBG in metastatic diseases. Better image resolution and shorter duration with PET are its advantages. Presence of normal surrenal involvement and the difficulty in obtaining it are its disadvantages. **F-18 DOPA (Fluoro-L-dihydroxyphenylalanine):** Endogenous catecholamine precursor is taken into the cell with large amino acid 2 (LAT-2). Signal/noise ratio is extremely high. It should be preferred as the first step in head–neck PGL (sensitivity is almost 100%). It can detect small lesions missed in conventional imaging. Its sensitivity is low in those with SDHB mutation. **F-18 fluorodeoxyglucose (F18-FDG) PET**: Similar to that in thyroid cancer and other NETs, there is FDG involvement associated with dedifferentiation. FDG involvement increases in some mutational PGLs also. **Brown adipose tissue (BAT) activation:** Increase in the amount of catecholamines in circulation provides alpha and beta receptor and BAT activation, and BAT increases beta receptors and GLUT activity. Consequently, FDG involvement increases. BAT should be suppressed with beta–alpha blockage before FDG imaging. **Ga-68 DOTA peptides**: Involvement of somatostatin analogs is associated with the receptor density on the cell surface and the internalization degree of the receptor–ligand complex. PGLs exhibit strong SSR-2 expression. SSR5 expression is low. There is strong SSR2 expression in some PGLs. Peptides used for this purpose are DOTATATEC, DOTANOC, and DOTATOC. With **somatostatin receptor imaging** (SSRI), there is less background activity, and a higher number of lesions can be detected. Patients can be selected for PRRT with SSR imaging. Owing to problems such as BAT activation and blood sugar increase in FDG, SSRI is more sensitive and specific. I-131 MIBG treatment, peptide receptor radionuclide treatment (PRRT) (Ytrium-90 or Lutesium-177), and meta211At-astato-benzylguanidine (At211-MABG) are among the available radionuclide treatments. **I-131 MIBG treatment**: it was used first in 1984 by Sisson et al. in metastatic pheochromocytoma. It is preferred in inoperable pheochromocytoma and PGLs which show adequate MIBG involvement in scintigraphy. **PRRT:** Ytrium-90- and Lutesyum-177-marked DOTA peptides are used. It also has indications for metastatic or inoperable pheochromocytoma/PGL which show SSR-2 expression. **MABG:** Astatine 211 shows effects with alpha emission. In cell culture and animal studies, pheochromocytoma results in quite a decrease in tumor volume without any severe side effects (25). Presence of

rarely malignant but SDHB mutation in MEN and VHL syndromes increases adrenal and extra-adrenal malignancy. Minimizing surgery in metastatic tumors, RT, cryoablation, or surgery in painful bone metastases, thrombotic therapy or radiofrequency ablation in unresectable liver metastases, radiotherapy or I-131 MIBG irradiation in unresectable tumors, and long effect octreotide if SSRI is tumor positive are preferred. Laparoscopic, robotic, and open methods are used in adrenalectomy (24).

4.11. Carcinoid syndrome (CS)

It is the most common among the ectopic hormone syndromes of NETs (20%). It was first defined with the name insulinoma in 1902; however, in 1954, it was named as carcinoid syndrome, and in 1955, it was named Zollinger–Ellison syndrome. It is a paraneoplastic syndrome which develops with the excretion of various humoral factors, is characterized by vasomotor changes (flushing and hypotension), diarrhea, bronchospasm, and fibrosis and has a poor prognosis (26). It primarily develops in well-differentiated midgut NETs with hepatic metastases. CS prevalence has increased by 72% from 2000 until 2011. According to the SEER database in USA, out of 9512 available patients aged >65 years, carcinoid syndrome was detected in 1786 (19%). It was found that compared with the non-CS NETs, carcinoid syndrome is more prevalent among females and in individuals of the white race; it has lower tumor survival at the advanced tumor stage, is associated with the use of octreotide; chemotherapy is more common, and its surgical treatment chances are lower (27).

The relationship between genetic changes (mutations) and cancer has been demonstrated in many studies; currently, it is known that there is a correlation between cancer and genomic indecision. It was reported that in the pathogenesis of NETs, mutations such as point mutation, deletion, methylation, chromosome loss, and missense can be effective factors. As a result of the inability to repair the damages occurring in DNA or their inaccurate repair, indecision in genome occurs. DNA damage can occur owing to the effect of exogenous or endogenous factors. Free radicals produced by endogens and the reaction products of these lead to some mutations such as oxidations and single- and double-chain fractures and deletions in the DNA. There are techniques used to determine the DNA damage. Comet method is a method commonly used in the determination of the fractures in the DNA single or double helix at a cellular level, and its validity is accepted (28).

The genes related to familial forms are RET, VHL, NF1, SDHD, SDHAF2, SDHC, SDHB, SDHA, TMEM 127, and MAX (28).

4.12. Prognosis

The 10-year survival rate in stage 1 and stage 2 is 100%, whereas in stage 3, the 5-year survival rate is 95% and the 10-year survival rate is 80%. The presence of specialized, experienced, multidisciplinary (endocrinology + gastroenterology + oncology + surgery + pathology + nuclear medicine + radiology) reference centers is one of the strongest factors improving the prognosis in NETs (11,12).

5. Neuroendocrine Cell Markers

There are 15 different types of neuroendocrine cells in the gastrointestinal system and pancreas. Histamine is excreted from the ECL cells in the stomach corpus; gastrin is excreted from the antral G cells of the stomach; serotonin and Substance P are excreted from the EC cells in the intestines; glucagon-like-peptide and peptide YY are excreted from the L cells; somatostatin is excreted from the D cells; and PP (pancreatic polypeptide), glucagon, insulin, and somatostatin are excreted from the langerhans islets of the pancreas (29).

5.2. Functional Imaging in NETs

Molecules used as radionuclides in somatostatin receptor imaging are In-111, Tc-99 m, and **Ga-68**. Those with chelating properties are DTPA, HYNIC, and **DOTA**. Agents with peptide structure are octreotide acetate, Tyr-3-octreotide (**TOC**), NaI-octreotide (**NOC**), Tyr3, Thr8-octreotide (**TATE**), and lanreotide. The somatostatin receptor SSTR2 is expressed the most in NET. SSTR1 has high specificity for prostate, SSTR3 has high specificity for hypophysis adenoma, and SSTR5 has high specificity for stomach cancer and hypophysis adenoma. **Superiorities of PET/CT performed with Ga-68-labelled peptides on receptor scintigraphy**: Imaging a multitude of lesions which are smaller and which have lower receptor expression with high spatial resolution. SSTR2 affinity is stronger, more selective, and has higher internalization ratios. Imaging starts in a short time (45–60 min. vs. 24–48 h). Imaging duration is shorter (12–15 min vs. 45–60 min). Absolute quantitative evaluation shows correlation with receptor density. A lower effective radiation dose is adequate. Ga-68 generator product (Ge-Ga68 generator, t1/2: 270.8 days) is used for this purpose (30). On PET/CT performed using Ga-68 DOTA peptides, tumor diameter of <5 mm, disease being masked owing to normal physiological involvement areas, atypical SSTR expression (such as insulinoma), being a poorly differentiated phenotype (can be imaged with FDG PET/CT), and receiving octreotide treatment may cause false negatives. Conversely, there may be false positives caused by varying SSTR expression in the pancreas, presence of accessory spleen, contamination,

inflammation (changes after reactive lymph node, prostatitis, and radiotherapy), increased osteoblastic activity (degenerative bone disease, fracture, and vertebral hemangioma), benign meningioma, and epiphyseal growth plaques (30).

5.2.1. Somatostatin receptor imaging

Diagnosis-first staging sensitivity is 91%, specificity is 94%, postsurgical staging restaging sensitivity is 78.3%–100%, and specificity is 83%–100%. Change in the treatment approach is observed in 45% cases. It is used in the detection of primary focus or metastases, staging, determination of presence and absence of SSTR, direction of treatment plans, restaging as a result of the selection of PRRT candidates, prognosis assignment, catheterization in masses for which endoscopic or percutaneous biopsy is not possible, and finally, in the evaluation of treatment response (31). **18F-FDG PET/CT**: It is transported into the cell via glucose transporters, where it is phosphorylated by hexokinase and then it stays in the cell. It does not enter the glycolytic pathway. **18F-FDOPA PET/CT**: Imaging is performed with 6-fluoro (18F)-L-3,4-dihydroxyphenylalanine; there is involvement in tumors of amine precursor uptake and decarboxylation (APUD) system, and tumoral involvement increases when the peripheral aromatic amino acid decarboxylase (AADC) transformation is inhibited with carbidopa. Physiological involvement areas are basal ganglions, myocardium, skeletal muscles, and urinary system. The involvement in pancreas in suppressed with carbidopa. Indications are glomus tumors, pheochromocytoma–paraganglioma, neuroblastoma, medullary thyroid carcinoma, jejunoileal NET, insulinoma, and congenital hyperinsulinism. False positivity causes are multiple myeloma, hepatocellular carcinoma, schwannoma, myxoid chondrosarcoma, cervical squamous cell carcinoma, adrenocortical carcinoma, epiglottic, pancreatic pseudopapillary tumor, poorly differentiated adenocarcinoma, and hurthle cell carcinoma. It shows better performance than ^{111}In-pentetreotide scintigraphy and poor performance compared with Ga-68 SSTR PET/CT (32). Its sensitivity is better with ileal NETs owing to serotonin production and apparent diffusion coefficient activity. Cl (−), SSR PET/CT (−) sensitivity in NETs without known primer is 44%, and it is a good alternative, particularly in well-differentiated tumors with high expression of biomarkers and production of serotonin. However, it cannot direct peptide receptor-targeted radiotherapy (PRRT) (32).

5.2.2. Imaging with GLP-1 analogs

The process is performed via the G-protein–mediated hormone receptor located on the GLP-1R pancreas islet cells. It is known that the numbers of islet cells are higher in insulinoma. Therefore, ^{111}In DOTA-exendin-4, In-111 DTPA-exendin-4,

and Tc-99 m HYNIC-exendin-4 agents are used. Moreover, the accuracy rate in the used Ga-68 DOTA-exendin-4 is 93.9%, and its contribution to the change in treatment approach is 42.3% (32). **PET/MR:** Complete body multiphase MR vs. Ga-68 PET/CT is preferred. Sensitivity and specificity of Ga-68 PET/CT is higher in all G1–G3 GEP-NETs. In lesion-based analysis, generally, Ga-68 PET/CT is more successful. In organ-based analysis of lymph node and lung metastases, Ga-68 PET/CT is more successful, whereas MR (dwMRI) (Ga-68 PET/MRI) is more successful in that of liver and bone metastases. It is reported that Ga-68 PET/MR is superior to Ga-68 PET/CT in especially <1 cm metastases of G3 tumors (33).

5.2.3. Imaging with somatostatin antagonists

These agents have characteristics such as greater bonding area, appropriate pharmacokinetic characteristics, strong bonding, and slower separating despite the weak internalization, higher tumor uptake and tumor/base activity ratio, and possibility of imaging tumors other than NETs. There are two generations. The first generation somatostatin antagonists were found in the year 1996 (In-111-DOTA-BASS, In-111-DTPA-TATE, In-111-DOTA-sst3-ODN-8, and In-111-DOTA-NOC). Tumor uptake with these increased 2-fold despite low affinity. The second generation antagonists started to be used in 2008 (Ga-68- NODAGA-JR11, Ga-68-DOTA-TOC), and their affinity and tumor uptakes are higher. **Cu-64-DOTATATE, GA-68 DOTATOC:** It has lower positron range and better spatial resolution; a higher number of lesions can be detected. Its sensitivity is similar on patient basis. Physical half life is 12.7 h, and shelf life is 24 h (34).

6. Current Imaging Methods in NETs

Multi-detector computed tomography (MDCT) is a method performed with contrast agent by taking multiphase thin slices without the use of oral contrast agent but with the use of intravenous (iv) 4–5 ml/s 100–120 ml contrast agent to perform late arterial, portal venous (30–35 s, 60–70 s) imaging. NETs in pre-examination are usually (hypervascular) lesions which get dense contrast in the arterial phase after the isodense contrast. Magnetic resonance imaging (MRI) is performed with 1.5 or 3 Tesla devices, multichannel surface coils are used, contrasted dynamic multiphase analysis, diffusion weighted imaging, and when needed, MR enterography are used, and for metastases, hepatocyte specific contrast agents are utilized. Apart from these, there are USG, MRI, PET, PET (Galyum 68) DOTATA, and endoscopic methods (Endoscopy+biopsy/colonoscopy+biopsy, capsule endoscopy, balloon enteroscopy, and EUS+ EUS-FNA/FNB) (35).

It is recommended in the preoperative period for each patient to undergo morphological imaging (MR or CT) and if possible, definitely undergo somatostatin receptor scintigraphy (octreoscan or PET gallium 68-DOTA-TOC/ NOC/ TATE) and in selected cases undergo PET F-DOPA, F-DG, and C-5HTP imaging. Moreover, endoscopy (upper endoscopy, colonoscopy, capsule endoscopy, and endoscopic USG) can provide additional benefits. CgA of the serum markers should be checked. CgA may be normal in undifferentiated tumors and in this case, NSE should be checked. In small intestine carcinoid tumors, in addition to plasma CgA levels, urine 5IAA level should be checked; moreover, if functional NET clinic is available, related hormone levels should be measured (11,12).

6.1. Endoscopic Sonography (EUS)

GEP-NETs are heterogeneous tumors characterized by highly variable clinical symptoms. Because the diagnosis is usually late, approximately 60% are advanced stage, and average survey duration is 33 months. Terminology classification differs, histological diagnosis is difficult, and treatment approaches are different.

GEP-NETs are the second most common GIS tumors, and their prevalence increases, particularly in the small intestines. Approximately 85% of the NETs exhibit gastrointestinal system involvement (1). EUS was developed in the beginning of 1980s. It is a version of endoscopes with ultrasound attached to the end. There are three different EUS devices (radial, linear, and miniprobe EUS). There are also EUS elastography, contrast-enhanced EUS, and 3-dimensional EUS methods. The objectives of elastography use are to evaluate the rigidity of the lesion and to discriminate between benign and malignant, to decrease the rate of unnecessary biopsy, to determine the area for biopsy, and to estimate the benign/malignant differentiation of the lymphatic glands. Tissue rigidity is malignant lesions/benign:100/1.

6.2. Contrast-enhanced EUS (Dynamic ultrasonography)

Vascularization of the mass shows whether it holds contrast. It differentiates between benign and malignant. In benign lesions, there is contrasting in the early vascular phase and late parenchymal phase. In the malignant lesions, although contrasting is present in the early vascular phase, there is no contrast holding in the late phase, and there is contrast defect in the parenchymal phase. Standard EUS devices are used in esophagus, stomach, ampulla of vater duodenum, and rectum pathologies, whereas miniprobe is used in colon and terminal ileum.

Moreover, standard EUS devices are used in the pancreatic NETs. Endoscopic sonography is beneficial in determining the size of the NETs, the layer from which they originate, their internal echo pattern, invasion depth, and lymph node involvement/biopsy to be performed from the lesion (EUS/FNA) (36).

7. Imaging methods used in the diagnosis of pancreatic NETs

USG, CT/Multi detector CT, MR, somatostatin receptor scintigraphy, PET, and angiography are used in the evaluation of metastatic cases (37-39). MDCT is quite effective and usually the primarily preferred method. In previous studies, sensitivity is approximately 69%–94%, and specificity is approximately 83%–100%. In the studies conducted using MRI, sensitivity is 74%–94%, and specificity is 78%–100%. It was found to be quite effective in small lesions. In the recent years, small pNETs are caught more frequently using the new imaging methods. Despite this, approximately 30% of the lesions cannot be diagnosed preoperatively using the current examination methods. This group includes those with negative somatostatin receptor, and 50% are nonfunctional small lesions (40).

8. Hepatic NET

It is quite rare. CT and MRI characteristics of the lesion are the same with metastases. Its radiological differentiation is difficult. When a lesion consistent with NET is detected, extrahepatic scan should be performed. Liver metastasis is one of the most frequent locations for metastasis in malignant NETs. CT generally reveals lesions showing washout in the arterial hypervascular venous phase (70%). However, there is also hypovascular contrasting observed at a 15% rate and hemangioma-like contrasting at a small rate. Sensitivity is 82%–100%, and specificity is 83%–100% (41).

9. Lung NET CT Results

Low- and high-grade differentiation is not always possible with CT results. They are more centrally located (hilar and parahilar). They are well circumscribed or slightly lobulated solid lesions. Atelectasis and consolidation may accompany. Punctate or diffuse calcification can be observed in 30% cases. Generally, contrasting of >30 Hounsfield units (HU) is observed. There are speculated non-circumscribed borders, surrounding tissue (vascular structure) invasion, and heterogeneous contrasting in higher grades (42).

10. Pheochromocytoma–Paraganglioma

In CT imaging, in the non-contrast series of >10 HU, it shows cystic degenerations, necrosis, and calcifications with heterogeneous dense contrasting and extended contrast washout time. In MR imaging, distinct T2 brightness and absence of signal suppression in phase-out phase are important. It is accepted that the diagnostic accuracy of endoscopic USG depends on the experience of the endoscopy operator in gastro–entero–pancreatic NETs based on major guidelines, and its advantages compared with some groups are as follows: it increases diagnostic precision of the morphological imaging in the T/N staging, it enables tissue diagnosis, it shows better respectability, and in the postoperative period, it is superior to MR and CT (43). In pNETs, endoscopic sonography is usually requested when there is pNET suspicion clinically or according to laboratory results and CT/MR is generally requested when the contrast holding of the lesion cannot be determined. EUS/EUS-FNA advantages: the probe allows very close examination of the pancreas from the GIS wall and acquiring high-resolution images, thereby helping in the detection of small lesions and assisting in cyto/histopathologic diagnosis (44-46). In pNET diagnosis during EUS, isoechoic, small, tail tumors with pedicle, false-negative results can be acquired in the additional presence of peripancreatic LAP (47). Diagnostic success of EUS is not dependent on the location of the tumor (48). Those located in tail and body are smaller and in the diagnosis of these pNETs, EUS is superior to other imaging methods (47). In the grading of pNET, Ki-67 index which is usually made of surgical materials is used. The correlation between EUS results and pNET grade is not fully known. Most of pNETs are of grade 1 and 2. According to EUS results, it is not possible to differentiate grade 1 and 2. EUS-FBN should also be performed (49). Ki67 index is important because it helps in prognosis and predicts the response to chemotherapy (50). The presence of K-ras mutation also shows that the lesion is malignant (51,52). EUS-FNA/FNB helps both IHC analysis and grading.

11. Imaging (Insulinoma)

68Ga-DOTA-peptid-PET/CT (increased SSTR 2 affinity), DOTATATE (DOTA, Tyr (3)-octreotate), DOTATOC (DOTA, D-Phe1, Tyr (3)-octreotide), and DOTANOC (DOTA, 1-Na(3)-octreotide) (the most SSTR5 affinity) do not differ from each other. In a previous study, 31 insulinoma cases were retrospectively examined. In total, 6/28 (21%) cases were diagnosed with USG, 17/31 (55%) with CT, 17/28 (61%) with MRI, 9/10 with 68Ga-DOTATATE PET/CT (90%), 29/31 (93.5%) with intraoperative USG, 28/30 (93% with selective arterial

Ca stimulation hepatic venous imaging, and 19/23 (83%) with intraoperative palpation. In this study, the level of 68Ga-DOTATATE PET/CT sensitivity was detected. Moreover, GLP-1 receptor expression is observed in several cancer cells (53). There are high levels of GLP-1 in insulinoma, and imaging using GLP-1 radioligands bonding with GLP-1 receptor is a new method. In malignant insulinoma, because there are usually no GLP-1 receptors, it was detected to be more effective in benign insulinoma. Apart from this, in the study conducted with the use of ^{111}In-labelled GLP-1R agonist, ^{111}In-DOTA-exendin-4 scintigraphies were diagnosed in 6/6 (54). In another study by the same group, 23/30 were diagnosed with ^{111}In-DTPA-exendin-4 SPECT/CT (55). In the study by Luo Y, insulinoma was diagnosed in 42/43 cases using 68Ga-NOTA-exendin-4 PET/CT (56).

In the recent years, selective angiography is performed less due to the increase of tumor detection in functional imaging methods and as it is an invasive method, and it is preferred in multiple lesions, in patients with negative scintigraphy results or after endoscopic ultrasonography (EUS) (57). Another method is intraoperative USG.

12. Postoperative Follow-Up and Treatment Response

It is recommended as a standard to perform biomarker and morphological imaging once every 3–6 months. Functional imaging is performed at 18–24 months. If there is rapid progression in the clinical picture of the patient and there is no progression in the morphological imaging or the CgA level, it is recommended to evaluate NSE and proliferative activity again.

13. GEP-NET Treatment

Surgery, local ablative treatments, radionuclide treatments, chemotherapy, and biotherapies [somatostatin analogs, interferon-alpha, the mTOR inhibitor Everolimus, the tyrosine kinase inhibitors Sunitinib and pazopanib (phase studies), and antihistamines diazoxide] are among the treatment alternatives. There is 98%–100% surgical cure rate in insulinoma. Cure rates are lower in vipoma and glucagonoma. If there is no widespread metastatic disease, operation is recommended. Malignant PNETs lead to liver metastasis at a high rate. Hepatic artery embolization, radiofrequency ablation, liver resections, and transplantations are performed in its treatment (58).

14. Surgery in Intestinal NET

Every patient is a candidate for curative surgery in small intestine NET.

Resection of the primary tumor and neighboring lymph-node metastases is a life-saving treatment option. Depending on the location of the primary tumor, intestinal resection or right hemicolectomy are the treatment options. In mesenteric and retroperitoneal explorations, because resection is mandatory in the case of metastasis, laparoscopic surgery is not suitable for this group of patients. Even in the case of far metastasis, surgery should be considered. Although it does not decrease mortality, mesenteric ischemia, fibrosis, and obstruction due to tumor can only be prevented with surgery. In these patients, postop mortality is evaluated to be <1% and postop complication <10% (58).

15. Surgery in Pancreatic NETs

The clinical picture of the patient is determined by assessing the tumor mass and the extent of the metastatic disease. Metastatic disease does not create a contraindication for surgery alone. As long as there is a respectable mass, surgical treatment should be considered even in the presence of hepatic metastases. Whipple, distal pancreatectomy, and enucleation are the treatment options. Because malignancy potential is higher among pancreatic NETs, regional lymph node dissection must also be performed. WHO-G3 (pancreatic neuroendocrine carcinoma) is considered to be inoperable as it is usually a widespread metastatic disease at the first diagnosis. There is enough evidence in literature supporting minimally invasive surgery for low-risk pNETs in the body and tail of pancreas. Conversely, minimally invasive surgery is not the recognized golden standard treatment for all pancreas resections (59). Overall, 35% of nonfunctional (NF) pNETs are incidental and asymptomatic. Approximately 26*61% comprise tumors of <2 cm. There are discussions regarding surgery or monitoring for the treatment plan of tumors of this diameter. There has been a 2-fold increase in pNETs in the last 16 years. There has been a 7-fold increase in incidental small NF-pNET (<2 cm). Tumors of >2 cm can be resected with surgery in NF-pNETs, and lymph dissection can be performed. It was believed that tumors of <1 cm could be monitored. According to some studies with short-term follow-up, it was stated that tumors of <2 cm can be monitored, and surgery would be appropriate in case they grew. Pancreatic enucleation is more appropriate for small pNETs. Follow-up is recommended once every 3 months in the first year and once every 6 months during the first 3 years. Moreover, surgery must be performed if there is a growth of >0.5 cm in a year (58).

16. Treatment in Advanced Stage Metastatic Disease

Gastro–entero–pancreatic NETs are the most prevalently observed tumors,

with grade 1-2 treatment indication. Liver metastases of NETs are the most important prognostic factor independent of the primary tumor region. Liver metastasis is observed in approximately 40%–60% of the GEP-NETs during diagnosis. Owing to its relatively slow course, diagnosis is frequently established at an advanced stage. They can be detected incidentally with radiology. In addition, they can present with a wide clinical picture such as symptoms caused by acid hypersecretion, serotonin syndrome, or carcinoid syndrome. Curative treatment of liver metastatic GEP-NETs is surgery. However, most of the cases exhibit metastasis at several locations in the body to be able to be removed surgically. Despite a large number of options, there is no general consensus regarding the optimal treatment sequence in patients with metastasis. Multidisciplinary approach is important (surgery, medical oncology, radiation oncology, interventional radiology, and nuclear oncology) (58).

If the metastatic disease is local or if at least 70% of the tumor can be resected, cytoreductive surgery is recommended. Local treatments alternative to the cytoreductive surgery include thermal ablation, radiofrequency (RF) ablation, laser thermotherapy, selective hepatic trans catheter arterial embolization, coembolization, and selective internal radiotherapy (SIRT) (60).

16.1.Thermal ablation: It is performed using heating (RF and MW), freezing (cryotherapy), and high voltage (IRE). It should be used in patients with NET metastasis ineligible for surgery. It is recommended to be used in patients with ≤5 lesions; however, the number also does not create absolute contraindications. Single lesion size of <3 cm should be preferred for the treatment to be effective. It can still be performed for lesions of up to 5 cm. Neighboring tissue (1 cm) should also be ablated during the ablation. Definitive contraindications include having extrahepatic diseases, Child C cirrhosis, active KC infection, uncorrectable coagulopathy, and main bile duct or major vein in invasion (60).

16.2. RF ablation (RFA): It is the most frequently used and the most prevalent ablation method. The purpose is to induce coagulation necrosis in the tissue as a result of increase in heat increase using electromagnetic energy and thermal damage. Tissue temperature of 50°C–60°C for 4–6 min causes irreversible necrosis. Between 60°C and 100°C, mitochondrial damage and coagulation necrosis occurs in the tissue. The tissue is carbonized and vaporized at 100°C –110°C. Cytotoxic damage is adequate in the breakdown of tumor tissue. Therefore, the temperature of 50°C–100°C for 4–6 min is sufficient. The most important factor negatively affecting the success of RF treatment is the blood

flow in the neighboring vein. Blood absorbs by carrying the heat in the tissue (heat sink effect). Various methods have been attempted to prevent this effect. For example, temporary portal vein flow can be prevented during laparotomy and laparoscopic processes (Pringle maneuver). Selective embolization of the nutritive artery can be performed prior to the process in hypervascular tumors. There are studies regarding the increase in the effectiveness of RFA performed along with coembolization (TAKE) (60).

16.3. Microwave ablation (MWA): It is an ablation method with increasing use despite its introduction after RFA. In MWA, thermal effect is provided by acquiring heat as a result of friction due to quick oscillation of the water molecules. Because, unlike RFA, electromagnetic effect is not used, there is no need to place grounding pads on the patient. Multiple electrodes can be placed for tissue ablation in a larger area. SF or CO_2 circulation is applied to prevent the heating up of the whole needle. Compared with RFA, heat sink effect in MWA is much less. Thus, it is much more effective in tumors in neighboring large vascular structures such as hepatic vein and VCI. Another advantage of MWA is that it can reach maximum ablation in a short span of time. The application is similar to RFA. One of the new needles is advanced up to the distal end of the lesion, and force is applied at the required level (Watt) and duration according to the desired ablation area. In the early period results of the studies, it is reported that RFA and MWA show similar results in the local control of tumor. Its complications are residue, relapse, post-ablation syndrome, abscess, biloma, hepatic necrosis, vascular occlusion, pseudoaneurysm, and biliary stenosis. Lesions close to the liver capsule can be treated despite the high complication risk. In the case of neighboring gastrointestinal system structures, the distance between them can be increased using various methods, and the operation can be percutaneously performed. The methods are hydrodissection, pneumodissection, and balloon interposition. Lesions close to hilus are of importance owing to relationship with the main bile duct. In these lesions, if there is <1 cm distance between the lesion and the duct, the protection of the duct from the heat effect can be provided by placing a nasobiliary catheter and administering fluid infusion through the catheter during the operation to prevent the development of biliary stricture (60).

16.4. Cryoablation: Cryoablation can also be potentially performed for liver tumors in addition to RFA and MWA. It is not much preferred owing to the high complication rates and high costs at the beginning and is performed at few centers. The only advantage of this method is that owing to the ice ball it creates

during the operation, the ablation area can be simultaneously observed with US, CT, and MR (which is not possible with other techniques) (60).

16.5. Irreversible electroporation (IRE): IRE is a non-thermal ablative treatment and is different from the others. High-intensity current passes through the tissue via the electrodes, disrupts the cell membrane functions, and causes apoptosis. Basic tissue structure is protected while the tissues die. Recurrence rate for tumors of >3 cm is high. Because it is a newly developing technology and owing to high cost, it is performed at very few centers (60).

16.6. Transarterial embolization/chemoembolization (TAE/TAKE): It is known that hepatic NET metastases are hypervascular compared with many other liver primary or secondary malignancies. A large portion of this vascularization is caused by hepatic artery. In the meantime, normal liver parenchyma has unique blood sources from both the hepatic artery (20%–40%) and the portal vein (60%–80%). The mean duration of survival is 3–4 years, and the objective treatment response reaches 75%. TAE is usually performed using lipiodol with the objective of achieving ischemia and necrosis of the neoplastic cells by selectively embolizing the hepatic artery feeding the tumor. It has been demonstrated that TAE improves biophysical markers and palliative symptoms and makes the tumor lesions smaller. Unlike TAE, TAKE combines the blockage of the tumor blood flow with the intra-arterial application of cytotoxic medication. TAKE is preferred in clinical practice. It is applied with an embolic agent such as doxorubicin, streptozocin, gemcitabine, mitomycin C, 5-FU, or cisplatin–lipiodol (60).

16.7. Selective Internal Radiotherapy (SIRT): It is a good alternative for patients with contraindications for TAKE or high risk owing to its early period low side-effect profile. Radiation (Yttrium-90) tolerance of hepatocytes is less than that of cancer cells. Two types of microspheres are used in radionuclide treatment: resin and glass. Resin microspheres are not metabolized and not secreted. They can be used together with adjuvant chemotherapy (60). Complete surgical resection is necessary in localized tumors, and it is believed that neoadjuvant chemotherapy may be a new approach in invasion toward surrounding tissues or metastatic tumors which cannot be resected (61).

17. Local treatments in GEP-NET

These types of treatments must be performed with medical treatment. Partial or full recovery rate in clinical symptoms and results vary between 70% and 100% (time: 14–20 months). Radionuclide is administered to tumor cells expressing SSTR in a target-oriented manner with this treatment method. The presence of

strong involvement in the somatostatin receptor scintigraphy is important for PRRT.

TARE is appropriate for patients who are ineligible for PRRT treatment or showing progression in metastatic NETs. In liver-limited or liver-dominant metastatic disease, in the case of biliary obstruction in liver capsule pain, in paraneoplastic syndrome, in the presence of symptoms (diarrhea, flushing, insulin/ glucose abnormalities, HT), in lobar/bilobar, solitary or multinodular, non-infiltrative or infiltrative diseases, and in a first-line/second-line or treatment refractory condition, it can be repetitively applied when necessary.

Yttrium- and Lutetium-labelled DOTATOC/DOTATATE (^{90}Y-DOTATOC, ^{177}Lu-DOTATATE) treatments are not within this group. It is a treatment alternative for functional or nonfunctional GEP-NET with involvement in the somatostatin-receptor scintigraphy regardless of primary tumor location. Objective treatment response rate is 20%–40%, and the rate in pancreatic NETs is higher than in intestinal NETs. Compared with alternative treatment options, there is a need for prospective randomized controlled studies for these treatment(62). Because the renal radiation exposure of these treatments are high, it is suggested to take renoprotective measures owing to long-term renal damage to prefer it in the patient group with high survival and perform at experienced centers. According to ENETS 2016 criteria, radionuclide treatment indications are as follows: tissue diagnosis of NET, positive somatostatin receptor expression, overall good condition of the patient (Karnofsky index > 60% or ECOG < 2), and the tumor not being differentiated (WHO grade ½ and Ki67 < 20%). It is believed that they can be affected in refractory hypoglycemia in malignant insulinoma. It is recommended when medical treatments fail in widespread PNET (63).

18. Somatostatin Analogs (Octreotide and Pasireotide)

Somatostatin analogs are the first choice in functional NET. They can be combined with chemoembolization and chemotherapy. It is particularly preferred in glucagonoma, vipoma, and carcinoid tumors. Although there is approximately 75% decrease in the glucagon level and recovery in all patients in flushing in glucagonoma, there is no response to weight loss, diabetes, and thromboembolia. The medication improves diarrhea within 24 h in patients with vipoma (11). Symptomatic and biochemical recovery with octreotide is at a 30%–75% rate, and >50% decrease is observed in the tumor mass. Stabilization rate of the mass size is 25%–50%. Although it is not observed in patients with acromegaly, in NET patients, tachyphylaxis symptoms such as growth, dedifferentiation, and desensitization in the tumor are observed at 6–24 months depending on

the treatment (11,64). Individual differences in the response to the octreotide treatment are owing to the variability of the somatostatin receptor expression from person to person which changes from tumor to tumor. It is believed that this will be improved by providing personal dose titration (65).

There are studies investigating the effects of somatostatin analogs on survival in nonfunctional GEP-NETs. In the prospective, randomized, placebo-controlled PROMID study conducted in 2009, it was reported that octreotide LAR increased survival without disease progression in patients with metastatic midgut NET. In CLARINET, another prospective, randomized, placebo controlled study conducted in 2014, 204 patients with sporadic nonfunctional metastatic or inoperable GEP-NET were subcutaneously administered 120 mg lanreotide autogel once a month. At the end of 96 weeks, not only was there no disease progression but also there was a 52% decrease in death or progression. This effect was observed in patients with both high and low tumor load in the liver. Side effect profile was similar to the previous somatostatin analog studies (66). Pasireotide Octreotide LAR having 40 times more affinity to SSTR 1, 3, 5 receptors are preferred in unresponsive cases (67).

When somatostatin analog treatment is started, the general approach is to continue treatment until disease progression stops or toxicity develops. Side effects are steatorrhea, diarrhea, pain in the injection site, gallstone, nausea, hyperglycemia, bradycardia, cholangitis, and septicemia (>1%). Side effects are usually temporary. Its safety and effectiveness data have been validated since a long time. Its long-term tolerability is extremely good.

19. Interferon

It was first used on carcinoid tumor in 1982 owing to stimulation of natural killer (NK) cells. Interferon-alpha (IF) is subcutaneously administered at 3–9 million U/3–7 times a week. Although the biochemical response is 40%–50% in carcinoid tumors, the decrease in tumor was detected at 10%–15% ratio. In pancreatic NET, biochemical response was 47%, and decrease in tumor was 12%. The highest treatment response is observed in vipoma, followed by gastrinoma, nonfunctional NET, and malignant insulinoma (68). Although a significant increase is observed in the biochemical response with the addition of IF in chemotherapy, there was a significant increase in the biochemical response and in the tumor diameter stabilization rate when IF was added to the somatostatin analogs (58,69).

20. Proton pump inhibitors

High dose omeprazole (80–240 mg) is used in gastrinoma. Significant suppression is achieved in acid secretion on the 2nd day; however, because acid breaks molecules in the medium, it is recommended to combine with H_2 receptor blockers for the first 2 days. Moreover, 100% recovery is observed at 1 month of treatment. Diarrhea, headache, skin reactions, and peripheral edema may occur as side effects (11).

21. Treatment in Insulinoma

Medical treatment involves the use of diazoxide, verapamil, somatostatin analogs (octreotide and lanreotide), and steroids. They are used in surgical preparation for patients with insulinoma or in conditions when surgery is contraindicated. Medical treatment is necessary in malignant insulinoma to prevent hypoglycemia and to decrease the tumor load. It suppresses direct insulin excretion, provides hypoglycemia control in 50%–60% of the patients, and quickens glycogenolysis. No response was noted in metastatic disease. It is administered at 5–15 mg/kg dose. Nausea, hirsutism, and fluid retention are among its side effects. Thus, it should be used in combination with diuretics. Somatostatin analogs are effective in 35%–50% of the cases, and its effect is dependent on SSR2 presence in tumor cells. In some cases, it should be used with care because it may increase hypoglycemia by inhibiting contraregulatory mechanisms (11). In the recent years, the success rates of laparoscopic surgery are similar to those of open surgery (70). Another method, EUS-guided ethanol ablation, is used in some cases (71). CT-guided radiofrequency ablation method was performed by Limmer (72). Moreover, it is suggested for intraoperative insulin measurement to be performed especially in the presence of multiple lesions such as MEN-1. It has affinity for pasireotide SSRT-1, SSRT-2, SSRT-3, and SSRT-5 receptors and it especially inhibits SSRT-2 and SSRT-5 receptors (73). It is believed that it can be a new treatment option for hypoglycemia in malignant insulinoma.

22. Medical treatment in carcinoid syndrome

Motility regulators (codeine and loperamide) and cyproheptadine are used for diarrhea, and ketanserin and ondansetron are used for flushing. Because histamine is a factor especially in the clinic of gastric carcinoid, antihistamines are used in the treatment. Telotristat is used as a new agent effective in carcinoid syndrome refractory to somatostatin analog treatment. Tryptophan hydroxylase 1 (THP1) has high affinity for peripheral tissue, whereas THP2 has high affinity

for brain. Telotristat cannot pass the blood–brain barrier. Recommended dose with somatostatin analog is 3×250 mg peroral. Side effects are nausea-vomiting, constipation, anorexia, transaminase, GGT increase, headache, depression, fever, and peripheral edema. Telotristat is accepted as a promising new class of agent which particularly provides symptomatic treatment in advanced stage carcinoid syndrome (74). In another study, telotristat was added to SSA in patients with carcinoid syndrome and diarrhea and found effective and safe (75). The use of telotristat in carcinoid heart disease was considered based on guideline recommendations in the presence of distinctly increased 5-HIIA and valve disease in ECHO (76). If symptomatic progression occurs despite SSA, tachyphylaxis develops and it is accepted as refractory carcinoid syndrome; SSA dose increase, locoregional treatments, surgical debulking, Everolimus + SSA, PRRT, interferon, and Telotristat should be considered as other treatment options.

23. Vipoma Medical Treatment

Diarrhea is important to treat because it may cause dehydration, severe electrolyte disorder, and renal failure. Although the symptoms were improved in 80%–90% of the cases with somatostatin analogs (octreotide-LAR, lanreotide-autogel) along with fluid and electrolyte treatment, vasoactive intestinal peptide (VIP) levels decrease in 60%–80% of the cases. It is believed that Sunitinib, a tyrosine kinase inhibitor, is effective in the symptoms and biochemical control of vipoma and can be used in somatostatin analog resistant tumors (77).

24. Carcinoid crisis and Vipoma crisis treatment

Severe diarrhea and/or peripheral vasodilatation and hypotension occur owing to extreme peptide excretion. Along with CVP monetarization, fluid resuscitation, appropriate electrolyte replacement, and octreotide subcutaneous divided dose or intravenous infusion (50–100 mcg/h) should be administered at 1500 mcg/day maximal dose.

25. Glucagonoma Medical Treatment

Using somatostatin analogs (octreotide LAR and lanreotide autogel), necrolytic migratory erythema (NME) is kept under control in 80%–90% of the cases. Although there is a 60% decrease in glucagon levels, weight loss, and improvement in diarrhea, diabetes mellitus usually does not improve.

26. Target oriented treatments

mTOR signaling pathway plays a role in proliferation and progression of cancer

cells. Inhibition of this pathway is advantageous in PNET treatment. Everolimus, as an mTOR inhibitor, has been approved for use in unresectable, widespread, and metastatic PNETs. In patients with malignant insulinoma or those who are ineligible for surgical resection, it is effective in controlling hypoglycemia and can be the first treatment alternative (78). Out of the RADIANT studies, only RADIANT-2 is approved for use in patients with carcinoid syndrome (79). Sunitinib, an oral multireceptor tyrosine kinase inhibitor, is utilized for its antitumoral activity in neuroendocrine tumors (74).

27. Conclusion

Neuroendocrine tumors, including insulinoma, vipoma, and glucagonoma are rare tumors, and their treatment modalities are significantly increasing. Development of imaging methods for these conditions is underway, and target-oriented treatments are on the agenda. Personalized treatment is important in the management of the disease, a multidisciplinary team, including endocrinology, pathology, radiology/nuclear medicine, surgical, and medical oncology/radiation oncology might play a critical role. It is also believed that there will be newer treatment approaches, including personalized treatment, based on the clinical and biological behavior of the tumor and immunotherapy in the future.

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Chapter 5

The Detrimental Consequence of Sleep Deprivation: Male Infertility

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1. Introduction

Sleep deprivation (SD) is becoming a prevalent health problem worlwide, and much research are being conducted to explore its effects on the world's population. SD is described as the partial or near-total lack of sleep, which causes a variety of negative health effects on an organism (1). There is no doubt that we live in a sleep-deprived world because it is proven by multiple studies demonstrating that average daily sleep duration is 6-8 hours, compared to 9 hours a century before, and that over 30% of adults today sleep for less than 6 hours per night (2). A previous report revealed that recent sleeping habits of humans notably contradict those of the preindustrial era when humans were able to sleep for longer hours (3). SD has such negative consequences that human evolution has yet to devise mechanisms to compensate for the accumulated sleep debts. The pressure to meet basic socio-economic needs imposed by normal employment, daily routines, studies, and social commitments is the cause of continuous sleep deprivation reported among many strata of individuals. According to research conducted by the National Sleep Foundation, about 39% of respondents reported receiving less than seven hours of sleep on weeknights (4). Furthermore, evidence suggests that over the last few decades, self-reported sleep durations have decreased by about 2 hours (4, 5). Although the ideal sleep length for an adult is debatable, it is widely assumed that roughly 8 hours of nightly sleep is sufficient and best for overall health and wellness (6).

SD are linked to a variety of health issues in human, including depression, hypertension, glucose dysregulation, cardiovascular disease, and anxiety disorders. Sleep disturbances in women have been linked to postpartum depression, pregnancy, menopausal transition, and premenstrual dysphoria (7). Animals' reproductive ability is influenced by changes in the circadian timing system brought on by irregular light-dark cycles and mutations in key biological clock genes. Female shift workers have infertility and problems in their menstrual cycle because of this. Infertility is defined as the failure to conceive after 12 months of regular, unprotected intercourse in women under 35, or six months in women over 35 (8).

This review will focus on the negative effects of SD on the male reproductive system and try to explain how circadian timing deprivation directly affects spermatogenesis and indirectly affects male fertility via affecting the expression levels of some circadian rhythm-related genes affecting the regulating and maintaining spermatogenesis. Male infertility, one of the many negative effects of circadian rhythm disorders on the male body, are increasingly becoming an important health problem. For this reason, many studies have been carried out

on experimental animals to elucidate the mechanism underlying the destructive effects of sleep disorder and to reduce its negative effects. This review will also evaluate current approaches by presenting experimental studies on sleep disorders.

2. Negative effects sleep deprivation on human health

When there is either a complete loss of sleep for a certain period or a deficiency of the projected normal sleep length, sleep deprivation (SD) occurs. SD is primarily induced by a person's modern lifestyle as well as work-related factors such as shift work. Shift workers have reported several occurrences of cardiometabolic and reproductive problems. Sleeplessness or sleep fragmentation can disrupt sleep timing patterns (9) and have similar implications to severe acute sleep deprivation, including cognitive functions, attention, and operant memory (10, 11). SD has been linked to a variety of serious physiological repercussions in both human and animal models, including endocrine disruptions, metabolic, immunological, and cardiovascular dysregulations. Short sleep duration is linked to a higher risk of mortality in people, and long-term sleep deprivation has been proven to cause the death of animals in an experimental animal model (12-15). As a result, SD is predicted to be one of the leading sources of harmful stress, which can have a negative impact on an organism's well-being and mental health (16).

3. Epidemiological evidence for the role of SD on male reproduction

The importance of the circadian clock in proper male reproduction in humans is supported by epidemiological studies. Several studies have found that annual variations in human conception rates are related to the season (17, 18), as well as photoperiod, monthly averages of daily sunshine hours, minimum and maximum temperature, and humidity (19). Furthermore, semen quality varied both diurnally and seasonally (20): early morning semen samples had the highest levels of sperm concentration, total sperm count, and normal morphology. In both man and mice, evidence for daily diurnal variation in sperm DNA fragmentation index was found (21). Seasonal variations in sperm parameters have been found in various investigations, in addition to diurnal variations. Summer was linked to the highest proportion of normal morphology and significant decreases in sperm concentration and total sperm count, while spring was linked to the highest percentage of normal morphology and significant decreases in sperm concentration and total sperm count (20). Other studies have found that normozoospermic and oligozoospermic men have better sperm parameters in the spring than in the winter (22, 23), and Giorgi et al. (24) found a higher proportion of samples with normal sperm pH in the spring and a higher sperm volume in the winter. Circadian rhythm disruptors,

such as SD, provide additional evidence for the importance of the circadian clock in male reproduction. The circadian system is linked to sleep/wake cycles, and multiple lines of evidence link sleep to male reproduction (25). Sleep length has been linked to testis volume (26), male fecundability (27), total sperm number and volume (28, 29), and sperm chromatin integrity (30), however there is no conclusive evidence that sleep duration affects testosterone concentrations (31, 32).

4. Human studies

There is no evidence that uncommon, functional genetic variation plays a role in male infertility. In a single Turkish family with nonobstructive azoospermia, whole exome sequencing identified a homozygous mutation in the *NPAS2* gene (33). Rare functional genetic variations in human circadian genes, such as Cryptochrome 1 (*CRY1*) (34), *CRY2* (35), *TIM* (timeless) (36), and *CKId* (36), have been found, resulting in delayed sleep phase disorder and familial advanced sleep phase syndrome.

4.1. Hormones and clock genes affecting male fertility

Initially, the molecular mechanism that governs clock gene machinery was discovered in cells from a variety of tissues and animals. Following that, several research found that clock mechanism had an impact on fertility and reproductive success (37, 38). This relationship is not one-way, because reproductive hormones can alter clock-gene expression (39), implying a complicated web of interactions (40, 41).

4.1.1. Gonadotropins

The hypothalamic–pituitary–gonadal axis (HPG axis) and two hypothalamic neuronal populations, Kisspeptin neurons and gonadotropin-releasing hormone (GnRH) neurons, delicately regulate fertility (42, 43). The LH surge is mediated by Kisspeptin neurons in the anterior ventral periventricular area, whereas the hypothalamic Kisspeptin neuron population in the arcuate nucleus feeds metabolic information to the HPG axis and releases GnRH (44, 45). The hypothalamus releases GnRH in a pulsatile manner, which works on the anterior pituitary lobe (Adenohypophysis), controlling the generation of gonadotropins and eventually releasing FSH and LH into the bloodstream. LH is required for testosterone production in males, whereas FSH is essential in sperm formation (46, 47).

4.1.2. Estrogens and androgens

Androgens have some effects on fertility. Androgen levels fluctuate throughout the day, with morning high values (48). Androgens are required for the development and maintenance of secondary sexual characteristics in men, including libido, growth, osteoporosis prevention, and spermatogenesis (49). Furthermore, androgens have direct and tissue-specific effects on *Per2* gene expression, which could explain the effects on the timing system's developmental program (50).

4.1.3. Glucocorticoids

Glucocorticoids (GCs) are endogenous clock synchronizers produced by the adrenal glands (51, 52). Their regulation follows a diurnal cycle, with maximal levels at the start of the activity phase (53). It has been shown that a rise in GCs levels caused by a stress-mediated response (54) or exogenous GCs injection can significantly affect several organs, including the immunological and reproductive systems (55, 56). GCs decrease the synthesis and release of LH and FSH in the pituitary gland, whereas they directly inhibit steroidogenesis and/or gametogenesis and induce apoptosis in the testis and ovary (57, 58). Studies have reported that steroidogenesis is impaired in male rats exposed to GCs prenatally, with lower FSH and testosterone levels and, as a result, lower sperm quality (lower sperm concentration, motility, and aberrant morphology). Betamethasone (BX) exposure induces anomalies in the testes and epididymis, including a decrease in anogenital distance and changes in other histomorphometric parameters (59). Furthermore, it has been shown that not only the F1 generation, whose fathers were exposed to the BX prenatally, but also the F2 generation, suffers from fertility problems (60).

4.1.4. Melatonin

Melatonin is a neurohormone produced by the pineal gland and regulated by dark–light and seasonal cycles, with pleiotropic effects on cell and organ growth, development, aging, and function in both male and female reproductive systems (61). Human melatonin receptors have been found in a variety of cells, including preovulatory follicle granulosa cells (62) and spermatozoa (63). Semen samples with low and poor fertilization rates have been found to have decreased melatonin levels (62). It's also known that light from electronic screens suppresses melatonin production, which has been associated to a drop in sperm motility and concentration in men (64). However, some evidence suggests that too much melatonin can cause infertility (65).

5. Animal studies

Animal models provide strong evidence for the role of the circadian clock in male fertility. In animal studies, mutations in some important regulators of the circadian clock are linked to lower male fertility, albeit the reasons are yet unknown. Male fertility has been impaired in mice with homozygous mutations in the *clock* gene (66, 67), and it has been suggested that the serine protease inhibitor *Serpina3k* is implicated in the pathogenic mechanism via regulating acrosin activity (68). *Clock* has also been shown to interact with *RANBP9* and bind numerous critical RNAs during mouse spermatogenesis (69).

Male infertility has been linked to mutations in Bmal1, another major circadian *clock* gene (70). Low testosterone and high luteinizing hormone levels were found in male knockout mice, indicating steroidogenesis impairment in the testes and other steroidogenic organs. Both *BMAL1* and *clock* proteins are involved in the assembly and function of the chromatoid body, a cytoplasmic organelle that plays a critical role throughout the late stages of germ-cell differentiation, according to Peruquetti et al. (71). The *BMAL1* protein has also been linked to the maintenance of brain circuits that control pheromone-mediated mating behaviors (72).

The cryptochrome 1 (*Cry1*) gene knockout animals also had impaired testicular function (73). *Cry1* loss was linked to increased testicular germ cell death and lower sperm count, as well as altered expression of 375 testicular genes. Loss of circadian clock function caused by mutant period (*per0*) and timeless (*tim0*) genes in Drosophila melanogaster males results in impaired reproductive function (74).

In a study on laboratory rats, a statistically significant reduction in sperm motility was observed after seven days of sleep deprivation when compared to the control group, but an insignificant reduction in sperm counts of the testis and cauda epididymis was observed between the control and SD groups (16). Sleep deprivation resulted in a considerable increase in corticosterone concentrations, with a matching significant fall in testosterone concentrations in the SD groups. These findings are consistent with those of other research (75-77) that looked at steroid hormone levels in the blood of sleep-deprived animals. The hypothalamus-pituitary-gonadal (HPG) axis may be suppressed by high amounts of corticosteroid released during SD triggered by stressful stimuli, resulting in lower testosterone secretion (78, 79). Testosterone levels in the blood have been demonstrated to rise throughout sleep (80), peaking during the first episode of rapid eye movement sleep (81). Several studies on human and animal subjects have found that SD is linked to lower levels of androgens in the blood, such as testosterone (82), which might impair gonadal and sexual functioning, potentially reducing fertility (83).

Several neurotransmitters and neuropeptides, including serotonin (5-HT), influence testicular activity (84, 85). In Leydig cells isolated from golden hamster testes, serotonin and its receptors have been identified (86). The quantities of serotonin in the brain increase during SD, according to animal studies (87, 88). Serotonin has been shown to suppress testosterone production (86), hence the decrease in testosterone levels reported during SD could be due to serotonin-related testosterone inhibition.

Conclusion

The importance of the circadian rhythm in male fertility is supported by epidemiological data as well as genetic research in humans and animals. Based on the available evidence, we can speculate that both circadian rhythm disruptors and genetic variation in genes encoding important regulators of the circadian clock may have a minor impact on male reproductive capacity. However, the mechanism of the devastating effects of sleep disturbance on male fertility has not been fully elucidated. In addition, the effect of sleep deprivation on the expression of genes involved in spermatogenesis and spermatogenic cell differentiation is still unclear. In conclusion, it is quite necessary that studies that will develop both clinical and prospective therapeutic and preventive approaches should be develop to determine the importance of circadian rhythm in male fertility.

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Chapter 6

Applications Of Dendrimer-Based Drug Delivery Systems in Breast Cancer

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1. Introduction

Cancer is a disease based on a series of sequential mutagenesis steps in which cells grow and multiply uncontrollably. Lung, stomach, liver, colon and breast cancers are the leading cancer types that cause death. Breast cancer (BC) is the most commonly diagnosed cancer in the world, and it accounts for more than 24% of new cancer cases according to the World Health Organization [1, 2]. Factors such as being a woman, having a familial history of BC, late menopause, early onset of menstruation, and not giving birth at all are seen as risk factors for breast cancer.

There are many drug delivery systems studied for BC treatment. Because drug delivery systems have come to the fore recently due to some advantages in drug administration. These systems provide an increase in drug solubility and drug loading efficiency, extended drug release, passive/active targeting (with the enhanced permeability and retention effect or pH, temperature, light sensitive structures), and enhance drug concentration in the desired region, reduce cytotoxic effects on normal cells, cause fewer side effects [1, 3, 4]. In this way, drug delivery systems improve treatment safety and patient compliance [5]. Drug delivery systems can be listed as follows; dendrimer, liposome, phytosome, transfersome, polymeric micelles, nanosuspension, nano/microemulsion, nano/microparticle.

In recent years, dendrimers have attracted great attention for BC treatment due to their nano-sized (5-100 nm), showing a narrow polydispersity index, and functionalization of the surface. In addition, their use in therapy has come to the forefront thanks to their advantages such as high drug loading capacity, easy synthesizing, stability, being functionalized as desired, being controllable in size, and effectiveness in active targeting [6]. In this chapter, dendrimers and studies on their use in BC treatment are reviewed.

2. Structure and General Properties of Dendrimers

In addition to linear polymer technologies, dendrimers are macromolecular with highly branched structures and differ from other conventional polymers. These multibranched molecules were discovered by Fritz Vogtle in 1978, by Donald Tomalia et al. in the early 1980s, and also but independently by George R. Newkome. The word “dendrimer” is a combination of two words, the Greek word “dendron” meaning tree and “meros” meaning unit. These are also called “cascade molecules”. Dendrimers are nano-sized, well-defined homogeneous and monodisperse systems consisting of tree-like atoms or branched structures as a new polymer. It has a narrow polydispersity as a drug delivery system and

provides ease in passing through biological barriers. (e.g. They are small enough to reach vascularendothelial tissues under extravasation) [7]. Dendrimers are macromolecules containing symmetrical or non-symmetrical branching units formed around a small molecule or a linear polymer core [7]. Dendrimers are preferred in a wide variety of biomedical and industrial applications. These are versatile polymers with similar dimensions and physicochemical properties to biomolecules such as proteins [7, 8].

Dendrimers are highly preferred in drug delivery systems and different drug administration routes, as they are water-soluble, highly bioavailable and biocompatible molecules. Since dendrimers are synthesized step by step from branched monomer units, it is possible to provide control over molecule size, shape, size, polarity, density, solubility, and flexibility by choosing different branching units and surface functional groups. Dendrimers provide encapsulation of hydrophobic drug molecules especially low generation forms as they have internal cavity spaces and open conformation. In addition, they also have a much higher density of surface functional groups compared to conventional macromolecules [7, 9].

Dendrimers are structures that are added through a repetitive series of chemical reactions, with the starting atoms of carbon and nitrogen and other elements forming a spherical branching structure. With the repetitive processes, layers are added and the desired size can be created. Its final form is the macromolecular structure that is spherical in size. These structures are similar to albumin and hemoglobin in blood [7, 10]. A typical dendrimer structure consists of three components:

- ✓ An initiator core determines the size and shape of the dendrimer;
- ✓ Generations of repeating units connected radially to the inner layers or inner core determine the amount of space that can be closed by the dendrimer;
- ✓ Any structure added to the surface allows dendrimer or other chemical modifications to grow [9].

3. Nomenclature of Dendrimers

In the production of dendrimers, the number of focal points is the production number when synthesized from the core to the surface. For example, a dendrimer that has four focal points on its way from the center to the periphery is called the 3rd generation and is denoted as “G3”. The core part of the dendrimer is shown in terminology as the “zero” generation “G0”. Therefore, the core structure is

not considered as focal points. In addition, dendrimers are also synthesized and named as half-generation (e.g. G3.5) [8, 9]. The general structure of dendrimers is shown in Figure 1.

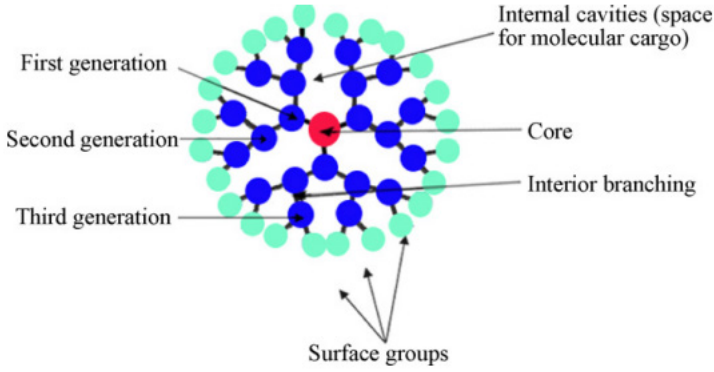

Figure 1. General structure of dendrimers [8]

4. Properties of Dendrimers As Drug Delivery Systems

Dendrimers, as drug delivery systems, must have certain properties in in vivo and in vitro applications. Minimizing the side effects of the drug can be achieved by changing the properties of the carrier. These properties;

- ✓ It should be non-immunogenic and non-toxic.
- ✓ It should be biodegradable.
- ✓ It must be able to cross barriers such as intestine, blood-tissue and cell membrane.
- ✓ It must be able to remain in circulation for the required time to achieve clinical effect.
- ✓ It should be targeted to specific structures.
- ✓ It must be compatible with the active substance and ensure its stability [9, 11].

The properties of dendrimers are being developed for pharmaceutical applications. By improving the pharmacokinetic and pharmacodynamic properties of a drug, an increase in bioavailability is also achieved. Dendrimer surfaces; It can be engineered with functional groups to increase or resist transcellular, epithelial, or vascular permeability. The space in the inner cavities allows for the encapsulation of small molecule drugs, metals or imaging agents. It contributes

to the reduction of toxicity and extended release of molecules encapsulated in this space [12]. Dendrimers with high generation neutral, apolar and cationic surface groups; It has been reported to be more biocompatible compared to the dendrimer with low generation, anionic or neutral polarity terminal surface groups. Dendrimer modified with small functional groups or polyethylene glycol (PEG) has less or no immunogenicity. Thanks to its nano-sizes, it is easier to be eliminated from the body [8, 11, 13].

Dendrimers have lower viscosity compared to linear polymers. As the molecular mass of these structures increases, their viscosity peaks in the fourth generation and then begins to decrease. This is different from linear polymers. Dendrimers have high solubility, miscibility and reactivity due to the presence of many chain ends. These drug delivery systems have solubility in many solvents. While dendrimers terminating in hydrophilic groups dissolve in polar solvents, dendrimers with hydrophobic end groups can also dissolve in non-polar solvents. Unlike conventional linear polymers, stepwise synthesized dendrimers have well-organized structures with a very low polydispersity. Because it has versatile functional groups, it plays a role in providing multiple interactions with biological receptor sites such as antiviral therapeutic agents [8, 11, 13].

5. Types of Dendrimer

PAMAM Dendrimers- Polyamidoamine (PAMAM) dendrimers are available in spherical or elliptical form. They are synthesized using ammonia or ethylenediamine as the core material. Their high solubility and reactivity is due to the presence of functional end groups and internal cavities on the surface. PAMAM dendrimers are commercially available, usually in methanol solution. Starburst trade name dendrimers are a PAMAM subtype dendrimer based on a trisaminoethylenimine core. The origin of the trade name is due to the star-like parts observed when looking at the structure of this type of high-generation dendrimer [8-10].

PAMAMOS Dendrimers- Polyamidoamine Organosilicon (PAMAMOS) is the first commercial dendrimer containing silicones. The inner part is made of hydrophilic and nucleophilic polyamidoamine (PAMAM), the outer parts are hydrophobic organosilicon (OS) and its structure is reversible non-molecular micelles. These dendrimers are extremely useful precursor molecules for the preparation of honeycomb-like networks with nano-sized PAMAM and OS domains [8, 10].

PPI Dendrimers- Polypropyleneimine (PPI) is one of the earliest known dendrimers originally developed by Vogtle. The core structure is based on

diamino butane (DAB) structure with primary amines as the terminal group and tertiary propylene amines inside. These dendrimers are also referred to as "DAB-dendrimers". PPI dendrimers are commercially available up to G5 and are widely used in materials science and biology. These AstramolTM are commercially available [8-10].

Tecto Dendrimers- Consists of a therapeutic agent-free core surrounded by other dendrimers, it has structures that can diagnose and treat simultaneously. With different compounds; It can perform various functions such as diagnosis of disease state, distribution of drugs and reporting of treatment results [8, 14, 15].

Liquid Crystalline Dendrimers- It is a highly branched oligomer or dendritic polymer containing mesogenic groups that can show mesophase behavior. These are mesogenic (liquid crystalline) monomers. For example, mesogen-functionalized carbosilane dendrimers can be given [8, 9, 15].

Multilingual Dendrimers- These dendrimers consist of multiple copies of a particular functional group on their surface [8, 9, 15].

Chiral Dendrimers- Chirality in these dendrimers is based on the attachment of structurally different but chemically similar branches to the chiral core. Chiral structures for enantiomeric resolutions and their potential use as chiral catalysts for asymmetric synthesis are known [8, 14, 15].

Hybrid Dendrimers- Copolymer structures containing both dendritic and linear polymers. Hybrid dendrimers are obtained by fully monofunctionalizing peripheral amines of the "zero generation" [8, 16].

Polyethyleneimine dendrimers- Structures in the form of columnar and cubic self-arranged lattices with various layers that can be structurally modified [8, 9, 15].

Amphiphilic Dendrimers- Consists of two separated regions of the chain end, one half electron donating and the other half electron withdrawing [8, 9, 15].

Micelles Dendrimers- Single-molecule micelles of water-soluble hyper-branched polyphenylene micelles [8, 9, 15].

Peptide Dendrimers- These dendrimers are made up of amino acids as a branching or internal unit. It is a dendron based on the polylysine skeleton. This type of dendrimer, introduced by J. P. Tam in 1988, has been predominantly used in biological applications such as vaccine and disease diagnostic research [8, 9, 15].

Frechet-Type Dendrimers- A newer type of dendrimer developed by Hawker and Frechet, based on a polybenzyl ether hyperbranched skeleton. These dendrimers usually have carboxylic acid groups for increasing the solubility of hydrophobic dendrimer types. [8, 17].

Glycodendrimers- It is formed by connecting carbohydrate groups to the dendrimer structure. These dendrimers exist as either carbohydrate-coated or carbohydrate-centered dendrimers. These structures are used in many intercellular biological studies such as protein-carbohydrate interactions [8, 14, 15]. Dendrimer types are shown in Figure 2.

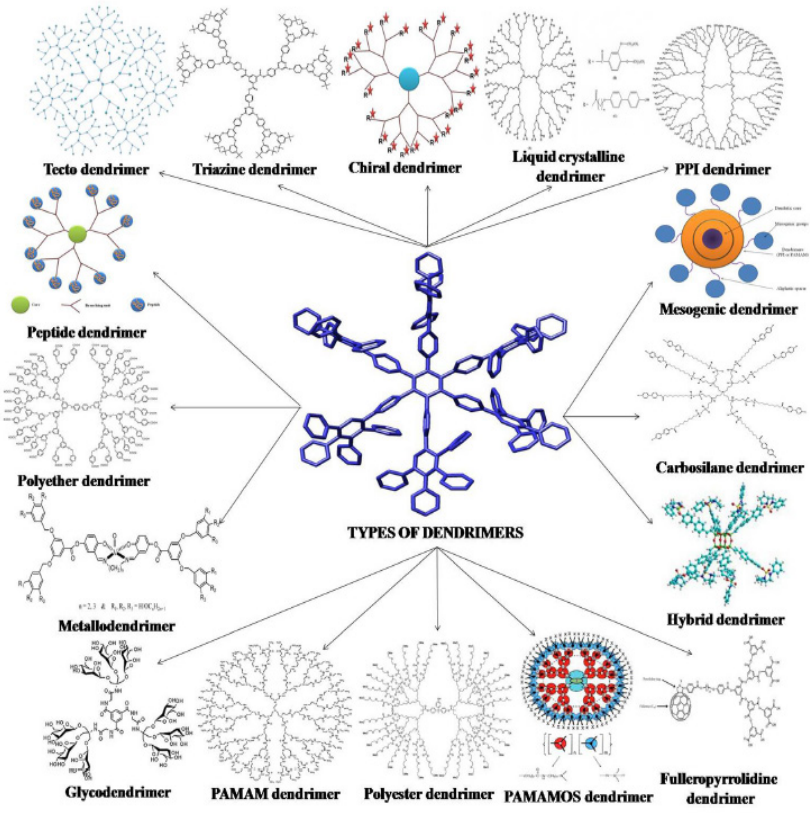

Figure 2. Types of dendrimers [18]

6. Synthesis Methods of Dendrimers

1) Divergent Dendrimer Synthesis Method

This method was first introduced by Tomalia. In the method, the growth of dendrimers starts from a core region. The core is reacted with two or more reagents containing at least two protective branching sites, followed by removal of the protecting groups to form first generation dendrimers. This process is repeated until the desired sized dendrimer is obtained. With this approach, the first synthesized dendrimers were polyamidoamines, also known as starbust dendrimers [8, 9].

2) Convergent Dendrimer Synthesis Method

Convergent dendrimer synthesis begins with what the final surface group will be. The dendrimer moves inward, connecting the surface units more and more. When the growing structures are large enough, several are added to a suitable core to give a complete dendrimer. The convergent growth method has several advantages such as being relatively easy to purify the desired product, minimizing the occurrence of defects in the final structure, and not suitable for high-generation dendrimer synthesis as stearic problems occur in the reactions of dendrons and core molecule [8, 9, 11].

3) Double Exponential and Mixed Dendrimer Synthesis Method

In this method, both convergent and divergent production monomers are involved and reacted together. The power of double exponential growth provides the opportunity to form large dendrimers in relatively few steps [8, 9].

4) Dendrimer Synthesis Method with Hypernucleated and Branched Monomers

This method involves preforming oligomeric species that can be linked together to form dendrimers in one branch, fewer steps or higher yields. The core is reacted with two or more reagents containing at least two protective branching sites, followed by removal of the protecting groups. Subsequent free reactive domains form the first generation dendrimers [8, 9].

5) Lego Chemistry

It is an approach developed to simplify the cost and synthesis time of dendrimers. Lego chemistry is based on the application of highly functionalized nuclei and branched monomers and is used in the synthesis of phosphorus dendrimers. Besides the high growth in the number of terminal surface groups in a few reactions, this method also enables the use of minimal volumes of solvents and purification with products such as water and nitrogen [18].

6) Click Chemistry

This method is very important in terms of fast, harmless by-product and high chemical yield. Moreover; simple reaction conditions, readily available reagents and solvents are the advantages of click chemistry. 2.0 G and 3.0 G triazole dendrimers are synthesized using Cu(I) catalyzed click chemistry reactions and the resulting dendrimers are easily purified by chromatographic methods [18]. Synthesis methods of dendrimers are shown in Figure 3.

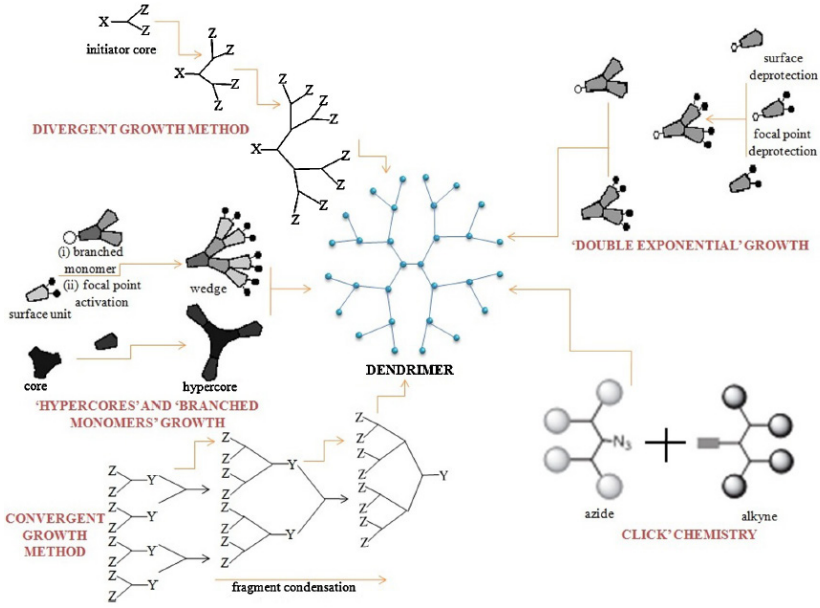

Figure 3. Synthesis methods of dendrimers [18]

7. Breast Cancer

There are two main types of tissue in the breast: the milk ducts (mammary ducts) and the breast lobules. According to the classification made in regards to the tissue of origin of BC, cancers originating from the milk ducts are called ductal carcinoma and correspond to 40-75% of diagnosed BCs. Conversely, the type of cancer originating from the breast lobules, which is classified as lobular carcinoma, constitutes 10-15% of diagnosed breast cases. Ductal and lobular carcinomas have specific oncoproteins and expression patterns [19]. In the molecular classification, five molecular subtypes are defined based on the gene expression profile and the presence of estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2): Luminal A (ER+, PR+, HER2-), luminal B (ER+, PR+, HER2+/-), HER2-enriched type (ER-, PR-, HER2+), triple-negative (basal-like, ER-, PR-, HER2-) and normal-like breast cancer [20].

Although histological and molecular factors greatly affect the treatment algorithm due to the heterogeneity of BC, mastectomy or lumpectomy is performed in approximately 90% of BC cases [21, 22]. Apart from surgical operation, radiotherapy, chemotherapy, neoadjuvant chemotherapy, hormone therapy and immunotherapy are also applied in the treatment of BC. Radiotherapy is usually recommended after surgery to reduce remaining cancer cells and the

chance of recurrence [23, 24]. Most patients receive neoadjuvant therapy before surgery to reduce tumor size, or adjuvant chemotherapy after surgery to destroy remaining cancer cells, prevent possible metastases, and prevent recurrence. Anticancer drugs that are frequently used in neoadjuvant therapy or adjuvant chemotherapy are presented in Table 1 [25]. Adjuvant endocrine therapy is administered to patients with ER+ and/or PR+ breast cancer for a period of five years to provide inhibition of estrogen synthesis. The most common endocrine therapy is tamoxifen and aromatase inhibitors such as anastrozole, letrozole and exemestane, which inhibit estradiol synthesis from androgens [23, 26].

Table 1. Anticancer drugs used in BC treatment

Drug	**Product name on the market**
Abemaciclib	Verzenio®, Yulareb®
Palbociclib	Ibrance®, Reampla®
Ribociclib	Kisqali®, Valamor®
Paklitaxel	Taxol®, Abraxane®, Genexol®,
Everolimus	Afinitor®, Votubia®
Capecitabine	Xeloda®
Cyclophosphamide	Cytoxan®, Endoxan®
Docetaxel	Taxotere®
Doxorubicin	Adriamyciin®, Myocet®, Dox®, Doxil®
Epirubicin	Ellence®, Epirubicin Ebewe®
5-Fluorouracil	Fluorouracil®, Biosyn®
Gemcitabine	Gemzar®
Lapatinib	Tykerb®
Vinorelbine	Navelbine®, Renovel®
Cisplatin	Platinol®

For the treatment of HER2+ breast cancer, tyrosine kinase inhibitors, monoclonal antibodies, and antibody-drug conjugates are used [19]. Trastuzumab (Herceptin®) is the first HER2-targeted monoclonal antibody (mAb) approved

for clinical use. HER2, which is a transmembrane glycoprotein, is overexpressed in cancerous breast tissue in contrast to normal cells. Binding of ligands to this receptor leads to heterodimer formation and activates signal transduction, which induces angiogenesis and tumor metastasis. Trastuzumab, on the other hand, inhibits this pathway by preventing dimerization by binding to HER2 and inducing apoptosis [27]. Pertuzumab was developed due to resistance seen in trastuzumab treatment. In December 2017, it was approved by the FDA with the trade name Perjeta® and released to the market. Except for trastuzumab and pertuzumab, other monoclonal antibodies approved by the FDA are presented in Table 2 [28]. In the treatment of BC, targeting to specific antigens is achieved by using mAbs directly (Tecentriq®, Margenza®, Keytruda®) or using immunoconjugate (mAb-drug conjugation) (Kadcyla®, Trodelv®) [1].

Table 2. Monoclonal antibodies approved by the FDA for BC

Name	Brand Name	Type	Treatment	Route	Year
Trastuzumab Hyaluronidase-oysk	Herceptin Hylecta	Combination of antibody and endoglycosidase	HER2+ BC	SC	2019
Atezolizumab	Tecentriq	mAb	Advanced or metastatic triple-negative BC combined with Abraxane	IV	2019
Ado-trastuzumab emtansine	Kadcyla	Antibody and microtubule inhibitor conjugate	HER2+ BC or metastatic BC	IV	2019
Pertuzumab, Trastuzumab, Hyaluronidase-zzxf	Phesgo	Combination of antibody and endoglycosidase	HER2+ BC	SC	2020
Margetuximab	Margenza	mAb	Metastatic HER2+ BC	IV	2020
Sacituzumab-govitecan	Trodelv	Antibody and topoisomerase inhibitör conjugate	Advanced or metastatic triple-negative BC	IV	2021
Pembrolizumab	Keytruda	mAb	Triple-negative BC combined with chemotherapy	IV	2021

SC: subcutaneous, IV: intravenous.

8. Applications of Dendrimers in Breast Cancer Treatment

One of the most major utilizations of dendrimers is their use as a nano-system for the diagnosis and treatment of BC. Thanks to its structures and characteristics that allow surface modification, it is suitable for encapsulation or conjugation on the surface or core of many different active pharmaceutical ingredients. As seen in Table 3, dendrimer-based drug delivery systems have been studied by different researchers on BC.

Table 3. Dendrimer-based drug delivery systems used in BC [29]

Polymers	Anticancer Drugs	Types of BC	Therapeutic Efficacy
PAMAM	Doxorubicin and cisplatin	MDA-MB-231 / MCF-7	Dendrimers modified with hyaluronic acid showed good anticancer activity compared to unmodified dendrimers.
PAMAM	Doxorubicin	T47D/ BT-549-Luc	High cellular uptake and binding.
PAMAM	Antisense oligodeoxynucleotide	MDA-MB-231	High cellular accumulation of the loaded drug.
PAMAM	Antisense oligodeoxynucleotide	-	Growth tumor inhibition
PAMAM	CpG oligodeoxynucleotide	SKBR3/ MDA-MB231	Decreased cell viability.
PAMAM	Trastuzumab	MDA-MB-231/ MDA-MB-453	Sustained drug release profile and reduced BC cell viability
polylysine	PHSCN peptide	MDA-MB-231 / SUM-149	High cytotoxicity
polylysine	Trastuzumab	SKBR3/ MCF-7	High cellular internalization
PAMAM	Florescein isothiocyanated	4T1	Good cellular uptake
PAMAM	Proscillaridin A and digoxin	MDA-MB-231 / MCF-7	High cell apoptosis
PAMAM	Tamoxifen	MCF-7	High cancer cell inhibitory effect

Perez et al. developed a methotrexate and glucose-loaded single-step PAMAM dendrimer (OS-PAMAM-MTX-GLU) in their study and evaluated the efficacy

of OS-PAMAM-MTX-GLU conjugate using triple-negative BC cells (MDA-MB-231). In the study, it was thought that the use of methotrexate (MTX), which has antimetabolite activity, with a nano-sized drug delivery system would be advantageous due to its side effects such as hepatoxicity, ulcerative stomatitis and reduction in leukocyte count. In addition, glucose conjugation to the PAMAM dendrimer was also performed due to the high glucose requirement of cancer cells and overexpression of glucose transporters (GLUT). According to the cytotoxicity study performed with the MTT assay, OS-PAMAM-MTX-GLU has been shown to reduce MDA-MB-231 cell viability by up to 20%. In MDA-MB-231 cells, MTX alone was reduced by 70%, OS-PAMAM-MTX conjugation was reduced by 45%, OS-PAMAM-MTX-GLU conjugation was reduced by up to 20%, and OS-PAMAM-MTX-GLU showed cancer-specific toxicity to the cells. The decrease in MDA-MB-231 viability was thought to be due to increased glucose uptake by glucose conjugation. Glucose conjugation increased its uptake into cancer cells, supporting the cytotoxicity study. PAMAM dendrimer and MTX/glucose conjugation have been observed to be promising in the treatment of triple-negative BC, as it increases accumulation in tumor cells and provides targeted therapy [30].

In the studies, PAMAM dendrimers acted as nano-sized drugs in some conditions. One of its most important biological effects is its ability to modulate gene expression patterns and affect cellular signaling pathways of HER family. Kheraldine et al. investigated the anticancer activities of different generations and surface properties of PAMAM dendrimers (G4NH2 (cationic), G6NH2 (cationic), G6OH (neutral), G5.5COOH (anionic)) in HER2+ BC cells (SKBR3 and ZR75 cell lines). In the study, cationic PAMAM dendrimers significantly reduced cell viability and colony formation in both cell. In the apoptosis study with Annexin V, it was observed that the G6 cationic PAMAM dendrimer had a higher apoptosis rate than the others. When pro-apoptotic (Bax, Caspase-3, 8, and 9) and antiapoptotic (Bcl-2) markers were examined by western blot method, in SKBR3 cell line treated with G6NH2 dendrimer, Bax, Caspase-3, 8, and 9 expressions increased 6-fold, 10-fold, 3-fold, 5-fold respectively, while Bcl-2 expression decreased 0.2-fold. Similarly, in the ZR75 cell line, expression of Bax (5.4-fold), Caspase-3 (7-fold), Caspase-8 (6-fold), and Caspase-9 (5-fold) increased, while Bcl expression decreased 0.4-fold. According to the results, the PAMAM dendrimers have the potential to be used as anti-HER2 agent [31].

PAMAM dendrimers have started to be used as non-viral vectors in drug and gene transport because they carry a large number of amino groups on their surface and form dendrimer-nucleic acid complexes as a result of electrostatic

interactions. Thang et al. developed the gene and drug delivery PAMAM system for microRNA-205 (miR-205) and 5-fluorouracil (5-FU). The aim of the study was to develop a partially acetylated PAMAM dendrimer conjugated with luteinizing hormone-releasing hormone (LHRH) (which provides targeting), miR-205 and 5-FU, and to reduce the side effects seen due to non-specific biodistribution and to increase the synergistic anti-tumor efficiency. For this purpose, the LNRH-G5NHAC-FUA/miR-205 PAMAM dendrimer was developed, claiming that LHRH conjugation and the combination of 5-FU and miR-205 would increase the therapeutic efficacy. In targeting specificity study, strong fluorescent signal was measured in MCF-7 and MDA-MB-231 breast cancer cell lines, but no fluorescence signal was detected in normal breast tissue cell MCF-10A. This study supported that LNRH provides targeting to BC cells. In the apoptosis study, it was observed that LHRH-G5NHAC-FUA/miR-205 (22.8%) induced apoptosis with a higher rate than LHRH-G5NHAC-FUA (16.1%) and LHRH-G5NHAV/miR-205 (14.7%). This has been interpreted as 5-FU and miR-205 playing a synergistic role in inducing apoptosis and inhibiting proliferation. To determine in vivo antineoplastic activity, female Balb/c mice were used and divided into nine groups (saline, G5NHAC, LHRH-G5NHAC, 5-FU, miR-205, 5-FU+miR-205, LHRH-G5NHAC-FUA, LHRH-G5NHAC/miR-205, LHRH-G5NHAC-FUA/miR-205). After the treatment, LHRH-G5NHAC-FUA/miR-205 was found to be the most effective structure for suppressing tumor growth and the least damaging to heart, kidney and liver tissue according to histology results. Therefore, the conjugated PAMAM dendrimer developed with miR-205 and 5-FU is considered to be a promising delivery system in the treatment of BC [32].

In aggressive breast cancers, the transcription factor TWIST1 is both overexpressed and involved in cell migration. In the study of Finlay et al., a significant decrease in metastasis and invasion was observed in SUM1315 TNBC cells by suppressing siRNA-based gene expression by forming the siRNA-PAMAM dendrimer complex [33].

In another study; by synthesizing nanoparticles modified with CD44 targeted thioaptamer (TA), BC was studied in MDA-MB-231 and MCF-7 cell lines in vitro and in Balb/c mice in vivo. In the study, polyethyleneglycol-PAMAM nanoparticles conjugated with Thioaptamer containing miRNA-145 were prepared. With the prepared delivery system, accumulation was observed only in cancerous tissues, and cell uptake efficiency was found to be high with the positive charge it carried. While no significant gene silencing was observed in MCF-7, MDA-MB-231 provided quite good gene silencing. It has also been reported to reduce invasion and metastasis [34].

In the study conducted by Liu et al., phospholipid-modified, siRNA-loaded PAMAM-siMDR1 dendriplexes were prepared targeting the multi-drug resistance gene (MDR1 gene) for MCF-7/ADR breast cancer cells. With this delivery system, high gene silencing was achieved by increasing cellular uptake. It has been stated that by decreasing P-gp expression, doxorubicin increases cellular accumulation and decreases tumor cell migration [35].

9. Conclusion

Dendrimers; It is one of the prominent drug delivery systems that are versatile and open to modification. Both the ability to encapsulate the active substance and the electrostatic and covalent bonding to surface groups provide strong therapeutic efficacy with multiple active substances, especially in the treatment of breast cancer. With this function, both a double effect can be achieved and side effects and adverse effects can be reduced in the treatment. In this respect, it may provide a contribution to the development of alternative treatments for many cancer treatments in the future.

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Chapter 7

Effects Of Plastic Exposure On Human Health Through Medical Devices And Materials Used In Health Care

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I. INTRODUCTION

Medical diagnosis and treatment approaches are developing every year.This development brings an increase in the use of plasticcontaining medical materials, and theseplasticcontaining medical materials and devices today constitute an important part of the medical market (1).These products preferred for reasons such as flexibility, durability, mechanical resistance, sterilizability and low cost, cause short or long-term exposure of patients to plastic products, and plastic exposure shows carcinogenic, toxigenic, mutagenic effects and it also has adverse effects on the gastrointestinal, urinary, hemopoietic and endocrine systems (1, 2).It emerges as a public health problem that cannot be ignored since it has been shown that exposures in the new born and prenatal periods, which are especially critical developmental periods, cause higher levels of effect (3, 4).

This paperprepared in order to find answers to some questions such as"What are the harmful plastic constituents the most in devices?", "How and in what way the ways of exposure is more?", "Which devices pose a greater risk to patient exposure?", "What are the general and primary health effects of exposure?" and "What could be the alternative methods that should be done in this subject?" by examining domestic and foreign research articles, scientific book chapters, case reports and review studies on the effects of the mentioned constituents of plastic on human health through medical materials and devices.Alternative methods researched by focusing on the medical equipment industry, health management, and what could be the changes in medical practice.

Exposure to all plastic products found in medical devices containing plastic occurs through respiratory, skin and mucosa.Medical products containing plastic used in health care are in the structure of tubes and catheters, blood bags, enteral feeding tubes, nasogastric tubes, dialysis equipment, branule, serum sets, injectors, extracorporeal oxygenation devices, medical pipes, injectors, serum bags, blood transfusion bags, bags and infusion sets used in dialysis and parenteral nutrition,urethral catheters, drains, aspiration catheters, nasal cannula, endotracheal tubes, dental filling materials used in dentistry, film tablets used in the pharmaceutical industry (6, 7, 8, 9).

Because plastic has a naturally hard and brittle structure, it is necessary to prevent punctures and flexions by providing the softness and flexibility needed in the medical market.Polyvinylchloride (PVC) added to the structure of the plastic provides this flexibility and accordingly, phthalate derivatives (dibutyl phthalate and di(2-ethylhexyl) phthalate) enter the structure of the material or device (6,10).Bisphenol-A (BFA) that is in the structure of plastic-containing medical materials and devices, Di-ethylhexyl phthalate (DEHP) that is one of the some

phthalate species added to the polyvinylchloride (PVC) structure are the main plastic constituents that have negative effects on human health (11, 12, 13).

II. BİSFENOL-A (BFA)

BFA is the most well-known constituent of impact resistant polycarbonate plastics and epoxy resins, as well as it is also often used as an additive to other plastics such as PVC (13).While the most common source of BPA exposure in adults and children in daily life is oral intake of canned food, polycarbonate baby bottles are more common in infants, but exposure occurs as a result of oral intake of canned food, liquid foods in polycarbonate bottles or feeding bottles.Although the materials as mentioned cause the transition of BPA to foods in any case, the transmission increases with damage to the plastic or exposure to heat (9, 14, 15).

II.I. PATHWAYS AND EFFECTS OF EXPOSURE THROUGH MEDICAL INTERVENTIONS

BPA exposure through medical equipment has shown to be possible from composites and other restorative materials used in dentistry, hemodialysis devices and equipment, cardio pulmonary bypass extracorporeal circulation circuits, BPA-containing catheters, laparoscopic trocars and cannulas, endoscopic instruments, polycarbonate containing walf, pacemaker, implants and long-term surgical procedures (16).

One of the most obvious negative effects of BPA is its endocrine disrupting effect.Although it does not take after to the endogenous estrogen hormone 17 B estradiol in terms of its chemical structure, it has similar properties with DES (Di Ethyl Stilbestrol), a strong synthetic estrogen (17, 18).This similarity explains the studies in which BPA administered to ovariectomized experimental animals produced estrogenic effects (18).Some studies in both animals and humans have shown that BPA has endocrine disrupting effects in both species.This effect is mostly through endogenous estrogen activity and effects on the androgenic system.In addition, it has been proven that it has an endocrine disrupting effect by causing changes in the number of hormone receptors and receptor gene activity in target tissues (19).Different animal experiments have shown that BPA triggers proliferation in breast cancer cells, reduces sperm production, and causes irregularities in sexual maturation in females and reproductive organs development in males (20).Some other studies have shown that it increases insulin secretion with pancreatic B cell stimulation and may pose a risk in terms of metabolic syndrome by suppressing the hormone called Adiponectin, which regulates insulin sensitivity in the body (21).Adverse effects on the Central

Nervous System (CNS) and thyroid function and in some studies suppressive effects on the immune system have been reported. Studies in animals have shown that BPA is closely associated with schizophrenia and other neurotoxic pathologies. Neuronal, behavioural and bipolar disorders have been observed in new-borns, infants and children as a result of BPA exposure (9, 19).BPA exposure during prenatal and early childhood periods is between 9 and 7 years of age. In another study investigating the effect on children in childhood, it was found that the depression and aggressive behaviour scores of boys exposed to BPA in the womb increased and this situation tended to turn into permanent behaviour; In girls, it has been reported that postnatal BPA exposure causes an increase in depression and aggressive behaviour scores (22). In this case, it turns out that BPAs exposed in the womb or after birth affect children in a gender-specific manner (23).

It is stated that BPA exposed in the second trimester of pregnancy causes early breast development (4).

It has been reported that phthalate levels exposed during specific periods of pregnancy significantly affect the birth weight, height, head circumference of the offspring and increase the risk of premature birth (24).

BPA reaches the highest concentrations in the placenta and fetus after it is taken into the body with the diet. This accumulation of BPA can cause easily repairable breaks in DNA, as well as multiple breaks that cannot be repaired. Although the liver can metabolize BPA by glucorinidation and sulfation pathways, the resulting intermediates adhere to DNA. It is stated that even low doses of BPA cause various signalling disorders in cells, deformities in the body and even tumorogenosis. Again, low levels of BPA exposure lead to calcium dysregulation (25).

Recent epidemiological studies have shown that human high urinary tractBFA excretion in angina, hypertension, heart attack, coronary and peripheral arterialassociated with different types of cardiovascular diseases such as patientsshows(26).

III. PHTHALATE

Chemical substances known as phthalates are added to Polyvinyl Chloride Plastics (PVC), which is widely used in medical materials, in order to provide sufficient flexibility.Although studies on the pathways of exposure to phthalates have focused on oral and inhaled exposure, it has been stated that dermal exposure is 10 to 20 times higher than inhaled exposure in recent years (27).

Although there are different types of phthalates in terms of chemical structure, exposure through medical equipment and devices has been shown to be generally via Di Ethyl Hexyl Phthalate (DEHP) (3, 8).

III.I. Di Ethyl Hexyl Phthalate (DEHP)

DEHP, which is an endocrine disrupting agent, has a lipophilic chemical structure and also is attached to the PVC structure with a non-covalent bond. These properties are two very important chemical properties for exposure to DEHP from medical equipment and devices.Because of its lipophilic feature and non-covalent bonds, its ability to separate easily from PVC to which it is attached and to easily pass into lipid-containing solutions increases the exposure level and rate (8, 6).

III.II. PATHWAYS AND EFFECTS OF EXPOSURE THROUGH MEDICAL INTERVENTIONS

Medical devices and materials that have been shown to leak DEHP are mainly IV fluid bags and infusion sets, nasogastric tubes, enteral and parenteral nutrition bags, chest tube, hemodialysis equipment, cardiopulmonary bypass equipment, endotracheal and ventilator tubes, urinary and aspiration catheters, nasal cannula, exchange transfusion materials and extra corporeal membrane oxygenation (ECMO) devices (28,29,30,31,10).

Although the degree of exposure through medical devices and materials depends on factors mainly such as substance that comes into contact with the device (faster and more intense transition with lipid-containing solutions), temperature of the device and solution if used (faster and denser transition at high temperature),storage time of the solution in devices used with any solution; it depends on manyfactors such asthe percentage of DEHP that the device contains, the duration of the treatment applied and the number of repetitions, and whether the treatment is used in critical periods(especially the new born period) or not. Insomuch as there are studies showing that even mechanical shaking of the device increases the DEHP level by 10-15% (10,32).

It has been shown that some chemotherapy drugs (especially docetaxel, paclitaxel, etoposide, teniposide) added to solutions in PVC containing infusion bags cause DEHP exposure depending on the duration (10,7,33).At the same time, some studies have found that leakage of DEHP into solutions prepared with physiological saline causes crystallization (10, 34).

Although animal experiments draw attention to the adverse effects of phthalate exposure on the liver, kidneys, lungs, and blood coagulation factors, the most vulnerable group in terms of phthalate exposure is infants and children in the developmental period.There are three main findings that the mentioned period is a special risk period.

a) It has been determined that there is a higher amount of absorption after exposure in children compared to adults, and it is thought that this may be due to the difference in children's metabolism.
b) Children are a group that is more sensitive in terms of pharmacodynamically to toxic substances.
c) Some medical procedures applied to children cause greater exposure (6).

Studies conducted on infants and children treated in neonatal intensive care units, in particular, have drawn attention to the fact that the toxicity caused by plastic medical materials is very high, mostly in terms of phthalates, and that the exposure of the children included in the studies may cause serious consequences in the later years of their lives (3,35).Many studies conducted in neonatal intensive care units found the level of DEHP high, especially in infants who were continuously transfused, but showed that the most intense exposure could be with exchange transfusion.The effects of prenatal exposure, especially on the male reproductive system, are worrisome.Shortness of the anogenital space and negativities in the development of the genital area have been associated with prenatal DEHP exposure (4,37,38).The study by Tellez Rojo et al. showed that phthalate exposure in the prenatal period may cause a decrease in the psychomotor development index, especially in girls (39).

IV. CONCLUSION AND RECOMMENDATIONS

It has been determined that the types of plastics that are exposed through the medical materials and devices currently used in health care are at a frequency that cannot be ignored, and that unconscious use can increase exposure.It has been also determined that they show their primary negative effects on human health, especially in newborn and childhood, as endocrine disruptors, and show their main effects through harmful effects on puberty, sexual development, mental and neurological function.In addition, non-human studies show that there are also neoplastic and mutagenic effects.Considering that exposure is inevitable in health care, it is important for public health to draw attention to the dimensions of the risk and to reduce exposure, to produce and use plastic materials that do not have harmful effects.We need more studies on exposure, critical exposure conditions, periods and harmful effects to be detailed the risks for health.In this regard, community based large scale, especially cohort and case control studies should be conducted.In addition, health personnel, medical equipment industry workers and health administrations should provide the most suitable product for the patient in cooperation.There should be warning information about the

types and rates of chemicals present in the product, the problems that may occur as a result of exposure, what can be done, storage conditions, and the features to be considered in order to eliminate or minimize exposure during use of the product.Government policies should be developed for the production and use of these products.Including the harmful effects of plastic products in the education curricula, organizing post-graduate in-service training programs and developing guidelines for the safe use of medical devices containing plastics are also ways to get over this public health problem.

Table-1: The results of different exposure scenario calculations are summarized in the study conducted by the Scientific Committee Committee on Emerging and Newly Identified Health Risks European Commission 2015 (16).

	BPA exposure estimation in ng/kg b.w./day			
Exposure scenario	Prematurely born infant	infant	child	adult
External contact with a medical device containing BPA (short-term)	1			0.08
Contact with dental material (acute exposure <24 h) (long term) Contact with orthodontic equipment (acute exposure <24 h) (short-term) (long-term)	na na	na na	 2 140 13.5 12	200 6 140 7.5 6
Contact with an implant (short-term) (long-term)			 11 0.8	 6 0.4
Hemodialysis (long-term)				57
Prolonged surgical procedures (short-term)		685	114	57
Prolonged exposure to different sources ofBPA in intensive care units (short-term)	3000			
Breast pump and collectionvessel made ofPC (short-term)		134		
Uses of PVC (short-term) (long-term)				

There is considerable uncertainty with respect to the chemical composition of medical devices and their release properties with respect to BPA. For each scenario considered, there are relatively few studies reported and in most cases the studies do not have much information on how the material under study is representative of the European situation (16). İn addition the variability of age and application period shows that it is important in terms of exposure.

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Chapter 8

Antimicrobial, Antiviral And Antifungal Activities Of Various *Cinnamomum* spp.

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Introduction

For thousands of years, people have used essential oils (EOs) obtained from plants not only for the content of perfumes or the aromatization of foods, but also for many different biological properties, including antimicrobial properties. Thanks to their antimicrobial properties, EOs are considered as an alternative to antibiotics in the management of the rapidly increasing number of antibiotic-resistant microorganisms today. According to the drug resistance report of the World Health Organization (WHO), third generation cephalosporins and carbapenems resistant Klebsiella pneumoniae, third generation cephalosporins and fluoroquinolones resistant *Escherichia coli*, methicillin resistant *Staphylococcus aureus*, penicillin resistant *Streptococcus pneumoniae* and *Salmonella* spp. are among the microorganisms that cause the most serious health problems. (Wińska, 2019).

To prevent the development and reproduction of pathogenic microorganisms, synthetic drugs are widely used in the treatment of microbial infections. Overuse of traditional antibacterial drugs causes an increase in the number of resistant bacterial strains and the development of antibiotic resistance during treatment. The antibiotics used can cause serious side effects, which poses a serious threat to global public health (Rad, et al., 2021). Therefore, there is a great need to discover inexpensive and natural antimicrobial compounds that can be an alternative to antibiotics, both with a strong effect on pathogenic microorganisms and easily accessible (Salehi et al., 2019). In this context, research on the cinnamon plant has shown the strong antimicrobial activity of essential oils obtained from various cinnamon species.

Cinnamon

Cinnamon is a natural herb that grows in Sri Lanka and tropical Asian countries. It is an important spice plant widely used in the world. It is known that the genus *Cinnamomum* has more than 250 species (Teles, 2019).

Cinnamomum verum is a small evergreen tree belonging to the Lauraceae family, known as the "true cinnamon tree" and the "Selylon cinnamon tree". *Cinnamomum cassia*, *Cinnamomum verum* etc. It is used to obtain cinnamon together with other cinnamon types such as (Ribeiro-Santos et al., 2017). The ancient botanical name of this tree - *Cinnamomum zeylanicum*- derives from Sri Lanka's ancient name, Ceylon (Ribeiro-Santos et al., 2017). *Cinnamomum cassia*, also known as cassia ıt is an evergreen tree species that is more common in the Southern China region (Bedigian, 2005).

Cinnamon species differ in appearance, taste and where they are grown. *C. cassia* cultivated in China is generally characterized by a deep reddish-brown color and a sharp and bitter taste, while *C. zeylanicum* grown in Sri Lanka is characterized by a bright to medium-dark reddish-brown color and sweet taste (Kawatra, 2015).

Chemical Composition of Cinnamon EO

Various studies on *Cinnamomum* species identify chemical compounds with potent biological activities. Depending on the EOs of the bark or cinnamon leaves, the ratio of the two main components (eugenol and cinnamaldehyde) differs significantly. For EO from cinnamon bark, it is characterized by a low amount of eugenol (5-10%) and an abundance of cinnamyl aldehyde (65-80%), while EO from cinnamon leaves is rich in eugenol (10-95%) and weak in cinnamyl aldehyde. (1–5%) (Wińska, 2019; Brochot, 2017).

Beneficial Effects of Cinnamon EO on Human Health

It has been determined that EOs obtained from different cinnamon species have beneficial effects on human health as well as antimicrobial properties and have different pharmacological properties that can be used in the treatment of diseases. The presence of catechins with antioxidant properties in the cinnamon plant can be effective on disorders such as dyspepsia, bloating, nausea, colic bowel, slow digestion, diarrhea and digestive disorders. Researchers have found in recent studies that cinnamon improves insulin's ability to metabolize glucose and helps control blood sugar levels (Qin et al., 2010). They stated that the potential antidiabetic mechanism of cinnamon is related to increasing the sensitivity of fat cells to the hormone insulin, which regulates glucose metabolism and controls blood sugar levels.

Cinnamon helps reduce pain due to its prostaglandin inhibiting effect, and it acts against the coagulation of platelets in the blood with cinnamaldehyde (Vallianou et al., 2019). Studies have also shown that cinnamon is effective in the treatment of inflammatory diseases such as rheumatoid arthritis (Shishehbor et al., 2018). Recent studies have shown that the smell and aroma of cinnamon can act as cognitive stimuli that can improve memory, visual motor capacity and virtual memory due to the compound cinnamic aldehyde (Nussbaum et al., 2017). Cinnamic aldehyde can also be effective in the treatment of behavioral and mental disorders and depression (Panickar et al., 2009).

Antimicrobial Activity of Cinnamon EOs

EOs obtained from various origins of the cinnamon plant have been investigated for their antimicrobial activity on many different pathogenic microorganisms in the last 20 years. It has been determined that studies on the anti-biofilm and anti-quarum sensing activities of cinnamon EOs, which are determined to have a very high antimicrobial potential, are limited.

The mechanisms of antibacterial action of *Cinnamomum* plants are quite complex. It has been reported that cinnamaldehyde and eugenoline inhibit the production of β-lactamase by bacteria and destroy the cell wall (Di Pasqua et al., 2007).

Secondary metabolites of cinnamon plants have the ability to pass through the lipid layer of the cytoplasmic wall and adhere to the microbial cell surface, thus accumulating in the bacterial cell wall. Therefore, phytochemicals change the structure of the membrane, increase its permeability, cause leakage of vital intracellular metabolites and finally cell death (Rhayour, 2003; Wu et al., 2009).

The number of studies on the antimicrobial activities of various *Cinnamomum* species and their main components is quite high. When we look at the studies carried out in this area, the antimicrobial activity against *Staphylococcus aureus* was high in studies using EOs obtained from the leaves of *C. zeylanicum*, which is rich in cinnamic aldehyde (Ferro et al., 2016), and in another study, *Enterococcus faecalis*, It was found to show better antimicrobial activity than antibiotics (ampicillin, chloramphenicol, tetracycline) against *Escherichia coli*, *P. auerginosa* and *S. aureus* (Aumeerudda-Elalfi et al., 2015).

In studies investigating the antimicrobial activity of EOs obtained from the bark of *C. zeylanicum*, it was found that cinnamon EOs had a better antimicrobial activity compared to clove EO, lemongrass, tea tree, ginger, basil EOs, and *S. aureus*, *E. coli*, *A. baumannii* and *P. aeruginosa* (Feng et al., 2017; Chouhan et al., 2017).

EO obtained from the bark of *Cinnamomum cassia,* which is rich in cinnamic aldehyde, was used to prepare an herbal toothpaste (91.8%) that inhibits the growth of *Streptococcus mutans*, which causes the development of dental caries. The toothpaste, which did not show antibacterial effect before the addition of EO, showed an inhibition zone of 39 mm on *S. mutans* ATCC 35668 after the addition of cinnamon EO (Karadagoglu et al., 2019).

It has been reported that the extract obtained from the bark of *C. burmannii* has antibacterial activity on the foodborne pathogens *Bacillus cereus*, *Listeria monocytogenes*, *S. aureus*, *E. coli* and *Salmonella anatum* (Shan et al, 2007). Regarding the EO of the leaves of *C. osmophloeum*, on 9 different test bacteria (*E. coli*, *P. aeruginosa*, *E. faecalis*, *S. aureus*, *Staphylococcus epidermidis*, methicillin-resistant *S. aureus* (MRSA), *Klebsiella sphemia*, and *Salmonella pneumoniae*. Minimum Inhibitory Concentration (MIC) values were investigated and MIC values were found to have a very strong inhibitory effect, ranging between 200 and 500 µg/ml (Chang et al., 2001). In another study, MIC values of EO of *C. cassia* leaves were determined as 500 ppm for *E. coli*, *B. cereus*, *Lactobacillus sakei* and 750 mg/ml for *Salmonella typhimurium* (Turgis et al., 2012).

In the study investigating the antimicrobial activity of *C. verum* EO, MIC was tested against 21 bacterial species. EO showed significant antibacterial activity against the strains tested. It showed higher activity against Gram-positive (*Enterococcus*, *Streptococcus*, *Staphylococcus*) and Gram-negative (*P. aeruginosa*) bacterial strains. These results reveal that cinnamon bark oil completely inhibits the growth of selected Gram-negative and Gram-positive bacteria Chao et al. 2000.

In a recent study, *C. verum* EO *S. aureus* showed remarkable inhibitory effects in multidrug resistant (MDR). Indeed, cinnamon oil at concentrations of 3.12% has been reported to potently inhibit all isolated *Streptococcus mutants*, with MICs of 12.8-51.2 and 64-256 mg/ml, respectively (Fani and Kohanteb, 2011; Rossi et al., 2019).

Wijesinghe et al. MIC and Minimum Bactericidal Concentrations (MBC) were investigated in the study conducted by *C. verum* leaf oil against *P. aeruginosa*, *S. aureus* and *K. pneumoniae* in vitro. Cinnamon EO exhibited 0.5 and 1.0 mg/mL MIC and MBC against all test strains. (Wijesinghe et al., 2020).

In the antibacterial screening of EO samples from *C. bejolghota*, disc diffusion method was used against zoonotic enteropathogens (*Salmonella* spp., *E. coli* O157, *Campylobacter jejunii* and *Clostridium perferingens*) and it was found that it showed promising antibacterial activity (Wannissorn et al., 2005).

Essential oils from the stem bark of *C. altissimum*, *C. porrectum*, and *C. impressicostatum* showed significant antibacterial activity against a wide variety of Gram-positive and Gram-negative bacteria, including MRSA, even when these essential oils were compared to methicillin-susceptible Staphylococcus aureus (MSSA). It has been reported to show better activity against MRSA (MIC: 19.5 µg mL). This study scientifically validated the traditional use of *Cinnamomum* species in the treatment of wound infections (Buru et al., 2014).

In the study evaluating the anti-biofilm activities of essential oils obtained from the stem bark of *C. zeylanicum* and *C. cassia*, as well as cinnamaldehyde on various clinical strains, biofilms of *Streptococcus pyogenes*, *P. aeruginosa* and *E. coli* showed sensitivity to all analyzed concentrations and substances and S. It has been found to exhibit bacteriostatic and bactericidal activity against *S. aureus* and *S. epidermidis*. MIC values were found in the range of 0.25 to 0.50 mg/ml (Firmino., 2018).

As can be seen in the studies, the antimicrobial activity of various cinnamon species investigated on many pathogenic microorganisms is quite high. Of course, not every *Cinnamomum* species is effective on every microorganism. For this reason, EOs obtained from both leaves and bark of each *Cinnamomum* species should be screened on microorganisms that cause serious clinical infections.

Antiviral Activity of Cinnamon EO

Cinnamomum plants have been the subject of many studies in terms of their phyto-components and pharmacological properties, as well as their traditional medicinal importance. It has been observed that studies on the antiviral effects of EOs obtained from the cinnamon plant are limited.

Cinnamon EOs from various origins have been used in antimicrobial and antiviral research on cinnamon EOs. The EO of *C. zeylanicum* was found to have a 100% inhibitory effect on influenza virus A1/Denver/1/57 (H1N1) with a 30-minute exposure at a concentration of 3.1 µL/mL. As a result of this study, Eugenol, the main component of *C. zeylanicum* EO, was reported to have the strongest anti-flu activity in both liquid and vapor phases (Vimalanathan at al., 2014).

In another study, silver nanoparticles obtained using *C. cassia* were found to be effective in the treatment of pathogenic avian influenza virus subtype H7N3 (Fatima, 2016). Brochot et al. In the study investigating the antiviral activity of a mixture consisting of equal parts of *Eucalyptus globulus* cineol (leaf) and *C. zeylanicum* cinnamaldehyde (bark), 3.00% *Rosmarinus officinalis* cineol (leaf), 1.04% *Daucus carotene* (each 3.52%). A reduction of more than 99% was observed on both viruses with a contact time of 60 minutes on H1N1 and HSV1 (Brochot et al., 2017).

Antifungal Activity of Cinnamon EO

The antifungal effects of various essential oils and plant compounds have been proven against human pathogenic fungi. Studies on the antifungal activity of *Cinnamomum* species are limited, and studies have been carried out especially on *Candida* species, which are the source of serious morbidity and mortality from cinnamon EOs. In a study evaluating the effect of EOs obtained from *C. zeylanicum* blume leaves on the roughness and hardness of acrylic resin used in dental prostheses, as well as the anti-Candida activity of the essential oil, it was reported that MIC = 625.0 µg/mL concentration showed anti-*Candida* activity (Oliveira, 2014).

In a study investigating the effectiveness of *Cinnamomum zeylanicum* EOs on the prevention of fungus and mycotoxin accumulation in cereals, it was stated that they had an inhibitory effect on *Fusarium* and *Aspergillus fungi* and *Pichia*, *Saccharomyces* and *Hyphopichia yeasts* (Sumalan, 2013).

In a study examining the in vitro effects of cinnamaldehyde against 18 fluconazole-resistant *Candida* isolates, it was determined that cinnamaldehyde was effective against different *Candida* isolates with a MIC (90) value in the range of 100-500 µg/ml (Shreaz et al., 2011).

In a study investigating the in vitro antifungal activity of *C. zeylanicum* bark and leaf EOs against *Candida albicans* and *Candida auris*, it was reported that essential

oils obtained from *Cinnamomum zeylanicum* Blume bark and leaf had lethal effects on both strains at very low concentrations (Tran, 2020).

In another study evaluating the effect of *C. verum* leaf oil against three virulence factors and in-vivo toxicity of *C. albicans*, *C. tropicalis* and *C. dubliniensis*, Eugenol, the main component of *C. verum*, *C. albicans*, *C. tropicalis* and They found the MIC values on *C. dubliniensis* to be 1.0, >2.0 and 0.34 mg/mL, respectively. These values indicate that *C. verum* leaf oil has a strong effect on the virulence factors of *Candida* (Wijesinghe, 2020).

Conclusion

Cinnamomum plants, extracts and chemical components have various activities that support human health. Antibacterial, antidiabetic, antioxidant, anti-inflammatory, antitumor and neuroprotective activities are the most studied biological properties of *Cinnamomum* plants. Unlike traditional therapeutic drugs, *Cinnamomum* species can be consumed daily without harmful effects in our diet and perhaps used as an anti-disease agent.

In this review, the antimicrobial, antiviral and antifungal properties of many species belonging to the genus *Cinnamomum* and the recent literature are summarized. In recent years, when antibiotic resistance has increased, more research is needed in this area in order to replace antibiotics with natural products that can be an alternative to antibiotics.

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Chapter 9

Properties of Metallic Biomaterials and Proton Therapy Applications

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1.1 Introduction

Hard tissues are usually caused by aging, accidents, congenital causes, genetic defects, etc. they are damaged for other reasons. The replacement of surgically damaged hard tissues with implants is a widely used method in practice. Implants according to regions, where they are placed and the functions they will perform differ according to the characteristics of the material used (Chunxiang et al., 2011). The evaluation of the implant, which is used instead of hard tissue, in terms of biocompatibility and biofunctionality in the region where it is placed is made with at least three criteria. These; biological compatibility (biocompatibility), mechanical compatibility and morphological compatibility (Oshida, 2000; Oshida et al., 1994).

Cranioplasty means surgical repair of a defect or deformity in the skull. History of cranioplasty applications dates back to BC. 7000. Archaeological finds show that inorganic materials began to be used much earlier than organic materials. In the 19th century, biomaterials such as rib bone and tibia were used. Although many different materials and methods have been defined, there has been no consensus on which method is better. It should have properties that can be given, cheap, ready to use, compatible with tissues and light, not carcinogenic or causing side effects, and can be easily sterilized (Aydın et al., 2011).

There may be different events that can lead to the formation of bone defects in the skull. One of these is the removal of the tumor tissue and skull bone together during the operation of a brain tumor that covers the skull bone. In bleeding after head trauma, multi-part fractures, the bone in that area can be removed to prevent brain pressure or the development of infection, and this region of the skull is filled with cranioplasty with different biomaterials. Small screw systems called miniplates or mushroom-like cranioplasty kits can be used to fix the material that will fill the bone cavity, or they can be fixed by suturing with a thick suture (Edwards et al., 1992; Alberktsson et al., 1985).

Metals are used as biomaterials due to their excellent electrical and thermal conductivity and mechanical properties. Since some electrons are independent in metals, they can transfer electrical charge and thermal energy quickly. The mobile free electrons act as a binding force to hold the positive metal ions together. This force is the reason for the metals' high specific gravity and a tight atomic structure that creates their high melting points. Because the metallic bond is essentially non-directional, the position of the metal ions can be changed without damaging the crystal structure, resulting in a plastically deformable solid (Park and Bronzino, 2003).

Because of their excellent mechanical properties and corrosion resistance,

some metals are used as passive substitutes for bone plates and fracture healing aids such as screws, spine fixation devices and dental implants, for hard tissue replacement such as full hip and knee joints. Some metallic alloys are used for more active roles in devices such as vascular stents, catheter guide wires, orthodontic arch wires and cochleal implants (Park and Bronzino, 2003).

The first metal alloy developed specifically for human use was “vanadium steel”, which was used to manufacture broken bone plates (Sherman plates) and screws. Used in the manufacture of Metallic Implants; Most metals, such as iron (Fe), chromium (Cr), cobalt (Co), nickel (Ni), titanium (Ti), tantalum (Ta), niobium (Nb), molybdenum (Mo), and tungsten (W), are eliminated by the body only Very small amounts can be tolerated. Sometimes, these metallic elements in naturally occurring forms are used in red blood cell functions (Fe) or the synthesis of a vitamin B_{12} (Co), but cannot be tolerated in the body in large quantities [Black, 1992]. The biocompatibility of the metallic implant is a major concern because these implants can corrode in vivo [Williams, 1982]. The consequences of corrosion are the spontaneous breakdown of the implant material, weakening the implant, and the harmful effects of corrosion products on surrounding tissues and organs (Park and Bronzino, 2003).

Considering the biocompatibility and human cross-sectional physiology of metallic biomaterials, their effects on the Bragg curves formed by the proton beams were investigated when they are used instead of cortical bone and cortical bone. Considering the variation range of cortical bone thickness according to humans, 0.4, 0.6, 0.8, 1.0 and 1.2 cm thicknesses were taken into account for these materials. These calculations were made on the Monte Carlo-based TRIM simulation system due to the absence of experimental proton beams.

1.2 Stainless Steels

The first stainless steel used for implant fabrication was 18-8 (type 302 in modern classification), which was stronger and more resistant to corrosion than vanadium steel. Vanadium steel is no longer used in implants due to insufficient in vivo corrosion resistance (Park and Bronzino, 2003). Later, 18-8sMo stainless steel was introduced with a small percentage of molybdenum to improve corrosion resistance in chloride solution (brine). This alloy came to be known as type 316 stainless steel. In the 1950s, the carbon content of 316 stainless steel was reduced from 0.08 to a maximum of 0.03 percent (all weight percent unless specified) for better corrosion resistance to chloride solution and to minimize sensitization, thus becoming known as type 316L stainless steel. (Park and Bronzino, 2003). The minimum effective chromium concentration is 11% to provide corrosion

resistance in stainless steels. Chromium is a reactive element, but chromium and its alloys can be passivated with 30% nitric acid to provide excellent corrosion resistance (Park and Bronzino, 2003).

Austenitic stainless steels, particularly types 316 and 316L, are most commonly used for implant fabrication. They cannot be hardened by heat treatment, but can be hardened by cold working. This group of stainless steels is non-magnetic and has better corrosion resistance than others. The addition of molybdenum increases resistance to pitting corrosion in salt water. The American Society for Testing and Materials (ASTM) recommends type 316L instead of 316 for implant manufacture. Technical specifications for 316L stainless steel are given in Table 1.1. The only difference in composition between 316L and 316 stainless steel is the maximum carbon content, i.e. 0.03% and 0.08%, respectively (Park and Bronzino, 2003).

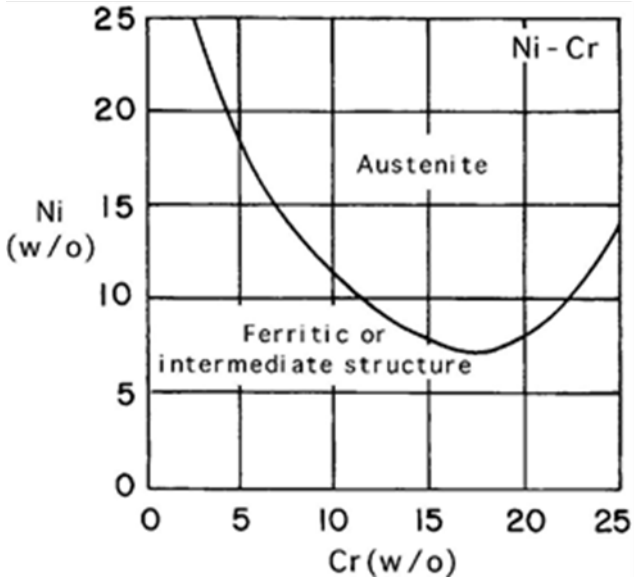

Figure 1.1 Effect of Ni and Cr contents on the austenitic phase of stainless steels containing 0.1% C [Keating, 1956]. Nickel stabilizes the austenitic phase [y, face-centered cubic crystal (fcc) structure] at room temperature and increases corrosion resistance. Austenitic phase formation can be affected by both Ni and Cr contents, as shown in Figure 1.1 for 0.10% carbon stainless steels. The minimum amount of Ni to sustain the austenitic phase is about 10% (Park and Bronzino, 2003).

Table 1.1 316L Stainless Steel Compositions [American Society for Testing and Materials, F139–86, p.61, 1992]

Element	Composition (%)
Carbon	0.03 max
Manganese	2.00 max
Phosphorus	0.03 max
Sulfur	0.03 max
Silicon	0.75 max
Chromium	17.00–20.00
Nickel	12.00–14.00
Molybdenum	2.00–4.00

As a result, the engineer must be careful when choosing such materials. Even 316L stainless steels can rust inside the body under certain conditions in a high-stress and oxygen-free area, such as the contacts under the screws of the bone fracture plate. Therefore, these stainless steels are only suitable for use in temporary implant devices such as broken plates, screws, and hip nails. Surface modification methods such as anodizing, passivation, and glow-discharge nitrogen implantation are widely used to improve the corrosion resistance, wear resistance, and fatigue strength of 316L stainless steel [Bordiji et al., 1996].

Percent comparisons of the proton beam conductivity of stainless steel with cortical bone in average thickness measures and at different beam energy ranges are given in Table 1.2. As the energy of the proton beam increases with the same thickness, it has been observed that the beam conductivity of the stainless iron approaches the cortical bone (Park and Bronzino, 2003). At the same proton beam energy value, it was observed that the difference between the conductivity and the cortical bone increased as the thickness increased. The biggest difference was found to be 69% in the 1.2 cm thick stainless iron in the 80 MeV energy proton beam, and the lowest difference was 3.3% in the 0.4 cm thick stainless iron in the 250 MeV energy proton beam. The mean difference between stainless iron and cortical bone in all energy and thickness values was 21.9% (Park and Bronzino, 2003).

Table 1.2 Percentage difference of Proton beam permeability in the 80-250 MeV energy range of Stainless Iron of different thicknesses with cortical bone

Thickness (cm)	Proton Beam Energies (MeV) and Percent Difference (%)										
	80	**100**	**120**	**140**	**160**	**180**	**200**	**220**	**240**	**250**	**Average**
0.4	30.6	20.8	14.0	11.2	9.4	8.1	6.3	5.4	4.9	3.3	11.4
0.6	47.8	34.2	23.0	17.9	13.6	11.0	9.1	7.8	7.5	5.7	17.8
0.8	73.9	43.7	31.0	24.2	18.0	20.7	12.9	11.5	9.3	8.2	25.3
1.0	27.9	21.9	39.2	29.2	23.4	19.4	15.7	22.3	11.7	10.1	22.1
1.2	69.0	67.6	38.5	35.7	28.1	22.9	19.4	20.5	14.6	12.1	32.9

They create different Linear Energy Transfer (LET) in the transition of proton beams between materials with different densities. Thus, they form a secondary peak similar to the Bragg peak. (Senrkentli et al., 2021; Ekinci and Bölükdemir, 2019; Ekinci, 2019) The higher this second peak value, the smaller the range of the LET and proton beam that the proton beam will transmit to the desired point. (Ekinci, 2019) In Table 1.3, the secondary peak values formed by different thicknesses of cortical bone and stainless iron at different proton beam energies were compared. It was observed that the difference increased as the stainless steel thickness increased at the same proton beam energy. It was observed that the difference decreased as the proton beam energy increased at the same material thickness. There was an average of 72.5% difference with cortical bone in all proton beam energy and material thickness values.

Table 1.3 The percentage difference of the LET formed by the proton beam in the energy range of 80-250 MeV in stainless iron of different thicknesses with the cortical bone

Thickness (cm)	Proton Beam Energies (MeV) and Percent Difference (%)										
	80	**100**	**120**	**140**	**160**	**180**	**200**	**220**	**240**	**250**	**Average**
0.4	72.3	71.1	70.2	72.4	70.8	71.5	62.0	66.2	69.2	67.1	69.3
0.6	75.8	75.2	71.7	73.2	70.9	70.2	72.2	70.1	67.4	69.4	71.6
0.8	81.4	77.8	73.4	72.0	73.4	72.7	72.2	72.1	70.2	70.1	73.5
1.0	72.2	73.0	74.7	73.1	72.2	73.6	71.2	72.9	72.5	70.1	72.6
1.2	87.5	84.6	74.3	74.4	75.0	72.6	71.7	73.2	69.8	69.7	75.3

1.3 CoCr Alloys

There are basically two types of cobalt-chromium alloys: (1) the castable CoCrMo alloy and (2) the CoNiCrMo alloy, which is usually forged by (hot) forging. Castable CoCrMo alloy has been used in dentistry for many years and relatively recently in artificial joint construction. The forged CoNiCrMo alloy is relatively new and is now used in the construction of prosthetic bodies for heavily loaded joints such as the knee and hip (Park and Bronzino, 2003). ASTM lists four types of CoCr alloys recommended for surgical implant applications: (1) cast CoCrMo alloy (F75), (2) wrought CoCrWNi alloy (F90), (3) wrought CoNiCrMo alloy (F562), and (4) wrought CoNiCrMoWFe alloy (F563). The chemical compositions of each are summarized in Table 1.4. Currently, only two of the four alloys, the castable CoCrMo and the wrought CoNiCrMo alloy, are widely used in implant manufacturing. As Table 1.3 shows, the compositions are quite different from each other (Park and Bronzino, 2003).

The two basic elements of CoCr alloys form a solid solution with up to 65% Co. Molybdenum is added to produce finer grains resulting in higher strength after casting or forging. Chromium increases the corrosion resistance of the alloy as well as solid solution strengthening (Park and Bronzino, 2003).

Table 1.4 Chemical Compositions of CoCr Alloys [American Society for Testing and Materials, F75–87, p.42; F90–87, p.47; F562–84, p.150, 1992]

	CoCrMo (F75)		CoCrWNi (F90)		CoNiCrMo (F562)		CoNiCrMoWFe (F563)	
Element	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
Cr	27.0	30.0	19.0	21.0	19.0	21.0	18.00	22.00
Mo	5.0	7.0	—	—	9.0	10.5	3.00	4.00
Ni	—	2.5	9.0	11.0	33.0	37.0	15.00	25.00
Fe	—	0.75	—	3.0	—	1.0	4.00	6.00
C	—	0.35	0.05	0.15	—	0.025	—	0.05
Si	—	1.00	—	1.00	—	0.15	—	0.50
Mn	—	1.00	—	2.00	—	0.15	—	1.00
W	—	—	14.0	16.0	—	—	3.00	4.00
P	—	—	—	—	—	0.015	—	—
S	—	—	—	—	—	0.010	—	0.010
Ti	—	—	—	—	—	1.0	0.50	3.50
Co	Balance							

CoNiCrMo alloy, originally called MP35N (Standard Pressed Steel Co.), contains about 35% Co and Ni each. The alloy is highly corrosion resistant to seawater (containing chloride ions) under stress (Park and Bronzino, 2003).

However, cold working on this alloy has a significant challenge, especially when making large devices such as hip joint bodies. Only hot forging can be used to produce a large implant with the alloy (Park and Bronzino, 2003).

The abrasive wear properties of the forged CoNiCrMo alloy are similar to that of the cast CoCrMo alloy (about 0.14 mm/year in co-simulation tests with an ultra-high molecular weight polyethylene acetabular cup); however, the former is not recommended for bearing surfaces of joint prostheses due to poor frictional properties with itself or with other materials. The superior fatigue and ultimate tensile strength of the wrought CoNiCrMo alloy makes it suitable for applications requiring long service life without fracture or stress fatigue (Park and Bronzino, 2003). This is also the case with the bodies of hip joint prostheses. This advantage is better understood when the implant needs to be replaced because the failed implant fragment embedded deep within the femoral medullary canal is very difficult to remove. In addition, revision arthroplasty is generally inferior in function to primary surgery due to weaker fixation of the implant (Park and Bronzino, 2003).

Experimental determination of nickel release rate from CoNiCrMo alloy and 316L stainless steel in 37°C Ringer solution showed an interesting result. Although the cobalt alloy had more nickel ion release into solution, the release rate was approximately the same for both alloys (3 x 10–10 g/cm2/day) [Richards Mfg. Company, 1980]. This is quite surprising as the nickel content of CoNiCrMo alloy is about three times that of 316L stainless steel (Park and Bronzino, 2003).

Metallic products released from the prosthesis due to wear, corrosion and abrasion can damage organs and local tissues. In vitro studies have shown that particulate Co is toxic to human osteoblast-like cell lines and inhibits the synthesis of type I collagen, osteocalcin and alkaline phosphatase in culture medium. However, particulate Cr and CoCr alloys are well tolerated by cell lines without significant toxicity. The in vitro toxicity of metal extracts showed that Co and Ni extracts at 50% concentration appeared highly toxic as all viability parameters were changed after 24 hours. However, Cr extract appears to be less toxic than Ni and Co [Granchi et al., 1996].

The modulus of elasticity of CoCr alloys does not change with changes in ultimate tensile strengths. Values range from 220 to 234 GPa, which is higher than other materials such as stainless steel. While the effect of increased modulus on the fixation and lifetime of implants is unclear, this may have some effect of different modes of load transfer to bone in artificial joint replacements. Low wear (mean linear wear on the MeKee-Farrar component was 4.2 μm/year) has been recognized as an advantage of metal-to-metal hip joints due to its stiffness and

durability [Schmalzried et al., 1996].

Percent comparisons of CoCrMo biomaterial with cortical bone in mean thickness measures of proton beam conductivity and at different beam energy ranges are given in Table 1.5. It was observed that the beam conductivity of the stainless iron approached to the cortical bone as the energy of the proton beam increased at the same thickness. At the same proton beam energy value, it was observed that the difference between the conductivity and the cortical bone increased as the thickness increased. The biggest difference was found to be 69% in the 1.2 cm thick stainless iron in the 80 MeV energy proton beam, and the lowest difference was 10.9% in the 0.4 cm thick stainless iron in the 250 MeV energy proton beam. The mean difference between stainless iron and cortical bone in all energy and thickness values was 24.1%.

Table 1.5 Percentage difference of proton beam permeability in the 80-250 MeV energy range of CoCrMo biomaterial of different thickness with cortical bone

Thickness (cm)	Proton Beam Energies (MeV) and Percent Difference (%)										
	80	**100**	**120**	**140**	**160**	**180**	**200**	**220**	**240**	**250**	**Average**
0.4	34.7	20.8	14.0	11.9	2.9	8.1	6.3	2.0	4.9	3.3	10.9
0.6	47.8	34.2	24.0	19.4	14.8	12.4	10.3	7.8	7.5	5.7	18.4
0.8	67.4	45.1	33.0	24.2	18.6	0.7	12.4	11.5	9.9	8.2	25.1
1.0	69.8	57.5	40.2	30.8	24.6	19.4	16.9	14.9	12.8	11.2	29.8
1.2	69.0	75.0	47.9	37.2	29.3	23.9	30.2	21.6	17.5	12.1	36.4

In Table 1.6, the secondary peak values formed by cortical bone and CoCrMo biomaterial at different thickness values and proton beam energies were compared. It was observed that the difference increased as the thickness of the stainless iron increased at the same proton beam energy. It was observed that the difference decreased as the proton beam energy increased at the same material thickness. There was an average of 72.5% difference with cortical bone in all proton beam energy and material thickness values.

Table 1.6 Percentage difference of the LET formed by the proton beam in the 80-250 MeV energy range of the different thickness CoCrMo biomaterial with the cortical bone

Thickness (cm)	Proton Beam Energies (MeV) and Percent Difference (%)										
	80	**100**	**120**	**140**	**160**	**180**	**200**	**220**	**240**	**250**	**Average**
0.4	73.2	72.0	71.1	73.2	41.2	72.4	63.0	37.8	69.9	68.0	64.2
0.6	76.7	76.0	72.6	74.0	71.8	71.1	73.0	70.9	68.3	70.2	72.5
0.8	78.0	78.7	76.6	72.9	74.2	73.5	72.4	72.9	71.0	71.0	74.1
1.0	83.2	82.1	75.7	74.0	73.1	74.4	72.1	73.5	73.3	71.0	75.2
1.2	88.7	85.9	77.9	75.3	75.8	73.5	73.3	74.0	71.5	68.8	76.5

Percent comparisons of the CoNiCrMo biomaterial with cortical bone in the mean thickness measures of proton beam conductivity and at different beam energy ranges are given in Table 1.7. It was observed that the beam conductivity of the stainless iron approached to the cortical bone as the energy of the proton beam increased at the same thickness. At the same proton beam energy value, it was observed that the difference between the conductivity and the cortical bone increased as the thickness increased. The biggest difference was 69% in the 1.2 cm thick stainless iron in the 80 MeV energy proton beam, and the lowest difference was 12.1% in the 0.4 cm thick stainless iron in the 250 MeV energy proton beam. The mean difference between stainless iron and cortical bone in all energy and thickness values was 24.4%.

Table 1.7 Percentage difference of proton beam permeability of different thickness CoNiCrMo biomaterial in the energy range of 80-250 MeV with cortical bone

Thickness (cm)	Proton Beam Energies (MeV) and Percent Difference (%)										
	80	**100**	**120**	**140**	**160**	**180**	**200**	**220**	**240**	**250**	**Average**
0.4	34.7	20.8	14.0	11.9	10.5	8.1	6.3	6.4	4.9	3.3	12.1
0.6	52.2	34.2	24.0	19.4	14.8	21.1	11.5	8.8	7.5	7.1	20.1
0.8	71.7	73.2	33.0	25.0	19.8	20.7	13.7	11.5	10.2	8.2	28.7
1.0	23.3	60.3	40.2	31.5	25.7	5.8	16.1	14.9	12.8	10.7	24.1
1.2	69.0	75.0	50.0	38.8	30.5	24.4	30.2	21.6	15.5	14.0	36.9

In Table 1.8, the secondary peak values formed by cortical bone and CoNiCrMo biomaterial at different thickness values and proton beam energies

were compared. It was observed that the difference increased as the thickness of the stainless iron increased at the same proton beam energy. It was observed that the difference decreased as the proton beam energy increased at the same material thickness. There was an average of 74.1% difference with cortical bone in all proton beam energy and material thickness values.

Table 1.8 The percentage difference of LET formed by the proton beam in the 80-250 MeV energy range of the different thickness CoNiCrMo biomaterial with the cortical bone

Thickness (cm)	Proton Beam Energies (MeV) and Percent Difference (%)										
	80	**100**	**120**	**140**	**160**	**180**	**200**	**220**	**240**	**250**	**Average**
0.4	74.1	72.8	71.7	73.7	72.4	72.9	63.8	68.0	70.6	68.7	70.9
0.6	77.4	76.6	73.5	74.5	72.4	72.4	73.5	71.8	69.0	70.8	73.2
0.8	84.7	86.3	74.9	73.5	75.0	74.0	73.8	73.5	71.8	71.6	75.9
1.0	63.9	82.7	75.9	74.4	73.7	66.1	72.3	74.0	73.9	71.8	72.9
1.2	90.1	86.9	78.6	76.2	76.4	74.1	73.9	74.8	74.1	72.0	77.7

1.4 Ti Alloys

1.4.1 Pure Ti and Ti_6Al_4V

Attempts to use titanium for implant manufacture date back to the late 1930s. Titanium, like stainless steel and Vitallium® (CoCrMo alloy), was found to be tolerated in feline femurs. Titanium's light weight (4.5 g/cm3; see Table 1.9) and good mechanochemical properties are outstanding features for implant application. As given in Table 1.6, there are four types of unalloyed commercially pure (cp) titanium for surgical implant applications. Pollution contents separate them; oxygen, iron and nitrogen must be carefully controlled. In particular, oxygen has a great effect on ductility and durability (Park and Bronzino, 2003).

Table 1.9 Specific Gravities of Some Metallic Implant Alloys (Park and Bronzino, 2003)

Alloys	**Density (g/cm^3)**
Ti and its alloys	4.5
316 Stainless steel	7.9
CoCrMo	8.3
CoNiCrMo	9.2
NiTi	6.7

A titanium alloy (Ti_6Al_4V) is commonly used in implant manufacturing and its chemical requirements are given in Table 1.10. The main alloying elements of the alloy are aluminum (5.5%~6.5%) and vanadium (3.5%~4.5%). Ti_6Al_4V alloy has approximately the same fatigue strength (550 MPa) as CoCr alloy after rotary bending fatigue tests [Imam et al., 1983].

Table 1.10 Chemical Compositions of Ti and Its Alloy [American Society for Testing and Materials, F67–89, p.39; F136–84, p.55, 1992]

Element	Grade 1	Grade 2	Grade 3	Grade 4	Ti_6Al_4V[a]
Nitrogen	0.03	0.03	0.05	0.05	0.05
Carbon	0.10	0.10	0.10	0.10	0.08
Hydrogen	0.015	0.015	0.015	0.015	0.0125
Iron	0.20	0.30	0.30	0.50	0.25
Oxygen	0.18	0.25	0.35	0.40	0.13
Titanium	Balance				

[a]Aluminum 6.00% (5.50~6.50), vanadium 4.00% (3.50~4.50) and other elements maximum 0.1% or total 0.4%. All are the maximum weight percentage allowed.

Table 1.11 Mechanical Properties of Ti and Its Alloys (ASTM F136) [American Society for Testing and Materials, F67–89, p.39; F136–84, p.55, 1992 and Davidson et al., 1994]

Property	Grade 1	Grade 2	Grade 3	Grade 4	Ti_6Al_4V	Ti13Nb13Zr
Tensile strength (MPa)	240	345	450	550	860	1030
Yield strength (0.2% offset) (MPa)	170	275	380	485	795	900
Elongation (%)	24	20	18	15	10	15
Reduction of area (%)	30	30	30	25	25	45

Titanium is an allotropic material with a hexagonal closed-pack structure (hcp, α-Ti) up to 882°C and a body-centered cubic structure (bcc, β-Ti) above this temperature. Titanium alloys can be strengthened and mechanical properties modified by controlled composition and thermomechanical processing techniques. The addition of alloying elements to titanium gives it a wide variety of properties: (1) Aluminum tends to stabilize the α-phase, that is, to raise the transformation temperature from α- to β-phase (Fig. 1.2); (2) vanadium stabilizes the β-phase by lowering the temperature of the transformation from α to β (Park and Bronzino, 2003).

The α-alloy has a single-phase microstructure (Fig. 1.3a), which promotes good weldability. The stabilizing effect of the high aluminum content of these

alloy groups provides excellent strength properties and oxidation resistance at high temperature (300~600°C). Since these alloys are single-phase, they cannot be heat treated for precipitation hardening (Park and Bronzino, 2003).

The addition of controlled amounts of β-stabilizers causes the higher strength β-phase to remain below the transformation temperature that results in a two-phase system. Precipitates of the β-phase will appear by heat treatment at solid solution temperature followed by quenching followed by aging at a slightly lower temperature. The aging cycle causes consistent precipitation of some fine α particles from metastable β; Giving an α structure can produce a local strain field that can absorb the deformation energy. In order for the hardness to be higher than the solid solution, cracks in the α particles are stopped or prevented (Figure 1.3b) (Park and Bronzino, 2003).

The higher percentage of β-stabilizing elements (13V% in Ti13V11Cr3Al alloy) results in a largely β microstructure that can be strengthened by heat treatment (Fig. 1.3c). Another Ti alloy (Ti13Nb13Zr) containing 13% Nb and 13% Zr showed a low modulus (E = 79 MPa) high corrosion resistance martensite structure after water quenching and aging [Davidson et al., 1994]. The formation of martensite plates causes significant elastic deterioration in the main crystal structure and increases the strength (Fig. 1.3d) (Park and Bronzino, 2003).

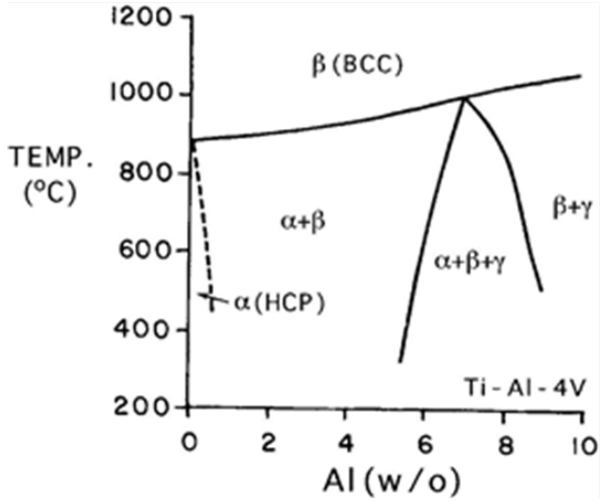

Figure 1.2 Part of phase diagram of Ti-Al-V at 4 w/o V [Smith and Hughes, 1966].

The mechanical properties of commercially pure titanium and its alloys are given in Table 1.11. Except for the 13Nb13Zr alloy, the modulus of elasticity of these materials is about 110 GPa. From Table 1.7 it can be seen that higher impurity content of cp-Ti results in higher strength and lower ductility. The strength of the material ranges from much lower than that of 316 stainless steel or CoCr alloys to approximately equal to that of annealed 316 stainless steel of cast CoCrMo alloy (Park and Bronzino, 2003). However, compared to specific strength (strength per density), titanium alloys outperform other implant materials. Titanium, however, has poor shear strength, making it less desirable for bone screws, plates and similar applications. It also tends to be damaged or jammed when in sliding contact with itself or another metal (Park and Bronzino, 2003).

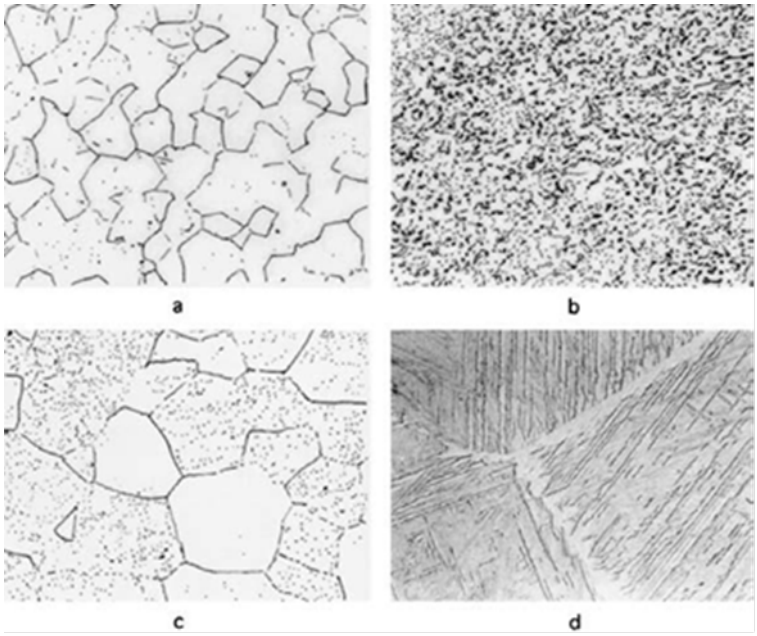

Figure 1.3 Microstructure of Ti alloys (all 500×) [Hille, 1966]. (a) Annealed α-alloy. (b) Ti_6Al_4V, α–β alloy, annealed. (c) β-alloy, annealed. (d) Ti_6Al_4V was heat treated and quenched at 1650°C [Imam et al., 1983].

Titanium provides its resistance to corrosion by forming a solid oxide layer up to a depth of 10 nm. Under in vivo conditions, the oxide (TiO_2) is the only stable reaction product. However, micro-movement is inevitable in cement-prosthesis and cement-bone; As a result, titanium oxide and titanium alloy particles are released in cemented joint prostheses (Park and Bronzino, 2003). Sometimes these wear debris build up as periprostic fluid collects and triggers a giant

cell response around implants. This cystic collection continues to expand and aspiration reveals “dark” heavily stained fluid containing titanium wear particles and histiocytic cells. Histological examination of the stained soft tissue revealed collagenous, fibrous tissue containing “fibrin necrotic residue” and histiocytic and foreign body giant cell infiltrate. Metallosis, the black staining of periprosthetic tissues, has been implicated in knee implants [Breen and Stoker, 1993].

Titanium implant surface consists of a thin layer of oxide and biological fluid of water molecules, dissolved ions and biomolecules (proteins with a water shell around it), as shown in Figure 1.4. The microarchitecture (microgeometry, roughness, etc.) and chemical composition of the surface are important for the following reasons:

1. The physical nature of the surface is based on the size of the biological units atomic, molecular or higher level, biomolecules, cells, etc. may cause different contact areas with Different contact areas can, in turn, produce different types of attachment and disruption of biological units, which can affect their conformation and function (Park and Bronzino, 2003).
2. The chemical composition of the surface can produce different types of bonding to biomolecules, which can also affect their properties and functions. Metals undergo chemical reactions on the surface depending on the environment, which causes difficulties in understanding the exact nature of the interactions (Park and Bronzino, 2003).

The surface-tissue interaction is dynamic rather than static, meaning it will evolve into new phases as time passes, especially in the first period after implantation. During the first few seconds after implantation, only water, dissolved ions and free biomolecules will be present in the closest vicinity of the surface, but no cells. The composition of the biofluid will then continually change as the inflammatory and healing processes progress, possibly resulting in changes in the composition of the biomolecule layer adsorbed on the implant surface until semi-equilibrium is established (Park and Bronzino, 2003). Eventually, cells and tissues will approach the surface and respond in specific ways that can further modify the adsorbed biomolecules, depending on the nature of the adsorbed layer. The type and activity of cells closest to the surface will change over time. For example, depending on the type of initial interaction, the end results may be fibrous capsule formation or tissue integration [Hazan et al., 1993; Kasemo and Lausma, 1988; Takatsuka et al., 1995; Takeshita et al., 1997; Yan et al., 1997].

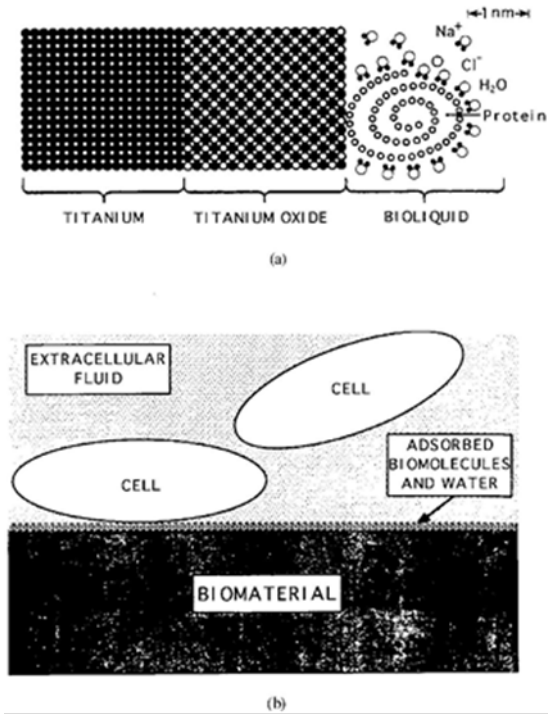

Figure 1.4 (a) Interface between a titanium implant and biofluid and (b) cell surface interaction [Kasemo and Lausma, 1988].

Osseointegration is defined as direct contact between living remodeled bone and an implant without soft tissue interference. The surface roughness of titanium alloys has a significant effect on bone implantation and bone implant interfacial tensile strength. The average roughness increased from 0.5 to 5.9 μm and the interface shear strength increased from 0.48 to 3.5 MPa [Feighan et al., 1995]. The highest levels of osteoblast cell adhesion are achieved on rough sandblast surfaces where cells are more differentiated than on smooth surfaces [Keller et al., 1994]. Chemical changes on the titanium surface after heat treatment are thought to form a TiO2 hydrogel layer on top of the TiO2 layer as shown in Figure 1.5. The TiO2 hydrogel layer can induce apatite crystal formation [Kim et al., 1996].

In general, rougher surfaces have lower cell counts, lower cell proliferation rate, and increased matrix production compared to smoother surfaces. Bone formation appears to be strongly associated with the presence of transforming growth factor β1 in the bone matrix [Kieswetter et al., 1996].

Percent comparisons of the Ti_6Al_4V biomaterial with cortical bone in the mean thickness criteria of proton beam conductivity and at different beam energy ranges are given in Table 1.12. It was observed that the beam conductivity of

the stainless iron approached to the cortical bone as the energy of the proton beam increased at the same thickness. At the same proton beam energy value, it was observed that the difference between the conductivity and the cortical bone increased as the thickness increased. The biggest difference was 38.1% in the 1.2 cm thick stainless iron in the 80 MeV energy proton beam, and the lowest difference was 0.8% in the 0.4 cm thick stainless iron in the 250 MeV energy proton beam. The mean difference between stainless iron and cortical bone in all energy and thickness values was 8.9%.

Table 1.12 Percentage difference of proton beam permeability of Ti_6Al_4V biomaterial with different thicknesses in the energy range of 80-250 MeV with cortical bone

Thickness (cm)	Proton Beam Energies (MeV) and Percent Difference (%)										
	80	**100**	**120**	**140**	**160**	**180**	**200**	**220**	**240**	**250**	**Average**
0.4	14.3	6.9	4.0	4.5	4.1	2.8	1.6	2.3	2.6	0.8	4.4
0.6	17.4	12.3	9.0	6.0	4.1	4.3	4.0	2.0	2.6	2.2	6.4
0.8	23.9	18.3	12.0	9.1	6.1	6.3	4.0	4.7	3.8	3.6	9.2
1.0	27.9	23.3	12.4	11.5	9.6	6.8	5.2	5.7	5.0	3.6	11.1
1.2	38.1	26.5	17.7	13.2	12.0	8.3	5.2	4.5	5.3	4.9	13.6

In Table 1.13, the secondary peak values formed by cortical bone and Ti_6Al_4V biomaterial at different thickness values and proton beam energies were compared. It was observed that the difference increased as the thickness of the stainless iron increased at the same proton beam energy. It was observed that the difference decreased as the proton beam energy increased at the same material thickness. In all proton beam energy and material thickness values, there was an average of 49% difference with cortical bone.

Table 1.13 Percentage difference of the LET formed by the proton beam in the 80-250 MeV energy range of the Ti_6Al_4V biomaterial with different thicknesses and the cortical bone

Thickness (cm)	Proton Beam Energies (MeV) and Percent Difference (%)										
	80	**100**	**120**	**140**	**160**	**180**	**200**	**220**	**240**	**250**	**Average**
0.4	47.7	47.1	43.7	50.4	46.0	49.6	39.7	46.5	44.9	43.5	45.9
0.6	49.6	50.3	47.6	51.0	45.0	46.8	50.4	46.8	41.6	47.2	47.6
0.8	51.6	54.7	49.0	47.7	51.1	49.2	49.3	50.3	46.7	46.7	49.6
1.0	55.6	57.0	49.1	48.5	47.9	51.2	47.1	50.7	50.5	47.0	50.5
1.2	71.8	56.7	50.2	49.2	52.2	47.5	48.0	51.5	40.7	47.0	51.5

1.4.2 TiNi Alloys

Titanium-nickel alloys show unusual properties, meaning that after being deformed, the material can return to its previous shape after being heated. This phenomenon is called shape memory effect (SME). SME of TiNi alloy was first observed by Buehler and Wiley at the US Naval Ordnance Laboratory [Buehler et al., 1963]. Coatomic TiNi or NiTi alloy (Nitinol) exhibits an exceptional SME near room temperature: if it deforms plastically below its transformation temperature, it returns to its original shape as the temperature rises. In general, SME may be related to a diffusionless martensitic phase transformation that is thermoelastic in nature, with thermoelasticity attributed to the ordering in the parent and martensitic phases [Wayman and Shimizu, 1972]. Another unusual property is superelasticity, shown schematically in Figure 1.6. As can be seen, after the initial elastic stress zone, the stress does not increase with increasing stress, and upon release of the stretch or strain, the metal springs return to their original shape unlike other metals such as stainless steel. Because traditional stainless steel wires are too hard and tough for teeth, super-elastic property is used in orthodontic arch wires. In addition, the shape memory effect can be used (Park and Bronzino, 2003).

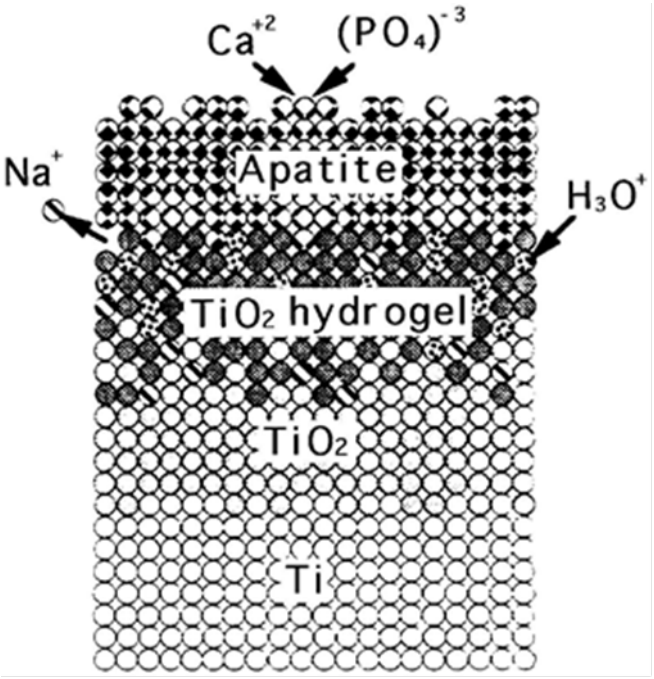

Figure 1.5 Chemical modification of alkali titanium implant surface after heat treatment [Kim et al., 1996].

Some possible applications of shape memory alloys are orthodontic dental arch wire, intracranial aneurysm clip, vena cava filter, contractile artificial muscles for artificial heart, vascular stent, catheter guide wire, and orthopedic staple [Duerig et al., 1990]. To develop such devices, it is necessary to fully understand the mechanical and thermal behavior associated with martensitic phase transformation.

A widely known NiTi alloy is 55-Nitinol (55% by weight or 50% atomic), which, in addition to single-phase and mechanical memory, has other properties such as high acoustic damping, direct conversion of heat energy to mechanical energy, good fatigue properties, and low temperature ductility. Ni). The deviation from 55-Nitinol (closer to stoichiometric NiTi) in the Ni-rich direction also yields a second group of alloys that are not completely magnetic but differ from 55-Nitinol in their ability to be thermally hardened to higher hardness levels (Park and Bronzino, et al. 2003). As the Ni content approaches 60%, the shape recovery capacity decreases and the heat treatability increases rapidly. Both 55- and 60-Nitinols have a relatively low modulus of elasticity and can be harder and more flexible than stainless steel, NiCr or CoCr alloys (Park and Bronzino, 2003).

The efficiency of 55-Nitinol shape recovery can be controlled by varying the

final annealing temperatures during preparation of the alloy device [Lee et al., 1988]. For the most efficient recovery, shape is fixed by confining the sample to a desired configuration and heating to 482~510°C. If the annealed wire deforms at a temperature below the shape recovery temperature, shape recovery occurs upon heating, provided the deformation does not exceed the crystallographic strain limits (~8% strain at stress). NiTi alloys also exhibit good biocompatibility and corrosion resistance in vivo (Park and Bronzino, 2003).

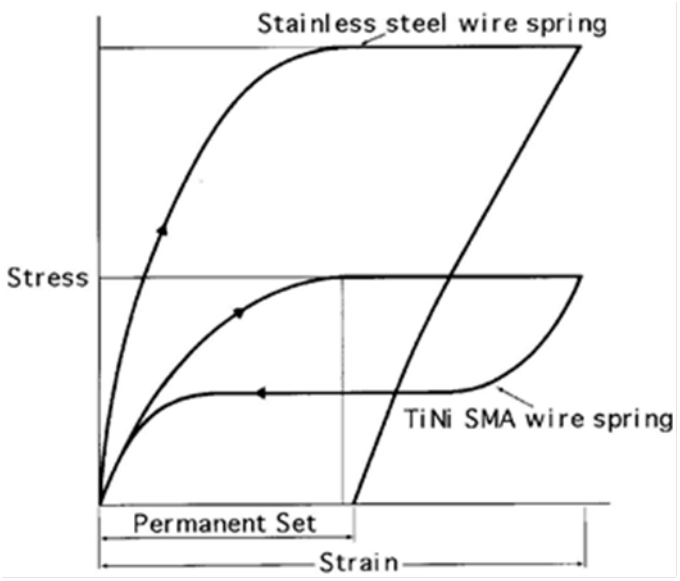

Figure 1.6 Schematic representation of stainless steel wire and TiNi SMA wire springs for orthodontic arch wire behavior (modified from [Wayman and Duerig, 1990]).

There is no significant difference between titanium and NiTi in inhibition of mitosis in human fibroblasts. NiTi showed a lower percentage of bone and bone contact area than titanium and Ti6Al4V alloy [Takeshita et al., 1997].

Table 1.14 Chemical Composition of NiTi Alloy Wire (Park and Bronzino, 2003)

Element	Composition(%)
Ni	54.01
Co	0.64
Cr	0.76
Mn	0.64
Fe	0.66
Ti	Balance

The mechanical properties of NiTi alloys are particularly sensitive to the stoichiometry of the composition (typical composition is given in Table 1.14) and to the individual thermal and mechanical history. Although much is known about the machining, mechanical behavior and properties related to the shape memory effect, much less is known about the thermo-mechanical and physical metallurgy of the alloy (Park and Bronzino, 2003).

Percent comparisons of the TiNi biomaterial with cortical bone in the mean thickness measures of proton beam conductivity and in different beam energy ranges are given in Table 1.15. It was observed that the beam conductivity of the stainless iron approached to the cortical bone as the energy of the proton beam increased at the same thickness. At the same proton beam energy value, it was observed that the difference between the conductivity and the cortical bone increased as the thickness increased. The biggest difference was 66.7% in the 1.2 cm thick stainless iron in the 80 MeV energy proton beam, and the lowest difference was 8.8% in the 0.4 cm thick stainless iron in the 250 MeV energy proton beam. The mean difference between stainless iron and cortical bone in all energy and thickness values was 18.1% (Park and Bronzino, 2003).

Table 1.15 Percentage difference of proton beam permeability of TiNi biomaterial with different thicknesses in the energy range of 80-250 MeV with cortical bone

Thickness (cm)	Proton Beam Energies (MeV) and Percent Difference (%)										
	80	100	120	140	160	180	200	220	240	250	Average
0.4	24.5	16.7	11.0	8.2	6.4	6.2	5.2	4.4	3.7	1.9	8.8
0.6	37.0	27.4	17.0	14.2	10.7	9.1	7.9	5.4	6.4	4.6	14.0
0.8	50.0	32.4	24.0	18.9	13.8	19.7	10.0	8.1	7.6	6.0	19.1
1.0	67.4	45.2	7.2	22.3	18.0	18.9	3.6	11.5	9.9	1.4	20.5
1.2	66.7	51.5	35.4	28.7	22.8	18.5	15.3	19.9	11.4	9.6	28.0

In Table 1.16, the secondary peak values formed by the different thickness values and proton beam energies of the cortical bone and TiNi biomaterial were compared. It was observed that the difference increased as the thickness of the stainless iron increased at the same proton beam energy. It was observed that the difference decreased as the proton beam energy increased at the same material thickness. There was an average of 66.7% difference with cortical bone in all proton beam energy and material thickness values.

Table 1.16 The percent difference of LET formed by the proton beam in the 80-250 MeV energy range of TiNi biomaterial with different thicknesses and cortical bone

Thickness (cm)	Proton Beam Energies (MeV) and Percent Difference (%)										
	80	**100**	**120**	**140**	**160**	**180**	**200**	**220**	**240**	**250**	**Average**
0.4	66.5	65.5	64.6	67.2	65.5	66.4	55.5	60.5	63.9	61.5	63.7
0.6	69.2	69.8	66.0	68.0	65.4	64.7	67.1	64.6	61.9	63.9	66.1
0.8	73.8	72.2	67.6	66.3	68.2	67.4	67.0	67.1	64.7	64.7	67.9
1.0	80.9	75.2	57.2	67.3	66.5	68.4	55.0	67.5	67.5	58.8	66.4
1.2	81.4	77.1	65.8	68.4	69.6	67.0	66.1	68.4	65.2	64.9	69.4

1.5 Conclusion

Because austenitic stainless steels harden quickly, they are processed with intermediate heat treatments. These heat treatments can cause corrosion as they induce the formation of chromium carbide (CCr4) at the grain boundaries. Therefore, welding is generally not recommended for austenitic stainless steel implants (Park and Bronzino, 2003). Another undesirable effect of intermediate heat treatment is the formation of surface oxide deposits. CoCrMo alloy is particularly susceptible to work hardening due to its special manufacturing procedure. However, the alloy must be formed by precision casting. Controlling the mold temperature will have an effect on the grain size of the final casting; rough ones formed at higher temperatures will reduce strength. However, a high machining temperature will result in larger carbide precipitates with greater distances between them, resulting in a less brittle material. Again, there is a complementary (compromising) relationship between strength and endurance (Park and Bronzino, 2003). Titanium is very reactive at high temperature and because it burns in the presence of oxygen, it requires an inert atmosphere for high temperature processing or is processed by vacuum melting. This can cause the oxygen to diffuse easily through the titanium and the dissolved oxygen to

make the metal brittle. Therefore, any hot working or forging operation should be done below 925 °C.

Bragg curves were calculated using the ideal cortical bone thickness of 0.4-1.2 cm of the metallic biomaterial used and intended to be used in cranioplasty in the head plate phantom. Biomaterial data were compared with reference to cortical bone. Bragg peak location in the proton beam compared to cortical bone at each selected thickness was 3-38 % in Ti_6Al_4V, 2-67 % in TiNi, 3-74 % in stainless steel, 2-75 % in CoCrMo and Co-NiCrMo' decreased by 3-75%. In their studies using the head plate phantom, they observed an increase in dose in the cortical bone region (Ekinci and Bölükdemir, 2019). It is named as the second peak because it is the second largest dose increase after the Bragg peak (Ekinci and Bölükdemir, 2019). It has been understood that the second peak, which is more pronounced with the density of the biomaterials used, is formed. The second peak amplitudes of the biomaterials were compared with reference to cortical bone. In the proton beam, 66-25% difference was observed in Ti_6Al_4V, 102-437% in TiNi, 163-699% in stainless steel, 61-786% in CoCrMo and 176%-909% in CoNiCrMo. Although stainless steel stands out in terms of ease of production and frequency of use, Ti_6Al_4V metallic biomaterial has been recommended considering its suitability for proton therapy.

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Chapter 10

Management of Sarcopenia in Older Adults

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A. Definition of Sarcopenia

Sarcopenia is the loss of muscle mass and function associated with age. It begins with atrophy and loss of type II muscle fibers and continues as a result of complex interaction of environmental and genetic causes.[1] It is common among older adults but can also occur earlier in life. In 1931, Critchley noted that muscle loss occurs with aging and is most common in muscles of the hands and foot.[2] Rosenberg used first the term sarcopenia in 1989. The term sarcopenia derives from the Greek "sarx (muscle)" and "penia"(loss).[3] Sarcopenia is a geriatric syndrome since 2010. In 2010, the European Working Group on Sarcopenia in Older People (EWGSOP) published a sarcopenia definition that aimed to foster advances in identifying and caring for people with sarcopenia and it was updated in early 2018 by the Working Group (EWGSOP2). Sarcopenia is a muscle disease defined as decreasing of muscle strength, muscle quantity/quality and physical performance according to the The European Working Group on Sarcopenia in Older People 2 (EWGSOP2) criteria. It is diagnosed by measuring muscle mass, strength and function. EWGSOP2 focuses on low muscle strength as a key characteristic of sarcopenia. Low muscle strength is the primary parameter of sarcopenia and suggests possible sarcopenia.The sarcopenia diagnosis is confirmed by the presence of low muscle quantity or quality. Muscle quality can be also impaired in sarcopenia; used to describe muscle composition. Due to technological limitations, measurement of muscle quantity and muscle quality as primary parameters to define sarcopenia is difficult. Severe sarcopenia is considered with a combination of low muscle strength, low muscle quantity/quality, and low physical performance. Poor physical performance is used for identifies severe sarcopenia. Detection of low physical performance predicts adverse outcomes.[4] (Table 1)

Sarcopenia is associated with poor health outcomes such as falls and fractures, functional decline, cardiorespiratory diseases, physical and cognitive capacity reductions, immobility, low quality of life, frailty and death.[5-11] . Sarcopenia increases hospitalization.[12] Sarcopenia is financially costly for healthcare systems.

Table 1. 2018 operational definition of sarcopenia

Probable sarcopenia is identified by Criterion 1.

Diagnosis is confirmed by additional documentation of Criterion 2.

If Criteria 1, 2 and 3 are all met, sarcopenia is considered severe.

1. Low muscle strength
2. Low muscle quantity or quality
3. Low physical performance

B. Prevalence

The prevalence of sarcopenia was 11% by EWGSOP2. It was 1-29% in community-dwelling populations, 14-33% in long-term care populations and 10% in the only acute hospital-care population examined.[4] It has been reported to range from 1.4% to 5.2% in men and from 1.6% to 7.2% in women.[13]

A progressive loss of muscle mass occurs from the age of about 40. This loss causes a 40% reduction in muscle circumference by the age of 60. After the age of 70, there is a faster loss of 25% to 40% per decade. The prevalence of sarcopenia was >50% in persons older than age 80.[14]

C. Risk Factors

Sarcopenia is considered to be an inevitable part of aging. Aside from aging, some risk factors may contribute to the sarcopenia. The most crucial risk factor for sarcopenia is lack of exercise.[15] The decline in muscle fiber and strength is more pronounced in patients with sedentary lifestyle.[16] Growth hormone, testosterone, thyroid hormone, insulin-like growth factor hormone concentrations decrease with aging. This leads to loss of muscle mass and strength. Muscle loss often results from decreasing hormonal anabolic signals and increasing catabolic signal inflammatory cytokines.[17] The ability to synthesize protein decrease with aging. Oxidized proteins are inadequately removed via the proteolysis system in skeletal muscle. This leads to an accumulation of dysfunctional protein in skeletal muscles and loss of muscle strength.[18] Moreover, the age-related decrease occurs in motor nerve cells that initiate movement. In addition, it has been suggested that low birth weight is associated with reduced muscle mass and strength in adult life.[19]

D. Screening Tools

The prevalence of sarcopenia increases with aging population. Sarcopenia is an overlooked and undertreated diagnosis in clinical practice. Sarcopenia is usually not noticeable in earlier stages. The evaluation of sarcopenia requires considerable time for objective measurement of muscle mass and function. Therefore, simple screening tools have been found. A wide variety of tests and tools are available for screening sarcopenia in practice. SARC-F is a very practical tool that is presented at 2013.[20] This screening tool was evaluated in the African American Health Study, Baltimore Longitudinal Study of Aging and the National Health and Nutrition Examination study.[21] The SARC-F questionnaire assesses 5 domains: strength, independence walking, rising from a chair; climbing stairs and history of falls. The total score was 10 points with each domain scoring two. A score of 4 or more indicates a risk of sarcopenia. (Table 2).

Table 2. SARC-F Score

Component	Question	Scoring
Strength	How much difficulty do you have in lifting and carrying 4.5 kg?	None = 0; Some = 1; A lot or unable = 2
Independence walking	How much difficulty do you have walking across a room?	None = 0; Some = 1; A lot or unable = 2
Rising from a chair	How much difficulty do you have transferring from a chair or a bed?	None = 0; Some = 1; A lot or unable = 2
Climbing stairs	How much difficulty do you have climbing a flight of 10 stairs?	None = 0; Some = 1; A lot or unable = 2
Falls	How many times did you fall in the past year?	None = 0; Some = 1; A lot or unable = 2

Studies have shown that the SARC-F has low sensitivity and high specificity. Therefore, it can generally detect severe cases. Alternatively, clinicians may prefer another screening tools. For example, the Ishii screening test is a method that estimates the probability of sarcopenia using age, grip strength and calf circumference.[22] In clinical practice, further testing for sarcopenia is recommended when a patient reports some symptoms (ie, falling, feeling weak, slow walking speed, difficulty rising from a chair, or weight loss/muscle wasting).

E. Measuring sarcopenia parameters

1. Muscle Strength

Measuring grip strength is easy and low grip strength is a powerful predictor of negative health outcomes such as longer hospitalization, functional limitations, poor quality of life, disability and death. A measurement of grip strength requires

use of a calibrated hand dynamometer. The Jamar dynamometer is validated and widely used for measuring grip strength. Low handgrip strength thresholds were defined according to the national data (<16 kg and <27 kg for females and males, respectively).[4]

The chair stand test can be used as a proxy for strength of leg muscles. (5-times sit-to-stand). It measures the time required to perform to rise five times from a seated position without using arms for a patient.[4] The cut-off threshold for the chair stand test is >15 seconds. It is a variation that 30-second chair-stand test.[23]

2. Muscle Mass

2.1. Magnetic Resonance (MR) and Computed Tomography (CT)

There are some muscle mass measurement techniques. MR and CT are gold standards techniques for the assessment of muscle mass and quality.[24] CT images of the lumbar (L3) vertebra are correlated significantly with whole-body muscle.[25] Mid-thigh imaging using MRI or CT is a good predictor of whole-body skeletal muscle mass and is very sensitive to change.[26] CT-based measurement of the psoas muscle has also been reported as simple and predictive of morbidities in certain conditions.[27] MRI and CT are used to assess muscle quality. However, these tools are not commonly used in practice.

2.2. Dual-energy X-ray absorptiometry (DXA)

Current diagnostic methods for sarcopenia include measuring muscle mass using dual-energy X-ray absorptiometry (DXA), ultrasound and bioelectrical impedance analysis (BIA). DXA is a more widely available instrument to determine muscle quantity (total body lean tissue mass or appendicular skeletal muscle mass: ASM) non-invasively. ASM index (ASMI) can be adjusted for body size in different ways, namely using height squared (ASM/height2), weight (ASM/weight) or body mass index (ASM/BMI). The cut-off thresholds for ASMI (height2) were designated as 7.0 kg/m^2 and 5.5 kg/m^2, in males and females, respectively.[4]

2.3. Bioimpedance analysis (BİA)

Bioimpedance analysis is a widely and successfully applied modality in clinical practice and research.[28] Fat-free mass is usually measured with bioimpedance analysis. (Fat-free mass cut-off points for men <20kg, for women <15 kg). Skeletal MM (SMM) is calculated using the formula: SMM (kg) = 0.566 × fat-free mass (kg). Muscle mass is related to body composition. Therefore, skeletal muscle mass index (SMMI) can be adjusted for using height squared, weight or

body mass index. The references vary by different population. In Turkey, the cut-off thresholds for skeletal muscle mass indexes (height2) were designated as 9.2 kg/m2 and 7.4 kg/m2, in males and females, respectively.[29]

2.4. Ultrasound

Ultrasound is a widely used research technique to measure muscle quantity, identify muscle wasting, and measure muscle quality. It is reliable and valid and is starting to be used at the bedside by trained clinicians. Assessment of pennate muscles such as the quadriceps femoris can detect a decrease in muscle thickness and a cross-sectional area within a relatively short time, thus suggesting potential for using this tool in clinical practice.[4]

2.5. Creatine dilution test

Creatine dilution test is a promising proxy measure for estimating whole-body muscle mass. Creatine is produced by the liver and kidney and is also ingested from a diet rich in meat. Creatine is taken up by muscle cells, where a portion is irreversibly converted each day to phosphocreatine, a high-energy metabolite. Excess circulating creatine is changed to creatinine and excreted in urine. For a creatine dilution test, an oral tracer dose of deuterium-labelled creatine is ingested by a fasting patient; labelled and unlabelled creatine and creatinine in urine are later measured using liquid chromatography and tandem mass spectrometry. Total body creatine pool size and muscle mass are calculated from deuterium-labelled creatine enrichment in urine.[30]

2.6. Anthropometric measurements

Anthropometric measurements are sometimes used to reflect nutritional status in older adults. Calf circumference is measured at the widest circumference of the calf while the subjects are standing. Middle arm circumference is measured at the middle of humerus while the arm is mildly elevated and internally rotated. Calf circumference has been shown to predict performance and survival in older people (cut-off point <31 cm).[31] However, it is not a good assessment technique to measure muscle mass.

3. Physical performance

Physical performance has been defined as an objectively measured whole-body function related to locomotion. Physical performance can assess by gait speed (GS), the Short Physical Performance Battery (SPPB), the Timed-Up and Go (TUG) and 400-m walk test. Gait speed is considered a quick and highly

reliable test for sarcopenia in practice. A commonly used gait speed test is called the 4-m usual walking speed test which is determined in meters per second. The abnormal test result is recognized as ≤0.8 m/s.[4] The SPPB is a composite test that includes assessment of gait speed, a balance test, and a chair stand test. The maximum score is 12 points, and a score of ≤ 8 points indicates the poor physical performance.[4] The TUG evaluates physical function. For the TUG test, individuals are asked to rise from a standard chair, walk to a marker 3 m away, turn around, walk back and sit down again. A score of 20 s and above indicates the poor physical performance. The 400-m walk test assesses walking ability and endurance. The abnormal test result is defined as the patient walking less than 400 meters in 6 minutes or cannot complete the test.[4]

F. Primary and Secondary Sarcopenia

Primary Sarcopenia is age-related muscle loss and has no specific cause, while in secondary sarcopenia, causal factors are evident than aging. Sarcopenia can develop as a result of inadequate intake of energy or protein such as anorexia, malabsorption, malnutrition, obesity and overnutrition. Physical inactivity, sedentary lifestyle, immobility also contributes to development of sarcopenia. Sarcopenia can also occur secondary to a systemic disease, rheumatological disorders, neurological diseases, inflammatory conditions (e.g. malignancy, organ failure). High levels of cytokines, age related changes in hormon levels, declines in neuromuscular junctions and mitochondria, apoptosis are contributed to the muscle loss. There are some potential biomarkers such as N-terminal propeptide, procollagen III, myostatin, activin causing sarcopenia.[32] Its etiology is multifactorial. The management of secondary sarcopenia should focus on treating the underlying primary condition.

G. Treatment

Early diagnosis and intervention are the keys to preventing poor outcomes in patients with sarcopenia. Treatment of sarcopenia includes exercise, nutritional support, some pharmacological, hormonal treatments and some alternative treatment options. The management of primary sarcopenia should focus on treating the some strategies to improve skeletal muscle strength and mass outlined below.

1. Non-Pharmacologic Treatment

Exercise

Exercise is the primary treatment for sarcopenia increases muscle mass and strength. Multicomponent exercise program including endurance, resistance,

coordination, balance, and flexibility exercises has been recommended for older adults to improve muscular strength, gait speed, balance and physical performance. Resistance training is a form of exercise that improves muscle strength and endurance. This is also known as strength training or weight lifting. During a resistance training workout, you move your limbs against resistance provided by your own body weight, gravity, bands, weighted bars, or dumbbells. Short-term resistance exercise increases ability and capacity of skeletal muscle to synthesize proteins.[33] Some researches have demonstrated that progressive resistance exercise to stimulate muscle protein synthesis, leading to improvements in muscle mass, strength, and functional capacity in sarcopenic older adults.[34,35] Aerobic exercise contributes to muscle protein synthesis. It can include activities like brisk walking, swimming, running, or cycling. Aerobic exercise causes ATP production in mitochondria within skeletal muscle, and improves aerobic capacity, metabolic regulation, and cardiovascular function.[36] Both resistance exercise and aerobic exercises are beneficial in improving age-related sarcopenia.

Nutrition

Maintaining or improving nutritional status in older adults is important for clinical course and quality of life. Nutrition is considered as a major point for the management of sarcopenia. Malnutrition is an essential component of sarcopenia. Nutritional interventions have proven efficacy regarding increased muscle mass and functional status.[37] Therefore, we should perform nutritional screenings to prevent sarcopenia that measure appropriate and adequate nutrition.

The consumption of protein nutrition enriched with qualified, sufficient and essential amino acides is also important. Using a combined approach of dietary supplements and exercise are known to have some benefits.[38] Protein supplementation (above the recommended daily amount 1.0-1.2 g/kg/day) combined with resistance exercise interventions provides an increase in muscle and bone mass as well as muscle strength, balance and functional capacity.[39,40] Older adults with acute or chronic diseases need higher dietary protein (i.e. 1.2–1.5 g/kg/day).[41] β-hydroxy β-methylbutyrate (HMB) is a leucine metabolite that has a role in regulating protein synthesis in muscle cells affects muscle mass and function. Essential amino acid supplements, including ~2.5 g of leucine, and HMB) supplements, show some effects in improving muscle mass, muscle strength and function parameters.[42]

In addition, vitamin D supplementation are essential. Vitamin D supplementation can be beneficial to improve muscle strength or physical performance when an older adult with sarcopenia has low baseline vitamin D

levels (<20 nmol/l).[43] The vitamin D supplementation has been shown to increase appendicular lean (muscle) mass and chair-stand speed and reduce markers of inflammation in sarcopenic older adults.[44,45]

2. Pharmacologic Treatment

Currently, there are no definitive pharmacologıcal therapy agents for the treatment of sarcopenia. Studies on hormonal treatments are also been investigated.

Growth hormone/ Insulin-like growth factor 1 (IGF-1)

Insulin-like growth factor 1 (IGF-1) has anabolic effects in adults. IGF-1 is produced primarily by the liver. Production is stimulated by growth hormone (GH). Growth hormone increases muscle protein synthesis and muscle mass but does not lead to gains in strength and function. This may be due to a lack of insulin-like growth factor 1 (IGF-1) activity, resistance to IGF-1 and inflammation in aging muscle.[46] Additionally, the side effects of IGF-1 are fluid retention, gynecomastia, orthostatic hypotension, carpal tunnel compression, hyperglycemia, arthralgia and general weakness. GH or IGF-1 supplementation are not recommended for improving muscle mass, muscle strength or physical performance in older people. Human growth hormone has little to no effect.[43]

Testosterone

Testosterone and anabolic steroids have a positive effect on muscle strength and mass. Age-related decline in testosterone leads to loss of muscle mass and strength. In older adults with clinical muscle weakness and low testosterone serum levels, testosterone has been shown to improve muscle mass, muscle strength and/or physical performance. However, they have limited use due to the adverse effects, such as the increased risk of prostate cancer in men, virilization in women, and overall increased risk of cardiovascular events.[46,47] Testosterone supplementation is recommended to improve muscle mass and strength in older men with low serum testosterone levels (<200–300 ng/dl) and have clinical muscle weakness. Hematocrit, lipid profile and prostate parameters should be monitored.[43]

Dehydroepiandrosterone

Dehydroepiandrosterone can be transformed to estrogen or testosterone in the body, could have some effect on muscle strength. But the results are inconclusive.

Selective Androgen Receptor Modulators (SARMs)

Selective androgen receptor modulators (SARMs) differentially bind to androgen receptors. SARMs result in anabolic cellular activity while avoiding the side effects of anabolic steroids. Selective androgen receptor modulators are hoped that they can achieve gains in skeletal muscle mass and strength without dose-limiting adverse events. However, its effects on muscle strength and function are lesser than testosterone.[47]

Angiotensin-Converting Enzyme Inhibitors (ACEI)

The renin–angiotensin–aldosterone system activity has been implicated in skeletal muscle via multiple different mechanisms. ACEI have been shown to have multiple potentially beneficial effects on skeletal muscle.[48] They may increase IGF-1 levels, regulation of angiogenesis and reduce inflammation.[49] Older people with heart failure or with severe sarcopenia might benefit from ACEIs.[50] ACEIs may be used to improve muscle mass, muscle strength or physical performance in older people.

Herbal Supplements

There is a great interest in using herbal supplements to promote muscle mass and health in patients with sarcopenia. Several botanicals (Phlebodium decamanum, Citrus aurantium, Coffea arabica, Zingiber officinale, Eugenia punicifolia, Panax ginseng, Go-sha-jinki-Gan, Vitis vinifera, and Curcuma longa) have a significant role in the prevention of sarcopenia.[51] It is recommended to use herbal supplements to promote muscle mass and health in patients with sarcopenia.[52] Using these supplements in humans is limited due to efficacy, potential drug interactions, and side effects. Hence, supporting the use of herbal supplements for the treatment and prevention of sarcopenia is limited until further research proves their safety and efficacy in humans.

H. Conclusion

Sarcopenia is a global health concern. Prevalence of sarcopenia has been reported 5-13% for aged 60 to 70 years and up to 50% for people over 80 years of age.[53] The number of older people 60 years and over was estimated to be 600 million in 2000 around the world. It is expected to rise to 1.2 billion by 2025 and 2 billion by 2050.[54]

The diagnosis of sarcopenia can be difficult. Measurements of sarcopenia components are not always practical in the clinic. Exercise remains the intervention of choice for managing sarcopenia. In addition, adequate protein intake,

replacing deficient nutrients and vitamins is recommended for the management of sarcopenia. Increasing awareness among healthcare providers, early screening and a multidisciplinary approach to treatment is current best practice to minimize the overall negative impact of sarcopenia.

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Chapter 11

Biological Effects Of Ionizing Radiation

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1. INTRODUCTION

X-rays began to be used for therapeutic purposes right after they were discovered in 1895. As a result of the applications, it was observed that healthy tissues were damaged and cancer occurred in the long term. The effect of radiation on living things is seen on cells, tissues, organs, systems or the whole body. The effects of radiation on living things occur in three stages: physical, chemical and biological.

Physical Stage: In this stage, the initiating reactions are ionization and excitation, and the occurrence time is 10-18-10-12 seconds.

Chemical Stage: Biomolecular disorders occur in this stage. Free radicals are formed. Damages occur in nucleic acids and proteins, lasting from 10-12 seconds to hours.

Biological Stage: It is the stage in which biological disorders occur. Cell death, death of the organism, mutations and cancer formation are possible.

2. RELATIONSHIP OF RADIATION DOSAGE AND RESPONSE

The radiation effect is not seen immediately after the irradiation. The time elapsed between the time of the effect and the time of the irradiation is called the latent period. Although the effects of different radiation and their dose levels are known, the limits cannot be determined precisely. There are two different dose-effect relationships that explain the possibility of a biological effect depending on the dose taken.

Threshold Dose-Effect Relationship (Deterministic Effect): In the thresholded dose-effect relationship, the effect first increases slowly and then rapidly with increasing dose and reaches saturation. There is a certain threshold dose and it is considered that no biological effects are seen up to this threshold dose. The dose rate has an impact on the extent of the damage. The effect that accepts the presence of a threshold dose is also called the nonstochastic effect. Figure 1 shows the dose-response graph showing the deterministic effect. The early and late response of somatic cells to radiation is expressed by the deterministic effect relation. A threshold dose is absolutely necessary for radiation to have an effect on somatic cells.

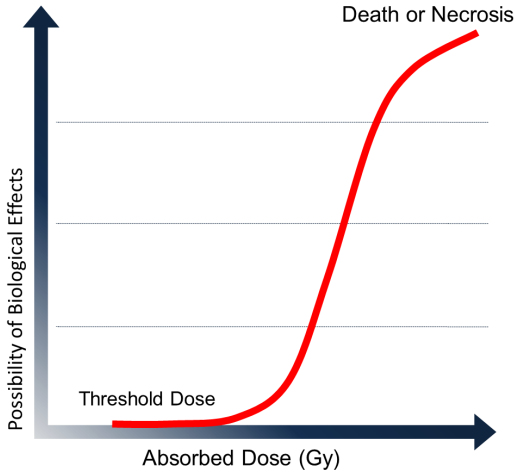

Figure 1: Threshold Dose - Effect Relationship (Sigmoid Curve)

Unthreshold Dose – Effect Relationship (Stochastic Effect): The probability of the effect of radiation without the need for a threshold dose directly depends on the dose of the radiation. Dose rate does not affect the curve. Very small doses are added in succession. Effects without threshold are called stochastic effects as well as nondeterministic effects. Figure 2 shows the dose-response curve showing the stochastic effect without a threshold dose.

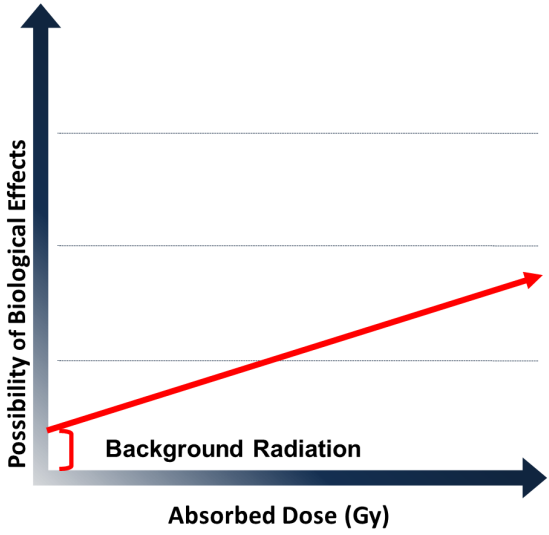

Figure 2: No Threshold Dose – Effect Relationship (Linear Effect)

The biological effects of radiation are divided into two as somatic (bodily) and genetic effects. The dose-effect relationship is different in the emergence of somatic and genetic effects. Figure 3 shows the biological effects of radiation schematically.

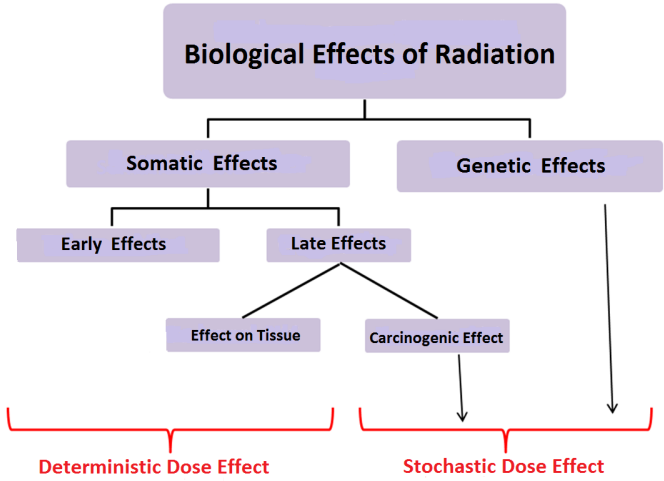

Figure 3: Diagram showing the biological effects of radiation

Factors determining the effects of radiation on living things:

- Radiosensitivity of the irradiated area
- Type of radiation exposed
- The amount of radiation exposed
- Radiation exposure time

3. RADIOSENSITIVITY OF TISSUE AND ORGANS

Sensitivity Classification of Tissues

In 1906, Bergonie and Tribondeau investigated the response of the rat testis to radiation in a study on this subject. It has been reported that the radiation sensitivity of the tissue is directly proportional to the mitotic activity and inversely proportional to the degree of differentiation.

Examples of Radiation Sensitive Tissues: Bone marrow, gonads, small intestine, epithelial tissue in the skin.

Examples of Radiation-Resistant Tissues: Liver, kidney, muscle, brain, bone, cartilage and connective tissue.

Radiation Sensitivity of Parenchymal Cells and Connective Tissue: Parenchymal cells perform special functions of tissues, they are supported by

connective tissue cells. Radiosensitivity of parenchymal cells varies according to organs, while radiosensitivity of connective tissue cells is moderate.

Mitotic activity is important in radiation sensitivity. Information on the relationship between the degree of differentiation and radiation sensitivity is still insufficient. Table 1 shows the sensitivity classification of tissues.

Table 1: Sensitivity Classification of Tissues

Cell Type	Cell Properties	Cell Examples	Radiosensitivity
I- Cells dividing without differentiation	They are constantly divided, they do not differentiate	Erythroblasts, Intestinal crypt cells, Germinal cells of the epidermis	High
II- Cells dividing by differentiation	They divide constantly, differing between divisions	Myelocytes	
Connective Tissue Cells			
III- Cells with the ability to divide	They are constantly indivisible, differentiated to varying degrees	Liver	
IV- Non-dividing cells	Hiç bölünmezler, yüksek düzeyde farklılaşma gösterirler.	Nerve cells, Muscle cells	Low

Effect of Radiation on Cell

The building block of the living organism is the cell. Different sensitivities of cells cause different sensitivities in organs. As the radiosensitivity of each cell is different, the cell nucleus, cytoplasm and cell membrane that make up the cell have different sensitivity. In figure 4 the structure of the cell is shown with a simple diagram.

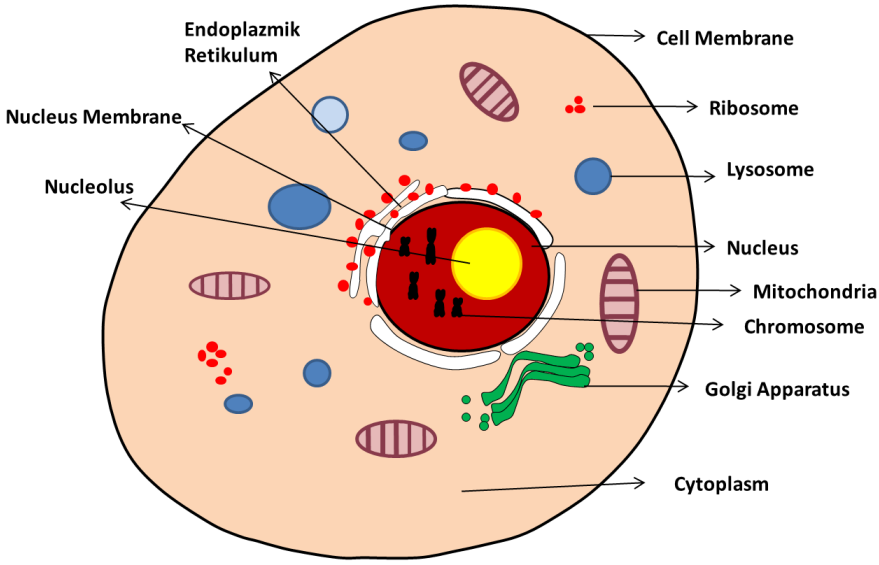

Figure 4: The structure of the cell is shown with a simple diagram

Cell Membrane: It is a transparent, fluid and flexible structure that surrounds the cytoplasm, protects the cell from disintegration and external factors, has selective permeability thanks to the holes on it. It is composed of lipid and protein. It provides the exchange of substances to the cell. It is the most resistant part of the cell to radiation.

Cytoplasm: It is a semi-fluid liquid composed of organic and inorganic substances such as protein, carbohydrates, fat, vitamins, minerals, enzymes and water, and is composed of organelles such as ribosome, endoplasmicreticulum, Golgi body, lysosome, and mitochondria. Although the sensitivity of the organelles to radiation is different, in general, the cytoplasm is moderately sensitive to radiation. It has been shown experimentally that a much higher dose is given with a polonium needle to kill a cell whose cytoplasm is irradiated only, compared to a cell irradiated with the cell nucleus.

Cell Nucleus: Many vital substances in the cytoplasm are made by the nucleus. Protein, enzyme, nucleic acid, DNA (Deoxyribo Nucleic Acid) and RNA (Ribo Nucleic Acid) synthesis are done here. It is the most sensitive part of the cell to radiation. DNA is more sensitive to radiation than RNA. The cell suffers the most damage when exposed to radiation. In DNA radiation damage, the target molecule is considered. One of the functions of DNA is to create identical DNA

by replication, and another task is to produce RNA. It carries genetic information vertically to future generations. Radiation causes structural and functional losses on DNA. Chromatid and chromosome damage may occur due to these damages. Structural Losses are chain breaks, base damage and base losses, formation of denaturation zones and crosslinking. Radiation damages can be in the form of single-chain fracture, double-chain fracture and fragmentation in the DNA molecule. Radiation affects DNA synthesis and accordingly leads to incorrect transmission of information.

Effects of Ionizing Radiation on DNA: As a result of the transfer of energy, the ejected electron directly affects the DNA molecule and causes damage by forming single or double chain breaks. The effect that occurs in this way is called the direct effect. The ejected electron interacts with the environment molecules in the first stage, causing the formation of free radicals, and in the second stage, it causes damage to the DNA molecule. Such an effect is called an indirect effect. Radiations with low LET transfer energies to matter through an indirect effect. Damages caused by this type of impact are generally considered to be single chain fractures and the resulting damage can therefore be repaired. Radiations with high LET are radiations with direct effect. It is thought that the radiations generated by this type of effect usually cause double chain fractures, therefore, it is not possible to repair the damage. In Figure 5, the direct and indirect effect mechanism of radiation is shown schematically.

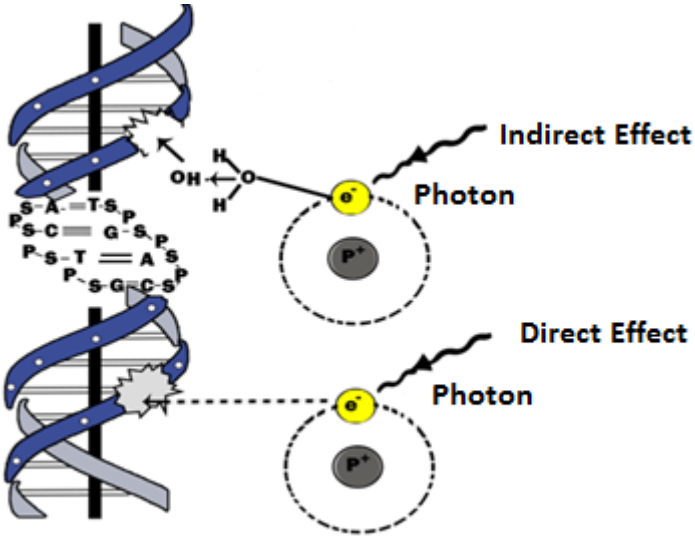

Figure 5: The direct and indirect effect mechanism of radiation is shown schematically.

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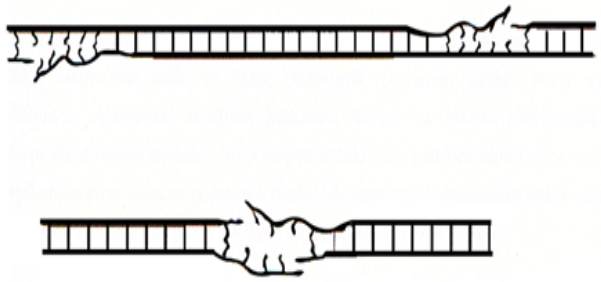

Figure 6: Single DNA chain breaks are shown at the top and Double strand breaks at the bottom

4. TYPE OF RADIATION EXPOSURE

The biological effects of ionizing radiation depend on the energy and type of radiation. The damage of radiation depends on the amount of ionization created by the energy transferred to the tissue; it is called Linear Energy Transfer (LET).

Linear Energy Transfer: It is the energy left in the environment along a unit length trace. LET is directly related to ionization and biological damage. It determines the quality of radiation.

LET (KeV/µm)= SI x W

SI = number of ion pairs per unit length

W = Energy released per ion pair

Table 2 shows the LET values according to the type and energy of the radiation.

Table 2: LET values by type of radiation

Type of radiation	LET keV/µm
Cobalt-60	0.25
250 kVp X-ray	2.0
Diagnostic X-rays	3.0
10 MeV protons	4.7
150 MeV protons	0.5
14 MeV neutrons	12.0
2.5 MeV alpha particle	166.0
Heavy Particles	1000.0

The biological effect is low in radiations with low LET and high in radiations with high LET. To express the biological effect, the concept of Relative Biological Efficiency (RBE) is used. **Relative Biological Efficacy:** Defined as the ratio of the reference radiation dose to the test radiation dose to achieve the same biological effect. The reference radiation is 250 kVp X-rays. The same biological effects or biological endpoint is fixed. But doses are not. Figure 7 shows the relationship between biological activity and LET.

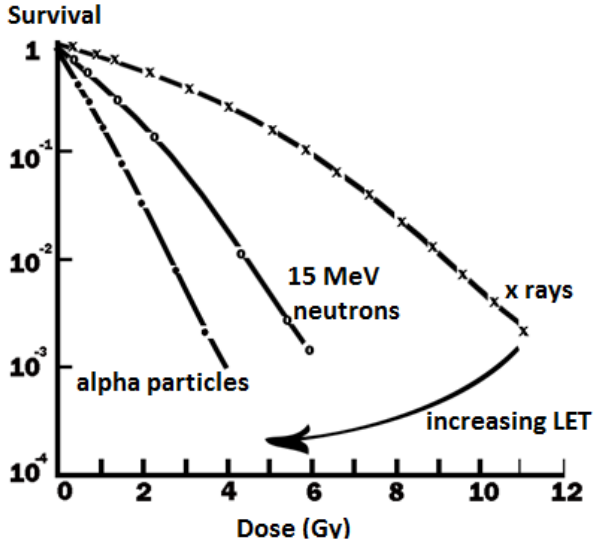

Figure 7: Relationship between biological activity and LET

In low LET radiation applications, the number of surviving cells for the same physical dose is higher than in high LET radiations. In low LET applications, the survival curve initially formed the shoulder, and the subletal injuries are repaired. In high LET radiations, there is no shoulder area, and subletal damage is not repaired. The number of surviving cells in the group of cells exposed to 1Gy photon rays is not the same as the number of surviving cells when exposed to 1 Gy α particle of the same number of cells. The doses are the same physically, but not biologically. The rays used in radiology are generally below 300 kVp, these rays have low LET. The RBE value is 1. Their biological activities are the same in all radiology applications.

5. FACTORS CHANGING THE EFFECT OF RADIATION

As the amount of radiation exposure of a living organism increases, deterministic and stochastic effects due to radiation increase. For this reason, care should be taken not to overdose the patient during radiation administration. The doses applied in radiotherapy are high. While giving the necessary dose to destroy the tumor, it should be ensured that the patient's healthy tissues receive the minimum dose.

The doses used in radiology applications are small, but small dose applications are added one after the other, causing an increase in the stochastic effect. It increases the possibility of genetic effects and cancer formation due to radiation. It is necessary to avoid film repetition in dental radiology and other radiology applications. In order to obtain the desired image, the patient should be prevented from overdosing by selecting the appropriate kV and mA. When necessary, a lead apron should be used so that the patient is not exposed to unnecessary radiation.

The atomic bomb was dropped on Hiroshima on August 6, 1945, and on Nagasaki on August 9, 1945. Our first information about the radiation dose was obtained as a result of this disaster. In addition to deaths due to exposure to doses, it has also caused mutations that will last for generations. The doses exposed in the bomb or reactor accidents are very high at the site of the accident, and decrease inversely with the square of the distance away from the center of the accidents. There have been many nuclear accidents to date. The most important of these accidents is the accident at the Chernobyl nuclear power plant in 1986, which is called the disaster. It has caused many direct deaths.

Regardless of the purpose, as the dose of radiation increases, the harmful effects of radiation will also increase.

Single or Fractional Radiation Applications

The amount of radiation dose exposed is as important as how long this dose is given. For example, the number of cells surviving when a radiation dose of 10 Gy is given at once is not the same as the number of cells surviving when this dose is given as 5+5 Gy in two times with a few hours intervals. Cells damaged by radiation repair themselves until the second dose is administered after a few hours. When the second dose is applied, some of the cells die again. Figure 8 shows the survival graphs showing the amount of cells that survived in single-dose and double-dose irradiation. The graph shows the amount of cells repaired in the shoulder area. Due to this repair mechanism, high doses applied in radiotherapy are not given all at once, but in fractions, allowing normal tissue to repair itself. The ability of the tumor tissue to repair itself is negligible. Radiation has both deterministic and stochastic effects in radiotherapy applications.

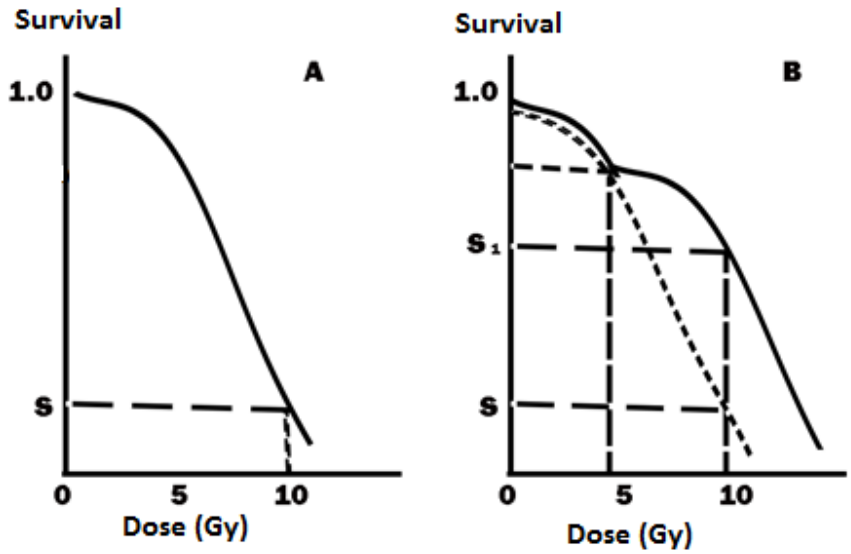

Figure 8: Applications of radiation dose in A) single and B) double fractions

The doses taken in dental radiology and other radiology applications are low, the application to be made is done in a single application so that the patient receives the least dose. The exposed doses are not doses that will create a deterministic effect with a threshold dose value that will change the function of organs in humans. These are the doses that do not require a threshold dose, which may increase the possibility of cancer formation and mutation.

In radiotherapy applications, the radiation dose received is much higher than the dose taken for diagnostic purposes. Both deterministic effects and stochastic

effects occur in people treated with radiotherapy. Depending on the radiation dose received in the early and late periods, chronic organ and tissue damage may occur. The probability of secondary cancer due to radiation also increases.

Persons working in radiotherapy and radiology applications are exposed to small doses for many years, as a result of which radiation has stochastic effects. Deterministic effects of radiation are not seen in workers. Genetic influence and the possibility of cancer may increase. For those working with radiation, the average and maximum doses they can be exposed to are limited by international commissions and national legal regulations.

6. CONCLUSION

As a result; Radiation has harmful effects on living things. Before deciding on radiation application, options that can be done without radiation should be evaluated. After the decision to application radiation, the patient should receive the lowest possible dose. The possible effects of radiation therapy should be explained to the recipients.

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Chapter 12

Biomaterials Used in Orthopedics and Their Properties

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1-Biocompatibility of Implants:

It is the ability of an implant to respond appropriately to the body. If it is biomaterial; It is defined as a material with biocompatibility. Biocompatibility is the most important feature of a biomaterial besides its strength. A biocompatible material does not harm the surrounding tissue in the body, does not develop a reaction and is integrative. Biocompatibility is the physical, chemical and biological compatibility of a biomaterial to body tissues and the

Implants can be classified as bioactive, biotolerant and bioinert according to their biocompatibility. Ceramics such as calcium hydroxyapatite are bioactive materials. Calcium hydroxyapatite coated implants are attached to the mineralized bone matrix through chemical reactions(1).For this reason, they are used as artificial bone in the construction of various prostheses, in the repair of cracked and broken bones, and in the coating of metallic biomaterials.

2-Types of Biomaterials:

a-Metallic Implants:

Changes that occur depending on the chemical environment in the body are called **corrosion**. Corrosion is a chemical reaction that weakens metals. In fact, all metals undergo these reactions in the physiological environment. This differs according to the chemical structure of the metals used. Stainless steel implants are more susceptible to corrosion than Cr-Co and titanium alloys. There are three types of corrosion in metals such as **fatigue, galvanic and crevice corrosion** (look table1)**.** Recently, it has been tried to prevent corrosion by changing the surface coatings of metals. However, repeated microtraumas can create scratches on the surface and lead to the wear process. This type of wear is the most common cause of implant failure. **Galvanic corrosion** refers to an interaction between the metal used in two different structures, which occurs with electric current. The corrosion caused by the accumulation and reaction of body chemicals in the destruction of the implant surface is called crevice corrosion. This type of corrosion can lead to implant failures(2,3).

Table-1: Problems of orthopedic implants

Implant related
• Corrosion • Fatigue • Break • Mechanical incompatibility • plastic deformation • load shield • Surface wear
Tissue related
• Osteopenia or Osteoporosis • Fibrous tissue formation • Infection • Pain • Metal allergy • Interface separation

It is known that the biological properties of **titanium and titanium alloys** are good. A dense oxide layer forms on the surface of these alloys at room temperature in a short time. This oxide layer prevents the intense flow of electrons and the passage of ions. Titanium and titanium alloys show high polarization resistance, so they are resistant to corrosive effects and have no negative effects on human cells. Toxic effects have been observed in cobalt, copper, nickel and vanadium. Nickel reduces the life of cells(3). Nickel-Titanium alloys, after being deformed, have the ability to return to their original shape when heated. This feature (Shape Memory Effect-SME) is called the ‘**‘shape memory effect”.** The SME effect of this alloy was first observed by Buehler et al(4). Some biomaterial applications where shape memory effect is required, production of dental bridges, vascular connections in the skull and orthopedic prostheses are used. **Titanium alloys have some advantages;** It shows the best compatibility in long-term implantation, the least chance of chemical reaction with the injected substances; non-magnetic, compatible for MR (Magnetic Resonance); due to its low density, it is light in weight; hypoallergenic.

Stainless steel and bone cement are biotolerant materials and fibrous tissue develops between the bone and implant. Materials such as chromium-cobalt and titanium alloys and aluminum oxide are bioinert materials. They attach directly to the bone without forming fibrous tissue at the bone-implant interface.

Bioceramic materials, which make up the majority of inorganic materials, are frequently used in medicine. For example; Glasses, test devices, thermometers,

tissue culture containers, fiber optics used in endoscopy, porous glasses (as enzyme, antibody and antigen carrier) are some of these areas. Their resistance to microorganisms, temperature, solvents, pH changes and high pressures provides great advantages for these applications.

Table 2: Biomaterials and their properties

Biomaterial	Advantages	Disadvantages	Usage areas
Metals	durable, solid, malleable	High intensity and corrosion resistant	Joint prostheses, plaques, dental implants
Polymers	It is elastic and easy to manufacture	It is not strong and deforms over time	Sutures, blood vessels, hip orthotic sockets
Ceramics	high biocompatibility, bioinert	Fragile, not elastic, difficult to manufacture	Hip replacement femur component, implant surface coatings
Composites	It is durable and specially designed	Difficult to manufacture	Joint implants and heart valves

The zirconia element has an inert effect in its physical environment. For this reason, it is preferred in hip replacement implants. There are radioactive elements (uranium, thorium, etc.) with a very long half-life in zirconia. Calcium phosphate-based bioceramics can be used to cover orthopedic implants(1) . One of the bioceramics and the most widely used in the clinic; Hydroxyapatite is a calcium phosphate-based ceramic that forms the inorganic structure of bone tissue, and is used as artificial bone in the construction of various prostheses, repair of cracked and broken bones, and coating of metallic biomaterials due to its biocompatibility. Calcium phosphate-based bioceramics have been used in medicine and dentistry for over 20 years. These materials are used as “bone powder” in orthopedic coatings and dental implants, facial bones, ear bones, hip and knee prostheses.

b-Biopolymers:

They are long chain and high molecular weight substances formed by the addition of multiple monomers to each other. Natural and synthetics are available. These include polyethylene (PE), polyurethane (PU), polytetrachloroethylene (PTFE), polyacetal (PA), polymethylmethacrylate (PMMA), polyethylene terephthalate (PET), silicone rubber (SR), polysulfone (PS), polylactic acid (PLA) and polyglycolic acid. (PGA) can be counted as many examples. Polymers can be produced in the form of fibers, films, gels, beads, nanoparticles. The structures of polymeric compounds can be adversely affected by sterilization processes(1). The peel resistance of cross-linked polyethylene, which is formed

as a result of irradiation, is higher than its non-cross-linked precursors, that is, it is durable. It shows high impact resistance, toughness and less friction wear. If radiation is used in the sterilization of polyethylene materials, it should not be used as it will increase the oxidation elastic modulus after irradiation and decrease the ductility and fracture toughness. Polylactic acid (PLA) polymers and polyglycolic acid (PGA) polymers undergo degradation in the body. This degradation is the disintegration and melting of polymers in the body with enzymes and hydroxylation.

c-Biocomposites:

They are materials consisting of biodegradable polymers (matrix) and biodegradable reinforcements. Biodegradable material is substances that can be degraded by living organisms. The fillers used in biocomposites are generally biofibers. Biocomposites made from natural biofibers are renewable, lightweight, biodegradable and environmentally friendly(5). Today, polymer science and technology is focused on composites made from renewable resources. Biocomposites produced from renewable resources have gained universal importance due to their biodegradable nature and have been the subject of extensive research for their use in various fields due to being environmentally friendly materials(6).

d-Bioceramics:

Another material used in orthopedics is ceramics. (7,8,9,10) Ceramics are generally known as inorganic, non-metallic materials. The main applications of ceramics in orthopedics are related to total hip and knee replacement. In addition, ceramic composites can be used alone or in combination with other materials with osteogenic, osteoinductive or osteoconductive properties to increase bone healing. The bioactivity of ceramic materials is limited by osteoconductivity. The main purpose of implanting porous degradable ceramics into bone is to provide natural tissue replacement in the defective area.

Bioceramics developed in recent years are divided into 2 groups as bioinert and bioactive. First generation ceramic materials, alumina and zirconia ceramics, are mostly considered bioinert. One of the pioneering applications of bioceramics is the use of high-density, high-purity alumina as a traditional metallic femoral head in hip replacement. Later, ceramic materials were also used as acetabular cups. Excellent wear resistance rate, excellent corrosion resistance, good biocompatibility and high toughness rates were observed. This meant that it showed a significant advantage over conventional polyethylene (PE) containers.

Second generation ceramic materials are bioactive ceramics, unlike Bioinert ceramics, and their main features are to provide a suitable surface for bone adhesion and growth.(7,8,9,11)

They are mainly used as bone defect fillers. The similarities between the bone mineral phase and their structural and surface properties are related to their good bioactive properties, providing attachment to bone without a fibrous connective tissue interface. Bioactive ceramics are biocompatible and osteoconductive materials.

Table 3: Types of orthopedic biomaterials

Metals	**Ceramics**
• Low carbon syntenitic stainless steels: 316L • Titanium and its alloys: Commercialpurity Ti (CP), Ti-6Al-4V et al. Ti-based alloys • Cobalt alloys: Co-Cr-Mo and other Co-based alloys	• Alumina Al 2 O 3 • Zirkonya ZrO 2 • Calciumphosphate (CaP) based ceramics: Hydroxyapatite , Ca 10 (PO 4)(OH) 2 • Bioglasses
Polymers	**Composites**
• Ultra highly cross linked polyethylene (UHMWPE) • Polymethylmethacrylate(PMMA) • Polyetheretherketone (PEEK) • Bioabsorbable polymers	• Fiber-doped polymers • PMMA-doped polymers

3-Biomechanical Properties of Implants:

It is defined as the mechanical properties of the material against the application of a force or weight in implants used in the body. Here, variables such as force, motion, direction and speed come into play. Mechanics deals with forces on bodies, motion, and relations between bodies. It can be divided into two groups, static and dynamic. It examines systems with static, internal and external forces in balance and fluids and systems moving at constant speed. The basic subjects of dynamics are kinematics(13). The science of biomechanics examines the external forces applied to a material to be used in the body and the internal effects and deformations caused by these forces. The forces acting on the object (compression, tension, torsion and shear) can cause deformation (deformation) in the object. This deformity can be temporary (elastic) or permanent (plastic).

The biomechanical properties of a material are measured by pressure and strain. Pressure is the force acting on the unit surface of a material. It is related to the internal resistance of the object against the applied load, its unit is pascal

(N/m). Strain, on the other hand, is the relative measurement of the deformation (deformation) of the object due to loading. Strain is a ratio so it has no unit. Basically, the force (stress) applied up to a certain limit and the strain are directly proportional (Hooke's Law). Young's Modulus is a measure of a material's hardness or capacity to resist deformation (elasticity). The pressure/strain curve of an elastic material is a straight line up to the breaking point. In other words, if the deformation that occurs when the force applied to a material is removed is completely reversible, this is a non-permanent deformation and this is called elastic deformation.

It has been biomechanically measured that the femoral head is loaded up to 3.5 times the body weight (80 kg body weight) during walking, showing that total hip prostheses must be able to withstand these loads. At the same time, these prostheses must be resistant to abrasion caused by friction in the joint. Today, vitalium (Co-Cr-Mo alloy), stainless steel, high-density polyethylene, polymethylmethacrylate and Al2O3 ceramics are used in the production of hip prostheses.

Due to the low mechanical strength of elements such as platinum, tantalum and zircon in modern technological production facilities, their use as implants is limited. The most commonly used metallic materials as load carriers; stainless steels (316L), vitalium alloys, and titanium and titanium alloys(1). Pure titanium and Ti6Al4V alloy are used in biomedical applications. Ti6Al4V is known as the most popular titanium alloy and is used as high as 50% in the world titanium market. The preference of Ti6Al4V alloy at this rate is mainly due to its high corrosion resistance, low density and high static and dynamic strength.

Fragile materials undergo a deformation that can fully recover when the force is removed prior to failure. Ductility is the ability of the material to permanently change shape (plasticity) without breaking when a force is applied. Expandable (ductile) materials show a large amount of irreversible deformation before fracture. Expansion is the measurement of deformation that develops after collapse. Toughness is a measure of the material's resistance to impact. The resistance of a material to rupture, breakage or deformation by impact is known as toughness. Toughness is the ability of the material to absorb the sudden load or impact load. The amount of energy absorbed in each unit volume of the material before failure (fracture, rupture, bending) is defined as the internal toughness of that material. The stress-strain behavior of viscous elastic materials (bone and ligaments) is time-speed dependent. The elastic modulus of a viscous elastic material increases as its stretch increases. As the loading speed increases, it exhibits a more elastic material behavior. Isotropic materials show the same

mechanical properties in all directions (eg golf ball). Mechanical properties of non-isotropic (anisotropic) materials change according to the direction of applied load (eg bone).(12,14,15,16)



Picture 1: Femoral stem removed from the femur as a result of aseptic loosening (Note: There are scratches and damage on the surface of femoral stem)

4-Result:

Biomaterials field; stems from a combination of disciplines such as life sciences, medicine, materials science, and engineering. The mutual compatibility, development and maintenance of biomaterials and tissues has been of interest to scientists for many years. The most important factor that distinguishes a biomaterial from any other substance is its ability to exist without harm when in contact with the tissues of the human body. In this context, materials with the ability to elicit specific biological responses are being developed. Many of the problems faced by orthopedic surgery fifty years ago have now been eliminated. Because new biomaterials and innovative devices are now widely used in many fields. Next generation biomaterials are new or modified materials that can meet these challenges currently faced by existing implants and offer biomaterials that will not only minimize the possibility of medical complications but also deliver more realistic, aesthetically pleasing results for patients. Materials engineering can add great value to future biomaterials, and next-generation implants can be fabricated using nanomaterials that can serve multiple purposes.

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Chapter 13

Microglial NLRP3 Inflammasome in Parkinson's Disease

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Parkinson's Disease

Parkinson's disease (PD) is named after James Parkinson because he was the first to describe the motor symptoms of this disease 200 years ago. (1). After Alzheimer disease (AD), PD is the second most prevalent neurodegenerative disease (ND) that affects 2–3% of the population ≥ 65 years of age. In addition, PD is the most prevalent movement disorder (2). The prevalence of PD is increasing day by day and predicted to double in the next 30 years (3). The majority of Parkinson's cases are sporadic, meaning the etiology is unknown. About 5-10% of PD cases are caused by genes such as SNCA, LRRK2, PARK 2, PINK1, DJ1 and VPS35 (4). At the histological level, progressive neuronal loss is associated with α-Synuclein (α-Syn) aggregation and the formation of intracytoplasmic proteinaceous inclusions, called Lewy Bodies (LBs), in which α-Syn is the primary component.

It has been demonstrated that in the course of progression of the PD, neurodegeneration develops primarily in the nigrostriatal circuits of the midbrain, then gradually in different regions of the brain. Since the nigrostriatal pathway is involved in the body's voluntary movement coordination, this condition results in typical motor symptoms. Indeed, this situation occurs afterward the damage of more than 80% of dopaminergic neurons in the substantia nigra pars compacta (SNpc) (5). Parkinson's disease is defined by impaired motor functions such as tremors, muscle stiffness, difficulty in optional movement types (bradykinesia, freezing and akinesia), and nonmotor disorders like cognitive and behavioral disorders (6).

Great progress has been made in understanding the neuropathology and progression of disease throughout the nervous system, as well as the molecular and neurophysiological mechanisms underlying the disease and its symptoms. Although the mechanisms causing neuronal degeneration have not been fully explained, many pathways and mechanisms such as disruption of α-Syn proteostasis, mitochondrial dysfunction, oxidative stress, calcium homeostasis, disruption of the ubiquitin-proteasome system, excitotoxicity, axonal transport, lack of neurotrophic factors, and neuroinflammation play a role in neuronal degeneration (2, 8).

Neuroinflammation

Neuroinflammation is an inflammatory reaction in the spinal cord or brain that occurs as a response to pathological conditions. With neuroinflammation, chemokines, cytokines, secondary messengers and reactive oxygen species (ROS) are produced. These molecules are produced by the local central nervous

system (CNS) glias (astrocyte and microglia), peripherally derived immune cells and endothelial cells. Microglia cells are the main and most important cellular mediators during the inflammatory response in inflammation in the brain.

These neuroinflammatory responses result in immune, biochemical, physiological and psychological outcomes. Also, the degree of neuroinflammation depends on the course of the primary stimulus, status and duration. It is known that controlled inflammation is generally beneficial for the living organism. Conversely, maladaptive inflammatory responses are harmful to the host organism. Chronic or uncontrolled inflammation is defined by increased production of cytokines such as tumor necrosis factor α (TNF-α) and interleukin 1 beta (IL-1β), ROS, and other inflammatory mediators. These markers are highly prominent following CNS trauma and accompany a substantial recruitment of peripheral neutrophils and macrophages to the injury area. Temporary inflammation afterward recurred social defeat stress additionally causes macrophage and monocyte recruitment, and leads to depression and anxiety. In addition, a low-level and chronic state of inflammation caused by IL-1 and interleukin 6 (IL-6) occurs as a result of aging. This condition comes after an acute phase of CNS trauma and causes decreased neuronal plasticity and cognitive impairments. Major indicator of NDs is the higher degree of chronic inflammation and this inflammation causes extensive damage to the nervous system. High-grade chronic inflammation in the brain can cause recruitment of immune cells, edema, neuronal injury and finally neuronal cell death (9, 10). Extensive experimental and clinical studies suggest that the association of neuroinflammation and microglial activation can be main organizers of dopaminergic neuron cell damage in PD (11, 12). Furthermore, pathogenic conditions such as increased motor symptoms and neuronal dysfunction, which are symptoms associated with PD, have been found to be more severe in patients with systemic inflammation (13, 14). Injured neuron cells activate microglia, and the activated cells form harmful molecules that further harm neurons leading a "vicious cycle" of neuroinflammation and neurodegeneration (Figure 1) (15-18).

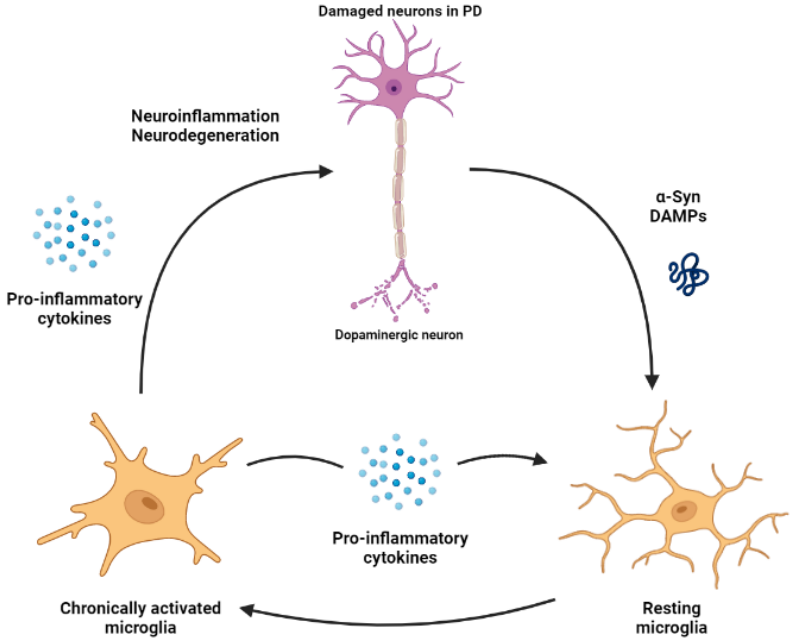

Figure 1. Neuroinflammation and neurodegeneration: A "vicious cycle". PD, Parkinson's disease; α-Syn, alpha-synuclein; DAMPs, damaged associated molecular patterns (24).

Microglia

Microglial cells described by Pio del Rio Hirtega in 1919, are available in large quantities in the CNS and constitute the innate immune system and defending of the CNS (19). Microglia are functionally and physically intertwined with neuronal cells and are in constant communication. Microglia cells perform a great variety of missions in pathological and physiological situations. These cells show different intensity in diverse brain areas, with distinct composition of parameters underlying their different functional acts and regional identity. This distributional diversity, unique cellular features, and dynamic behavior have made these cells important CNS cells that deserve considerable care and detailed investigation.

In normal circumstances, microglia cells serve various brain tasks like synaptic remodeling and pruning. The signaling that takes place between neuronal cells and microglia is particularly critical for brain connectivity and neural circuitry. In a pathological condition, microglia emigrate to the damaged area and serve to alleviate or make the injury worse. Studies have shown that the morphology of microglia cells, which undertake the tasks, can be distinguished by their shapes in two main states: resting and activated. While microglia are in a resting state and

frequently wandering around, they become active when they detect any pathology in the nervous system. The transition of microglia from the resting state to the active state has a complicated adjustment. This situation allows the emergence of active microglia to be very tightly controlled. In spite of this widespread binary categorization, it is in general belief that distinct receptors and targets adjust microglial response in a constant mode and plural types of activation status exist.

Surprisingly, microglial activation is frequently related with neuronal cell degeneration, a degenerative course seen in the eventual pathology of PD. In contrast to resting microglia, activated microglia usually have a large soma, amoeboid morphology, and have increased expression of their receptors located on their surface. Microglia cells tend to sense the pathology that occurs around them, swallow waste, as well as excrete potential pro- and anti-inflammatory mediators that intensify or alleviate disease development. Therefore, the activated microglial cells are usually a significant sign and mirrors neuronal dysfunction in PD.

How microglia work in the neurodegeneration and pathogenesis of PD remains an important area of research for researchers. Although no definite link has been established between DA neuron death and the activation of microglial cells, it is widely believed that these cells are involved in the disease process (20).

NLRP3 Inflammasome

A considerable increase in both microglia and nucleotide-binding domain, leucine-rich repeat, and pyrin domain 3 (NLRP3) inflammasome activation has been detected in the tissue of the SNpc in patients with PD (21).

Dr. Jurg Tschopp and his colleagues were the first scientists to use the term “inflammasome”. Firstly, they defined the inflammasome as a “caspase activating complex” including caspase-1, caspase-5, Pycard (caspase interacting domain), and NALP1 (an old name for NLRP1). It was later referred to as the NLRP1 inflammasome and this complex is essential for the formation of the mature form of IL-1β. After two years, the same research group also discovered NLRP3 inflammasome. Studies of this group have found increased NLRP3 inflammasome activation in macrophages isolated from patients with Muckle-Wells syndrome. Since the NLRP3 inflammasome is involved in both adaptive and innate immunity, it has become the best characterized inflammasome. It is activated by either all pathogens including viruses, bacteria and fungi or by host-derived molecules like α-Syn, amyloid-β (Aβ) peptide, glucose and extracellular adenosine triphosphate (ATP).

Inflammasomes are cytosolic protein complexes that formed in response to damage-associated molecular patterns (DAMPs) and pathogen-associated

molecular patterns (PAMPs). To date, several types of inflammasomes have been described, including the NLRP1, nucleotide-binding oligomerization (NOD)-, leucine-rich repeat (LRR)-, and pyrin domain-containing 3 (NLRP3), NOD-, lRR-, and CARD-containing 4 (NLRC4), NLRP6 and AIM2 inflammasomes. However, the NLRP3 inflammasome is the best characterized inflammatory sensor molecule (22).

The NLRP3 inflammasome consists of three parts: sensor molecule (NLRP3), an adaptor molecule (ASC; apoptosis-associated speck-like protein containing a CARD, also known as PYCARD) and an effector molecule (caspase 1). The NLRP3 inflammasome has a tripartite domain architecture composed of amino-terminal pyrin domain (PYD), a central NACHT domain and a C-terminal area containing leucine-rich repeat (LRR) motifs. The NACHT domain has ATPase activity that is crucial for NLRP3 function and self association. ASC consists of two domains: an amino-terminal PYD and a carboxy-terminal caspase recruitment domain (CARD). Full-length caspase 1 composed of an amino-terminal CARD and two catalytic domains (a central large catalytic domain (p20) and a carboxy-terminal catalytic small subunit domain (p10)). During activation, NLRP3 molecules are oligomerized via NACHT domain homotypic interactions. Oligomerized NLRP3 recruits ASC through homotypic PYD-PYD interactions and promotes ASC speck formation through the assembly of multiple ASC filaments. ASC then recruits caspase-1 through CARD-CARD interactions and activates caspase-1 through proximity-induced self-cleavage between p20 and p10 of caspase-1 (23). The active heterotetrameric caspase-1 mediates the maturation and secretion of IL-1β and interleukin 18 (IL-18) cytokines and stimulates their release from the cell (Figure 2).

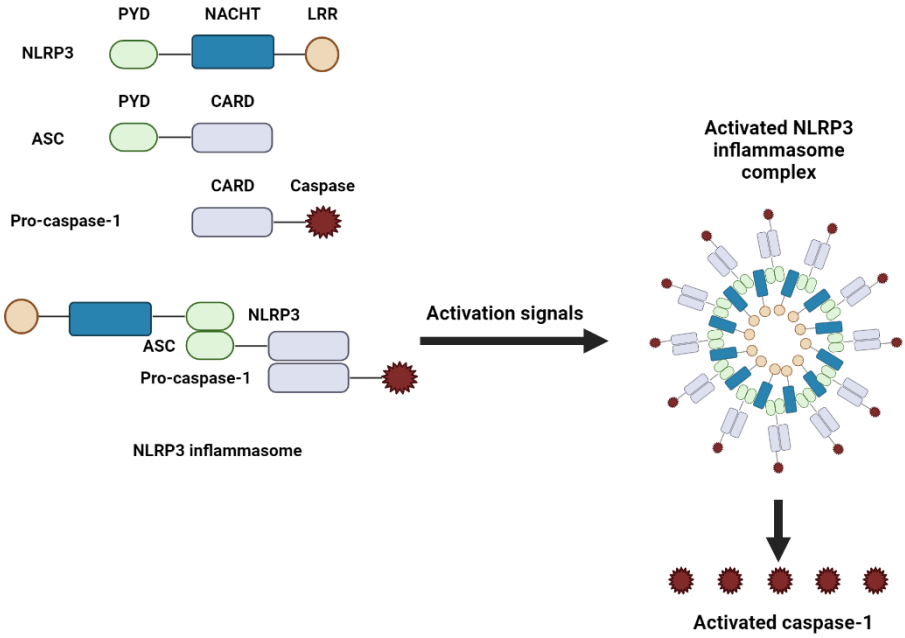

Figure 2. Components of NLRP3 inflammasome (24).

NLRP3 inflammasome activation requires two signals as follows: priming signal and activation signal. Priming signals increase in NLRP3, pro-IL-1β, and pro-IL-18 transcription through the nuclear factor kappa light chain enhancer of activated B cells (NF-κB). The activation signals initiate the activation, assembly, and NLRP3 inflammasome complex formation (24). Among the diverse exogenous and endogenous stimulatory molecules, PAMPs and DAMPs, PRR ligands and IL-1α act as priming signals. ATP, misfolded proteins, pore forming toxins, crystalline substances, and viral RNA are the activation signals. In NDs in microglia, various endogenous proteins or signals, such as α-Syn, Aβ, prion protein, superoxide dismutase and ATP activate the NLRP3 inflammasome pathway. In addition, several environmental toxins or exogenous stimuli can activate the NLRP3 inflammasome by increasing ROS production, impairing mitochondrial and lysosomal function in PD. Recently, neurotoxins that generate PD-like effects in both *in vitro* and *in vivo* models, such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 1-methyl-4-phenylpyridinium (MPP^+), paraquat and 6-hydroxydopamine (6-OHDA) have been found to activate the NLRP3 inflammasome (Figure 3 and Figure 4) (24).

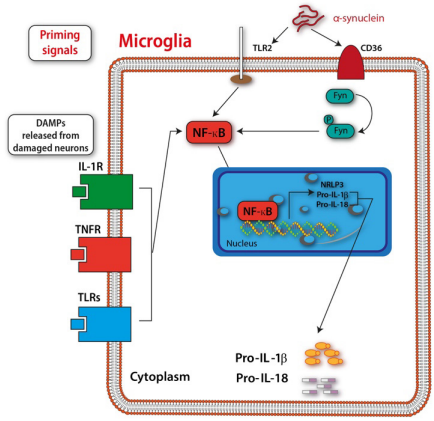

Figure 3. Priming signals of the NLRP3 inflammasome. TNFRs, tumor necrosis factor receptors; CD36, cluster of differentiation 36. (24)

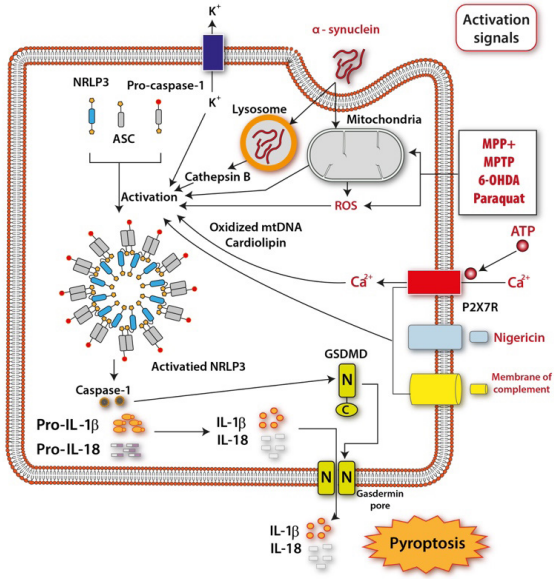

Figure 4. Activation signals of the NLRP3 inflammasome, and pyroptosis process in PD (24).

NLRP3 inflammasome activation causes pyroptosis pro-inflammatory programmed cell death. The NLRP3 inflammasome leads to auto-proteolytic activation of caspase-1, which afterwards causes the cleavage of the pro-inflammatory cytokines IL-1β and IL-18 and the pyroptotic substrate gasdermin D (GSDMD). The N-terminus of GSDMD (N-GSDMD) oligomerizes and forms plasma membrane pores that mediate cell death and the secretion of the mature forms of IL-1β and IL-18 (Figure 4) (25). However, it has been discovered in recent studies that releasing of IL-1β is both GSDMD-dependent and -independent. IL-1β released into the extracellular space plays a crucial role in maintaining the integrity of the blood brain barrier (BBB). IL-1β activates microglia and astrocytes. This situation can result in the activation of CNS-infiltrated T cells and the next release of TNF-α, IL-6, and other neurotoxicity mediators. Also, IL-18 acts as two different roles in the regulation of neurodegeneration and neuroinflammatory pathways. When microglia are activated by IL-18, it stimulates metalloproteinase levels, caspase-1 expressions, and generates pro-inflammatory cytokines that increase neuroinflammation in the CNS (24).

NLRP3 Inflammasome-Mediated Inflammatory Pathways in PD

α-Syn activates the NLRP3 inflammasome

PD has three characteristic features. These are advancing loss of dopaminergic neuron cells in the striatum and SNpc, aggregation of α-Syn and Lewy bodies (α-Syn-rich protein aggregates) in certain parts of the brain, and neuroinflammation (27). The α-Syn molecule functions as a DAMP and modifies the expression of TLR (toll like receptor) which is located on the surface of microglia. In a mouse model overexpressing the α-Syn molecule, a rise in the count of activated microglia was shown before neuron cell injury (28). The α-Syn molecule can activate glial cells by being released into the extracellular space through different mechanisms (29). Lately, it has been argued that cutting of α-Syn, which supports its aggregation, may happen via caspase-1 (30). Caspase-1 is activated by classical activation of NLRP3 inflammasome by triggers like lipopolysaccharide (LPS), nigericin, or other specific compounds (30, 31).

α-Syn may be found in the brain as both monomeric and fibrillar types (32). In human primary monocytes, both monomeric and fibrillar forms of α-Syn are able to activate pro-IL-1β expression in microglia cells via the TLR2 receptor. However, just the fibrillar types of α-Syn can release mature IL-1β via activation of the NLRP3 inflammasome. After neuronal degeneration, fibrillar α-Syn is released into the extracellular space. Since fibrillar α-Syn is a pathological protein, it must be phagocytosis by microglia. After phagocytosis, fibrillar α-Syn induces

intracellular cathepsin B release and ROS production. Release of cathepsin B and production of ROS stimulate NLRP3 activation and caspase-1. This situation enables them to subsequently produce IL-1β (33). It is thought that the fibrillar structure of α-Syn is recognized by the innate immune system due to its fibril-specific cross-β structure and activates NLRP3 inflammation (34). Gordon and colleagues found in 2018 that α-Syn-mediated dopaminergic neuronal cell death by activation of the microglial NLRP3 inflammasome can be initiated by both the availability of fibrillar α-Syn and the lack of α-Syn. Tissue samples obtained from SNpc of PD patients› brains were found to have high levels of ASC and cleaved caspase-1. Also, NLRP3 expression in activated microglia was found to be elevated in postmortem tissue samples. This research group also performed research for PD on mice and they found that microglial NLRP3 activation via α-Syn causes lagged but strong IL-1β and ASC release without causing pyroptosis (21). In 2019, Panicker and colleagues published a study showing that α-Syn misfolding in PD causes activation of microglial NLRP3 inflammasome. This research group previously described that in microglial cells, Fyn kinase (FK) is fastly activated via TNF-α and LPS. However, they showed in both in vivo and in vitro studies that FK, non-receptor tyrosine kinase, arranges the recruitment of misfolded α-Syn into microglia and subsequent activation of the NLRP3 inflammasome. FK can arrange LPS-dependent priming microglial NLRP3 inflammasomes via controlling the PKCδ-dependent nuclear translocation of NF-κB p65 and activation through facilitated importing of α-Syn, which supports ROS production and the next activation of NLRP3 inflammasome (35, 36).

Generally, during PD pathogenesis, α-Syn aggregation and activation of the microglial NLRP3 inflammasome generate a ‘’vicious cycle’’ of neuronal cell death via production of the elevated levels of cytokine. The information indicates that α-Syn activates the microglial NLRP3 pathway, suggesting that it is a sustained source of neuroinflammation in PD that can cause advancing neuron cell injury and loss.

Mitochondrial impairment and mitophagy arrange the NLRP3 inflammasome

Mitophagy is defined as a mitochondrial autophagy pathway, and it has been shown that mitophagy is a negative regulator of NLRP3 inflammatory-mediated hyperinflammation (37). Studies show that disruption of mitochondrial dynamics and mitophagy directly attend to dopaminergic neuron cell injury and death in PD (38). Heat shock protein 60 (HSP60) has been found to impair mitochondrial membrane potential and increase ROS levels, which has been reported to activate

NLRP3 inflammasome and caspase-1 and cause IL-1β release from activated microglia (39). In 2017, Sarkar and colleagues found treatment of primary mouse microglia primed with LPS with rotenone-inhibited complex-I mitochondria. This has been shown to induce ASC speck formation and activate NLRP3 inflammasome and pro-IL-1β formation. Moreover, mature IL-1β release was found to be ameliorated when primary microglia were treated by mito-aposine, which is a specific mitochondria antioxidant. This proves that mitochondrial ROS has an important role in the activation of NLRP3 inflammation and release of IL-1β. Furthermore, conditioned media from rotenone-treated primary microglial cells primed with LPS was used for culturing human dopaminergic neuronal cells. This resulted in NLRP3 inflammasome mediated dopaminergic neurodegeneration. In a similar study, in a PD model induced by chronic administration of rotenone to rodents, mitochondrial damage was induced in microglia cells, which in turn activated the NLRP3 inflammasome pathway and induced dopaminergic neuronal cell death (8). The P2X7 receptor is abundantly expressed in microglial cells and is involved in NLRP3 inflammasome activation and the release of cytokines (40). BzATP is a P2X7 receptor agonist, and activation of the P2X7 receptor by this compound causes CaMKK- and ROS-dependent AMPK activation, dysfunction of mitochondria, autophagy, and partial cell death. In microglial cells, cytotoxicity occurred as a result of P2X7 mediated mitophagy and cathepsin B activation (41). In addition, Parkin and PINK1 are regulators of mitophagy, and their loss of activity is clearly related to microglial NLRP3 activation in PD (42, 43). Activation of AIM2 and NLRP3 inflammasomes in bone marrow-derived macrophage (BMDM) has been discovered to cause caspase-1 connected mitochondrial dysfunction through reduction of mitophagy. Extensive exercise induces mitochondrial DNA (mtDNA) mutations in Parkin knock-out mice. Also, loss of dopaminergic neurons and motor functions was observed in the SNpc of parkin knockout mice (44). Furthermore, treatment with Z-VAD-FMK, a caspase inhibitor, was found to protect parkin-free microglial cells from necroptotic cell death (43). HtrA2 is a serine protease found in mitochondria which acts as a crucial part in the quality control of mitochondrial protein folding and cell survival. Like parkin and pink1, HtrA2 has been associated with the inflammasome pathway in PD (45). In 2018, Rodrigue-Gervais and colleagues reported that in BMDMs, HtrA2 can impede ASC dependent activation of the AIM2 and NLRP3 inflammasome. The HtrA2 may prevent ASC accumulation. Thus, it can regulate the inflammasome pathway and autophagy (46).

Stimulation of dopamine receptors can activate the NLRP3 inflammasome

Dopamine receptors (DRDs) are expressed on nearly entire glial and immune cells (47, 48). Activation of DRDs can modulate immune cells. It can do this in cells by activating the production, proliferation and release of cytokine molecules from the cells (49). Studies have shown that dopamine (DA) activates dopamine D1 receptors (DRD1), and this activation negatively arranges NLRP3 inflammasome. This inhibition has been shown to be due to cAMP binding to NLRP3 and promoting its degradation via MARCH7 (50). Another study reported that the DRD1 agonist A-68930 improved neurological functions by inhibiting the NLRP3 inflammasome (51). A similar in vitro study found that DRD2 receptors inhibit activation of the NLRP3 inflammasome via β-arrestin2. In addition, activation of DRD2 in the MPTP-induced PD model has also been shown to reduce caspase-1 and IL-1β levels in the midbrain (52). Therefore, there is evidently a relationship between DA levels and activation of microglial NLRP3 inflammasome in PD.

K^+ efflux activates NLRP3 inflammasome

Various studies have indicated that one of the important cellular events triggering NLRP3 inflammasome activation is K^+ efflux (53-55). Almost all of the many activators that activate the NLRP3 inflammasome conduce to potassium efflux. Thus, the K^+ concentration in the cytoplasm is reduced. However, the ways by which K^+ efflux by different activators occur are distinct. The ATP molecule located outside the cell binds to a ligand-gated ion channel, P2X7R. After, the ion channel opens, allowing K^+ to pass through to form a K^+ efflux (54, 56). Bacterial toxin molecules like valinomycin, and nigericin constitute membrane pore in the cell membranes to support K^+ efflux (56). However, the certain connection between activation of the NLRP3 inflammasome and reduced content of intracellular K^+ still uncertain.

Moreover, to comprehend the act of K^+ efflux in NLRP3 inflammasome activation, it would be helpful to know the NEK7 protein, which is called the downstream protein of K^+ efflux. NEK7, a NIMA-related kinase, with the help of the downstream role of K^+ efflux, allows activation of NLRP3 inflammasome (57-59). A NEK7 contributes to the activation of the NLRP3 inflammasome, especially by taking part in the oligomerization of NLRP3, ASC speck generation and caspase-1 activation. In the failure of NEK7, activation of caspase-1 and releasing of IL-1β are decreased (58). This makes NEK7 an important regulatory molecule in NLRP3 inflammasome activation.

NLRP3 inflammasome as a therapeutic target for PD

There is a lack of information regarding how the activation and structure of the NLRP3 inflammasome is directly involved in the loss of dopaminergic neurons in PD. This has hampered the discovery of selectively targeting the microglial NLRP3 inflammasome for the treatment of PD and potential modulators that could be used as therapeutic agents (Table 1).

Table 1. Small-molecule inhibitors of the NLRP3 inflammasome (24).

Nicotinamide Adenine Dinucleotide Phosphate
BAY 11-7082
OLT1177
VX-740
VX-765
Glyburide
JC-171
16673-34-0
3, 4-methylenedioxy-β-nitrostyrene
MCC950 or CRID3 or CP 456,773
BOT-4-one
Parthenolide
Mefenamic Acid
Flufenamic Acid
Fc11a-2
Dopamine
β-hydroxybutyrate
IFN-39

Additionally, most of the former research had related to regulation of the NLRP3 inflammasome pathway in distinct neurodegenerative diseases. PD is also a neurodegenerative disease and studies on this subject are closely related to the neuroinflammation process caused by microglial NLRP3 in PD. Thus, considering all of these situations, future research using these methods may be tailored to pharmacologically target the NLRP3 inflammasome pathway in PD pathogenesis (Table 2).

Table 2. Potential therapeutic pathways in the pathogenesis of PD for NLRP3 inflammasome (24).

Inhibition of IL-1β signaling pathway
Inhibition of NLRP3 transcription and posttranslational modification
Small-molecule inhibitors of the NLRP3 inflammasome
MicroRNA-based inhibition of the NLRP3 inflammasome

Conclusion

Former researches have revealed that immune abnormalities and inflammation always act together in the formation and progress of PD. Microglial cells are innate immune cells found in the CNS and can be activated via clustered α-Syn, a pathological condition that is one of the most important elements in the pathogenesis of PD. After α-Syn is taken into the cell in various ways, it is perceived as an endogenous danger signal. This causes the activation of the NLRP3 inflammasome and subsequently the formation of caspase-1, that ultimately transforms the inflammatory cytokine pro-IL-1β to an active form and also induces pyroptosis. As a result of pyroptosis, both IL-1β and α-Syn are released from the pores opened in the cell membrane, causing further damage to dopaminergic neuron cells. In this way, a ‘’vicious circle” is formed that strengthens the inflammatory response. α-Syn spreads very rapidly and severely in the CNS, causing irreversible damage to dopaminergic neuron cells. For this reason, we must turn to strategies that can explain and treat the relationship between microglial NLRP3-induced neuroinflammation and PD (10). In this context, using selective potential NLRP3 inflammasome-targeting compounds will help to treat dopaminergic neuronal loss and microglial NLRP3 inflammasome-mediated neuroinflammation in PD. In the context of the complicated mechanisms of NLRP3 inflammasome activation and the pathogenesis of PD, the NLRP3 inflammasome selectivity, efficacy and safety of the compounds to be used for treatment should be investigated. This can aid scientists to discover orally active and BBB permeable therapeutic molecules that target the NLRP3 inflammasome. Thus, new horizons will be opened for the pathogenesis and treatment of PD (24).

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Chapter 14

URETHRAL MEATUS STENOSIS IN BOYS

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INTRODUCTION AND DEMOGRAPHICS:

The urethral meatus is the final part of the urinary system. Meatal stenosis (MS) is a disease characterized by narrowing of the urethral meatus and can cause difficulty in urine flow in men. Acquired causes account for the majority of the patients (1). The incidence of MS is less than 1% (2) After circumcision if it is less than 5 mm is accepted as MS, the incidence can reach up to 20% (3). MS can be seen in 27% of patients with balanitis xerotica obliterans (BXO) (4).

ETIOPATHOGENESIS

The etiology of MS is unclear, meatitis and meatal ischemia after circumcision has been suggested as possible causes of MS. Some studies have suggested exposure of the glans penis and meatus with environmental factors such as faeces, urinary ammonia, underwear and diapers, chronic irritation, and increased adhesion to the epithelial layer as possible causes of MS (5–8). Studies suggest that MS also develops due to allergic factors (9). Infection, inflammatory diseases such as BXO, failed hypospadias surgery or urethral instrumentation is also other causes of meatal stenosis. The exact cause of the development of meatal stenosis after circumcision is not well-known (Figure 1). Ischemia due to the damage of the frenular artery and chronic irritation of the meatal mucosa with the removal of the protective effect of the preputium on the meatus is the suggested mechanisms. (10). Although the pathogenesis of BXO is not fully known, genetics, autoimmunity, cellular damage caused by trauma, infection, and chronic irritation from urine are suggested theories. Some studies have determined that BXO can be seen together with other autoimmune diseases(11). Although many of the BXO cases are sporadic, up to 12% of the cases have a family history. HLA DR11, DR12 are detected more frequently (12). Urethral involvement usually starts from the meatus and may involve the entire anterior urethral mucosa, causing spongiofibrosis and voiding symptoms(13).

SYMPTOMATOLOGY:

Deviated, misdirected high-velocity and long-distance urine stream (low stream, splitting or spraying, intermittency, hesitancy, straining, and terminal dribble) is the hallmark symptoms of MS Some parents also note that the flow of the urine is lateral and misdirected due to rotational torsion of the penis.

- Pain at the beginning of micturition
- The need to stand back or sit on the toilet while urinating
- Burning in the meatus

- Blood on underwear
- Urgent, frequent and prolonged emptying of the bladder; are other symptoms that can occur.

Rarely, obstructive findings such as hematuria, vesicoureteral reflux, and hydronephrosis may occur(3).

PHYSICAL EXAMINATION

Due to the presence of a small meatus on examination, meatus stenosis may be suspected if the ventral edges of the meatus appear to be fused, especially with lateral traction. The most common finding is the presence of a narrow flow deviated upwards during urination; this represents the clinical finding that can be corrected with surgery. The calibration of the urethra is important. Litvak et al reported that the meatus in children younger than 1 year is compatible with a lubricated 5-French feeding tube. They also reported that in children aged 1 to 6 years, an 8-French feeding tube could pass without difficulty(14).



Figure 1: Meatus stenosis after hypospadias repair

TREATMENT APPROACH:

The treatment of MS that can occur after circumcision in children is meatotomy or meatoplasty. The reconstruction and secondary operation rate was detected 2% after meatotomy under local anesthesia meanwhile it was found to be 0.2% after formal meatoplasty (15). In the standard meatoplasty technique, the urethral meatus is incised ventrally, and sutures are placed for the eversion of the mucosa.

The situation is more complicated in BXO patients. Different techniques were used in the treatment of these patients. Malone meatoplasty can be described with a small ventral meatal incision is followed by a large dorsal incision to improve the aesthetic appearance of the glans, a V-shaped incision is made ventrally than sutures are placed. The success rate of this technique in meatal stenosis after BXO is 83.3% (16). The circular buccal mucosal graft technique is another surgical option in BXO-induced MS and the success rate has been reported as 100% in a series of 15 patients. (17). In another series, success was achieved in all MS patients due to BXO with the single-stage buccal mucosal inlay graft technique(18) In BXO related strictures with a viable urethral plate, single-stage dorsal onlay buccal mucosal urethroplasty provides excellent intermediate-term results(19). Two-session repairs are standard treatment in complex distal urethral strictures with obliterating the lumen (after hypospadias surgery, in the presence of lichen sclerosis or advanced fibrosis) (19). Penile fascia cutaneous flaps should not be used in BXO-related cases because of the high recurrence rates (20).

In medical treatment, preventive measures should be explained to the patient. Irritants should be minimized, sick skin should be moistened with emollient creams, the potential irritant of urine should be avoided. The traditional medical treatment of BXO related MS is topical steroids. Steroids are inhibiting the chronic inflammatory response in the early stage of the disease, reducing initial symptoms and slowing disease progression(21). The use of clobetasol propionate (0.05%) twice a day for 3 months and gradually tapering off is the most valid form of medical treatment. However, the treatment response does not exceed 50-60% (21). The advantages of oral mucosal are its thick epithelium, dense subdermal vascularization, early healing, easy acquisition, and preparation of tissue of sufficient width and length, and acceptable cosmetic results. Today, mucosal grafts should be used primarily in cases where inflammatory diseases such as lichen sclerosis are the etiology of meatal and/or distal anterior stenosis. The use of penile skin flap in these patients will result in high recurrence(22,23).

CONCLUSION:

Meatal stenosis usually develops after circumcision. Treatment of MS that develops after circumcision is relatively easier. A formal meatoplasty is usually sufficient for treatment. BXO associated meatal stenosis is more complicated to treat. The most frequently preferred tissue for the replacement of the urethra is the oral mucosa in current practice.

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Chapter 15

CLINICAL FEATURES OF THE ATYPICAL PARKINSONIAN SYNDROMES

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INTRODUCTION

Parkinsonism is a clinical condition of resting tremor, rigidity, and bradykinesia or akinesia. The most common primary neurodegenerative disease which causes parkinsonism is Parkinson's disease (PD). However, although it is rare, there are patients with early dementia, frequent falls, ocular motor dysfunctions, ataxia, or severe dysautonomia with parkinsonian signs. This group of patients is generally considered within the atypical Parkinson's syndromes (APS). Progressive supranuclear palsy (PSP), multiple system atrophy (MSA), corticobasal degeneration (CBD), and Lewy-body dementia (DLB) are the most common causes of APS.

Although it is sometimes challenging to distinguish them clinically, especially in the early stages of diseases, a common feature of all APS is the lack of efficacy of dopaminergic treatments. Since the progression is faster than classical PD and the response to treatments is not well enough, it is essential to distinguish them from PD and get the correct diagnosis early to make the prognosis expectation correctly.

In this section, we aimed to define APS with different clinical features and emphasize the important points in the differential diagnosis.

1. Progressive Supranuclear Palsy

PSP was first described 50 years ago and is the most common cause of APS (Richardson et al., 1963). Parkinsonian signs seen in PSP are generally symmetrical, with a predominance of bradykinesia rather than tremor (Williams et al., 2005). The youngest age of onset reported in PSP is 41, with a mean age of 60-63 (Höglinger et al., 2018). Most patients develop the disease sporadically, but few cases of familial PSP are associated with the MAPT gene (Im et al., 2015).

The definitive diagnosis of PSP, which is pathologically formed by tau accumulation, can be made by neuropathological examination. However, over the years, diagnostic criteria have been developed for clinical diagnosis. According to these criteria, if a patient over 40 years of age has a sporadically developed progressive neurological deficit and does not have episodic memory disorders, severe autonomic disturbances, visual hallucinations, signs suggestive of motor neuron disease, and sudden or step-wise worsening, the patient can be clinically evaluated for PSP. In these clinical criteria, ocular motor dysfunctions, postural instability, akinesia, and cognitive disorders are core features (Hoglinger et al., 2017).

Vertical gaze palsy and slow velocity of vertical saccades are detected in PSP as ocular motor dysfunctions. Upgaze limitation is not specific to PSP and can be found in other neurodegenerative diseases as well as in physiologically aging. Downgaze limitation is the most sensitive gaze palsy seen in the PSP and can be noted in the early stages of the disease. Procerus sign or vertical wrinkling of the forehead develops in PSP patients with hyperactivity in the frontal muscle and eyelid retraction. Thus, the characteristic facial appearance, called surprised or anxious face, occurs in patients with PSP (Batla et al., 2010). It can be easily differentiated from the hypomimia seen in PD.

Most PSP patients have a history of frequent and spontaneous falls. These falls are often serious and cause injuries, even fractures (Williams et al., 2006). Frequent falls are prominent in the early stages of the disease and can distinguish PSP from

other atypical parkinsonian diseases. Therefore, having such falls in the last three years is included in the diagnostic criteria of PSP. Another feature stated in PSP patients is progressive gait freezing. Gait in PSP is stiff and broad-based. This impairment in gait is also resistant to levodopa therapy. Speech/language functions are also frequently impaired in PSP patients. These disturbances are; progressive apraxia of speech, nonfluent/agrammatic variant of primary progressive aphasia, or frontal-type cognitive/behavioral problems (Hoglinger et al., 2017).

Different clinical phenotypes emerge depending on the combination of different signs and symptoms included in the diagnostic criteria. For example, ocular motor dysfunctions and postural instability are seen in PSP-Richardson syndrome (PSP-RS), which was first described and accounts for approximately 24% of PSP cases (Respondek et al., 2014). Ocular motor dysfunctions and akinesia constitute PSP with progressive gait freezing (PSP-PGF), while frontal type cognitive deterioration with ocular motor dysfunctions compose the PSP with frontal presentation (PSP-F). Ocular motor dysfunctions with speech/language disorders generate the PSP with predominant speech/language disorder (PSP-SL). PSP-P subtype refers to patients with akinetic symptoms together with ocular motor dysfunctions (Hoglinger et al., 2017).

Pseudobulbar palsy develops in almost all patients during the course of the disease, sometimes even at the beginning. On the other hand, dysarthria is also noted in PSP patients. Characteristically, dysarthria of PSP patients has features of both parkinsonian dysarthria and strained, strangled, hypophonic properties. Unfortunately, intelligible word output gradually decreases as the disease progresses.

Although the life expectancy in PSP varies between 2 and 28 years, it has been reported to be 8.7 years on average. PSP-RS phenotype has the shortest (7.3 years), and PSP-P phenotype has the longest (12.8 years) average life expectancy (Respondek et al., 2014). The most common causes of death in PSP patients were found to be pneumonia and pneumosepsis (Litvan et al., 1996).

2. Multiple System Atrophy

MSA is a rapidly progressive neurodegenerative disease where parkinsonian and cerebellar signs and dysautonomia can coexist in different combinations. In the 1960s, it was known by various names, such as Shy-Drager syndrome, olivopontocerebellar atrophy, and striatonigral degeneration, according to clinically involved areas. However, after the pathological data were obtained, it was seen that all of them represented the same disease, and this terminology was preferred due to the involvement of different areas of the brain.

The mean age of onset in MSA has been reported as 60. However, the age range of onset varies between 34 and 83. Men and women are affected at a similar rate (Wüllner et al., 2007). The type with severe autonomic disturbances together with parkinsonian signs is called MSA-P, while the type with cerebellar signs is called MSA-C. While approximately 60% of MSA patients in Europe and America are MSA-P type, more than 80% of the cases reported in Japan are MSA-C type (May et al., 2007; Yabe et al., 2006). The reason for this significant difference in terms of ethnic variety is not yet clearly known.

Parkinsonian signs seen in MSA-P patients are predominantly rigidity and bradykinesia and are more symmetrical than classical PD. Even if there is a tremor, unlike the tremor of PD, it has a higher frequency, lower amplitude, and has myoclonic components (Köllensperger et al., 2008). Falls in the early period are ten times more common in MSA than in PD but are less common than in PSP (Tison et al., 2002). Pyramidal findings such as Babinski reflex and brisk tendon reflexes are more common in MSA-C, but can also be seen in MSA-P (Köllensperger et al., 2010).

Urinary dysfunction and orthostatic hypotension is frequently detected in MSA patients, even in the initial period, regardless of subtype (Köllensperger et al., 2010). It has even been reported that autonomous dysfunctions may begin a few years before the onset of motor symptoms (Kaufmann et al., 2017).

REM sleep behavior disorders (RBD) occur in approximately 80% of MSA patients. About half of MSA patients report the onset of RBD symptoms before the onset of motor symptoms.

Cerebellar ataxia, dysarthria, and extremity ataxia are the main cerebellar signs detected in MSA-C patients together with autonomic dysfunction. Cerebellar ataxia seen in MSA is not significantly different from other sporadic ataxias. The presence of autonomic dysfunction and parkinsonian signs are clues for correct diagnosis.

Cognitive impairment is usually not found in the early stages of MSA. However, dementia may develop in approximately 15% of patients in advanced stages (Brown et al., 2010).

The average life expectancy in MSA patients is reported to be 7-9 years. It has been determined that the presence of severe autonomic dysfunction in the early stage is the most important marker for poor prognosis (Low et al., 2015).

3. Corticobasal Degeneration

CBD is a rare, progressive neurodegenerative disease with atypical parkinsonian signs. Although it was first described as corticodentatonigral degeneration in the 1960s, it was found that there was no cerebellar degeneration in the following years (Rebeiz et al., 1967). Neurodegeneration in CBD develops mainly in cortical structures and basal ganglia. CBD, which has many different phenotypic presentations due to the huge size of the affected anatomical region, is pathologically a tauopathy like PSP. The mean age of onset was reported as 63. Age of onset varies between 45 and 77 (Wenning et al., 1998). Men and women are equally affected. The average life expectancy has been reported as six years (Litvan et al., 2000).

Determining the true incidence of CBD is a challenging issue. First, it has a high ante-mortem misdiagnosis rate because it has different phenotypic features and is sometimes confused with other neurodegenerative diseases (Armstrong et al., 2013). Second and most importantly, there are no population-based epidemiological studies in the literature.

The classically known CBD phenotype is progressive and usually involves one limb, resulting in different combinations of akinesia, severe rigidity,

dystonia, myoclonus, apraxia, and alien limb phenomenon (Rebeiz et al., 1967). However, four different phenotypes were observed in a large study examining the clinical features of pathologically confirmed CBD patients. These are corticobasal syndrome (CBS), frontal behavioral-spatial syndrome (FBS), nonfluent/agrammatic variant of primary progressive aphasia (naPAA), and progressive supranuclear palsy syndrome (PSPS) (Armstrong et al., 2013). Therefore, diagnostic criteria have been developed for CBD, which can be clinically confused with many different APS and other neurodegenerative diseases. According to these criteria, the signs/symptoms for the diagnosis of CBS were divided into two groups. The first group includes rigidity/akinesia, limb dystonia, limb myoclonus, and the second group includes orobuccal or limb apraxia, cortical somatosensory deficits, and alien limb phenomenon. For the diagnosis of probable CBS, at least two findings from each group must be detected asymmetrically. A possible CBS diagnosis can be made if one sign from each group is observed (Armstrong et al., 2013). Definite diagnosis of CBD requires neuropathological examination.

Asymmetric limb rigidity and bradykinesia are the most common parkinsonian signs in CBD (Wenning et al., 1998). CBD is the most common disease with asymmetric parkinsonian findings among APS. Levodopa therapy has little or no effect on these findings (Kompoliti et al., 1998). Tremor was detected in 39% of CBD patients at any point during the course of the disease. It typically comes out in the combination of action, postural, and resting tremors and differs from the classical resting tremor seen in PD.

Limb dystonia typically develops within two years of disease onset and usually begins in the upper extremity. After it starts in the upper extremity, it can jump to the other side or pass to the lower extremity and form a hemidistonia. However, it is infrequent to start in the lower extremities (Stamelou et al., 2012).

The incidence of limb myoclonus during the course of the disease has been reported to be 27% (Armstrong et al., 2013). It is typically limited to one limb, usually the arm, and is triggered in response to sensory stimuli or by voluntary action (Thompson & Shibasaki, 2000).

Apraxia is seen at admission in about half of CBD patients. The much more common apraxia subtype in CBD is ideomotor apraxia. In this type of apraxia, specifically, voluntary imitation of gestures or mime of tool use is impaired (Armstrong et al., 2013).

The alien limb phenomenon is the most striking finding in CBD. This phenomenon can be described as involuntary but purposeful hand movements. Patients usually describe this condition by stating that their arms have their own will (Carrilho et al., 2001). Interestingly, the alien limb phenomenon is not a CBD-specific finding but may develop in some other lesional disorders of the brain.

Another diagnostic sign of CBD is cortical somatosensory losses such as agraphesthesia, neglect, and astereognosis, developed secondary to parietal lobe pathology. In addition, cortical somatosensory losses are often associated with limb apraxia (Reich & Grill, 2009).

4. Lewy-Body Dementia

LBD is another disease with atypical parkinsonian signs. However, unlike other APS, dementia is noted in the early stages of the disease and is the most important cause of disability. Pathologically alpha-synuclein accumulations take place in the brain as well as in MSA and PD. However, unlike MSA and PD, alpha-synuclein accumulations occur in the limbic and neocortical regions, even in the early stages of LBD.

Autopsy and clinical studies have been showing inconsistent results regarding the frequency of LBD. Its frequency among dementias was found to be 4% in a community-based epidemiological study. However, it is thought that its true incidence may be higher because, in some cases, LBD is misdiagnosed as Alzheimer's dementia or Parkinson's dementia (Thomas et al., 2017). In addition, it is more common in men than in women (Vann Jones & O'Brien, 2014).

Different diagnostic criteria have been developed for the diagnosis of LBD. Ultimately, the last criteria revised in 2017 are detailed in Table 1.

Tablo 1: Diagnostic criteria of Lewy-body dementia (McKeith et al., 2017)

Essential criteria • Progressive cognitive decline strong enough to interfere with daily activities
Core clinical features • Fluctuating cognition with pronounced variations in attention and alertness • Recurrent visual hallucinations • Rapid eye movement (REM) sleep behavior disorder (may precede other symptoms) • Parkinsonism (defined as one or more spontaneous cardinal features: bradykinesia, rest tremor, rigidity
Supportive clinical features • Severe sensitivity to antipsychotic agents • Postural instability • Repeated falls • Syncope or other transient episodes of unresponsiveness • Severe autonomic dysfunction • Hypersomnia/excessive daytime sleepiness • Hyposmia • Hallucinations in other modalities • Apathy, anxiety, and depression
Indicative biomarkers • Reduced basal ganglia dopamine transporter uptake (SPECT or PET) • Abnormal (low uptake) iodine 123-MIBG myocardial scintigraphy • Confirmation of REM sleep without atonia on polysomnography

A probable LBD diagnosis can be made when two or more core clinical features present, or at least one indicative biomarker is present together with a core clinical

feature. A diagnosis of possible LBD can be made when there is only one core clinical feature but no indicative biomarker or where there are only two indicative biomarkers but no core clinical feature (McKeith et al., 2017). Supportive clinical features do not have a role in the definite diagnosis of LBD but are further supportive in clinically suspected cases, and therefore, it is important to question them.

Attention, visual processing, and executive functions are cognitive capabilities that deteriorate early in LBD. However, unlike Alzheimer's dementia, naming and memory loss are not evident in the early stages (Ferman et al., 2006). Stroop tasks, trail-making tests are functional tests to evaluate executive functions and attention. Therefore, it is helpful to use them in a patient with suspected LBD.

Although fluctuation in cognitive functions is seen in advanced stages in other dementias, it is essential to observe it in LBD in the early stages. The fluctuations seen in LBD are typically delirium-like. There are waxing and waning episodes of cognition, attention, and arousal. Disturbances in speech, staring gaze, and daytime sleepiness may occur during these episodes (McKeith et al., 2005).

Repetitive, well-formed visual hallucinations (people, animals, passage hallucinations) play an essential role in the diagnosis of LBD. Hence, 80% of LBD patients have visual hallucinations even in the early stages. Patients often speak with their surroundings about what they are experiencing while they perceive hallucinations. In addition, patients may also show emotional reactions to hallucinations they perceive (McKeith et al., 2017).

Spontaneous parkinsonian signs with no secondary cause are one of the core clinical features for the diagnosis of LBD. It occurs in approximately 85% of patients. They can be found in different combinations of bradykinesia, rest tremor, and rigidity. However, the presence of one of the three signs is considered sufficient for diagnosis. Parkinsonian signs in LBD are usually observed asymmetrically and may develop in the other extremity in the later stages.

REM sleep behavior disorders (RBD) are also common in LBD. While asleep, patients act as if they live the dream content, and polysomnography shows the disappearance of REM atony, which should be physiologically present in REM sleep. Patients may have a history of injuries or involuntary injury to their bed partner due to this condition. That's why it's essential to question the bed partner for RBD. In addition, RBD may occur years before other motor and cognitive disturbances (Postuma et al., 2012).

CONCLUSION

The approach to atypical parkinsonian syndromes requires careful evaluation and detailed consideration of the patient's history and signs/symptoms. The necessary clues for the correct diagnosis can often be obtained as a result of detailed evaluation. The application of diagnostic criteria when diagnosing atypical parkinsonian syndrome is essential because different phenotypes of diseases and overlap syndromes are described. Early and accurate diagnosis is also of great importance in terms of predicting the progression of the disease, establishing the correct expectation of survival, and determining treatment strategies.

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