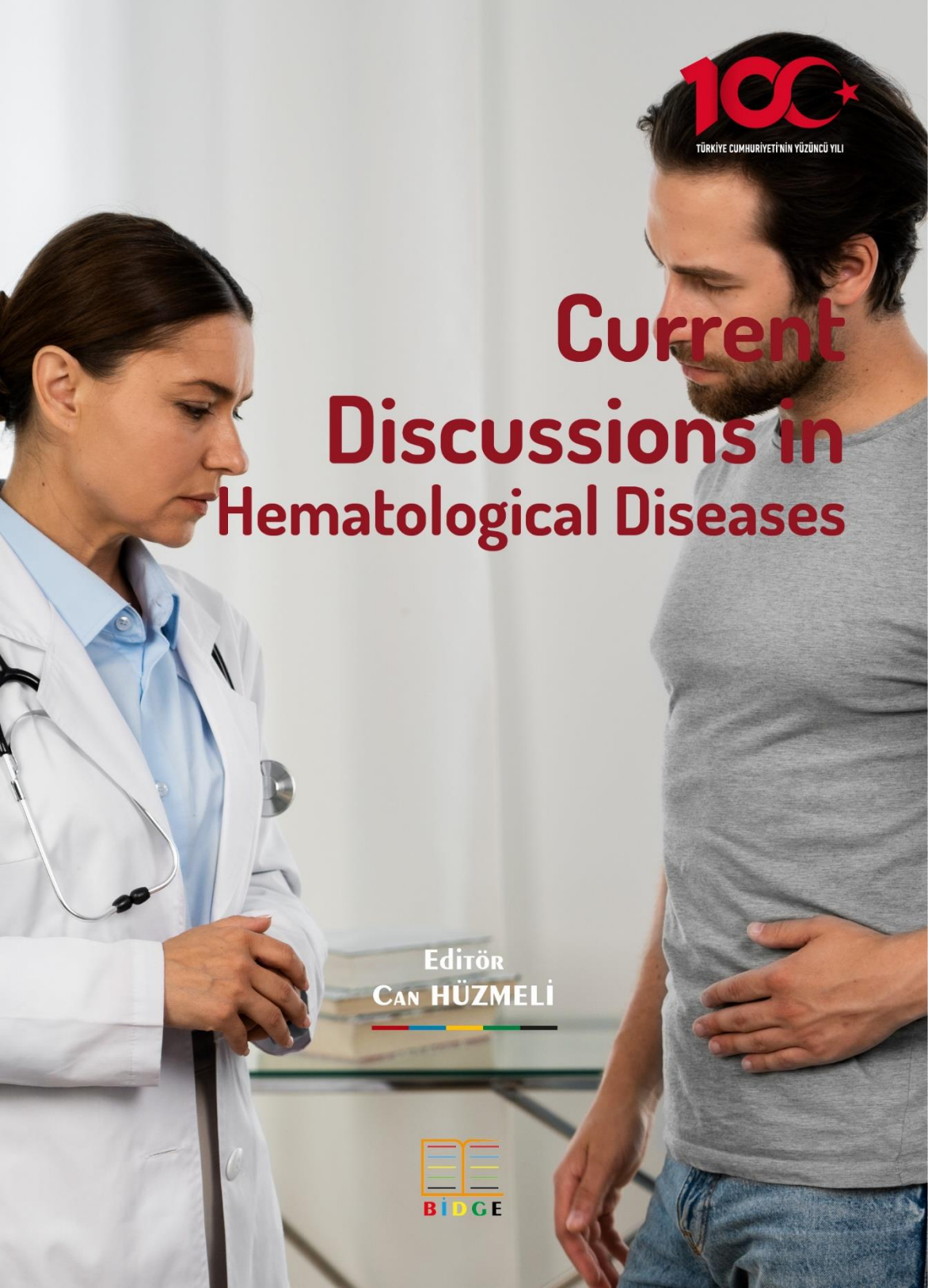

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CHAPTER I

Primary Hyperaldosteronism

Oğuzhan AKSU

Introduction

The clinical manifestation that develops due to pathologies originating from the adrenal gland and occurs as a result of excessive aldosterone secretion is called primary hyperaldosteronism. The clinical manifestation of primary hyperaldosteronism was first described in 1955 by the American endocrinologist Jerome W Conn (1). Postmortem adrenal-secreting adrenal adenomas have been demonstrated in patients with hypertension, hypernatremia, and hypokalemia. Excessive aldosterone release due to pathologies originating from the adrenal gland is called primary hyperaldosteronism, while excessive aldosterone release due to adrenal gland stimulation by non-adrenal pathologies is called secondary hyperaldosteronism. The figure shows the physiology of a normal renin aldosteronism and the pathophysiology of primary hyperaldosteronism (2).

Approximately 90% of all hypertensive patients have essential hypertension, and 10% have secondary hypertension. In previous studies, it was stated that primary hyperaldosteronism constituted 1% of all hypertensive patients, but recent studies have shown that patients with essential hypertension are actually patients with primary hyperaldosteronism with adrenal hyperplasia (3-4) this rate increases up to 17-23% in patients with severe hypertension (5). Another study investigated the etiology of secondary hypertension in patients with resistant hypertension and found that 61.5% of patients with secondary hypertension had primary hyperaldosteronism (6).

Aldosterone-secreting adenoma accounts for 65-70% of primary hyperaldosteronism cases. Meanwhile, bilateral adrenal hyperplasia is the second most common (20-25%). Other rare causes include glucocorticoid-correctable hyperaldosteronism (due to duplications of genes encoding 11-beta hydroxylase and aldosterone synthase), adrenal carcinoma, and ectopic aldosterone-producing tumors (ovarian, renal) (7).

Clinical Manifestations

Patients with primary hyperaldosteronism most commonly present with hypertension and symptoms secondary to hypopotassemia. However, it should be noted that in 20% of patients, serum potassium levels will be normal. Symptoms such as weakness, fatigue, polyuria, polydipsia, paresthesia, and headache may be observed due to spontaneous or diuretic-induced hypopotassemia. Moreover, U waves and cardiac arrhythmias may be seen on ECG. Severe and resistant hypertension (patients with systolic blood pressure above 160 mm Hg and diastolic blood pressure above 100 mm Hg despite triple antihypertensive therapy). In addition, metabolic alkalosis may be observed (8). Patients with primary hyperaldosteronism do not show edema despite sodium retention. Due to the escape phenomenon caused by the tubule receptor problem, fluid accumulation does not continue beyond a certain point, and edema does not develop. However, edema is seen

in heart failure or renal failure (9). It is well-known that patients with primary hyperaldosteronism are more likely to suffer from metabolic diseases, and cardiovascular events occur more frequently and increase mortality as compared to patients with primary hypertension (10).

Patients to be Investigated for Primary Hyperaldosteronism (SEMT Guideline 2022) (11).

1. Presence of hypokalemia,
2. Severe, resistant, or relatively acute hypertension,
3. Presence of adrenal incidentaloma, In the presence of hypertension (Aldosterone-secreting adenoma rate is 2.5%, this rate is 6% after surgery),
4. Presence of hypertension in young patients under 30 years of age, without obesity and familial hypertension,
5. In patients with hypertension, if a family member has had a stroke under the age of 50,

Diagnosis

The most important step in the diagnosis of primary hyperaldosteronism is the evaluation of the renin-angiotensin-aldosterone axis. For this reason, plasma renin activity and aldosterone levels should be measured first. First, the ratio of Plasma Aldosterone (PA) to Plasma Renin Activity (PRA) should be looked at simultaneously. For Primary Hyperaldosteronism, the ratio of PA (ng/dl) to PRA (ng/ml/h) should be 20/1 or higher (Step 1 tests). Blood samples should be collected between 2-3 hours after waking up in the morning and after resting for at least 10-15 minutes. Extreme salt-restricted diets may increase aldosterone levels and cause false positivity (Table SEMT guideline). In the presence of spontaneous hypokalemia, the presence of PRA or plasma renin at an unmeasurable level, and a PA level of ≥20 ng/dL in the treatment-naïve individual, there is no need for 2nd-line tests (5). When PRA

is very low, even if aldosterone levels do not increase much, the PA/PRA ratio may be above 20 or even 30, which may cause false positivity. Hence, at least a PA level above 15 ng/dl is expected. However, these parameters may still not be sufficient for diagnosis, and confirmatory tests may be needed. Drugs that alter aldosterone and renin secretion should not be used before starting second-line tests (ACEI, Beta-blockers, spironolactone, and eplerenone should be discontinued 2-4 weeks before and alpha blockers and calcium channel blockers without effect should be used), hypokalemia inhibits aldosterone secretion and should be corrected (12).

Step 2 tests

Saline suppression test, NaCl, 2 liters, 500 cc/hour, infused IV for 4 hours. Patients should have normal cardiac function. In normal individuals, it is below 6 ng/dl but increases above 10 ng/dl in the presence of autonomic aldosterone production. It is the most commonly used test, but oral salt loading test, fludrocortisone suppression test, and Captopril challenge test (CCT) are also used (5).

Localization

Adrenal glands should be imaged in a patient diagnosed with primary hyperaldosteronism. Although both computed tomography and magnetic resonance imaging can be used, the use of computed tomography should be preferred over magnetic resonance imaging (13). Adrenal vein sampling may also be performed in patients with bilateral adrenal abnormalities on imaging or in patients with suspected adrenal hyperplasia. Right and left adrenal vein samples are compared with inferior vena cava samples; aldosterone ratios from right and left adrenal veins greater than 4 suggest unilateral adenoma, while ratios less than 3 suggest bilateral hyperplasia (13). In the presence of unilateral adenoma (>10 mm), adrenal venous sampling may be omitted in young patients (<35 years) with PA levels above 30ng/dl and spontaneous hypokalemia.

Treatment

The treatment for unilateral adrenal adenomas with aldosterone secretion is unilateral adrenalectomy. If it cannot be localized or a bilateral disease is present, and the patient is not suitable for surgical intervention, medical treatment can be performed with mineralocorticoid receptor antagonist (14).

Figure: Physiology of a normal renin aldosteronism and pathophysiology in primary hyperaldosteronism

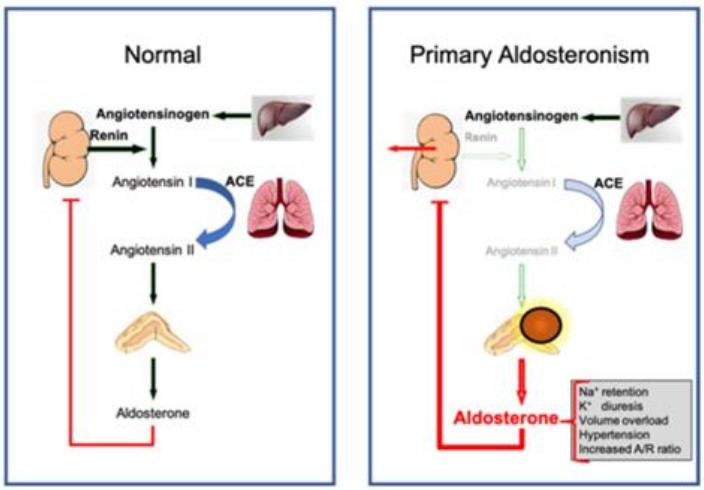

Table : Initial evaluation according to the ratio of PA to PRA

PA/PRA (ng/dl/ml/h)	Diagnosis
<20	Normotensive or essential hypertension
≥	PH 90% sensitive 90% specific
≥	The probability of PH is very high

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CHAPTER II

Frequency of MEFV Gene Mutation in Patients with Chronic Spontaneous Urticaria and Its Clinical Significance

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Introduction

Urticaria describes a heterogeneous group of disorders, characterised by erythematous skin lesions. Urticaria is classified according to the duration and clinical features of the disease. In acute

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urticaria, the duration of recurrent urticaria attacks is shorter than six weeks, whereas in chronic urticaria, lesions occur almost every day or close to every day in a period longer than 6 weeks (1). While an etiological cause can frequently be found in patients with acute urticaria, no triggering cause has been found in approximately 70% of patients with chronic urticaria (2-3). In addition, chronic urticaria develops in 1-2% of patients (4).

Investigation of genetic pathologies in the etiology of chronic spontaneous urticaria (CSU) has been increasing especially in the last decade. The frequency of urticaria was found eight times more frequently than expected in first-degree relatives of patients with CSU. This situation supports the idea of familial transmission (5). A strong association between HLA-DR4 and in vitro basophil histamine release activity has recently been shown and it has been associated with the DQ8 allele in patients with positive CSU (6). In a different study, it was reported that the CT allele of TGF-Beta1 was higher than the CC allele in patients with CSU and this increases inflammation (7). In a recent study, changes in HLADQA1 and ITPKB loci were found to be associated with KSU in a genome-wide association analysis of 679 KSU patients (8). Although the number of studies in this field is small, the finding of significant associations suggests that genetic pathology may be underlying. This situation has paved the way for further studies. Despite all studies, studies to be performed on the etiopathogenesis of CSU are still important because of the limited information on its etiology and pathogenesis.

Pirin protein consisting of 781 amino acids encoded by the MEFV gene is released by many cells; mainly from neutrophils, eosinophils, dentritic cells, synovial fibroblasts, and monocytes with cytokine activity (9,10). In studies conducted in experimental models to explain the mechanism of action of pirin protein on inflammation, it was shown that pirin both activates and suppresses the caspase 1/IL-1 signalling pathway (11). The pirin protein involved in the NF-κB cell signalling pathway regulates apoptosis and suppresses the inflammatory response by suppressing proinflammatory molecules and increasing the transcription of anti-

inflammatory proteins (12). Structural changes in the pirin molecule as a result of mutations in this gene lead to increased leukocyte migration and prolongation of the response to inflammatory stimuli. Defect in pirin protein, i.e. MEFV gene mutation, has always been a subject of research especially in the pathogenesis of diseases characterised with increased inflammation.

In this study, we aimed to determine the frequency of MEFV gene mutation in patients with chronic spontaneous urticaria and to determine whether it plays a role in its aetiology.

Material and Metods:

The study included 42 patients older than 18 years who were diagnosed with chronic spontaneous urticaria between 2010 and 2012 in a single centre. Exclusion criteria were chronic inflammatory diseases such as systemic lupus erythematosus, Behçet's disease, rheumatoid arthritis, ankylosing spondylitis, vasculitis, and familial Mediterranean fever (FMF), which have been shown to increase the frequency of MEFV genetic mutations in various studies. In addition, patients with autoimmune thyroid disease, helicobacter pylori positivity, parasitosis, urticaria persisting for more than 24 hours, patients diagnosed with physical urticaria, and patients in whom a cause in the etiology of chronic urticaria such as drug- and food-induced urticaria was detected were excluded from the study. Studies on MEFV gene mutation carriage in healthy Turkish population were determined as control group. Patients with CSU were evaluated in terms of mutation frequency and compared with studies on MEFV gene mutation carriage in healthy Turkish population in terms of mutation frequency. In addition, patients with CSU with and without MEFV gene mutation were compared in terms of age at diagnosis, disease duration, eosinophil count, sedimentation level, and frequency of urticaria in first-degree relatives.

Our study was a single-centre, case-control study and was approved by the GATA Ethics Committee on 22 November 2010 (22 November 2010 and number 162).

Statistics:

SPSS version 20.0 was used for the analysis. The distribution of the data was evaluated using one-sample Kolmogorov Smirnov test. Normally distributed continuous variables were expressed as mean ± SD, skewed continuous variables as median (minimum-maximum), and categorical variables as number and percentage. Fisher exact test or chi-square test was used to compare categorical variables. Differences between numerical variables were tested by Student's t-test or, where appropriate, a Mann-Whitney U-test. Percentages of categorical variables in the mutated and non-mutated groups were compared with the "z-test for significance of the difference between two unrelated percentages". P value below 0.05 was considered statistically significant.

Results:

A total of 42 patients aged 20-60 years, 16 females (38%) and 26 males (62%) were included in the study. Heterozygous MEFV gene mutation was found in 6 of 42 patients with CSU included in the study. E148Q mutation was found in 3 patients, P369S mutation in 2 patients and M694V mutation in 1 patient. The mean age was 28±6.928 years in the group in which mutation was determined, and 35.14±12.527 years in the group in which mutation was not determined. The mean age at diagnosis was 25.50±7.204 and the mean duration of disease was 2.5±2.074 (1-6) in the mutation-detected group, while the mean age at diagnosis was 29.50±15.711 and the mean duration of disease was 5.63±6.80 (1-26) in the non-mutation-detected group. A history of urticaria in first-degree relatives was present in 2 (33.3%) patients in the mutation group and in 5 (12%) patients in the non-mutation group. No statistically significant difference was found between the patients with CSU who had MEFV gene mutation and those who did not in terms of age at

diagnosis, disease duration, eosinophil count, sedimentation level, and frequency of urticaria in first-degree relatives (Table-1).

Patients with CSU whose first-degree relatives had urticaria, were considered to have a positive family history. It was found that there was no correlation between the presence of MEFV gene mutation and family history. Family history did not lead to an increase in mutation frequency (Table 2).

The mutation frequency was found to be 9.5% (for five mutations including M694V, M680I, V726A, E148Q, E694I). No statistically significant difference was found when compared with the study on MEFV gene mutation carriage in healthy Turkish population in which carriage was found to be 20% (Table-3). The "Z test for significance of the difference between two unrelated percentages" was used to compare the MEFV gene mutation rates of patients with CSU and healthy individuals in the study of Yılmaz et al. The critical ratio Z value calculated for the 10.5% difference between the percentage of individuals with MEFV mutations in patients with CSU and healthy Turkish population was 1.54. Since this value is less than 1.96 at .05 significance level, the difference between the percentages is not significant.

Table 1: *Comparison of Age at Diagnosis, Disease Duration, Family History, Eosinophil Count, Sedimentation Level and Presence of MEFV Gene Mutation*

	CSU patients with MEFV Mutation (n=6)	**CSU patients without MEFV Mutation (n=36)**	**P value***
Gender **Female(n,%)** **Male(n,%)**	 2 (33.3%) 4 (66.6%)	 14 (38.9%) 22 (61.1%)	p>.05
Age	28±6.928 (21-39)	35.14 ±12.52 (20-60)	p>.05
Age At Diagnosis	25.50±7.204 (17-38)	29.50 ±15.71 (1-57)	p>.05
Disease Duration	2.5±2.07 (1-6)	5.63±6.80 (1-26)	p>.05
Family History	2 (33.3%)	5 (12%)	p>.05
Total Eosinophil Count ($x10^3$/microL)	0.128 ± 0.10	0.153 ± 0.12	p>.05
Sedimentation Level (mm/hr)	10.33 ± 8.61	13.50 ± 11.25	p>.05

* Mann Whitney U test

Table-2:*Comparison of Participants with Positive Family History in Terms of MEFV Gene Mutation Frequency*

	CSU patients with MEFV Mutation (n=6)	**CSU patients without MEFV Mutation (n=36)**	**Total**	**P value***
Family History	2 (33.3%)	5 (12%)	7 (16.7%)	p>.05
No Family History	4 (66.6%)	31 (88%)	35 (83.3%)	

* Fisher Exact test

Table-3: *Comparison of MEFV Gene Mutation in Patients with CSU with Carriers in Healthy Turkish Population*

			Mutation Frequency				
	N	MEFV mutation frequency	M694V	E148Q	M680I	V726A	M694I
CSU	42	9.5% (14.2%)*	1/84	3/84	0/84	0/84	0/84
Control Group**	100	%20	3/200	12/200	5/200	2/200	0/200
p value		p>.05	p.>05	P>.05	p>.05	p>.05	p>.05

(*) Carrier frequency when 2 patients with P369S mutation, who were not studied in the control study and therefore their frequencies could not be compared, were added.
(**)The study on MEFV gene mutation frequency in healthy Turkish population by Yılmaz et al.

Discussion:

Chronic urticaria is more common in young adults and lasts for 3-5 years on average. However, there are cases lasting longer than 20 years. One of our pre-study predictions was that patients with MEFV gene mutation would be diagnosed at an earlier age and have a longer duration of disease. However, the age at diagnosis was 25.50±7.20 (17-38) in patients with mutations and 29.50±15.71 (1-57) in patients without mutations. Although this result seems to support our predictions, the difference was not statistically significant due to the small number of patients. In addition, the duration of the disease was 2.5±2.074 (1-6) in those with mutations, while the duration of the disease was 5.63±6.80 (1-26) in the group without mutations. The probable reason for the difference was that no mutation was detected in the three patients who stated that the disease continued for more than 20 years. In addition, the frequency

of disease persistence over 20 years in our patient group was above our expectations.

One of the topics that has been investigated recently is the association of chronic urticaria with autoimmune thyroid disease (1). In recent studies, antithyroid antibodies and antimicrosomal antibodies were found in 0-70% of patients with chronic urticaria (13). Although some studies have shown a decrease in the frequency of attacks in patients with chronic urticaria who were rendered euthyroid with thyroid replacement therapy, there is no definite evidence that thyroid disease plays a role in the pathogenesis of urticaria. In our study group, 3 (7%) patients were receiving thyroxine treatment with a diagnosis of hypothyroidism. It was observed that urticaria attacks in euthyroid patients continued despite treatment. Thyroid autoantibodies were higher than the upper limit of normal in 12% of the participants. This result was lower than the autoantibody positivity found in previous studies.

As expected, no statistically significant difference was found between the whole blood, renal function, liver enzymes and sedimentation levels of patients with MEFV gene mutation and those without MEFV gene mutation. The reason for this is that diseases with chronic inflammation were determined as exclusion criteria in our study in order not to affect the frequency of MEFV gene mutation. In addition, the majority of the patients included in the study did not have an urticaria attack at the time of hospital admission and examination.

In a study conducted by Saçkesen et al., the frequency of asthma, allergic rhinitis and eczema in pediatric patients diagnosed with FMF disease was compared with the normal population and found to be lower than the frequency in the normal population. As a possible mechanism, it has been reported that MEFV gene mutation leads to an increase in Th1 activity and this leads to an increase in IFNγ synthesis (14). The finding that the prevalence of atopic diseases decreased in rheumatoid arthritis patients with increased Th1 activity supports this mechanism. It is also known to decrease

the number of IFNγ, IgE and eosinophils and inhibit eosinophil-dependent reactions. Therefore, it may be interpreted that the presence of the mutation may have a protective effect for atopy and asthma. The finding that the eosinophil count was 0.128 ± 0.10 in the group with MEFV mutation and 0.153 ± 0.12 in the group without MEFV gene mutation supports this mechanism. However, the difference was not statistically significant probably due to the small number of patients. In addition, the frequency of MEFV gene mutation was found to be 9.5% (for five mutations including M694V, M680I, V726A, E148Q, E694I) in our study. When we compared it with the study conducted by Yılmaz et al. on MEFV gene mutation carriage in healthy Turkish population in which carriage was found to be 20%, it was found to be lower although no statistically significant difference was found (15). This finding also supported the study of Saçkesen et al. In addition, one of the findings supporting this study was the duration of disease. While the disease duration was shorter in the group with mutation, it was found to be longer in the group without mutation. However, the difference was not statistically significant, probably due to the small number of patients. Although our pre-study predictions were that the frequency of MEFV gene mutation was increased in patients with CSU, these results suggest that MEFV gene mutation may be a protective factor for chronic urticaria. However, further studies with larger patient groups are required to support this idea.

E148Q was detected most frequently in the participants as in healthy individuals. However, the P369S mutation, which was detected in two patients and could not be included in the comparison. Its rate was found in FMF patients nearly 10%. Whether the high frequency of this mutation, 33% in our study group, is a feature of CSU could not be clearly demonstrated due to the small number of patients.

In our study, the frequency of participants with a history of urticaria in first-degree relatives was compared between those with and without MEFV gene mutation. However, 33% of those with MEFV gene mutation had a history of urticaria in first-degree

relatives, whereas this rate was 12% in those without mutation. In fact, this result supports the idea that genetic pathologies may play a role in the etiology of CSU, which has been frequently investigated recently. However, this difference was not statistically significant due to the small number of patients. One patient with M694V heterozygous mutation was excluded from the study because the biopsy result was compatible with urticarial vasculitis. As known, M694V mutation is the most common mutation found in patients with FMF, while the presence of M694V homozygous mutation is associated with a low response to colchicine treatment and an increased risk for the development of amyloidosis. In addition, in Behçet's disease, which is characterized by increased inflammation, the M694V mutation is most commonly associated with the disease. Therefore, this suggests that studies with large patient participation are needed to more clearly evaluate the MEFV gene mutation association in urticarial vasculitis.

Although there are no studies on the association of urticaria with FMF carrying MEFV mutation, there are cases reported (16). However, since the main aim of our study was to evaluate the frequency of MEFV gene mutation in the etiopathology of urticaria and FMF was an exclusion criterion, we do not have clear data on the association. However, in the long-term follow-up of patients with MEFV mutation, no clinical finding related to FMF developed.

The most important factor limiting our study was the small number of patients. In addition, the fact that the included patient group was predominantly young male patients may have led to heterogeneity in the study group. Therefore, further studies in new patient groups with large participation are needed.

Conclusion:

Although MEFV gene mutation was found to be lower in patients with chronic spontaneous urticaria than in carriers in the healthy population, no statistically significant difference was found. In addition, MEFV gene mutation was not found to contribute to

familial inheritance, duration and severity of the disease in patients with chronic spontaneous urticaria.

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CHAPTER III

Histopathological Changes in The Jejunum at Day Fifteen and Thirty After Total Gastrectomy

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Emel KOPTAGEL[2]

Introduction

Gastric cancer is a very common cause of death worldwide (1-2) and part or all of the stomach is removed for treatment (3-4), thus increasing the survival chances of patients with gastric cancer. After total gastrectomy, nutritional, metabolic and hormonal disorders, osteopenia, pancreatic and liver cancer, anemia, and demyelination occur. The stomach synthesizes hydrochloric acid and pepsinogen for protein digestion, intrinsic factor for vitamin B12 absorption and

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gastrin for calcium absorption. It is also active in the control of cholecystokinin, insulin and glucagon release (5-8). Considering all these, osteopenia, excessive weight loss and anemia are inevitable if an effective treatment and nutrition method is not applied after total gastrectomy.

Since determination of the morphologic changes that may occur in the digestive tract after surgery may be important in terms of establishing an effective treatment and nutrition method, we aimed to determine the histopathologic changes in the jejunum in the early period after total gastrectomy.

Material And Methods

Experimental Technique

In this study, 24 adult Wistar albino male rats with an average weight of 350-400g, which were bred at Sivas Cumhuriyet University Experimental Animal Center and fed with standard pellet feed and running water, were used. The animals were fasted the night before surgery and then ketamine chloride (90 mg/kg body weight) and xylazine (3-10 mg/kg body weight) were administered intraperitoneally for anesthesia. Gastrectomy was performed by ligation of blood vessels and end-to-end anastomosis of the esophagus and duodenum followed by total resection of the stomach. The esophagus and duodenum were ligated with 6/0 atraumatic chrome catgut. The abdominal cavity was closed with 3/0 silk sutures. For the control group, the abdominal cavity of the rats was opened, waited for 45 minutes for total gastrectomy and closed. After the operation, both groups were not given food and water for 24 hours. Pasteurized cow's milk followed by standard feed and water were given for 3 days after the first 24 hours and no additional nutrients were given. The study was conducted with the permission of the Cumhuriyet University Animal Experiments Local Ethics Committee numbered 65202830-050.04.04-382.

Light Microscopy

Jejunum tissue samples were taken from the experimental and control group animals on the 15th and 30th days after the operation and some of them were fixed in 10% neutral formaldehyde for 48 hours, some of them were fixed in Bouin's fixative for 7 hours and blocked in paraffin after routine tissue follow-up procedures. Immunohistochemical methods for carcinoembryonic antigen (CEA) with hematoxylin-eosin, mallory azan staining methods, James's silver precipitation method, PAS reaction and immunohistochemical methods were applied to sections taken from paraffin blocks with a thickness of approximately 5μ.

Transmission Electron Microscopy (TEM)

Jejunum tissue samples from the experimental and control group animals were dissected into approximately 1mm3 pieces in 0.1 M phosphate buffer on the 15th and 30th days after the operation. The tissues were fixed at +4 C0 for 48 hours with 3% glutaraldehyde solution prepared with Milloning phosphate buffer, pH 7.4, and kept in 0.1 M phosphate buffer for 15-20 minutes. As a second fixative, 1% OsO4 was applied to the tissues for 2 hours. Then they were washed in phosphate buffer, passed through an ethyl alcohol series and transparently fixed in propylene oxide twice for 20 minutes each. They were kept overnight in a one-to-one mixture of Araldite CY-212 and propylene oxide and then blocked in Araldite CY-212. Semi-thin sections taken from these blocks were stained with toluidine blue and evaluated under a light microscope and thin sections were taken from the appropriate areas. Thin sections were contrasted with uranyl acetate and lead citrate and photographed under Zeis-109T electron microscope.

Results

No pathologic findings were found in the light and electron microscopic examinations of the tissue samples of the control group (Figure 1, 2). The villi formed by single-layered prismatic striated-edged epithelial cells and lamina propria are quite long. There are goblet cells filled with acid glycoproteins and containing mucin

granules between the absorptive cells. PAS positive images of the microvillus structures that give the epithelial surface its striated edge characteristic show that the striation is not interrupted (Figure 1b). There are dense reticular (Figure 1a) and collagen fibers (Figure 1c) in the lamina propria and submucosa. CEA immunolocalization was negative in all layers of the organ (Figure 1d). In electron microscopy, microfilament bundles inside the microvillus extend along the microvillus. Epithelial cells are seen with oval shaped nuclei and abundant mitochondria with smooth crystals (Figure 2a-c). Mast, plasma, lymphocyte and eosinophil cells in the lamina propria preserve their normal morphologic structures (Figure 1d).

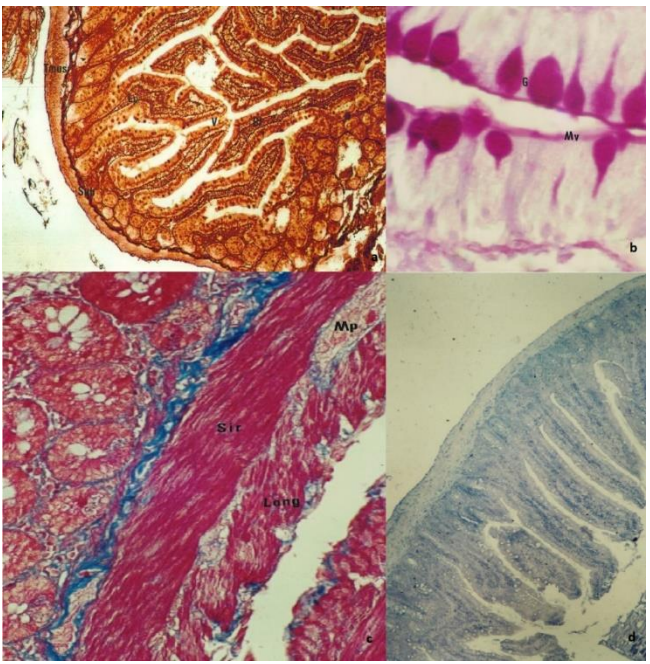

Figure 1a-d: Control group villus structures (V), epithelial cells (E), basal lamina (Bl), lamina propria (Lp), submucosa (Sub), tunica muscularis (Tmus). a) James Technique, X10, b) PAS, X100, c) Mallory-azan, X40, d) CEA, X10

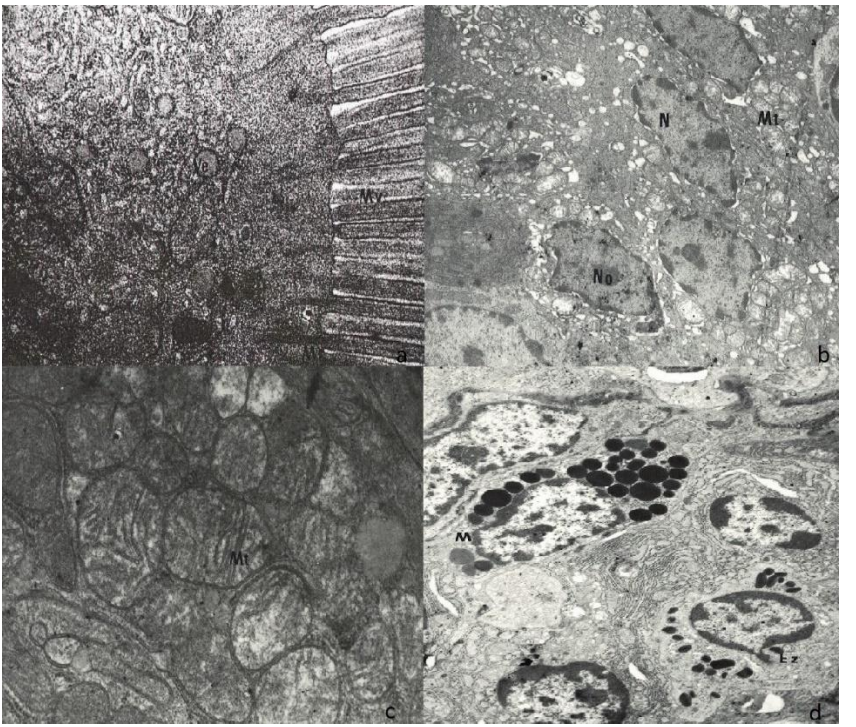

Figure 2: Fine structure of control group absorptive cells. a) Microvillus (Mv), microfilaments (Mf), vesicles (Ve), X20000, b) Nucleus (N), nucleolus (No), X3000, c) Mitochondria (Mt), X20000, d) Eosinophil (Ez), mast (M) and plasma (P) cell, X3000

When the 15th day samples of the experimental group were compared with the control group, pathologic changes were observed at the light and electron microscope level (Figure 3-5). It was observed that villus lengths were shortened and thickened and erosion was observed on the mucosal surface in the 15th day samples of the experimental group (Figure 3a). A decrease in the amount of mucin granules of goblet cells (Figure 3b), irregular and scattered structuring of reticular fibers in the lamina propria, and intense lymphocyte infiltration were observed (Figure 3c). Dense collagen fiber bundles were observed in the submucosa layer (Figure 3d). CEA immunolocalization was positive in the lamina propria,

submucosa, and tunica muscularis, especially in the longitudinal muscle layer (Figure 4a, b).

In the electron microscope examinations, there was no difference in the microvillus structure and arrangement of the epithelial cells compared to the control group, but there was a decrease in the number of mucin-containing granules of vesicles and goblet cells, and increased vascularization in the lamina propria layer (Figure 5a-c).

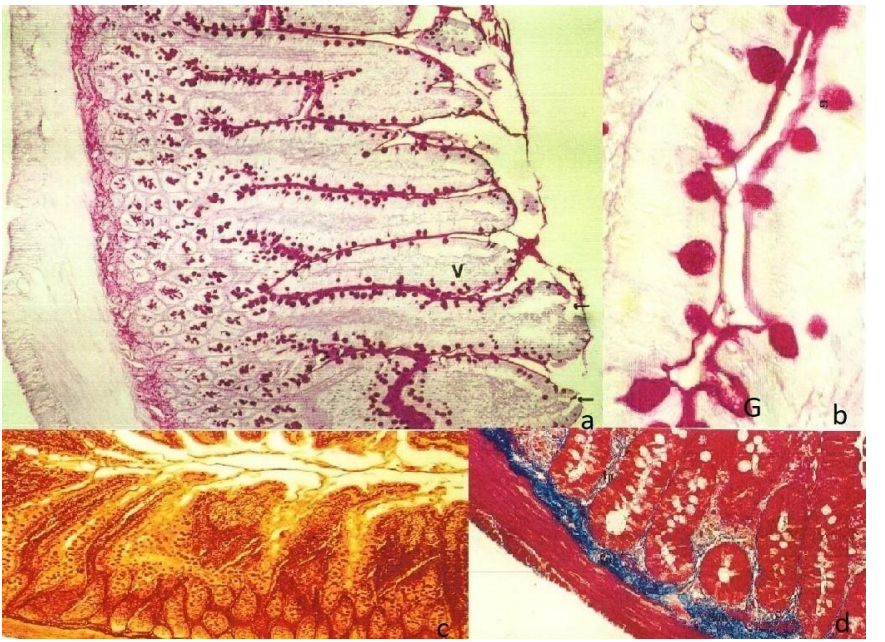

Figure 3: a) Villi with shortened and thickened length and epithelial erosion (←) on the surface 15 days after total gastrectomy (V), PAS, X10, b) Goblet cells with decreased secretion (G), PAS, X100, c) Dense lymphocyte infiltration in the lamina propria (*), James Technique, X10, d) Dense collagen fiber bundles in the submucosa (Kol), Mallory-azan, X40

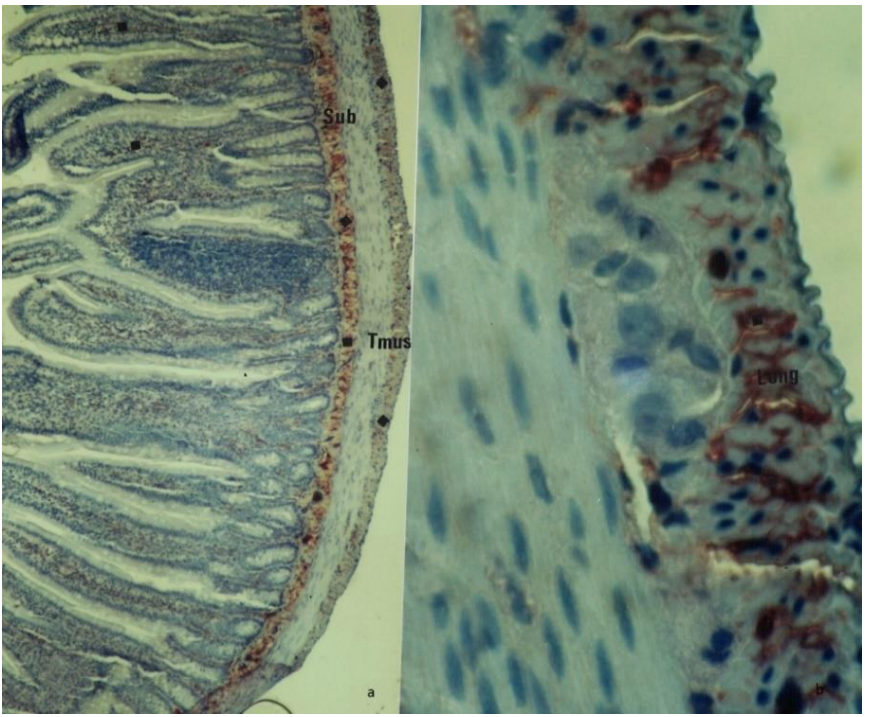

Figure 4a, b: Intense CEA (■) immunolocalization in the lamina propria (Lp), submucosa (Sub), tunica muscularis (Tmus) and especially in the longitudinal (Long) area 15 days after total gastrectomy, CEA, X10, X100

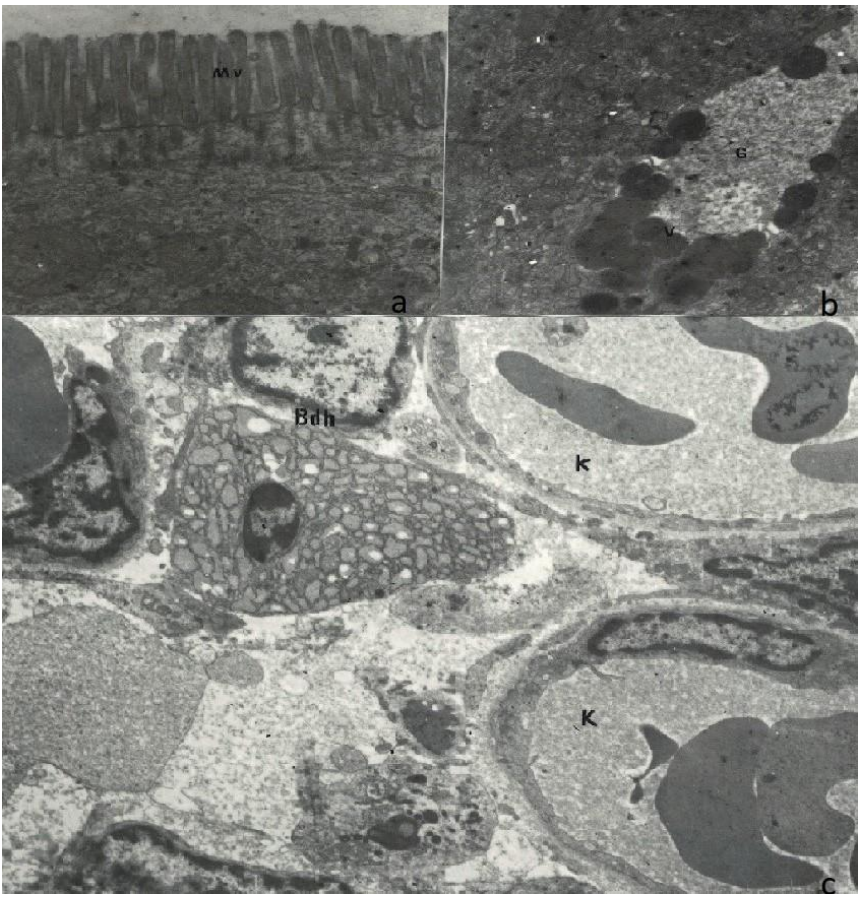

Figure 5: a) Regular and densely arranged microvilli of absorptive cells (Mv) 15 days after the operation, X20000, b) Goblet cell (G) secretory vacuoles (V), X7000, c) Numerous capillaries (K), connective tissue cells (Bdh) in the lamina propria, X3000

In jejunum specimens taken one month after total gastrectomy, villi atrophied in many parts of the intestinal lumen, adjacent villi fused with each other and intense epithelial erosion occurred on their apical surfaces, and the amount of goblet cell secretion decreased (Figure 6a-d). The apical of some villi were enlarged due to increased connective tissue fiber and lymphocyte infiltration (Figure

6a-c, 7a-d) and debris cells and tissues were observed in the intestinal lumen (Figure 6a,c,d). In the lamina propria, reticular fiber increase and edema occurred with lymphocyte infiltration (Figure 7a, b). CEA immunolocalization was strongly positive in the lamina propria, submucosa, and the circular and longitudinal muscle layer of the tunica muscularis (Figure 7c-e).

In electron microscopy, polychromatophilic erythroblast cells were densely located among the absorptive cells and enlargement of endoplasmic reticulum cisternae, disruption of mitochondrial cristae structure and abundant glycogen accumulation in the cytoplasm were detected in the absorptive cells (Figure 8a-c). At the apex of microvilli, the glycocalyx cover increased and was observed as dark-colored accumulation. Secretory vacuoles of goblet cells were observed at the base of the cell and in small numbers (Figure 8d, e). Lymphocyte infiltration and connective tissue fiber increase in the lamina propria and dense myofilaments in the hypertrophic smooth muscle cells of the tunica muscularis were determined. In addition, enlargement of pinocytotic vesicles localized in the periphery of the cells was remarkable (Figure 8f, g). No pathologic changes were observed in the ganglion cells of Auerbach's myenteric plexus (Figure 8h).

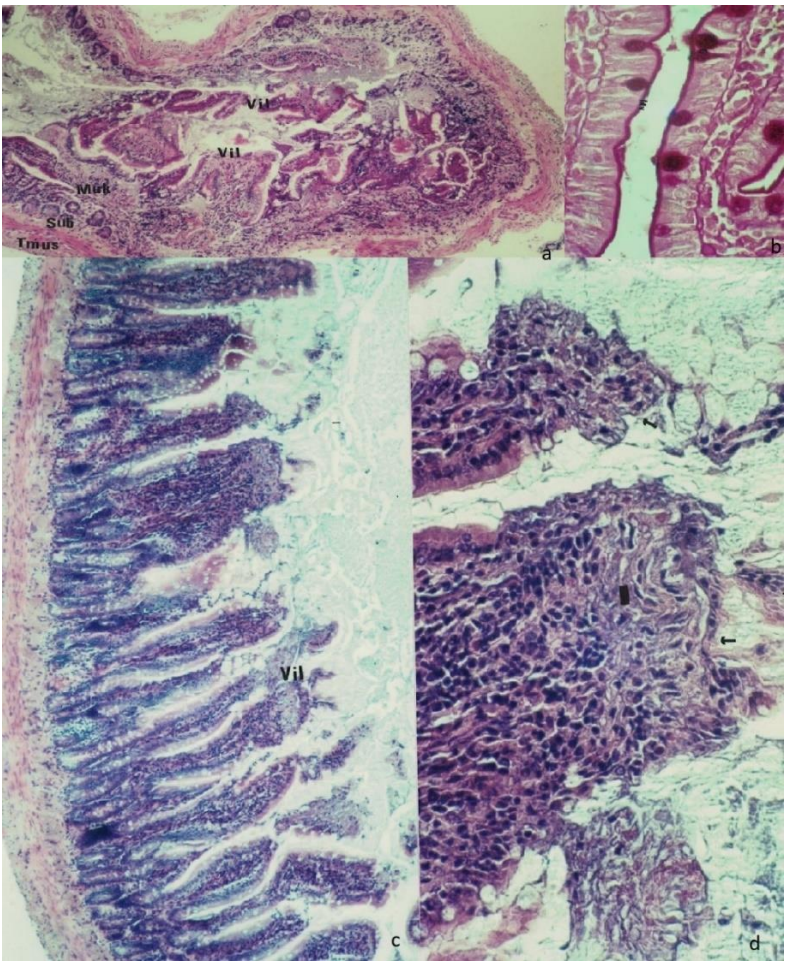

Figure 6: a) All affected layers of the jejunum 1 month after total gastrectomy, Hematoxylin-eosin, X3,2, b) Microvillus (Mv) reacting PAS positive and goblet cells with decreased secretion, PAS, X100, c), d) Mucosa (Muk), submucosa (Sub), tunica muscularis (Tmus), epithelial erosion (←) in atrophied villi (Vil) and increased connective tissue fibers (▬) in the apices of adjacent villi that fuse in places, and debris cells and tissues in the intestinal lumen with intense mononuclear cell increase in the villus centers (*), Hematoxylin-eosin, X10, X40

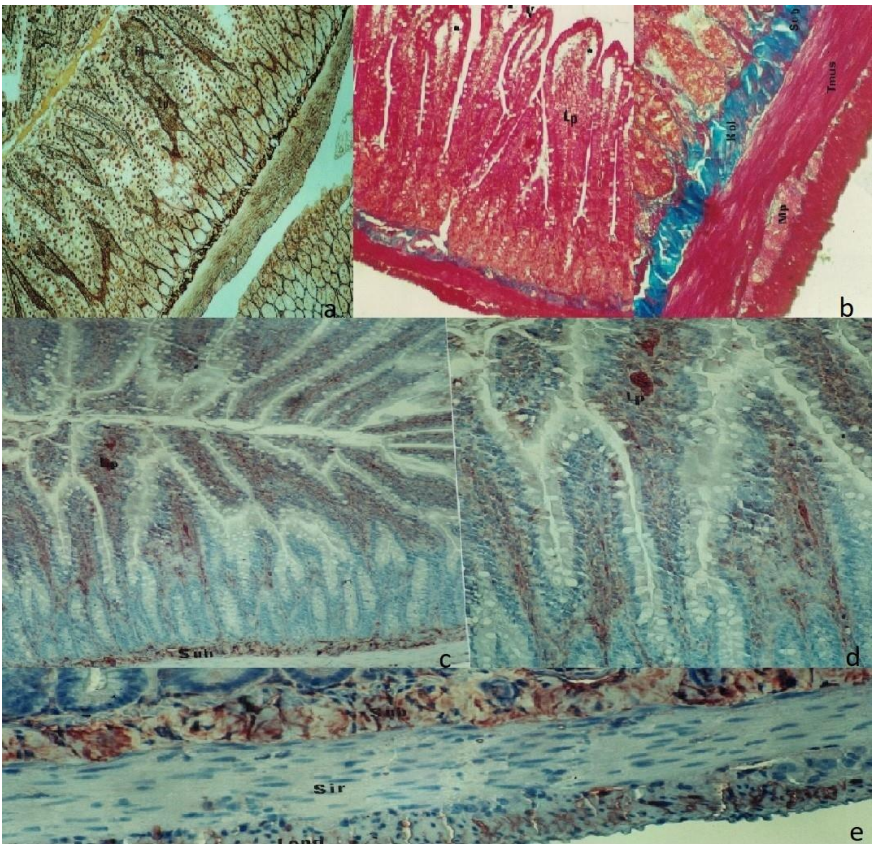

Figure 7: a) Intense lymphocyte infiltration in the lamina propria with increased reticular fiber (Rl) in the lamina propria (Lp) and submucosa 1 month after total gastrectomy (*),James Technique, X10, b) Edematous areas in the lamina propria (Lp) in the apical region of the villi (■), dense collagen fiber bundles (Kol) in submucosa (Sub) and Auerbach's myenteric plexus (Mp) in tunica muscularis externa (Tmu), Mallory-azan, X10, X40, c-e) Intense CEA immunolocalization in all layers. CEA, 10X, 20X, 40X

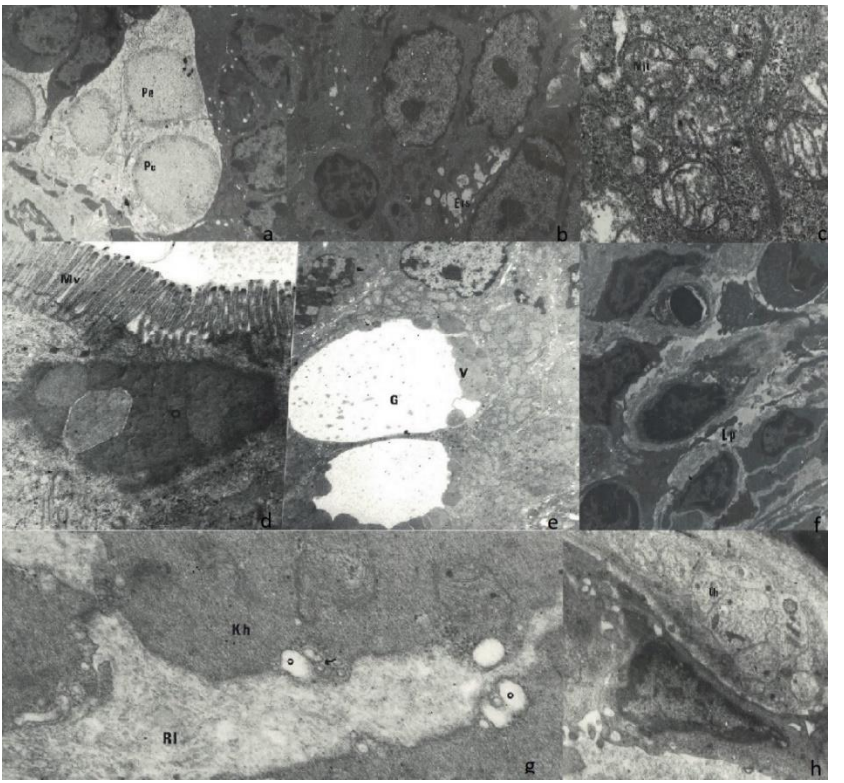

Figure 8: a) Polychromatophilic erythroblast (Pe) cells among epithelial cells (E) 1 month after total gastrectomy, X3000, b) Expansion of endoplasmic reticulum cisternae (Ers) in epithelial cells, X3000, c) Mitochondria (Mit) with disrupted cristae structure and dense glycogen accumulation, X20000, d) Glycocalyx structure observed in the form of accumulations at the apex of microvilli (Mv) and concentrated secretory accumulations in the cytoplasm of enterocytes (o), X12000, e) Basally located, small amount of secretory vacuoles in goblet cells (V), X3000, f) Numerous connective tissue cells and fibers in the lamina propria (Lp), X3000 g) Dense accumulation of reticular fibers (RI) between cells with pinocytotic vesicles (←) and terminal sacs (o) in hypertrophic muscle cells (Kh), X20000, h) Ganglion cells (Gh) in Auerbach's myenteric plexus, X12000

Discussion

The stomach is an organ that stores food, performs digestion and produces hormones and secretions necessary for the body. The stomach secretes mucus, pepsinogen, hydrochloric acid, intrinsic factor and gastrin. The stomach, which undertakes these important functions, has a high rate of cancer involvement and mortality. The only treatment method for malignant tumors of the stomach is surgical intervention. Surgery is performed as partial or total gastrectomy. Depending on the location of the tumor, methods such as Billroth I, Billroth II, Roux-en-Y, esophagoduodenostomy and esophagojejunostomy are used (7-10). Esophagojejunostomy may have complications such as bile reflux, gastritis, afferent loop and marginal ulcer; therefore, gastroduodenostomy is mostly recommended. In our previous studies on esophagoduodenostomy, the anastomosis site, pancreas, liver and ileum sites were evaluated histopathologically (7-70). In our study in the jejunum region, shortening and thickening of the lengths of the vilus, fusion of the vilus with each other, intense epithelial erosion on the apical surfaces and loss of microvillus undoubtedly affected absorption in a bad way. The decrease in the number of membranous vesicles in absorptive cells in the experimental groups supports this idea. It is known that in gastrectomized mice and rats, pathologic changes in the pancreas and disturbances in the cytumulation of insulin secretion and suppression of glucagon secretion (7-11).

Glycogen accumulation in the cytoplasm of absorptive cells may be explained by this mechanism. The changes observed in the cristae structure of the mitochondria of epithelial cells may have been caused by postoperative pancreatic dysfunction. As is known, mitochondria are a vital organelle in many cellular processes such as energy conversion, calcium homeostasis, and reactive oxygen species (ROS) production. Alterations in mitochondria cristae structure may be associated with cancer, diabetes, and many neurodegenerative diseases (12). During gastrectomy, gastric mucosa blood flow and mucin secretion decrease as a result of devascularization and denervation of the gastric mucosa (13). In our

study, the decrease in the amount of vacuoles containing mucin in goblet cells in the jejunum region was determined by light and electron microscope examinations and this may have developed due to regional devascularization and denervation during gastrectomy.

CEA is a determinant antigen for adenocarcinomas in the digestive system (14). In our study, CEA immunolocalization was strongly positive in the lamina propria, submucosa and tunica muscularis layers, especially on the 30th postoperative day. Intense lymphocyte infiltration, plasma and eosinophil cells in the lamina propria and submucosa layers on the 15th and 30th postoperative days may be related to the great surgical stress. The large number of polychromatophilic erythroblast cells detected among the absorptive cells in our electron microscopic examinations that supports the development of pernicious anemia after total gastrectomy due to the absence of intrinsic factor that would provide B12 absorption (15). Hypertrophy in the tunica muscularis externa layer with increased myofilament and enlargement of pinocytotic vesicles, calcium deficiency with postoperative malnutrition, irregular bowel movements caused by rapid food passage into the digestive tract with the disappearance of the pyloric sphincter and denervation may have been effective. No pathologic finding was found in Auerbach's myenteric plexus structure in our electron microscopic examinations. In fact, B12 deficiency is observed after total gastrectomy and is known to cause neurodegeneration in both central and peripheral systems. The lack of pathologic findings in ganglion cells due to B12 deficiency is due to the early postoperative period.

In conclusion, it is important to determine the early morphologic changes that may occur in the digestive tract after total gastrectomy in order to develop an effective treatment and nutrition system. In this way, postoperative patient loss can be prevented. For this, histopathologic evaluation of the entire digestive tract is necessary. In our previous studies, we have examined the esophageal and duodenal anastomosis region, pancreas, liver and ileum histopathologically, but the colon and anal canal regions should also be studied.

Disclosure statement

The authors declare no conflict of interest.

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CHAPTER IV

Investigating the Need for Emergency Haemodialysis and Causes of Progression in Patients with Compensated Chronic Kidney Disease

Mine KARADENİZ[1]

Introduction

Chronic kidney disease (CKD) is a serious public health problem with increasing incidence and prevalence worldwide. Since the early stage of CKD is usually asymptomatic, it is difficult to estimate the true incidence and prevalence in the community. According to the Chronic Kidney Disease Prevalence Study of Turkey (CREDIT) conducted in our country, the prevalence of CKD in our population was 15.7% and the rate of those with GFR < 60

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ml/min/ 1.73m² was 5.1%, which means that one in every 6 adults has CKD and one in every 20 adults has critical CKD. Chronic kidney disease is a general term used for diseases that last 3 months or longer, affect the structure and function of the kidney, and lead to chronic inflammatory and degenerative changes in the renal parenchyma (1). Structural impairment is demonstrated by imaging-proven renal disease or tissue diagnosis, whereas functional impairment is demonstrated by blood and urine parameters indicating renal dysfunction. A glomerular filtration rate (GFR) of less than 60 ml/min/1.73 m² for more than 3 months without renal damage is accepted as a second definition of CKD. In CKD, GFR may be normal or decreased. In the staging of chronic kidney disease, serum creatinine level alone is insufficient to determine decreased renal function (2).

End-stage renal disease (ESRD) is a clinical condition characterised by progressive and irreversible loss of renal function and requires renal replacement therapies (RRT) in order to survive **(**3). The most common causes of ESRD are diabetes mellitus (DM), hypertension (HT), chronic glomerulonephritis, chronic interstitial nephritis, hereditary/congenital diseases and malignancies (4**)**. Common complications of chronic kidney disease include hypertension, anaemia, dyslipidemia, disorders in bone and mineral metabolism, metabolic acidosis, malnutrition, hyperkalemia and endocrine abnormalities, especially secondary hyperparathyroidism. Peripheral neuropathy, intestinal bleeding and bleeding disorders are some of the other complications affecting other systems (5).

The multi-disciplinary approach to CKD includes specific treatment depending on the aetiology of kidney disease, treatment of comorbid diseases, treatments to prevent disease progression and kidney damage, adjustment of drug doses according to GFR, treatment of complications and preparation of patients for renal replacement therapy (6).

Emergency haemodialysis (HD) treatment is defined as haemodialysis usually administered with a temporary catheter as a

result of acute kidney injury or progression of compensated CKD. Indications include hyperkalemia that is resistant or depresses cardiac functions, anuria, volume overload, development of uremic complications, and severe metabolic acidosis (7).

The factors that may influence progression in chronic kidney disease can be categorised as non-modifiable and modifiable factors. Non-modifiable factors include age, genetics, gender and race, while factors include uncontrolled diabetes and hypertension, proteinuria, obesity, smoking, low body mass index, hyperuricemia, infections and dyslipidemia. The conditions thought to reduce progression of the kidney injury include weight control, quitting tobacco and its derivatives, restriction of protein intake in the diet, glycemic control, control of hypertension, preferably the use of drugs that block the renin-aldosterone-angiotensin system, maintenance of high bicarbonate levels, correction of anaemia and metabolic acidosis and lipid-lowering treatments (8-9). However, no consensus has been reached about the net effects of these factors (6).

Our aim in this study is to determine the causes of progression and emergency dialysis requirement in patients with compensated renal failure, to contribute to the literature and to share with you.

Patients and methods

Sixty patients aged 18 years and older, diagnosed with chronic kidney disease or diagnosed after admission to our hospital, and undergoing emergency haemodialysis were included in this study. Acute kidney injury and acute exacerbations of the patients in the scheduled-haemodialysis program were excluded. Demographic characteristics, comorbid conditions, treatment protocols and laboratory results required for the study were collected through history-taking, hospital records and ministry of health registration system.

K-DOQI guideline for staging and MDRD formula was used for GFR calculation All patients were continuously monitored during dialysis. Blood pressure measurements were performed at 10

min intervals and a blood pressure below systolic 90 mm Hg and/or diastolic 60 mm Hg was recorded as a hypotensive episode, and systolic 180 mm Hg and/or diastolic 110 mm Hg as a hypertensive episode. Blood glucose was measured at 30 min intervals and hyperglycaemic and hypoglycaemic attacks were considered if the measurement was above 200 g/dl and below 70 g/dl, respectively. In blood biochemistry, a uric acid level of 7 mg/dl and above is defined as hyperuricemia and a phosphorus level of 4.5 mg/dl and above as hyperphosphatemia. A blood pH below 7.35 and/or HCO_3 below 22 mEq/l were considered as metabolic acidosis and a potassium level of 4.5 and above is considered hyperkalaemia in arterial blood gas evaluation. 24-hour urine volume below 100 ml/day indicates anuria.

Data were analysed using SPSS (Statistical Package for The Social Sciences, Chicago, IL, USA) for Windows. Descriptive statistics are presented as number of cases and percentage (%). In comparisons between independent groups, Chi-square and Likelihood ratio tests were used for categorical data, and statistically significant correlation between groups was investigated by calculating the Eta (η) coefficient. A value of $p < 0.05$ was considered statistically significant in all statistical analyses.

The informed consent obtained from all the participants on a voluntary basis. Ethical approval obtained from the hospital ethics committee.

This research article was based on a thesis.

Results

The study included 60 patients who required emergency haemodialysis due to progression of chronic kidney disease. 10% of the patients were under 40 years of age, 60 % were over 65 years of age. 38.3% of the patients were female and 61.7% were male. In the study group, 54 patients had at least 1 or more comorbidities. The most common comorbidity was HT (n=35, 58.3%). DM and cardiac pathologies were present as comorbidities in 23 and 13 patients, respectively. Six patients had malignancy and 6 patients had renal

pathology. Demographic characteristics and comorbid conditions of the study group are shown in table 1.

Only 24 (40%) of all patients were followed up by a specialist physician for renal disease. Of these 24 patients, RRT was recommended to 7 patients but only 1 of them accepted this recommendation. Of the remaining 6 patients, 5 stated that they felt well and dialysis would decrease their quality of life. One patient completely refused to start dialysis. Of the 17 patients for whom RRT was not recommended, 11 patients received medical support (antihypertensive agents, anti-acidosis, anti-potassium, phosphate binders, oral iron, active vitamin D preparations) and for 6 patients specialists decided to continue follow-up. Of the 36 patients who were not followed up by a nephrologist, 6 had previously been advised by another physician that they had renal disease and should go to the nephrology department. In this patient group, 4 patients did not pay attention to this recommendation as they did not have any complaints, 1 patient did not accept this diagnosis, and the last patient presented with acute dialysis needs before the appointment date. In the patient group, it was determined that 6 patients in the last 1 month and 23 patients in the last 1 to 6 months (1-3 months 12 patients, 3-6 months 11 patients) were admitted to the health institution, while 24 patients had no examination and hospital admission in the last 1 year.

The blood urea level at the time of HD was between 101-200 in 55% and above 200 in 51.7%. Creatinine level was between 5-10 in 68.3% and above 10 in 18.3%. Haemoglobin (Hb) level was below 7 in 13.3% of the study patients, and higher than 11 in 20%. The 24-hour urine protein level was between 300-2999 mg in 53.3% of all cases and 3000 mg or more in 26.7%. The albumin level was below 2.5 in 8.3% of the cases, between 2.5- 4.0 in 75%. The pre-dialysis HCO_3 level of 20% of the patients was below 10. Hyperuricemia was noted in 38.3% and hyperphosphatemia in 65% of the patients. HbA1c was above 6.5 in 13 patients without a diagnosis of diabetes mellitus at the time of presentation to the emergency department.

Some important blood parameters before HD are summarised in table 2.

Urinary tract infections were the most common cause of progression (n=30, 50%) and E.coli was the most responsible microorganism (n=14). Other main causes were pneumonia, contrast exposure and NSAID use. The causes of progression of kidney disease are presented in table 3.

Table 1. Demographic characteristics and comorbid conditions of the study group

variables	***n, (%)***
Age	
40>	6, (10.0)
40-65	18, (30.0)
65<	*36, (60.0)*
Age	
40>	6, (10.0)
40-65	18, (30.0)
65<	36, (60.0)
Gender	
Female	23, (38.3)
Male	37, (61.7)
Comorbidity	
HT	35, (58.3)
DM	23, (38.3)
Cardiac problems	13, (25.0)
COPD	3, (5.0)
Renal pathology	6, (10.0)
Malignancy	6, (10.0)
Alzheimer	2, (3.3)
Other	2, (3.3)
No	6, (10.0)

Abbreviations: COPD: chronic obstructive pulmonary disease, DM: diabetes mellitus, HT: Hypertension

Table 2. Some blood and urine parameters evaluated in patients before emergency haemodialysis

variables	n, (%)
urea (18-50 mg/dl)	
50-100	2, (3.3)
100-200	27, (45.0)
200<	31, (51.7)
creatinine (0.7-1.2 mg/dl)	
<5	8, (13.3)
5-10	41, (68.3)
10<	11, (18.3)
albumine (3.5-5.2 g/dl)	
2.5>	5, (8.3)
2.5-4	45, (75)
4<	10, (16.7)
Hb (11.6-18 g/dl)	
7>	8, (13.3)
7-9	15, (25.0)
9-11	25, (41.7)
11<	12, (20.0)
24-hour urine protein (0-150 mg/day)	
anuria	4, (6.7)
150 >	2, (3.3)
150-300	5, (8.3)
300-3000	32, (53.3)
3000 <	16, (26.7)

Abbreviations: Hb: haemoglobin

Table 3. Identifiable aetiology of progression of kidney disease in patients.

Identifiable aetiology of progression	n, (%)
urinary tract infections	30, (50.0)
pneumonia	5, 88.3)
light chain diseases	3, (5.0)
contrast and NSAID	5, (8.3)
glomerulonephritis	3, (5.0)
septicemia	5, (8.3)
TTP	1, (1.7)
progression of metastatic cancer	1, (1.7)
other (CHF, DKA)	2, (3.3)
total	60, (100)

Abbreviations: CHF: congestive heart failure, DKA: diabetic ketoacidosis, NSAID: non-steroidal anti-inflammatory drugs, TTP: thrombotic thrombocytopenic purpura

The most common indication for emergency haemodialysis was hypervolemia (n=25, 41.7%) and the second was metabolic acidosis (n=21, 35%). All patients had to undergo haemodialysis with a temporary central venous catheter, but no complication or infection was observed in the acute phase. HD-related acute complications were observed in 11 patients (18.3%). Among these, hypoglycemic and hypotensive attacks were the most detected. Indications for emergency haemodialysis and HD-related acute complications are shown in table 4 and table 5.

Table 4. Emergency haemodialysis indications and acute complications

Emergency haemodialysis indications	**n, (%)**
hypervolemia	25, (41.7)
metabolic acidosis	21, (35.0)
anuria	9, (15.0)
hyperkalemia	3, (5.0)
uremic encephalopathy	2, (3.3)

Table 5. Acute complications of emergency haemodialysis

HD-related acute complications	**n, (%)**
no complication	49, (81.7)
hypoglycemic attack	4, (6.6)
hypotensive attack	4, (6.7)
hyperglycemia	2, (3.3)
hypertensive episode	1, (1.7)

Discussion

Chronic kidney disease often presents at an advanced stage or with the need for acute dialysis because of its asymptomatic course for a long time or because most patients have vague symptoms. CKD, with mortality rates reaching 20%, is predicted to be the 5th

most common cause of death in 2040 (10-11). The prevalence of CKD varies between 11 and 13%; but real prevalence is not known because many patients are asymptomatic (12-13). According to the CREDIT study, the prevalence of CKD in Türkiye is about 15% (14). There are studies showing that it is more common in females, as well as contrary articles (13,15-16). In our study, approximately 2/3 of the patients were male.

In an article, HT was the most common comorbidity in the study group (51.9%) and diabetes was observed in the second frequency (34%) (17). The most common comorbidity in another study consisting of 9 men and 6 women was DM (53%) and the mean age was 51 years (18). Many studies declared that HT, diabetes and obesity are risk factors for CKD and aggravate GFR decline (19-21). In this study, data supporting the literature were obtained and HT was the most common comorbidity and DM was the second most common comorbidity. 60% of our patients were 65 years of age or older.

There is no consensus or guideline data on when patients with chronic kidney disease should be referred to a nephrologist. But the general approach can be as follows; if there is a problem in the diagnosis, blood pressure cannot be controlled, signs of fluid overload, anaemia resistant to iron therapy, GFR is between 30-45 ml/min and the annual GFR decrease is more than 5 ml/min, nephrology department follow-up should be recommended (22). In a cohort, it was stated that 36% of patients with end-stage renal failure were never evaluated by a nephrologist before starting haemodialysis, and it was emphasised that complications, mortality rate were higher and hospital stay was longer in these patients (23-27). In our study, the rate of patients without follow-up by a nephrologist was 60%, which was considerably higher than in the literature. In another study, patients who were followed up for less than 6 months had a higher need for urgent haemodialysis (28-29). It was determined that 82% of the patients who received emergency haemodialysis (41%) had chronic kidney disease, most of these patients had no haemodialysis preparation and their mortality rate

was 23%. As an explanation for this situation, late referral to the specialist physician is shown (17). In a cohort of 15 patients consisting of 9 men and 6 women who underwent emergency haemodialysis, all of the patients were found to have unawareness of kidney disease, 5-fold increase in 1 year and 14-fold increase in 5 years mortality, worse morbidity and higher carecost (18, 29-32). Similar studies reporting the unawareness status of patients are also available in the literature (33-35). Preparation of patients for haemodialysis program contributes to the prevention of hemodialysis-related complications, mortality, sepsis and bleeding, and improvement of quality of life (23, 29-31, 36-37). Also, in the literature, some studies stated that HD patients have poor self-management and this is related to patient outcomes (38-40). It was also observed in our study that the majority of patients who were recommended HD agreed with this idea and they refused HD. The most important result of this study is that the majority of patients with renal failure are not aware of their disease, have problems accepting the situation, could not access the necessary treatment stages at the appropriate time, experience delays in referral to a specialist physician, and even lack expert opinion and follow-up.

Anaemia in chronic kidney disease is associated with poor outcomes and increased mortality (41-43). Correction of anaemia provides an increase in quality of life (44). In another literature data, it was emphasised that albumin level and anaemia had no effect on mortality (28-29). There are also studies emphasising that lower haemoglobin and higher creatinine levels are detected in patients undergoing emergency hemodialysis (45-48) and lower prealbumin levels have been associated with a lower survival rate (47, 49-50). In our study, findings supporting the literature data were also obtained. Haemoglobin level was below 11g/ dl in 80 % of our patients. Creatinine and urea levels were also considerably higher compared to patients who started dialysis pon a scheduled basis, when we evaluated them with clinical experience. Urea level was above 200 mg/ dl in more than half of our patients. Creatinine level was above

5 mg/ dl in about 80 %. Only 10 patients had albumin levels above 4 mg/ dl.

Another issue is metabolic acidosis. It accelerates progression and (51-52) controlling metabolic acidosis in end-stage renal disease may contribute to slowing the progression process (53). Some of the patients in our study were on anti-acidosis therapy. In 20% of the patients, the HCO_3 level in the pre-dialysis blood gas evaluation was at a level indicating deep metabolic acidosis (below 10 mEq/ l). In the literature, in a book, it is mentioned that the degree of proteinuria correlates with disease progression (54). In our study, laboratory significant proteinuria was observed in 90% of patients (n=54). Another important point is that 26.7% (n=16) of these patients had proteinuria of 3 g/day or more.

There are also studies evaluating the predictability of GFR decline and annual GFR change with functional magnetic resonance imaging are ongoing (55). The results of these studies may provide data that can contribute to faster detection of asymptomatic and high-risk cases and enable precautions to be taken in a timely manner for the patients.

This study has some limitations. It is a single-centre, cross-sectional and retrospective study with a small number of patients. Morbidity and mortality data could not be presented because long-term follow-up of the patients could not be performed. The acute process and its effects could be evaluated. Habitual behaviour and dietary information that may be effective on the progression of patients could not be obtained in short-term follow-up.

Conclusion

Chronic renal failure is not only an important health problem for the whole world due to its morbidity and mortality, but also causes a serious decrease in the quality of life of patients and high financial loss in all countries. The need for emergency haemodialysis and the stage of its administration increase both the mortality, morbidity and complication rates and so that the financial burden

many times over. Increasing the awareness of the community and primary health care personnel in terms of kidney disease and the precautions that can be taken at an early stage, monitoring these patients with regular controls by a specialist and timely dialysis preparation is the most important factor that can contribute to a significant decrease in emergency dialysis indications, a decrease in morbidity, mortality and complications.

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CHAPTER V

Evaluation of Renal Function of Diabetic Patients with Neutrophil Gelatinase Associated Lipocalin (NGAL)

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Introduction:

Today, the prevalence of diabetes is increasing worldwide. According to World Health Organisation data, in 2003 there were at least 191 million people with diabetes worldwide (2.8% of the total

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world population) and it is estimated that approximately 330 million people will have diabetes in 2030. The incidence of diabetes inevitably increases along with the incidence of its complications. Diabetic nephropathy is the leading cause of end-stage renal failure worldwide. According to the National Registry System of the Turkish Society of Nephrology, while 4.7% of patients undergoing haemodialysis in 1991 had renal failure due to diabetes, this rate increased to 16.5% in 1999 and 35% in 2009.

Glomerular filtration rate (GFR) measurement is important in diabetes mellitus, which is a risk factor for chronic kidney disease and cardiovascular disease. Currently, a reliable, precise, accurate and easily applicable GFR measurement method has not yet been established. Inulin clearance, which is the gold standard GFR measurement method, is a time-consuming, impractical method that requires 10-12 blood samples from the patient during the test and is only used for research purposes(1,2). GFR measurements performed with radionuclear substances (diethylene triamine penta-acetic acid, ethylene triamine tetra-acetic acid, iodothalamate) given exogenously with a single injection are generally used for research purposes due to the limited number of centres, the long duration of the procedure and the fact that radiopharmaceuticals are not always available(3,4). Currently, the most commonly used marker is serum creatinine level measurement, but this examination alone is not sufficient to determine the early stages of renal failure. It has been reported that the sensitivity of serum cystatin-C level measurement, which has been on the agenda in recent years, is less than creatinine clearance(5,6,7). Chronic kidney disease guidelines recommend the use of creatinine-based formulae (such as Cockgroft-Gault, MDRD) in the determination of GFR. However, it has been reported that the results obtained from these formulae are also affected by changes in body content.

Neutrophil Gelatinase Associated Lipocalin (NGAL) is a 25kDa molecular weight protein originating from neutrophils and renal tubulus cells and covalently bound to neutrophil gelatinase, which gives it its name. It is expressed in very low amounts in

various tissues (lung, stomach and colon). NGAL expression is markedly induced in epithelial damage (8,9). It has been shown that NGAL levels in blood and urine are increased approximately 100-1000-fold in the early period of toxic and ischaemic acute kidney injury in humans, and there are studies suggesting its use as an indicator of acute kidney injury(9,10,11,12). There are limited publications on how plasma and urine NGAL levels show a profile in diabetic patients and chronic kidney disease. In addition, reference GFR methods (such as inulin, radionuclear methods) were not used in these studies to compare with NGAL and the number of patients was limited(10,12-16).

Objective:

The aim of this study was to investigate the place of serum and urine NGAL levels, which have been used in the early diagnosis of acute kidney injury in recent years, in the determination of renal dysfunction in diabetic patients and their relationship with endogenous creatinine clearance, estimated GFR (eGFR) (calculated by MDRD-4, MDRD-7, Cockcroft-Gault formulae), serum cystatin-C and GFR determined by double plasma sampling ^{99m} Tc-DTPA. In this study, diabetic patients who have not yet developed urea-creatinine retention were investigated in terms of the use of serum and urinary NGAL levels as GFR markers and these results were compared with healthy subjects.

Materials and Methods:

A total of 228 people, including 186 patients with serum creatinine values within normal limits, proteinuric or non-proteinuric, diagnosed with diabetes mellitus according to WHO criteria, 186 patients selected according to exclusion criteria and 42 age and gender matched controls, who applied to the Internal Medicine, Endocrinology and Nephrology outpatient clinics of Gülhane Haydarpaşa Training and Research Hospital (now Sultan

Abdülhamithan II EAH) between December 2008 and December 2010, were included in the study. In the study, serum and urine NGAL levels, endogenous creatinine clearance, estimated GFR (eGFR) (calculated by MDRD-4, MDRD-7, Cockcroft-Gault formulae), serum cystatin-C and its relation with GFR determined by double plasma sampling 99mTc-DTPA were analysed. The study was designed as a cross-sectional, controlled, prospective clinical study. Serum and urine NGAL levels were determined by ELISA method. 24.11.2008 Ethics Committee approval was obtained.

Exclusion criteria:

1. Patients with serum creatinine levels above normal,
2. Those with abnormal thyroid hormones,
3. Those who use nephrotoxic drugs (such as NSAIDs, contrast agents, aminoglycosides, diuretics, cyclosporine, corticosteroids) within 1 week before the examination,
4. Acute infection, those with signs of inflammation, (fever, leucocytosis, elevated CRP, pyuria, pyodermia, respiratory tract infection, gastroenteritis, arthritis, etc.)
5. Those with known malignancy,
6. Patients with pyuria, bacteriuria or urine culture growth in complete urine examination were excluded from the study.

Evaluation of selected patients:

The following procedures were performed in type 1 and 2 diabetic patients who were excluded from the above exclusion criteria and in the control group:

1. Identity information (name-surname, age, automation number, address and telephone number) was received,
2. Drugs used were recorded,
3. Medical history (coronary artery bypass graft operation, peripheral artery disease, chronic kidney disease, hypertension, ischaemic heart disease detected by coronary angiography, history of cerebro vascular accidents) was recorded,
4. Anthropometric measurements and physical examination were performed (height, weight, body mass index, body surface area,

blood pressure, pulse rate, fever were measured, general systemic examination was performed).

5. Haemogram, AKC, urea, creatinine, albumin, T.cholesterol, triglyceride, LDL cholesterol, HDL cholesterol, CRP, sT4, TSH, HbA1c, complete urine test were performed,

6.24-hour urine creatinine clearance, proteinuria, microalbuminuria were measured,

7. e-GFRH was calculated with MDRD-4, MDRD-7, Cockcroft-Gault formulae,

8. Samples were taken for serum cystatin-C measurement and NGAL measurement in serum and urine. Urine samples were centrifuged at 3000 rpm for 8 min and serum samples were centrifuged at 5000 rpm for 5 min and then taken into epandorf with the help of micropipette and stored in -80 degree cooler until the test day.

9. GFR was measured with ^{99m} Tc-DTPA with double plasma sampling.

(***Estimated GFR was measured in the control group).***

Statistical Evaluation:

For statistical analysis, SPSS 15.0 programme compatible with Microsoft Windows 2003 was used and the data were prepared in Microsoft Office Excell and transferred to SPSS programme. Descriptive statistics for numerical variables were presented as mean, standard deviation, minimum, maximum. Spearman correlation coefficient, co-interaction regression analysis, and risk logistic regression analysis were used to test the interaction in variables that did not fit the normal distribution. Statistical significance level was accepted as a "p" value less than 0.05. Roc analysis was performed to determine the sensitivity and sensitivity levels.

Findings:

The mean age of the study and control groups was 56.4 years, the mean age of the patient group was 59.4±13.81 years, and the

mean age of the control group was 52.52±15.77 years. No statistically significant difference was found between the mean age, gender status, and body surface area of the subjects according to the groups ($p>0.05$). Out of 166 patients with 24-hour urine microalbumin levels, 131 were normalbuminuric, 33 were microalbuminuric and 2 were macroalbuminuric. Of 185 patients with 24-hour urine proteinuria, one had nephrotic and one had nephritic proteinuria. When 24-hour urine proteinuria levels were compared with serum and urine NGAL levels, no correlation was detected, whereas urine NGAL showed weak positive correlation when compared with albuminuria levels ($p>0.05$). According to albumin excretion level, patients were divided into 3 groups as normal albuminuria, microalbuminuria and macroalbuminuria. When each group was compared with serum and urine NGAL levels, a weak correlation was found between urine NGAL level and albuminuria level in the normalbuminuric group ($p<0.027$), while a moderate positive correlation was found between serum and urine NGAL level and albuminuria level in the microalbuminuric group ($r=0.340$ $p<0.018$, $r=0.388$ $p<0.009$)(Table 1.1). Since there were 2 patients in the macroalbuminuric group, correlation analysis could not be performed. When the mean serum and urinary NGAL levels of normoalbuminuric and microalbuminuric patients were compared, both urinary NGAL and serum NGAL levels showed a statistically significant difference ($p<0.02$)(Table 1.1) When the patient and control groups were compared in terms of fasting blood glucose, haemoglobin, haematocrit, serum urea, serum creatinine, HbA1c level, GFR measured by endogenous creatinine clearance, MDRD-4, MDRD-7, eGFR measured by Cockcroft Gault method, serum Cystatin-C level, serum-urine NGAL level, albuminuria levels, a statistically significant difference was found for each parameter ($p<0,005$). When serum NGAL levels were compared with GFR, eGFR (MDRD-4, MDRD-7, Cockcroft-Gault, Tc99m-DTPA) results measured by endogenous creatinine clearance in both control and patient groups, no correlation was detected ($p>0.05$), but when compared with serum Cystatin-C levels, correlation was

detected in the patient and control groups (r=0.35, p<0.0001). When urinary NGAL levels were compared with eGFR (MDRD-4, MDRD-7, Cockcroft-Gault, DTPA) results in both control and patient groups, negative correlation was detected. In the patient group, no correlation was detected when compared with GFR and serum NGAL levels determined by 99mTc-DTPA (p>0.05), while a weak correlation was detected with urinary NGAL level (r=-0.17, p<0.04). GFR was not measured with 99mTc-DTPA in the control group. In both patient and control groups, no correlation was detected when GFR measured by endogenous creatinine clearance was compared with urine-serum NGAL levels (p>0.05). In the patient and control groups, no correlation was detected when eGFR measured by MDRD-4 was compared with serum NGAL levels, while a negative correlation was observed with urinary NGAL levels (r=-0.212 p<0.05). In the GFR subgroup analysis, when urine and serum NGAL levels and GFR results measured by various methods were compared with the GFR results of those with a GFR above 90ml/min in the patient and control groups, urine NGAL levels and Cockcroft-Gault eGFR showed correlation in both groups (r=-0,189, p<0,046, r=-0,435, p<0,04), while no correlation was detected with other methods (p>0,05) (Table 1.2). In the age subgroup analysis, correlation was detected between serum NGAL and serum cystatin-C levels in both the over 65 and under 65 age groups (r=0.377 p<0.000*, r=0.306 p<0.009). When the tests used in GFR measurement were compared with each other, the highest correlation was found in MDRD-4 formula in both patient and control groups. In the subgroup analysis according to the degree of albuminuria, when the tests used in the measurement of GFR were compared with each other in normoalbuminuric and microalbuminuric groups, the highest correlation was found in the MDRD-4 formula in the patient and control groups. MDRD-4 was accepted as the gold standard test for serum and urine NGAL sensitivity and specificity tests (Table 1.3). In the subgroup analysis of the tests used in the measurement of GFR, when those with creatinine clearance greater than 120 ml/min were compared with each other, the highest correlation was

found in the MDRD-4 formula in both the patient and control groups. In the subgroup analysis of the tests used in the measurement of GFR, the highest correlation was found in the MDRD-4 formula in both patient and control groups when those with creatinine clearance between 90-119.9 ml/min were compared with each other. In the subgroup analysis of the tests used in the measurement of GFR, the highest correlation was found in the MDRD-4 formula in the patient group when those with creatinine clearance between 60-89.9 ml/min were compared with each other. Correlation could not be analysed in the control group because of insufficient number of patients. In the subgroup analysis performed with patients with creatinine clearance between 45-59.9 ml/min and 30-44.9 ml/min, correlation analysis could not be performed due to insufficient number of patients. When urine and serum NGAL were compared with GFR measured in subgroup analysis according to MDRD-4 in the patient group, urine NGAL showed negative correlation with MDRD-4 in all groups but not with creatinine clearance and DTPA, serum NGAL showed strong negative correlation with DTPA above GFR 120 ml/min, while MDRD-4 showed negative correlation with GFR 45-60 ml/min. According to the assumption that MDRD-4, endogenous creatinine clearance, DTPA and GFR measurement methods were accepted as gold standard, NGAL sensitivity, selectivity and predictivity results in various GFR values were as shown in Table 1.4. When GFR measurement with MDRD-4 was accepted as the gold standard, urine NGAL showed 95.4% sensitivity and 55% specificity in the range of 60-89.9 ml/min, while serum NGAL showed 99.4% sensitivity and 64% specificity in the range of 45-59.9 ml/min. When GFR measurement with MDRD-4 was accepted as the gold standard, urine NGAL sensitivity increased in the microalbuminuric group as GFR decreased, while serum NGAL sensitivity decreased according to albuminuria subgroup analysis. When albuminuria was accepted as the gold standard and microalbuminuria limit was taken as the cut-off point, MDRD-4 showed 25% sensitivity and 98% specificity, urine NGAL showed 23% sensitivity and 90.5% specificity, and serum NGAL showed

94% sensitivity and 21% specificity. In GFR subgroup analysis of urinary NGAL according to MDRD-4, urinary NGAL averages increased inversely proportional to GFR decrease in control and patient groups (Table 1.5).

Discussion:

Diabetic nephropathy ranks first among the factors leading to end-stage renal failure all over the world. According to the national registry system of the Turkish Society of Nephrology, while 4.7% of patients undergoing haemodialysis had diabetes-related renal failure in 1991, this rate increased to 35% in 2009 (17). It is known that renal failure is an important cause of morbidity and mortality and early diagnosis can slow down the development or progression of diabetic nephropathy (18-20). The GFR we normally measure is a level below the maximal attainable rate, the value between the maximal attainable GFR and the normal GFR is defined as renal reserve, in other words, it can be defined as the capacity of the kidney to increase GFR as a result of various stimuli. In progressive renal diseases, GFR does not change significantly without depletion of renal reserve (at least 50% loss is required). Current methods used for GFR basically do not assess renal reserve and no ideal marker to assess renal reserve has been found yet.

The definitive diagnosis of diabetic nephropathy can be made by biopsy. However, this is not performed in clinical practice. Microalbuminuria, serum creatinine level, creatinine clearance and various GFR measurement formulae are mostly used (20). In a diabetic patient, an increase in serum creatinine or a decrease in creatinine clearance even if serum creatinine is normal is considered as progression to chronic renal failure. However, it is difficult to say that serum creatinine and clearance are good indicators of GFR. Creatinine being affected by extra-renal factors (muscle mass, rhabdomyolysis, excessive meat intake, heavy exercise, etc.) and tubular secretion are negative factors for these two tests (21,22). Since elevated serum creatinine level occurs after loss of renal reserve, it cannot evaluate renal reserve (serum creatinine

concentration does not change unless GFR falls below 50 ml/min/1.73m²)(22). Incomplete urine collection by patients and some analytical problems in the methods used in creatinine determination can significantly reduce the sensitivity and reproducibility of the creatinine clearance test(53).The diabetic nephropathy model was developed in the 1980s based on the finding of microalbuminuria, which was considered an early marker of progressive renal disease in diabetes. The diagnosis of diabetic nephropathy is made with the detection of >300 mg/day or >200 μg/min albuminuria in at least 2 urinalyses in a period of 3 to 6 months or >300 μg/mg albumin/creatinine ratio in spot urine (20). However, the fact that albumin excretion rate is affected by various factors (standing, exercise, increased diuresis, vprotein diet, ACE-I and NSAIDs, circadian rhythm, urinary infections, heart failure, fever, etc.) and the difficulty of urine collection in elderly and paediatric patients are factors affecting the sensitivity of the test(20). Inulin clearance, which is the gold standard GFR measurement method, is a time-consuming, impractical method that requires 10-12 blood samples from the patient during the test and is used only for research purposes(1,2).GFR measurements performed with exogenously administered radionuclear substances (diethylene triamine penta-acetic acid, ethylene triamine tetra-acetic acid, iodothalamate) are generally used for research purposes because of the limited number of centres, the long duration of the procedure and the fact that radiopharmaceuticals are not always available(3,4).Cystatin-C, which is presented as a new marker for the diagnosis of nephropathy and measurement of GFR, has a constant production rate, is not affected by inflammatory, immunological and neoplastic disorders, has no diurnal rhythm, is easily filtered from glomeruli due to its low molecular weight and basic pH (approximately 9.0), and is catabolised by reabsorption from proximal tubules. Cystatin-C serum levels do not vary with age and sex. Therefore, it has been claimed to be more significant than microalbuminuria in the detection of renal reserve as an early marker of the process underlying progressive diabetic nephropathy in the

initial period of renal dysfunction(23,24). In studies performed with exogenous substances (51Cr-EDTA, 99mTc-DTPA, iohexol, inulin, etc.), which are accepted as gold standard, high correlation was found between Cystatin-C and GFR; therefore, it is in question to be used as a new marker for GFR (25-30).NGAL is a 21 kD member of the lipocalin superfamily. It was first demonstrated in the granules of neutrophils surrounding E. Coli bacteria in culture medium. NGAL also shows a 100-1000 fold increase in tubular damage, independent of its bacterial effect, and is used as an early marker of acute renal failure. The known effects of NGAL on cell proliferation, apoptosis and differentiation have enabled it to be used as a marker of renal function in chronic kidney diseases such as diabetes, in which inflammation and morphological changes in renal tissue continue continuously(31-42).Studies on the use of NGAL in acute kidney injury are concentrated in two areas; the use of NGAL as an early marker of acute kidney injury and its relationship with dynamic GFR changes that develop in the short term (use in GFR prediction).In models of renal ischaemia during major cardiac surgery (32,33), contrast agent-induced nephropathy (34,42), renal ischaemia during and after AMI (43), septic shock-induced renal ischaemia (52), NGAL increases have been associated with acute kidney injury and dynamic GFR changes. When the studies on the use of NGAL in chronic kidney injury are examined, instantaneous and prospective NGAL levels were associated with GFR measured instantaneously or prospectively (in various time periods). When the studies on chronic kidney injury are examined, there is no mention of the period of renal reserve loss (44-51).

In our study, we aimed to investigate the extent to which changes in serum and urine NGAL levels reflect renal reserve and damage in diabetic patients. In a study by Nishida et al. in a population of children with steroid-resistant or sensitive nephrotic syndrome, tubular dysfunction, glomerulonephritis and glomerulonephritis with eGFR <90ml/min, serum and urine NGAL levels of the patients were compared with the control group. It was found that serum and urinary NGAL levels increased as GFR values

decreased and the degree of proteinuria and urinary NGAL levels were positively correlated. It was reported that the increase in urinary NGAL level was more significant than the increase in serum NGAL level. It was concluded that urinary NGAL was a better marker of CKD. In our study, a negative correlation was found between urinary NGAL level and eGFR (MDRD-7 ($r=-0.19$, $p<0.01$), MDRD-4 ($r=-0.21$, $p<0.0036$), Cockcroft-Gault ($r=-0.19$, $p<0.01$), double sampling Tc99m-DTPA ($r=-0.17$, $p<0.04$)) results measured by various methods in the patient group (Table 1.6, 1.7). In addition, similar to the results of Nishida et al., albuminuria level correlated with normoalbuminuric urinary NGAL level ($r=0.254$, $p<0.027$); serum ($r=0.34$, $p<0.018$) and urinary ($r=0.388$, $p<0.009$) NGAL levels of microalbuminuric patients correlated, the increase in urinary NGAL level was greater than the increase in serum NGAL level.

In the GFR subgroup analysis, no correlation was detected between urine and serum NGAL levels and eGFR measurements (DTPA, MDRD-4, MDRD-7, C-G) in the group with GFR above 90ml/min in both patient and control groups ($p>0.05$) (Table 1.3). In a study conducted by Przybylowski et al. with 165 patients with various stages of chronic renal failure who underwent allogeneic heart transplantation, patients were divided into 2 groups as under and over 65 years of age, and the relationship between Cystatin-C, MDRD-4, eGFR and serum NGAL levels was examined, and it was observed that serum NGAL levels correlated with eGFR and Cystatin-C levels measured with MDRD-4 in the group over 65 years of age, and it was stated that it could be used as a renal function marker over 65 years of age (53).

In our study, when the patients were divided into two groups as below and above 65 years of age, correlation was found between serum NGAL level and serum Cystatin-C level in both age groups (below 65 years of age $r=0.377$ $p<0.000$*, above 65 years of age $r=0.306$ $p<0.009$) (Table 1.8)(Table 1.8). Szewczyka et al. investigated the relationship between serum Cystatin-C, serum creatinine and NGAL levels in a study conducted with 71 heart

transplant patients and 7 lung transplant patients. Serum NGAL level was found to be higher in all transplant groups compared with the control group, but no correlation was found with serum creatinine and Cystatin-C levels(54). In our study, no correlation was found between serum creatinine and serum NGAL levels in the control and patient groups ($p>0.05$)(Table 1.6). In addition, a weak correlation was found between urinary NGAL level and serum creatinine level in the patient group ($p<0.04$)(Table 1.7).Bachorzewska et al. In their study with 26 non-diabetic patients with normal urea-creatinine levels who underwent conventional coronary angiography due to coronary heart disease, the correlation between eGFR, serum Cystatin-C, serum and urine creatinine results and serum-urine NGAL levels with MDRD and Cockcroft-Gault formula was investigated by taking serial serum and urine samples(55). He found that serum-urine NGAL levels started to increase at the 2nd hour of angiography, serum and urine creatinine and plasma Cystatin-C values were normal at the 24th hour, while NGAL levels increased and started to decrease at the 48th hour. They stated that the control of urea-creatinine levels only in patients who were kept in the hospital for 48 hours at most, such as patients who underwent coronary angiography, was insufficient for the diagnosis of contrast nephropathy, and NGAL serum and urine levels should be examined in the early period after angiography in risky patients. Since our study was not performed in patients with acute kidney injury and elevated serum creatinine was a screening criterion, it does not provide information about the relationship between dynamic GFR change and NGAL level. In our study, no correlation was found when urine and serum NGAL levels were compared with serum urea-creatinine levels in the patient and control groups ($p>0.05$, Table 1.6-1.7).Bolignano et al.'s study with 56 type 2 diabetes mellitus patients divided into three groups as normoalbuminuric, microalbuminuric and macroalbuminuric, the relationship between albuminuria, proteinuria levels and eGFR, urine-serum NGAL levels was investigated and it was observed that serum NGAL level increased in normoalbuminuric patients. In addition, it was found

that NGAL levels increased in direct proportion to the degree of the disease, with serum NGAL level being more prominent, and urinary NGAL levels showed positive correlation with proteinuria and albuminuria and negative correlation with eGFR and serum albumin level in Pearson correlation test(56). In our study, serum and urine NGAL levels were found to be approximately 3-4 times higher in the normoalbuminuric group compared to the control group (Table 1.1). Albuminuria level was positively correlated with urinary NGAL levels ($r=0.254$, $p<0.027$) in normoalbuminuric patients and with serum ($r=0.34$, $p<0.018$) and urinary ($r=0.388$, $p<0.009$) NGAL levels in microalbuminuric patients (Table 1.1). There was no correlation between 24-hour urine protein level and serum ($p<0.56$) and urine ($p<0.15$) NGAL levels (Table 1.9). In a study conducted by Malyszko et al. with 92 non-diabetic patients with stage 2-4 CKD, the correlation between urine-serum NGAL levels and serum creatinine, MDRD and eGFR, serum Cystatin-C, haemoglobin, haematocrit, leukocyte values were examined(51). Urinary NGAL levels were correlated with haemoglobin, haematocrit, serum creatinine and eGFR. In the subgroup analysis of the study performed in healthy volunteers, serum NGAL levels were positively correlated with age, serum creatinine, serum Cystatin-C levels, leukocyte count and negatively correlated with eGFR (51). In our study, urinary NGAL levels were positively correlated with haemoglobin ($r=0.2$, $p<0.01$), haematocrit ($r=0.21$, $p<0.000$*), serum creatinine ($r=0.15$, $p<0.04$) in the patient group (Table 1.6).In a 4-year prospective study conducted by Stine Elkjaer et al. in 78 type 1 diabetic patients with various stages of diabetic nephropathy, urinary NGAL levels and GFR levels measured by Cr51 were compared at certain intervals. It was found that there was no correlation between GFR and urinary NGAL levels(49). Although our study provides limited information about its use in the follow-up of CKD because it was not prospective in terms of its design, when compared with nuclear medicine methods in terms of instant GFR measurements, a weak negative correlation was found between measurements made with double plasma sampling Tc99m-DTPA

and urinary NGAL level (r=-0.17, p<0.04) in contrast to the measurements made by Stine Elkjaer et al. with Cr51 (Table 1.7). In a study conducted by Bolignano et al. with 69 patients with CKD (glomerulonephritis, polycystic disease, mild proteinuria) due to various causes, the relationship between urinary and serum NGAL levels and serum creatinine level, eGFR(CG), eGFR(MDRD-4) and estimated GFR was examined(47). The relationship between serum NGAL levels and serum creatinine (r=0.345, p<0.001), Cockcroft-Gault (r=-0.739, p<0.0001) and MDRD-4 (r=-0.732, p<0.0001) and estimated GFR, urinary NGAL levels and serum creatinine (r=0.499, p<0.005), Cockcroft-Gault (r= -0.771, p<0.0001) and MDRD-4 (r=-0.769, p<0.0001) and estimated GFR. NGAL serum level was found to be 26.2 ng/ml (9.2-46.6) in the control group and 409.9 ng/ml (52.6-1215.4) in the patient group, and NGAL urine level was found to be 7.5 ng/ml (3.3-12.9) in the control group and 195 ng/ml (73.9-963.1) in the patient group. In our study, the mean urinary NGAL level was 0.45 ng/ml in the control group and 5.02 ng/ml in the patient group, and the mean serum NGAL level was 0.65 ng/ml in the control group and 3.69 ng/ml in the patient group. When the difference between the control and patient groups was correlated with GFR, MDRD-4 (r=-0.21, p<0.0036), MDRD-7 (r=-0.19, p<0.01), Cockcroft-Gault (r=-0.19, p<0.003) correlations were detected only in the urinary NGAL arm (Table 4.8).In a study by Mark Mitsnefes et al. in a paediatric age group of 46 patients with CKD between stages 2-4, serum NGAL levels were compared with eGFR obtained by ioversol clearance, serum Cystatin-C levels were compared with eGFR measurements obtained by Schwartz formula. Serum Cystatin-C and serum NGAL levels were correlated (r=0.74, p<0.000). When plasma NGAL and Cystatin-C levels were compared with eGFR measured by Schwartz formula and GFR measured by ioversol, both levels showed correlation (Cystatin-C r=0.62, p<0.0000*, NGAL r=0.71, p<0.0000*) (48). In our study, serum NGAL and cystatin-C levels showed correlation (r=0.35, p<0.00*)(Table 1.6). However, when GFR measured by various methods (MDRD-7, MDRD-4, Cockcroft-Gault, DTPA with double

plasma sampling, endogenous creatinine clearance) was compared with serum NGAL levels, no correlation was detected with any measurement method ($p>0.05$) (Table1).In a study conducted by Bolignano et al. in 33 patients with chronic kidney disease due to macroproteinuric, membranous and membranoproliferative nephropathy, the degree of proteinuria and urinary NGAL levels were compared. A correlation was found between the degree of proteinuria and urinary NGAL levels ($r =0.294$, $p<0.01$). The mean urinary NGAL level in the group with macroproteinuria was 378.28 ± 111.13 ng/ml, while this ratio was 7.38 ± 3.26 ng/ml in the control group(57). In our study, no statistical study was performed because the number of macroproteinuric patients was two in total, but the mean urinary NGAL of the two macroproteinuric patients was approximately 11 times higher than the mean urinary NGAL of the control group.Bolignano et al.'s study with 96 patients (25 glomerulonephritis, 19 diabetic nephropathy, 25 autosomal dominant polycystic kidney disease, 21 various kidney diseases, 6 undiagnosed kidney disease) with stage 2-4 CKD, stable renal function, not receiving immunosuppressive treatment, patients were selected as the endpoint of "2-fold increase in basal creatinine level or development of end-stage renal failure"(8). Correlation was found between MDRD-7-eGFR measurements and haemoglobin ($r=0.24$, $p<0.02$), blood pressure ($r= -0.026$, $p<0.01$), urinary NGAL ($r= -0.41$, $p<0.0001$), serum NGAL ($r= -0.44$, $p<0.001$) levels. When certain peak values were obtained by ROC analyses in serum and urine NGAL levels, it was found to be more significant in predicting the development of ESRD independent of age and eGFR, and it was stated that it could be used in the follow-up of the loss of renal reserve period. In our study, when double plasma sampling GFR measurement with Tc99m-DTPA was compared with MDRD-4, MDRD-7, eGFR(CG), endogenous creatinine clearance, a statistical correlation was found for all four measurement methods ($r>0.5$, $p<0.001$)(Table 1.10), while a weak negative correlation was observed with urinary NGAL level ($r=-0.17$, $p<0.04$)(Table 1.7). Since our study was not prospective in terms of its design,

correlation analysis could not be performed between the change in GFR value over time and the change in NGAL level, but when urinary NGAL averages were examined in various GFR ranges, urinary NGAL levels increased as GFR decreased and showed negative correlation with MDRD-4 in all GFR ranges, indicating that it can be used in the follow-up of chronic kidney disease (Table 1.11, 1.12). In a study conducted by Meijer et al. in 102 patients with autosomal dominant polycystic kidney disease, urinary NGAL, serum KIM-1, N-AG, urinary beta-2-microglobulin, cardiac fatty acid binding protein, MCF-1 (Monocyte Chemotactic Protein-1), MMIF-1 (Macrophage Migratory Inhibitory Factor-1) levels were compared with GFR and urinary albumin excretion rate measured with iodothalamate 125. Correlation was found between GFR measured with iothalamate 125, urinary NGAL, urinary beta-2-microglobulin, and cardiac fatty acid binding protein levels (46). In our study, correlation was found between radionuclide method (DTPA) and urinary NGAL ($r=-0.169$, $p<0.04$)(Table 1.7). In a study conducted by Ding et al. with 70 patients with IgA nephropathy, urinary creatinine, urinary NGAL and urinary N-Acetyl increased in parallel with the progression of the disease and correlation was found between NGAL accumulation in the tissue and the degree of disease in the evaluation made by staining of tissue samples(58). While a weak correlation was found between urinary NGAL level and albuminuria level in the normoalbuminuric group ($p<0.027$), a moderate positive correlation was found between serum and urinary NGAL level and albuminuria level ($r=0.340$ $p<0.018$, $r=0.388$ $p<0.009$) in microalbuminuria, which is accepted as one of the earliest markers of diabetic nephropathy (Table 1.1) and the fact that serum and urine NGAL levels in the normoalbuminuric group were approximately 3-4 times higher than serum and urine NGAL levels in the control group showed that NGAL could be used in the follow-up of diabetic nephropathy (Table 1.1). While there was correlation between urinary NGAL level and GFR measurements determined by various methods (MDRD-4, MDRD-7, Cockcroft Gault, Tc99m-DTPA with double plasma sampling), serum NGAL level correlated

only with plasma Cystatin-C level(Table 1.7). The negative correlation of urinary NGAL levels with eGFR methods in the patient and control groups and the fact that there was no statistical difference between the results of other GFR measurement tests, while the difference of NGAL levels between the control and patient groups was approximately 5-6 times showed that urinary NGAL can be used as a marker of kidney damage(Table 1.7). When the tests used in GFR measurement were compared with each other including subgroups, the highest correlation was found in MDRD-4 formula in both patient and control groups. MDRD-4 was accepted as the gold standard for specificity and sensitivity measurements. When urinary and serum NGAL were compared with GFR measured in subgroup analysis according to MDRD-4 in the patient group, urinary NGAL showed a negative correlation with MDRD-4 in all groups, but not with creatinine clearance and DTPA, serum NGAL showed a strong negative correlation with DTPA above GFR 120ml/min, while MDRD-4 showed a correlation between GFR 45-60 ml/min. The results showed that urinary NGAL can be used to estimate GFR in all groups (Table 1.11, 1.12). When the tests used in GFR measurement were compared with each other, the highest correlation was determined by MDRD-4 formula in all subgroups (Table 1.10). In our evaluation by ROC curve using MDRD-4 as a reference, urinary NGAL showed high sensitivity as GFR decreased in terms of estimation of clearance, while serum NGAL sensitivity did not change according to GFR values (Table 1.13). When urine and serum NGAL specificities and sensitivities were compared in certain GFR ranges, urine NGAL showed the highest sensitivity in the range of 60-89.9 ml/min and serum NGAL showed the highest sensitivity in the range of 45-59.9 ml/min(Table 1.14). In albuminuria subgroup analysis, urinary NGAL sensitivity did not show a significant change in the normoalbuminuric group, whereas sensitivity increased as GFR decreased in the microalbuminuric group (Table 1.4). When albuminuria was taken as the gold standard and microalbuminuria limit was chosen as the cut-off point, MDRD-4 showed 25% sensitivity and 98% specificity, urinary NGAL showed 23%

sensitivity and 90.5% specificity, serum NGAL showed 94% sensitivity and 21% specificity(Table 1.15). In the GFR subgroup analysis of urinary NGAL according to MDRD-4, urinary NGAL averages increased inversely proportional to the decrease in GFR in the control and patient groups, indicating that it can be used in the follow-up of chronic kidney disease.

Conclusion:

In this study, urine and serum NGAL levels were found to be higher in diabetic patients with normal urea-creatinine levels compared to the control group (Table 1.5 The causes of this elevation may include aging-related remodelling and diabetes-related inflammation and morphological changes in renal tissue. The fact that urinary NGAL level is higher in the patient group than in the control group and that it is measured higher in normoalbuminuric diabetic patients than in the non-diabetic control group suggests that it may be used as a marker of early kidney damage in patients with diabetic nephropathy who are still in the renal reserve period. Urinary NGAL level was negatively correlated with MDRD-4, MDRD-7, DTPA and eGFR measurements. Since the study was not prospectively designed, no information was obtained about the use of NGAL level change as a risk marker in the progression to chronic renal failure, but when urinary NGAL averages were examined at various GFR ranges, urinary NGAL levels increased as GFR decreased and showed negative correlation with MDRD-4 at all GFR ranges, suggesting that it can be used in the follow-up of chronic kidney disease.

Table 1.1: *Albuminuria NGAL correlation according to subgroups.*

	Albuminuria					
	Normalb. Number of patients	r,p	Number of microalb. patients	r,p	Macroalb. number of patients	r,p
Patient serum NGAL	131	r=0,03 p<0,943	31	r=0,340 p<0,018*	2	-**
Patient urine NGAL	131	r=0,254 p<0,027*	31	r=0,388 p<0,009*	2	-**
Control serum NGAL	38	r=0,186 p<0,048	2	**	0	-
Control urine NGAL	38	r=0,021 p<0,955	2	**	0	-
	Albuminuria					
	Normalb. Mean ng/ml		Microalb. mean ng/ml		p	
Patient serum NGAL	1,63		4,05		p<0,02	
Patient urine NGAL	1,48		6,71		p<0,001	
Control serum NGAL	0,59		0,61		***	
Control urine NGAL	0,42		0,58		***	

** Correlation analysis could not be performed because the number of patients was two.
*** Statistical difference could not be analysed because the number of patients was two.

Table 1.2: *Correlation between GFR measurement and other parameters in patients with endogenous creatinine clearance >90/ml/min.*

Urine/Serum NGAL	Patient group		Control group	
	Urine NGAL	serum NGAL	Urine NGAL	serum NGAL
MDRD-7 eGFR	r=0,094 p<0,327	r=0,37 p<0,698	r=-0,271 p<0,095	r=0,228 p<0,163
MDRD-4 eGFR	r=0,130 p<0,171	r=0,3 p<0,75	r=-0,277 p<0,088	r=0,183 p<0,265
Cockcroft-Gault eGFR	r=-0,189 p<0,046	r=0,027 p<0,778	r=-0,435 p<0,04	r=-0,331 p<0,04
eGFR by Tc99m-DTPA double plasma sampling method	r=0,017 p<0,877	r=0,118 p<0,271	-	-
GFR with endogenous Creatinine clearance	r=-0,96 p<0,316	r=0,096 p<0,314	r=-0,039 p<0,813	r=0,98 p<0,555

Table 1.3: *Correlation of the tests used in GFR measurement with each other in subgroup analysis according to the degree of albuminuria.*

			Cockcroft-Gault	MDRD-4	MDRD-7	Creatinine cl.	Tc99m-DTPA
Patient	Normoalbuminuric group	Cockcroft - Gault	*	r=0,878, p<0,0001	r=0.846, p<0.0001	r=0.468, p<0.0001	r=0,425 p<0,0001
		MDRD-4	r=0,878, p<0,0001	*	r=0,984, p<0,0001	r=0.450, p<0.0001	r=0.428, p<0.0001
		MDRD-7	r=0.846, p<0.0001	r=0,984, p<0,0001	*	r=0.479, p<0.0001	r=0.398, p<0.0001
		Creatinine cl.	r=0.468, p<0.0001	r=0.450, p<0.0001	r=0.479, p<0.0001	*	r=0.360, p<0.0001
		Tc99m-DTPA	r=0,425 p<0,0001	r=0.428, p<0.0001	r=0.398, p<0.0001	r=0.360, p<0.0001	*
Control		Cockcroft - Gault	*	r=0.944, p<0.0001	r=0.938, p<0.0001	r=0.439, p<0.004	-

		MDRD-4	r=0.944 p<0.0001	*	r=0.992, p<0.0001	r=0.356, p<0.001	-
		MDRD-7	r=0.938, p<0.0001	r=0.992, p<0.0001	*	r=0.348, p<0.003	-
		Creatinine cl.	r=0.439, p<0.004	r=0.356, p<0.001	r=0.348, p<0.003	*	-
		Tc99m-DTPA	-	-	-	-	*
			Cockcroft-Gault	MDRD-4	MDRD-7	Creatinine Kl.	Tc99m-DTPA
Patient	**Microalbuminuric group**	**Cockcroft - Gault**	*	r=0,956, p<0,0001	r=0,950, p<0,0001	r=0.483, p<0.0001	r=0,610 p<0,0001
		MDRD-4	r=0,956, p<0,0001	*	r=0,993, p<0,0001	r=0.507, p<0.0001	r=0.349, p<0.037
		MDRD-7	r=0,950, p<0,0001	r=0,993, p<0,0001	*	r=0.492 p<0.0001	r=0.323, p<0.055
		Creatinine cl.	r=0.483, p<0.0001	r=0.507, p<0.0001	r=0.492 p<0.0001	*	r=0.457, p<0.0001
		Tc99m-DTPA	r=0,610 p<0,0001	r=0.349, p<0.037	r=0.323, p<0.055	r=0.457, p<0.0001	*
Control		**Cockcroft - Gault**	**	**	**	**	**
		MDRD-4	**	**	**	**	**
		MDRD-7	**	**	**	**	**
		Creatinine Kl.	**	**	**	**	**
		Tc99m-DTPA	**	***	**	**	**

**Correlation analysis was not performed because there were four patients in the microalbuminuric control group.

Table 1.4: *Urine-serum NGAL specificity and sensitivity values in subgroup analysis according to albuminuria grades when the gold standard test MDRD-4 is accepted.*

Urine NGAL			**Sensitivity %**	**Specificity %**	**Positive predictive value %**	**Negative predictive value %**
MDRD-4	Normoalbuminuric group	GFR>120	99	6	51,4	85,7
		GFR>90	86,5	48,5	62,8	78,2
		GFR>60	83,3	42,8	59,2	71,9
		GFR>45	87,8	26,6	54,4	68,5
	Microalbuminuric group	GFR>120	64,2	97	88	72,6
		GFR>90	65,7	60	61,4	63,6
		GFR>60	80	35	55,1	63,6
		GFR>45	90,9	28,1	55,8	75,5
Serum NGAL			**Sensitivity %**	**Specificity %**	**Positive predictive value %**	**Negative predictive value %**
MDRD-4	Normoalbuminuric group	GFR >120	99	16	54	94,2
		GFR>90	83,3	57,1	66	68,1
		GFR>60	87,8	26,6	54,1	68,5
		GFR>45	73,1	53,2	60,9	66,4
	Microalbuminuric group	GFR>120	88	22	53	64,7
		GFR>90	81,5	60	67	76,4
		GFR>60	66	98	97	74
		GFR>45	45,4	90,6	82,8	62,3

Table 1.5: *Urine NGAL averages according to GFR values.*

MDRD-4		**GFR >120**	**GFR 90-120**	**GFR 60-89,9**	**GFR 45-59,9**
Patient	Urine NGAL mean ng/ml	1,56	2,61	8,79	14,2
Control	Urine NGAL mean ng/ml	0,38	0,42	0,53	-

Table 1.6 : *Comparison of serum NGAL level*

Serum NGAL	**Patient**			**Control**		
	Correlation coefficient	P*	N	Correlation coefficient	P*	N
Age	0,08	0,26	186	-0,19	0,24	42
Height	0,08	0,29	186	-0,07	0,65	42
BMI(Body Mass Index)	-0,03	0,70	186	-0,07	0,67	42
BSA(Body Surface Area)	0,10	0,18	186	-0,25	0,11	42
WBC	0,20	0,01*	186	0,02	0,88	42
Hgb	0,06	0,43	186	-0,06	0,72	42
Htc	0,04	0,55	186	0,00	0,99	42
Urea	0,03	0,66	186	-0,19	0,23	42
Creatinin	0,02	0,77	186	-0,14	0,36	42
Fasting Glucose	-0,06	0,41	186	-0,18	0,25	42
Trigliseride	-0,05	0,52	186	0,21	0,19	42
LDL-Cholesterol	-0,13	0,09	186	0,01	0,97	42
HDL- Cholesterol	-0,08	0,30	186	-0,19	0,24	42
Total Cholesterol	-0,14	0,06	186	0,09	0,56	42
TSH	-0,04	0,55	186	-0,19	0,24	42
sT4	-0,04	0,60	186	0,26	0,10	42
HbA1c	-0,04	0,57	186	0,02	0,90	42
24 Hour Urine.Protein	0,11	0,15	185	-0,05	0,73	42
24Hour Urine.Microalb.	0,05	0,51	166	-0,18	0,28	38
24 Hour Urine Cr. Cl.	0,04	0,54	185	-0,06	0,69	42
MDRD-7 e-GFR	-0,01	0,84	186	-0,07	0,67	42
MDRD-4 e-GFR	-0,02	0,83	186	-0,04	0,82	42
Cockcroft-Gault e-GFR	-0,04	0,60	186	-0,14	0,36	42

DTPA e-GFR	-0,11	0,20	148	.	.	0
Serum Cystatin-C	0,35	0,00*	186	0,40	0,01*	42

Table 1.7 : *Comparison of urinary NGAL level*

Urine NGAL	**Patient**			**Control**		
	Correlation coefficient	P*	N	Correlation coefficient	P*	N
Age	-0,12	0,12	186	0,45	0,003*	42
Height	0,05	0,49	186	-0,44	0,003*	42
BMI(Body Mass Index)	0,10	0,17	186	0,32	0,04*	42
BSA(Body Surface Area)	0,12	0,11	186	-0,16	0,32	42
WBC	0,02	0,75	186	-0,08	0,61	42
Hgb	0,20	0,01*	186	-0,36	0,02*	42
Htc	0,21	0,00*	186	-0,25	0,10	42
Urea	-0,05	0,46	186	0,08	0,61	42
Creatinin	-0,15	0,04	186	0,09	0,55	42
Fasting Glucose	0,03	0,71	186	0,35	0,02*	42
Trigliseride	-0,10	0,15	186	0,07	0,68	42
LDL-Cholesterol	-0,05	0,50	186	0,28	0,07	42
HDL- Cholesterol	-0,06	0,38	186	0,09	0,59	42
Total Cholesterol	-0,05	0,49	186	0,23	0,14	42
TSH	0,02	0,80	186	-0,26	0,10	42
sT4	-0,04	0,63	186	0,12	0,44	42
HbA1c	0,05	0,47	186	0,04	0,79	42
24 Hour Urine.Protein	-0,04	0,56	185	0,01	0,96	42
24Hour Urine.Microalb.	0,07	0,038	166	0,21	0,20	38
24 Hour Urine Cr. Cl.	-0,12	0,11	185	-0,21	0,17	42
MDRD-7 e-GFR	-0,19	0,01*	186	-0,37	0,02*	42
MDRD-4 e-GFR	-0,21	0,0036*	186	-0,37	0,02*	42
Cockcroft-Gault e-GFR	-0,19	0,01*	186	-0,53	0,0003*	42
DTPA e-GFR	-0,17	0,04*	148	.	.	0
Serum Cystatin-C	0,05	0,52	186	0,19	0,24	42

Table 1.8: *Correlation between Serum Cystatin-C and other methods in age subgroup examination.*

Serum Cystatin-C	**Under 65 years old**		**Over 65 years old**	
MDRD-7 eGFR	r=0,163	p<0,081	r=0,027	p<0,82
MDRD-4 eGFR	r=0,187	p<0,046	r=0,072	p<0,55
Cockcroft-Gault eGFR	r=0,210	p<0,024	r=0,003	p<0,98
Serum NGAL	r=0,377	p<0,000	r=0,306	p<0,009
eGFR by Tc99m-DTPA double plasma sampling method	r=0,117	p<0,271	r=-0,058	p<0,667
GFR with endogenous cryopreservation	r=0,055	p<0,559	r=0,011	p<0,928

Table 1.9: *Serum-urine NGAL, proteinuria-albuminuria correlation*

	Urine NGAL	Serum NGAL
24-hour urine proteinuria	r=-0.04 p<0.56	r=0.11 p<0.15
24-hour urine albuminuria	r=0.07 p<0.038	r=0.15 p<0.51

Table 1.10: *Correlation of tests used in GFR measurement with each other*

		Cockcroft-Gault	**MDRD-4**	**MDRD-7**	**Creatinine cl.**	**Tc99m-DTPA**
Patient	**Cockcroft - Gault**	*	r=0,906, p<0,0001	r=0.897, p<0.0001	r=0.616, p<0.0001	r=0.525 p<0.0001
	MDRD-4	r=0,906, p<0,0001	*	r=0,984, p<0,0001	r=0.593, p<0.0001	r=0.545, p<0.0001
	MDRD-7	r=0.897, p<0.0001	r=0,984, p<0,0001	*	r=0.616, p<0.0001	r=0.545, p<0.0001
	Creatinine cl.	r=0.616, p<0.0001	r=0.593, p<0.0001	r=0.616, p<0.0001	*	r=0.578, p<0.0001
	Tc99m-DTPA	r=0.525 p<0.0001	r=0.545, p<0.0001	r=0.545, p<0.0001	r=0.578, p<0.0001	*
Control	**Cockcroft - Gault**	*	r=0.751, p<0.0001	r=0.692, p<0.0001	r=0.439, p<0.004	-
	MDRD-4	r=0.751, p<0.0001	*	r=0.967, p<0.0001	r=0.496, p<0.001	-
	MDRD-7	r=0.692, p<0.0001	r=0.967, p<0.0001	*	r=0.444, p<0.003	-
	Creatinine cl.	r=0.439, p<0.004	r=0.496, p<0.001	r=0.444, p<0.003	*	-
	Tc99m-DTPA	-	-	-	-	*

Table 1.11: *Serum and urine NGAL correlation with the tests used to measure GFR in subgroup analysis according to MDRD-4.*

MDRD-4 >120 ml/min	**MDRD-4**	**Creatinine cl.**	**Tc99m-DTPA**
Urine NGAL	r=-0.382 p<0.018	r=0.013 p<0.992	r=-0.176 p<0.041
Serum NGAL	r=-0.208 p<0.792	r=-1,000 p<0,0001	r=-1,000 p<0,0001
MDRD-4 90-119 ml/min	**MDRD-4**	**Creatinine cl.**	**Tc99m-DTPA**
Urine NGAL	r=-0.109 p<0.038	r=0.225 p<326	r=0.276 p<472
Serum NGAL	r=-0,090 p<0,699	r=-0.08 p<0.979	r=-0.133 p<0.432
MDRD-4 60-89 ml/min	**MDRD-4**	**Creatinine cl.**	**Tc99m-DTPA**
Urine NGAL	r=-0.148 p<0.023	r=-0.033 p<0.754	r=-0.004 p<0.934
Serum NGAL	r=-0.113 p<0.285	r=-0,218 p<0,036	r=-0.482 p<0.0001
MDRD-4 45-60 ml/min	**MDRD-4**	**Creatinine cl.**	**Tc99m-DTPA**
Urine NGAL	r=-0.226 p<0.014	r=-0.032 p<0.826	r=-0,379 p<0,017
Serum NGAL	r=-0.380 p<0.007	r=-0.09 p<0.952	r=-0.007 p<0.968

Table 1.12: *Urine NGAL averages according to GFR values.*

MDRD-4		GFR >120	GFR 90-120	GFR 60-89,9	GFR 45-59,9
Patient	Urine NGAL mean ng/ml	1,56	2,61	8,79	14,2
Control	Urine NGAL mean ng/ml	0,38	0,42	0,53	-

Table 1.13: *Urine-serum NGAL specificity and sensitivity values according to various GFR measurement levels.*

Urine NGAL		**Sensitivity %**	**Specificity %**	**Positive predictive value %**	**Negative predictive value %**
MDRD-4	GFR>120	38	87,5	75,2	58,5
	GFR>90	84,9	40,8	58,9	72,9
	GFR>60	88,4	25,7	53,1	68,9
	GFR>45	94,7	19,1	53,9	78,2
Endogenous Creatinine Clearance	GFR>120	85,6	40	51,3	73,5
	GFR>90	90,7	27,8	55,6	74,9
	GFR>60	77	42	57	64
	GFR>45	100	55	68,7	100
DTPA	GFR>120	93,3	15,5	52,4	70

	GFR>90	90	13,2	51	56,8
	GFR>60	66,6	57,9	61,2	64,4
	GFR>45	*	*	*	*
Serum NGAL		**Sensitivity %**	**Specificity %**	**Positive predictive value %**	**Negative predictive value %**
MDRD-4	GFR>120	80,4	62,5	68,1	76,1
	GFR>90	87,2	51	64	79,9
	GFR>60	79,7	47,1	60,1	69,8
	GFR>45	84,2	58,8	67,1	78,8
Endogenous Creatinine Clearance	GFR>120	83,8	43,3	59,6	72,7
	GFR>90	94,7	25,8	56	82
	GFR>60	55	26	42	36
	GFR>45	100	28	78,4	100
DTPA	GFR>120	13	96	76,4	52,4
	GFR>90	8	97	72	51,3
	GFR>60	33,3	80	62,4	54,5
	GFR>45	*	*	*	*

*No GFR measurement result below 45 ml/min with DTPA.

Table 1.14: *Urine-serum NGAL specificity and sensitivity values at various GFR measurement intervals.*

Urine NGAL		**Sensitivity %**	**Specificity %**	**Positive predictive value %**	**Negative predictive value %**
MDRD-4	GFR 90-120	84,4	43,9	64,6	73,7
	GFR 60-89.9	95,4	55	67	92
	GFR 45-59.9	34	82	65	55,4
	GFR 30-44.9	67,4	52,7	55,7	59,1
Serum NGAL		**Sensitivity %**	**Specificity %**	**Positive predictive value %**	**Negative predictive value %**
MDRD-4	GFR 90-120	85,5	48,7	62,5	77
	GFR 60-89.9	29,6	33,7	46,7	55
	GFR 45-59.9	99,4	64	73,1	99
	GFR 30-44.9	11	84	40,7	48,5

Table 1.15: *MDRD-4, urine and serum NGAL sensitivity, selectivity and predictivity results when microalbuminuria limit is taken as cut-off point.*

Albuminuria 30+ <30	Sensitivity %	Specificity %	Positive predictive value %	Negative predictive value %
Urine NGAL 2.2 ng/ml	23	90,5	70	54
Serum NGAL 1.56 ng/ml	94	21	54	77
MDRD-4 118 ml/min	25	98	92	56,6

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CHAPTER VI

Endobronchıal Ultrasonography Procedure In Accordance Wıth The Lıterature

Savaş GEGİN [1]

Introduction

EBUS is the most commonly used minimally invasive method in determining the pathological diagnosis of mediastinal LAP and masses. In addition to lung cancer staging, it enables the evaluation of mediastinal cancers, non-endobronchial mass lesions and benign pathologies. As time goes by, there is an increase in the number of centers where EBUS is performed and the number of specialists. Its diagnostic sensitivity is similar to mediastinoscopy, and it is more advantageous when evaluated in terms of complications and hospital stay. While mediastinoscopy is performed with general anesthesia, EBUS can be performed with local or deep sedation, which reduces the complication rate. One of the most commonly used lung

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diagnostic imaging methods is bronchoscopy, which allows airway evaluation and biopsy of endobronchial lesions. In addition, the bronchoscopy method was inadequate in the evaluation of masses without endobronchial formation and medacytinal lymph nodes. In such cases, it is possible to make a diagnosis by endobronchial ultrasonography (EBUS) and transbronchial needle aspiration biopsy (TB-IAB). While the airway can be visualized with the EBUS procedure, at the same time, due to its ultrasound feature, structures adjacent to the airway, vascular structures, masses and lymph nodes can be evaluated and biopsied. It plays an important role in determining treatment by allowing staging of lung cancer, especially by sampling mediastinal lymph nodes. (1–4)

EBUS Procedure:

EBUS device is made with a device that provides video image and ultrasonic image at the same time (Figure 1). Ultrasound-graphic imaging of the endobronchial structure can be achieved up to a depth of 4 cm. Unlike the flexible bronchoscopy device, it contains a convex ultrasound probe at its tip. (Picture 2) While the ultrasonic image is taken with the probe, the video image is also watched at the same time. It is also possible to perform a needle aspiration biopsy during imaging. In thorax computed tomography (CT), needle aspiration biopsy can be performed from pathologically sized lymphadenoids (4R, 4L, 7, 10R, 10L, 11L, 11R) and the mass with an EBUS device. During the procedure, deep anesthesia with propofol or conscious sedation with midazolam can be applied. During the procedure, after determining the station where needle biopsy will be performed, negative aspiration is performed with a 22 gauge needle and biopsy is performed from the lymph node or mass. (Picture 3-4) Aspiration is recommended at least 4 times from each station. Aspirates are spread on glass slides, air dried, and immediately fixed with 95% alcohol. Histological aspirate tissues are fixed with 10% neutral buffered formalin. (4) Pathological qualification (ROSE) can be performed per case during the EBUS procedure. Although there is no difference in pathological diagnosis between cases in which ROSE was performed and those in which it

was not performed, there are studies proving that it shortens the procedure time and allows fewer samples to be taken. (5) The samples were sent for pathological examination. Figure 1 shows the lymph node map that can be biopsied with EBUS. Picture 5 shows the ultrasound image of the lower right paratracheal lymph node biopsied with EBUS, while picture 6 shows the image of needle aspiration biopsy from subcarinal (number 7) lymphadenopathy. (3,4)

Figure 1:EBUS device and unit.

Figure 2: convex ultrasound probe

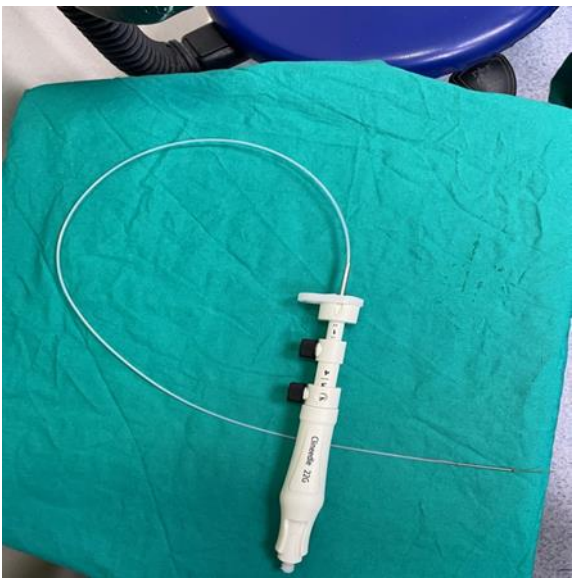

Figure 3:EBUS needle

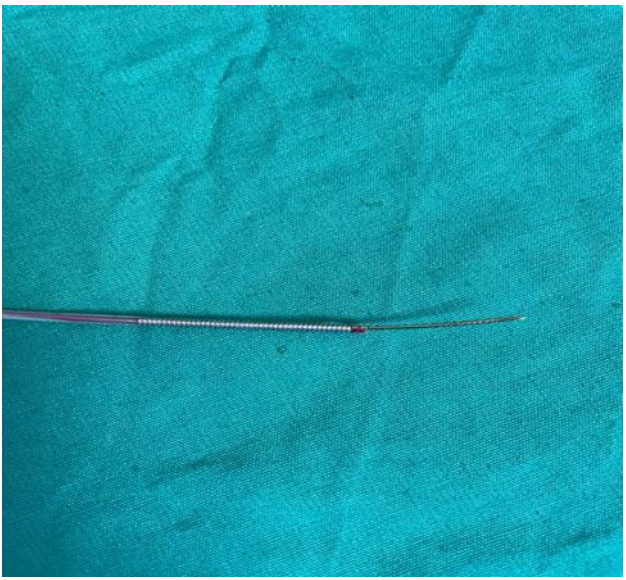

Figure 4 : EBUS needle point

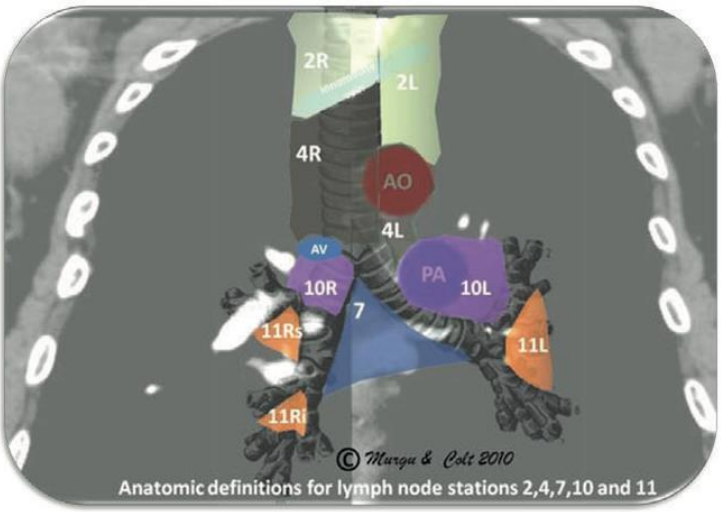

Figure 1: Lymph Node Stations Sampled with EBUS (Quoted from source 3)

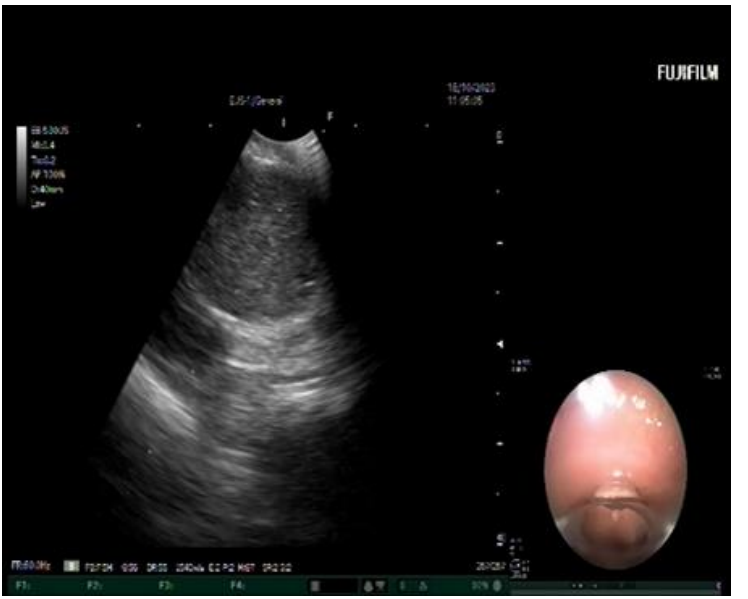

Figure 5: lower right paratarchael lymph node view

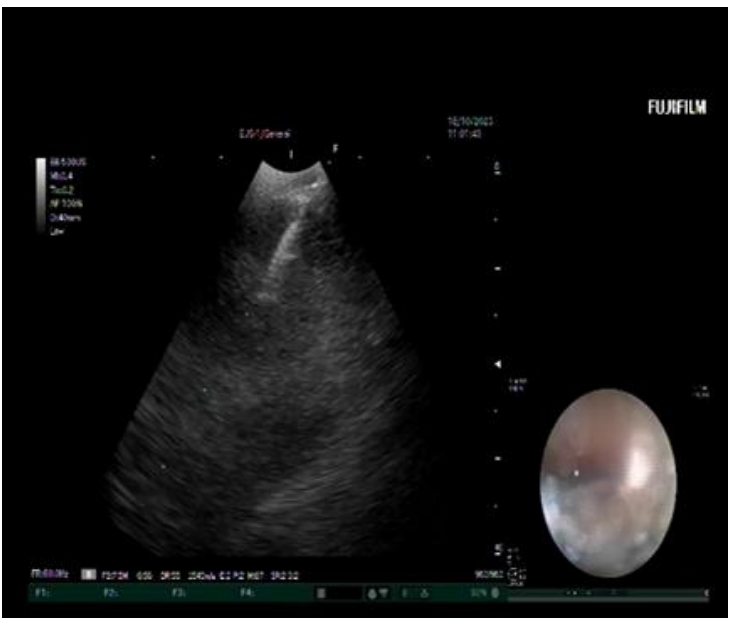

Figure 6: Image during biopsy

Indications and examples of studies conducted;

With EBUS, lung cancer is staged, as well as sarcoidosis, tuberculosis and other mediastinal diseases are diagnosed. There are many published articles about EBUS around the world. The number of patients undergoing EBUS has increased over the years. While EBUS constituted 8.8% of all bronchoscopies performed in 2009, it was found that this rate increased to 21.4% in 2014. Today, this rate is higher and it is thought that the increase towards EBUS will increase over time. (5)

Since tissue can be monitored with EBUS compared to the traditional transbronchial needle aspiration method, the biopsy accuracy rate is higher. In a study, 123 lymph nodes of 64 patients who could not be diagnosed with traditional methods were biopsied with EBUS. While malignancy was detected in 24 patients, benign pathology was detected in 26 patients (sarcoma, tuberculosis, anthrocosis). 14 patients could not be diagnosed. As a result of 6-month follow-up or medaistinoscopy, 9 patients were diagnosed with reactive lymph nodes, 3 patients were diagnosed as malignant, one patient was diagnosed with lymphoma, one patient was diagnosed with tuberculosis and one patient was diagnosed with sarcoidosis. With these findings, it was concluded that biopsy with EBUS gives more accurate results than traditional methods. (6) In another study, bronchoscopic transbronchial biopsy and EBUS method were compared. Although no significant superiority of the EBUS method was found over the traditional method, it was suggested that traditional methods and EBUS should be preferred in a stepwise manner in significant lesions for cost effectiveness. (7). In the study where single center data were evaluated, EBUS was applied to a total of 167 patients. While lung cancer was detected in 90 patients, metastasis was detected in two patients. Sarcoidosis was detected in 41 patients, tuberculosis was detected in one patient, and reactive lymph nodes were observed in 33 patients. The most frequently biopsied lymph node was reported as a subcarinal focus. (8)

In addition to the diagnosis of primary lung cancer, EBUS provides the opportunity to evaluate metastases of extrathoracic malignancies. In studies, the most common extrathoracic malignancies were found to be head and neck malignancies, breast carcinoma, urological malignancies and gastrointestinal system tumors. (9–12)

In countries where tuberculosis is endemic, tuberculosis should be excluded as a diagnosis in mediastinal lymphadenopathy and masses. In a study, 49 patients with suspected tuberculosis were evaluated prospectively. After EBUS and biopsy, tuberculosis was detected in 41 patients, lung cancer in 5, inflammation in 7, and sarcoidosis in 5. (13) In another study, biopsies were taken with EBUS from 102 patients in the region where tuberculosis is endemic. The diagnostic rate for sarcoidosis, tuberculosis, and lung cancer was stated as 80.9%, 84.8%, and 75%, respectively. (14) Sarcoidosis is a lung disease and the most common finding is mediastinal lymphadenopathy. EBUS-guided needle biopsy is a minimally invasive method that can be applied diagnostically in the diagnosis of sarcodosis. In a study, transbronchial lung biopsy was performed in 180 patients. Sarcoidosis was diagnosed in 79 (43.9%) patients. Endobronchial needle biopsy was performed in 340 patients. Diagnosis was made in one hundred and one patients (29.7%). Among 549 patients who underwent EBUS biopsy, 84% were diagnosed with sarcoidosis. The diagnosis rate was found to be higher compared to other biopsy methods. According to these results, EBUS was considered to be a suitable method for the diagnosis of sarcoidosis. (15) Although mediastinoscopy is still the gold standard in lung cancer staging, EBUS is increasingly used as a minimally invasive method considering the complications of the procedure. In a study, EBUS was performed on 270 patients and 24 metastases were diagnosed, while 125 were diagnosed with adenocarcinoma, 31 with large cell carcinoma, 28 with squamous cell carcinoma and 29 with small cell carcinoma. (16)

Complications ;

Rare complications related to the EBUS procedure have been reported in the literature. One of these was a needle breakage and the broken tip was removed with a flexible bronchoscope. (17) Case reports of pneumodiastineum developing after urination have been reported. (18) In another study, 5 serious complications were observed in 3123 cases. These have been reported as fever lasting more than 24 hours, bronchogenic cyst infection, mediastinal abscess, pericarditis and pneumomediastinitis with empyema. (19) Apart from this, bleeding and anesthesia-related problems are among the possible complications.

Result ;

EBUS is a minimally invasive method that is becoming increasingly common and is used in primary lung cancer, extrathoracic malignancy metastases, lung cancer staging, and mediastinal involvement of inflammatory diseases.

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CHAPTER VII

Multiple Myeloma-Related miRNAs and Their Therapeutic Potentials

Osman AKIDAN
Sema MISIR

Multiple Myeloma

Multiple myeloma (MM) is the second most frequent hematologic malignancy, characterized by the unchecked proliferation of clonal plasma cells (PCs) (Dima et al., 2022) (Figure 1). Multiple myeloma (MM) diagnosis needs one or more of the following myeloma-defining events (MDEs) in addition to ≥10% of clonal plasma cells in the bone marrow or a biopsy-proven plasmacytoma. The underlying plasma cell proliferation is responsible for the CRAB characteristics, which include hypercalcemia, renal insufficiency, anemia, or bone lesions. Clonal bone marrow plasma cells are ≥60%, involved to uninvolved serum free light chain (FLC) ratio ≥ 100, or more than one focal lesion measuring 5 mm or more on MRI (Goel et al., 2022). Multiple myeloma, the second most common hematologic malignancy,

accounts for 1.8% of all new cancer cases and 18% of all hematologic malignancies in the United States (Mikhael et al., 2022). At diagnosis, the average age of patients is approximately 66-70 years, with 37% of patients under 65. While MM is reported to be between 0.02% and 0.3% in people under 30, it is stated that it is slightly more common in men (Kazandjian, 2016). The cause of myeloma is not fully known. Chromosomal abnormalities involving the immunoglobulin heavy chain critical region (on the long arm of the chromosome) have been identified as the most common cause. Additionally, although reasons such as radiation and exposure to industrial/agricultural toxins or viruses have been shown, there is not enough evidence (Rajkumar, 2022). In most cases of MM, the disease begins with asymptomatic pre-malignant stages and eventually progresses to symptomatic intramedullary/extramedullary MM. Monoclonal gammopathy of undetermined significance (MGUS), smoldering multiple myeloma (SMM), and active MM form a disease continuum characterized by progressive clonal evolution (Dima et al., 2022; Dutta et al., 2019; Landgren et al., 2009). Approximately 1% of patients diagnosed with MGUS develop MM or other hematological malignancies (Kyle R.A., Therneau T.M., Rajkumar S.V., Larson D.R., Plevak M.F., Offord J.R., Dispenzieri A., Katzmann J.A., 2006; Kyle R.A., Therneau T.M., Rajkumar S.V., Offord J.R., Larson D.R., Plevak M.F., 2002; Rajkumar S.V., Gupta V., Fonseca R., Dispenzieri A., Gonsalves W.I., Larson D., Ketterling R.P., Lust J.A., Kyle R.A., 2013). Within the first five years following diagnosis, about 10% of SMM cases progress to MM (Rajkumar S.V., Gupta V., Fonseca R., Dispenzieri A., Gonsalves W.I., Larson D., Ketterling R.P., Lust J.A., Kyle R.A., 2013). Common symptoms of MM include bone pain and pathological fractures, anemia (bone marrow failure), recurrent infections (due to immune paresis), hypercalcemia, renal failure (multiple etiologies: hypercalcemia, light chain deposition, non-steroidal drugs, anemia, infections), abnormal bleeding (due to platelet dysfunction) are observed (Dutta et al., 2019). Treatment for MM aims to eliminate myeloma cells, control tumor growth, control

pain, and enable the patient to continue her active life (Maral et al., 2018). Various methods such as stem cell transplantation (Landgren et al., 2009), high-dose chemotherapy, new therapeutic agents proteasome inhibitors (bortezomib), immunomodulatory drugs (IMiDs) (lenalidomide and thalidomide), and monoclonal antibodies are used in the treatment of patients (Figure1). These treatment strategies have transformed this once fatal disease into a chronic and manageable disease and extended MM patient survival (Kyle R.A., Therneau T.M., Rajkumar S.V., Offord J.R., Larson D.R., Plevak M.F., 2002). Despite advances in the treatment of the disease in recent years, drug resistance and relapse remain an essential problem. Therefore, new targeted and safer approaches are needed to combat MM disease.

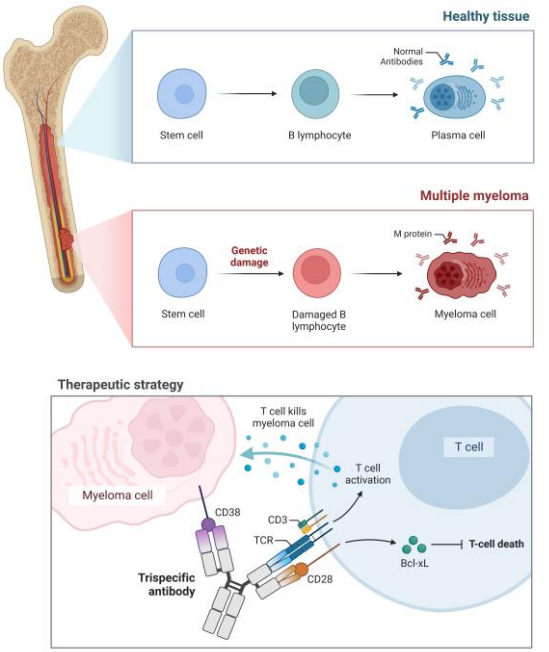

Figure 1. Multiple myloma biogenesis and a therapeutic target Created with BioRender.

microRNA(miRNA)

Functional proteins are only created from a relatively small portion of the human genome (about 1%) of the whole. The remainder of the genome was thought to be of minor importance; however, this view disappeared with the discovery of small RNA molecules (Kim et al., 2011; B. Zhang et al., 2007)**.** miRNAs transcribed from DNA but encoded by non-protein-coding genes are small RNA molecules, usually 20-22 nucleotides long (Lynam-Lennon et al., 2009). miRNAs are highly conserved non-coding RNA molecules involved in the post-transcriptional regulation of gene expression. These molecules are crucial for various cellular and developmental processes and pathological conditions in eukaryotic organisms (García-López et al., 2013). Abnormal expression of miRNAs is associated with many diseases, including cancer, and provides essential clues in elucidating its molecular pathology (O'Brien et al., 2018). miRNAs are stable molecules in body fluids such as plasma, serum, urine, saliva, and tears. They are being investigated as new therapeutic targets for the early diagnosis and treatment of many diseases (X. Chen et al., 2008).

miRNA Biogenesis and Function

miRNA biogenesis begins with the transcription of RNA polymerase II/III transcripts (O'Brien et al., 2018). About half of the miRNAs identified are intragenic, while the other half are intergenic. A host gene is transcribed independently and regulated by its promoters (Brien et al., 2018). The biogenesis process of miRNAs consists of many post-transcriptional post-modification steps that begin in the nucleus and end in the cytoplasm (Huang et al., 2011). Biogenesis of miRNA is classified into canonical and non-canonical pathways (Figure 2) (O'Brien et al., 2018).

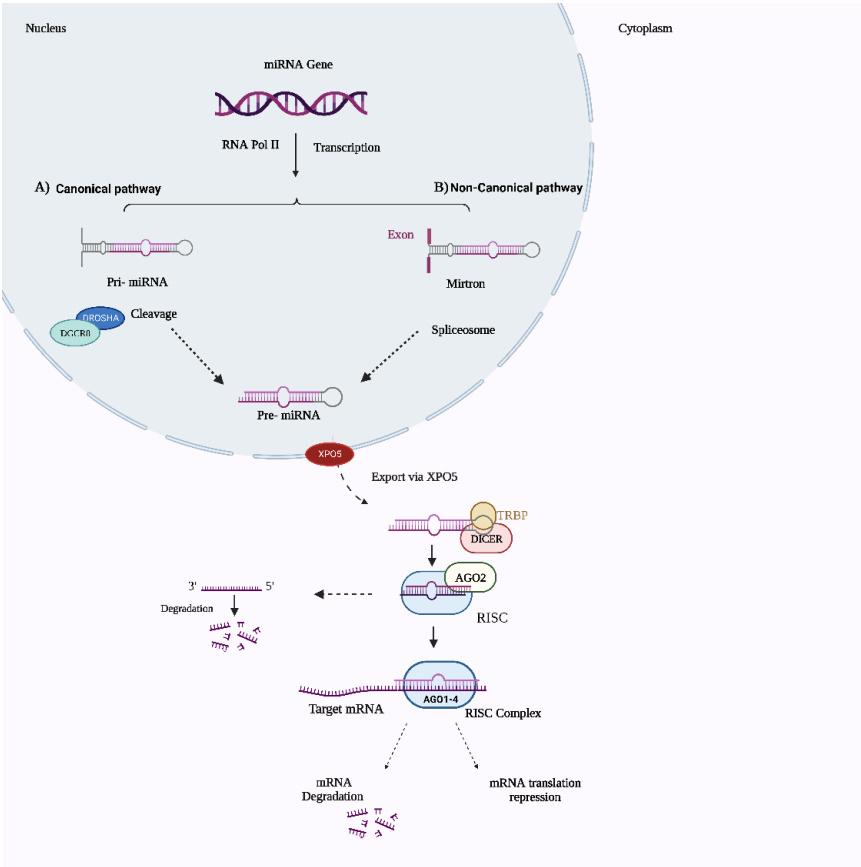

Figure 2. *miRNA biogenesis is divided into canonical and non-canonical pathways Created with BioRender.*

Canonical

The predominant pathway of miRNA biogenesis is the canonical biogenesis pathway. It begins with the transcription of a long primary miRNA transcript, characterized by a hairpin structure (pri-miRNAs), by RNA polymerase II (in some cases, RNA polymerase III) (Peixoto da Silva et al., 2022). In the nucleus, pri-miRNAs are cleaved by a microprocessor complex composed of Drosha (RNase III), an RNase III endonuclease, and the RNA-

binding protein DGCR8(DiGeorge syndrome critical region gene 8: Pasha). A stem-loop structure (pre-miRNAs) of ~70 base pairs long is formed. Pre-miRNAs are then transported to the cytoplasm by the (XPO5)/RanGTP complex, which consists of a transmembrane protein exportin-5 (XPO5) and RanGTP cofactor (O'Brien et al., 2018). The Dicer-TRBP complex cleaves pre-miRNAs in the cytoplasm, resulting in a 19-25 nucleotide long miRNA:miRNA* duplex (Saliminejad et al., 2019). The RISC complex only contains one miRNA from each duplex. The more stable strand is chosen and added to the RISC complex by the protein argonaute (Ago 1-4) family (Peixoto da Silva et al., 2022). Once miRNAs are incorporated into the active RISC complex, they either cause suppression of protein translation or cause mRNA degradation (Acunzo et al., 2015).

Non-canonical

Non-canonical miRNA biogenesis is the process of miRNA synthesis that is not dependent on Drosha or Dicer (Saliminejad et al., 2019). There are some examples, such as non-canonical miRNAs, mirtrons, endogenous short hairpin RNAs (endo-shRNAs), and Small nucleolar RNAs (snoRNAs) miRNAs (Peixoto da Silva et al., 2022). Mirtrons, premiRNAs produced from introns, are the most well-known mechanism of non-canonical miRNAs. This pre-miRNA-like form is transferred to the cytoplasm by XPO5 to continue via canonical pathways. Pre-miRNAs are produced that are cut into ~22 nt RNAs by DICER (Saliminejad et al., 2019). Another group of DROSHA-independent miRNAs are small nucleolar RNA-derived miRNAs (snoRNAs) and tRNA-derived miRNAs (Kotowska-Zimmer et al., 2021). Endogenous short hairpin RNAs (endo-shRNAs) are produced by direct transcription and require XPO1 for nuclear transport. After cleavage by DICER, ~20 nt RNA is produced, and only 3p-miRNAs are loaded into AGO proteins (Kotowska-Zimmer et al., 2021; O'Brien et al., 2018).

miRNAs Molecular Mechanism of Action

miRNAs have the ability to recognize target genes that are complementary to their nucleotide sequences. Depending on the degree of complementarity between the miRNAs and their target mRNAs, miRNAs' gene expression regulatory impact results in the inhibition of mRNA translation and/or degradation (Peixoto da Silva et al., 2022). miRSC can bind to the untranslated region (UTR) at the 3' end of the target mRNA or to the ORF (open reading frame) region and 5' end of the target mRNA under the guidance of the mature miRNA in its structure (Szudy-Szczyrek et al., 2022). Each miRNA can regulate the expression of multiple mRNAs, and different miRNAs may target the same mRNA (Kent & Mendell, 2006).

Multiple Myeloma and miRNAs Relationship

While multiple myeloma treatments have advanced, there are still uncharted territories in medicine, particularly in cancer treatment (Lancman et al., 2021). The changes in the expression levels of miRNAs in different cancer types have been investigated, and the difference between normal and pathological tissues has begun to be investigated extensively. miRNAs are dysregulated in MM cases and are essential in disease progression or suppression (Handa et al., 2019). According to reports, miRNAs are involved in cell proliferation, invasion, apoptosis, and metastasis in MM cells (Szudy-Szczyrek et al., 2022). Depending on the molecular pathways of the mRNA they target, miRNAs exhibit either oncogenic or tumor-suppressive properties (Otmani & Lewalle, 2021). Tumor suppressors are miRNAs that regulate the production of an oncogene, and oncogenes are miRNAs that repress tumor suppressors (Peng & Croce, 2016). Oncogene and tumor suppressor miRNAs that play a role in different biological processes of MM development and the diagnosis/treatment of the disease are shown in Figure 3.

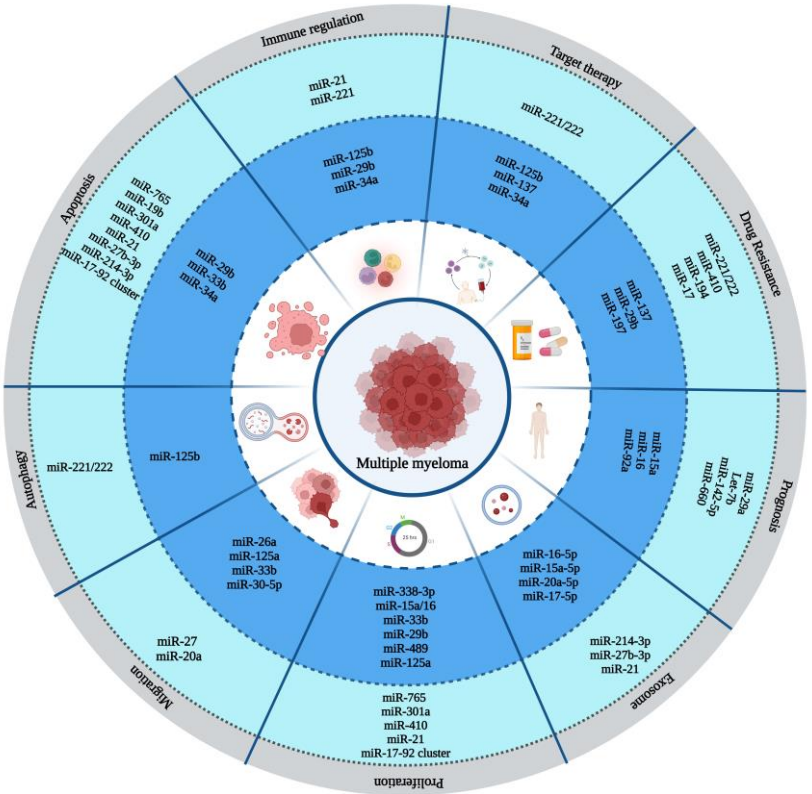

Figure 3. *Multiple myeloma development has been related to miRNAs. The diagram shows the roles of miRNAs in multiple myeloma. The blue colored layer indicates downregulated miRNAs, the third layer in green shows upregulated miRNAs in multiple myeloma. Created with BioRender.*

Tumor Suppressor miRNAs in Multiple Myeloma

Tumor suppressor miRNAs are down-regulated in MM. These miRNAs function, among other ways, to prevent the formation of tumors by blocking important oncogenes (Soliman et al., 2020).

The miR-34 family (miR-34a, miR-34b, and miR-34c) consists of three miRNAs and functions as tumor suppressors

(Dimopoulos et al., 2013). Various malignancies, including MM, exhibit dysregulated and downregulated expression of miR-34a, which has a potent tumor suppressor effect. miR-34a targets molecules such as MYC, BCL-2, and CD44, negatively regulating cancer stem cells (Bader, 2012). In animal models, miR-34a analogs have been shown to suppress MM growth by activating apoptosis and mediated by CDK6, BCL2, and NOTCH1 kinases (Szudy-Szczyrek et al., 2022). Di Martino et al. reported that overexpression of miR-34a in MM cells leads to the downregulation of BCL2 and NOTCH1 and induces apoptosis (Maria T Di Martino et al., 2015).

The miR-15a/miR-16-1 cluster has tumor suppressor effects by targeting oncogenes such as BCL2 and CCND1 (Aqeilan et al., 2010). In cells transfected with miR 15a/16-1, it has been observed that apoptosis was induced and tumor development was inhibited. Both miR-15a and miR-16-1 have been shown to inhibit a variety of pathways, including mitogen-activated protein (MAP)-kinases, AKT serine/threonine-protein-kinase, and ribosomal-protein-kinases (Roccaro et al., 2009).

It has been stated that miR-15a/16-1 targets VEGF and may contribute to angiogenesis during MM development (Sun et al., 2013). In addition to their role in angiogenesis, miR-15a/16-1 has been reported to promote the migration of MM cells in vivo models together with miR-192, miR-194, and miR-215 (Pichiorri et al., 2022; Roccaro et al., 2009).

miR-29b has been reported to function as a tumor suppressor in MM. Overexpression of miR-29b has been shown to induce MCL-1 downregulation and trigger apoptosis through caspase 3 activation (N. Amodio et al., 2012; Y. K. Zhang et al., 2011). Additionally, it has been reported that miR-29b suppresses cell proliferation by targeting CDK6 (N. Amodio et al., 2012) and inhibits tumor development by contributing to cell differentiation (Kapinas & Delany, 2011) methylation (Nicola Amodio et al., 2012), and drug resistance (N. Amodio et al., 2012).

miR-155 expression is downregulated in MM patients, and miR-155 replacement has pro-apoptotic and anti-proliferative effects in MM cells (Nicola Amodio et al., 2019). It has also been stated that it is effective in drug resistance and increases the sensitivity of drug-resistant MM cells to Bortezomib by targeting miR-155, tumor necrosis factor alpha-induced protein 8 (TNFAIP8) (Rastgoo et al., 2020).

miR-125a and miR-125b also have tumor suppressive effects in MM. MM cell viability and colony formation capacity are reported to be decreased by miR-125a (L. Wu et al., 2020), whereas miR-125b inhibits interferon regulatory factor 4 (IRF4), reduces tumor growth, and promotes apoptosis and cell death (Hamasaki et al., 2005; Soliman et al., 2020).

miR-137/197 expression is downregulated in MM cells and patients. It has been demonstrated that these molecules target MCL-1 and contribute to the migration, colony formation, viability inhibition, and induction of apoptosis in MM cells. Additionally, it was stated that inhibition of miR-137/197 would improve MM cell survival and decrease their sensitivity to bortezomib (Y. Yang et al., 2015).

Upregulation of miR-33b has been shown to reduce cell viability, migration, and colony formation and promote apoptosis in MM. It has been stated that miR-33b blocks the binding between BCL2 agonist (Bad) and BCL2/1-XL, which causes inhibition of apoptosis by targeting PIM-1 kinase (Szudy-Szczyrek et al., 2022; Tian et al., 2012).

Hu et al. reported that overexpression of miR-26a reduces proliferation and cell migration and triggers apoptosis in MM cell lines. It was demonstrated that miR-26a directly targets the CD38 protein (Szudy-Szczyrek et al., 2022).

According to research, miR-489 is downregulated in MM cells, which prevents cell growth and survival. miR-489 has been

shown to reduce glucose uptake, thus ATP production, and target lactate dehydrogenase-A (LDHA) (H. Wu et al., 2020).

The contact between MM cells and bone marrow mesenchymal stromal cells (BMSCs), which in turn promotes the production of BCL9, a transcriptional co-activator of the Wnt signaling pathway known to enhance MM cell proliferation, has been proven to be the cause of this (Chu et al., 2014).

miRNA-338-3p has been reported to be a tumor suppressor in many types of cancer. It has been stated that miR-338-3p functions as a tumor suppressor in MM by inhibiting CDK4 and affecting cell proliferation, apoptosis, caspase 3, and caspase 8 activities in MM cells (Cao et al., 2018).

Oncogene miRNAs in Multiple Myeloma

miR-30-5p downregulation is a frequent pathogenetic occurrence in MM. Many miRNAs with oncogenic effects have been identified and associated with the development or spread of MM. According to reports, the expression of oncogenic miRNAs is increased in MM (Szudy-Szczyrek et al., 2022).

miR-21 targets genes involved in proliferation, apoptosis, and metastasis and is overexpressed in many types of cancer. It has been shown that miR-21 supports the development of MM, and due to overexpression of miR-21, the expression of PTEN, BTG2, Rho-B mRNA, and PTEN protein levels decreases (Leone et al., 2013). In the first study revealing the effect of miR-21 in MM, it was stated that miR-21 was upregulated by the IL-6/STAT3 pathway, and its overexpression inhibited apoptosis (Löffler et al., 2007). In 41 human multiple myeloma cells and 15 patient samples (10 with MM and five with MGUS), Pichiorri et al. (Pichiorri et al., 2008) analyzed miRNA expression sequences. The expression of miR-21 was elevated in both MGUS and patients with MM (Dimopoulos et al., 2013). It was also revealed that miR-21 is associated with drug resistance(Munker et al., 2010) and bone marrow microenvironment in MM (Dimopoulos et al., 2013; Wang et al., 2011)

The miR-17-92 cluster consists of six miRNAs, including miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1, and miR-92a-1 (Pichiorri et al., 2008). Abnormally increased expression of the miR-17-92 cluster is involved in the malignant progression of MM (Pichiorri et al., 2008; Szudy-Szczyrek et al., 2022). It is believed that miR-19a/19b molecules increase the growth of MM cells by downregulating the SOCS1 cytokine signaling protein's expression (L. Chen et al., 2011; Szudy-Szczyrek et al., 2022).

The expression of miR-181a/b is elevated in MGUS and MM tumor plasmocytes, and it has been suggested that miR-181a/b plays a significant role in the control of P53 (N. Liu et al., 2019; Pichiorri et al., 2008). It has been stated that miR-181a/b can negatively regulate P-300-CBP-related factor (PCAF) expression, antagonize the positive effect of PCAF on P53, and decrease P53 expression (Pichiorri et al., 2008). miR-181a is overexpressed in MM cells, regulating the expression of neuro-oncological ventral antigen-1 (NOVA1). Silencing of miR-181a has been stated to trigger cellular apoptosis. Additionally, it has been demonstrated to reduce the expression of the miR-181a inhibitor NOVA1 and inhibit tumor growth (N. Liu et al., 2019).

miR-221 is another known oncogenic miRNA and is overexpressed in many types of cancer, including MM. It was stated that increased expression of miR-221/222 in MM caused suppression of PTEN, PUMA, p27Kip1, and p57Kip2 genes and tumor cell growth (Maria Teresa Di Martino et al., 2013). miR-221/222 has been shown to mediate the inhibition of autophagy and increase drug resistance in plasma cells of MM patients (Xu et al., 2019). It can also promote the secretion of proinflammatory cytokines IL-6 and TNF-a, promoting MM cell growth (Sehgal et al., 2015).

Other oncomiRs that are increased in MM cells include miR-19b and miR-20a. miR-19b/20a inhibits apoptosis and increases plasma cell proliferation and migration. miR-19b/20a transfection

has been shown to reduce PTEN protein expression (Yuan et al., 2019).

miR-27 is also overexpressed in MM and is associated with shorter overall survival. It has been shown to promote MM growth by increasing cell proliferation, migration, and invasion through its Sprout 2 (SPRY2) homolog (Che et al., 2019).

miR-214-3p increases resistance to apoptosis in myeloma fibroblasts by targeting the apoptotic pathways of FBXW7 and PTEN/AKT/GSK3 (Frassanito et al., 2019).

It has been reported that miR-765 has increased expression in MM patients and cell lines, and it exhibits oncogenic properties in MM by regulating cell proliferation and apoptosis(Long et al., 2019).

Furthermore, it was demonstrated that miRNAs could affect crucial cellular pathways of MM cells: miR-410 suppresses apoptosis. It encourages cell cycle progression and proliferation (N. Yang et al., 2017); mir-301a targets TIMP2 in MM, promoting cell proliferation and inhibiting apoptosis. miR-301a may be a novel molecule in MM and provide helpful therapeutic strategies for the treatment of MM (Liang et al., 2015).

miRNAs as a Biomarker in Multiple Myeloma

In recent years, the number of studies focusing on guiding clinical practice and discovering reliable prognostic biomarkers in cancer has increased (D. Chen et al., 2021). miRNAs are regarded as essential diagnostic and prognostic molecules because of their stability in bodily fluids like plasma, serum, and urine and their relative simplicity for regular measurement. The association of miRNAs with different stages of disease offers significant promise for facilitating early diagnosis of disease, monitoring disease progression, and treatment response (Ahmad et al., 2014; D. Chen et al., 2021). Many miRNAs have been reported to be strongly correlated with the prognosis of MM patients (D. Chen et al., 2021). When examining, the research in the literature among the markers

were increased plasma miR-125b-5p, serum miR-29a, serum miR 4449, and decreased serum miR-30d and miR-203 (Gupta et al., 2019; Jiang et al., 2018; Sevcikova et al., 2013; Shen et al., 2017; Zhu et al., 2018). Kubiczkova et al. reported that increased miR-34a and decreased let-7e could distinguish MM from control with 80% sensitivity and specificity (Kubiczkova et al., 2014). Compared to healthy controls, MM patients' serum showed increased expression of miR-142-5p, miR-660, and miR-29a. It has been stated that miR-29 can be a biomarker with 88% sensitivity and 70% specificity (Sevcikova et al., 2013). Another study reported that newly diagnosed MM patients had increased levels of six miRNAs (miR-148a, miR-181a, miR-20a, miR-221, miR-625, and miR-99b) in their peripheral blood compared to healthy donors. MiR-20a and miR-148a have been reported to be prognostic and linked to shorter relapse-free survival (Federico et al., 2019). Let-7 family members let-7c, miR-20a, miR-103a, miR-140, and miR-185a were found to be significantly lower in MM, while miR-4505 and miR-4741 were found to be higher in this disease. These serum exosomal miRNAs have been suggested to be used as biomarkers for disease progression (Z. Zhang et al., 2019).

Therapeutic Importance of miRNAs in Multiple Myeloma

The oncogenic or tumor-suppressive properties of miRNAs by targeting multiple signaling pathways offer new approaches for miRNA-based therapies for MM (D. Chen et al., 2021; Szudy-Szczyrek et al., 2022). There are two essential approaches for miRNA-based therapeutic strategies. These are the inhibition of oncogenic miRNAs and the replacement of tumor suppressor miRNAs. The main goals of both strategies are to inhibit uncontrolled cell proliferation and inhibit the oncogenic properties of cancer cells by inducing apoptosis (D. Chen et al., 2021). Technology such as complementary RNA molecules (antagomirs), miRNA sponges (Ebert et al., 2007), oligonucleotide MASK, and CRISPR/Cas9 can be used to eliminate the impact of highly expressed oncogenic miRNAs (Szudy-Szczyrek et al., 2022). miR-

221/222, for instance, has proven to be a promising therapeutic target candidate. According to research, miR-221/222 inhibitors have an anti-proliferative effect by upregulating miR-221/222 targets such as p27Kip1, PUMA, PTEN, and p57Kip2 in MM cells (Maria Teresa Di Martino et al., 2014; Handa et al., 2019). The second goal is to restore down-regulated tumor suppressor miRNAs in MM cells. It reactivates the potential inhibitory effects of these molecules on tumor growth and metastasis, interfering with the oncogenic properties of the cell, thereby enabling the development of potent therapeutic tools for the treatment of MM (Ahmad et al., 2014; Soliman et al., 2020). Different techniques can replace tumor suppressor miRNAs, including viral vectors, non-viral vectors (inorganic compounds and lipid-based carriers), and miRNA mimics (Y. P. Liu & Berkhout, 2011; Soliman et al., 2020). According to reports, miR-15a/16-1 has been used to reduce tumor cells in vivo xenograft models (Sun et al., 2013). It has been shown that MM cell growth is inhibited, and apoptosis is induced in vitro by lentivirus-based permanent miR-34a expression or transient miR-34a synthetic mimic expression. This molecule has been shown to downregulate the mRNA and protein levels of its targets BCL2, CDK6, and NOTCH1 (Handa et al., 2019; Maria T Di Martino et al., 2015). According to Yang et al., there was a significant decrease in the expression of miR-137/197 in both MM cells and patients compared to control groups. Overexpression of miR-137/197 by transfection of synthetic mimics induced apoptosis and inhibited proliferation, colony formation, and migration ability of MM cells by targeting MCL-1 (Y. Yang et al., 2015). Another study stated that overexpression of miR-125b by transfecting with lentiviral vectors or synthetic mimics triggers apoptotic and cell death by directly targeting IRF4 (Morelli et al., 2015). There are some challenges to realizing miRNA therapeutic applications; they are an efficient delivery system for maintaining miRNA stability, possible undesirable toxicities, and delivery to target tissue (Handa et al., 2019; Soliman et al., 2020). miRNAs are crucial molecules for

creating novel therapeutic approaches, but more research is required before miRNAs may be used safely in the clinic.

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CHAPTER VIII

Diyabetin Karaciğer Üzerine Etkisi

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1. Giriş

Karaciğer, pek çok fizyolojik süreç için kritik bir merkez olarak tanımlanan bir organdır. Bu fizyolojik süreçler; makro besin metabolizması, kan hacminin düzenlenmesi, bağışıklık sistemi desteği, büyüme sinyal yollarının endokrin kontrolü, lipid ve kolesterol homeostazı ve mevcut birçok ilaç da dahil olmak üzere ksenobiyotik bileşiklerin parçalanması şeklinde ifade edilebilmektedir (Trefts vd., 2017:1141-1155). Karaciğerde diyabet

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kaynaklı glikojen ve lipid metabolizmasını etkileyen çeşitli yapısal ve fonksiyonel bozukluklar şekillenmektedir (Schmatz R vd., 2012). Karaciğer de dahil olmak üzere birçok organda diyabet sonucu oksidatif stresin arttığı belirtilmiştir (Manna vd., 2010:1464-1484). Hiperglisemiden kaynaklı oksidadif stres sonucunda karaciğer hepatositlerinde belirgin şekilde şişkinlik, nekroz, kromatin yoğunlaşması ve apoptotik cisimler meydana geldiği bildirilmiştir (Manna vd., 2010:1464-1484). Bunun yanısıra oksidadif strese en duyarlı yapılardan birisi olan pankreas β hücrelerinde oluşan hasarın hipergliseminin toksik etkilerinden dolayı gözlendiği düşünülmektedir (Oishi vd., 2018:141-146). Diabetes mellitus pankreasın Langerhans adacıklarındaki β hücrelerinden insülin salınımı, insülin hormonu duyarlılığı ya da her ikisinde meydana gelen defektler sonucu oluşan kronik hiperglisemi ile karakterize edilen metabolik bir hastalıktır (Özdenoglu ve Ünver Saraydin, 2016:742-751). Diyabette, karbohidrat, yağ ve protein metabolizmasında anormallikler meydana gelmektedir. Ayrıca glukogenolizis ve glukoneogenez yolları ile glukozun karaciğerde üretimi artış göstermekte, kas ve yağ doku gibi perifer dokular tarafından glukozun alınması da azalmaktadır (Saraydın vd., 2022:1-11).

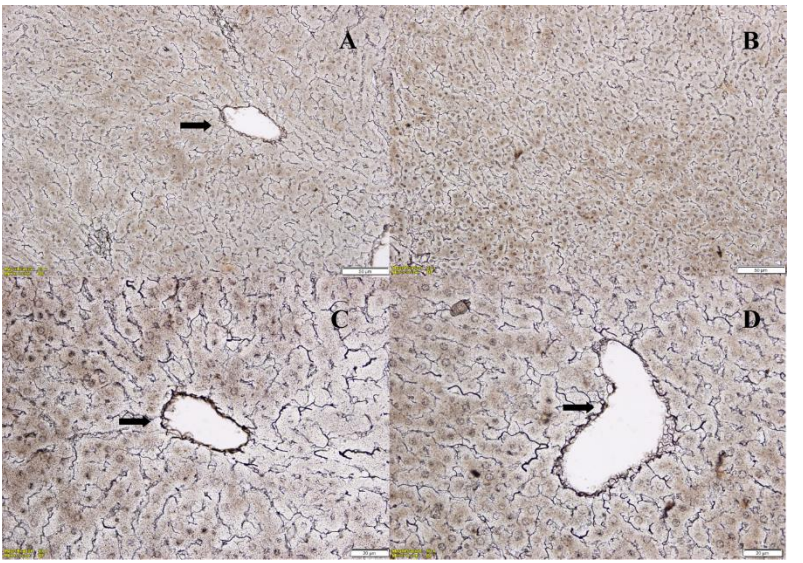
2. Yöntem

Çalışmamızda ağırlıkları yaklaşık 250-350 gram ağırlığında 8-10 haftalık Wistar Albino cinsi erişkin dişi rat kullanıldı. Hayvanlar standart pellet yem ve çeşme suyu ile beslendi ve kontrol ve diabetik olmak üzere iki gruba ayrıldı. Hayvanlar östrus siklusuna göre; östrüs dönemlerinde alındılar. Sıçanlardan, kontrol grubu için serum fizyolojik (%0.9 NaCI), deney grubuna ise 60 mg/kg derişimlerde hazırlanan streptozotosin, intraperitonal yoldan enjekte edildikten 30 gün sonrasında hayvanların karaciğer dokuları çıkarıldı. Doku örnekleri %10 tamponlanmış nötral formalin çözeltisinde tespit edildikten sonra farklı konsatrasyonlarda alkoller ile dehidrasyon ve ksilol ile şeffaflandırma işlemi yapılarak sonrasında parafin bloklara gömüldü. Devamında parafin bloklardan 3 μm kalınlığında kesitler

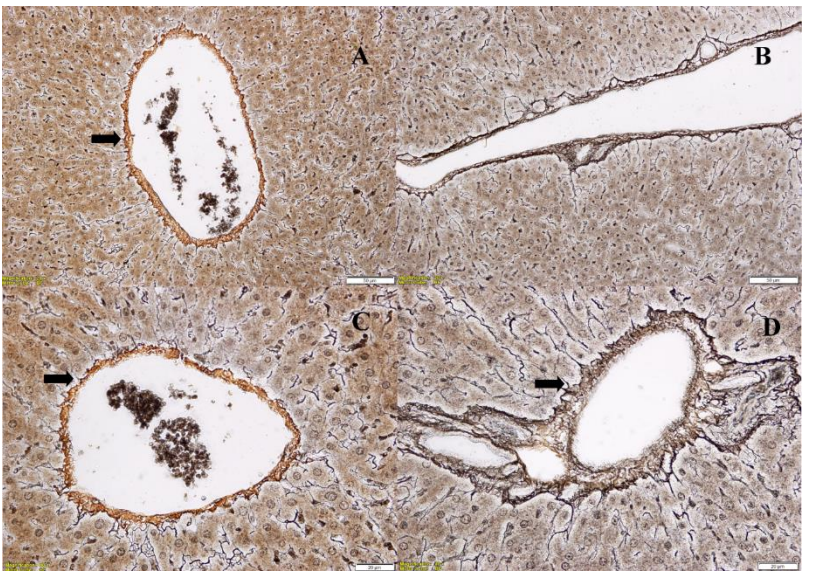
alındı. Daha sonra karaciğerde, retiküler lifleri gösterebilmek için gümüş çöktürme, MMP-9 proteinini göstermek için ise immünohistokimyasal teknikler yöntemi uygulandı. Mikroskobik değerlendirme için alınan kesitler ışık mikroskobunda değerlendirilerek uygun alanlardan fotoğraflar çekildi.

3. Bulgular

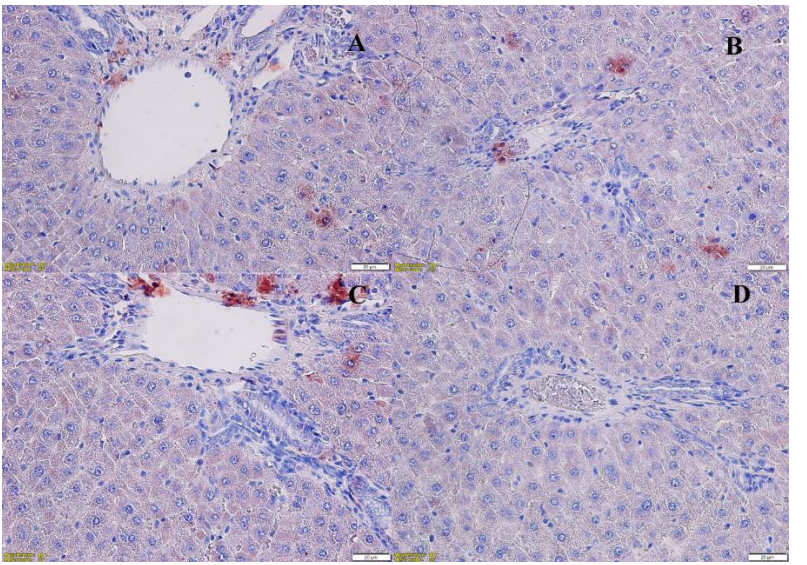
Kontrol grubu ile deney grubu karaciğer doku örnekleri karşılaştırıldığında, iki grup arasında anlamlı farklılıklar bulundu. Doku örneklerine karşılıklı bakıldığında, deney grubunun lif dağılımı açısından kontrol grubuna oranla daha yoğun olduğu belirlendi (Şekil 1,2). Özellikle vena centralis çevresinde retiküler lif yoğunluğu dikkat çekti (Şekil 2). Karaciğer örnekleri immünohistokimyasal olarak MMP-9 boyanma yönüyle incelendiğinde ise, deney grubunda özellikle vena centralis çevresine yakın konumlanmış hepatositlerde MMP-9 lokalizasyonu görüldü. MMP-9 lokalizasyonu hepatositlerin sitoplazmik alanlarında izlendi (Şekil 3). Kontrol grubunda ise MMP-9 ekspresyonu, yok denecek kadar az yoğunlukta izlendi (Şekil 4).



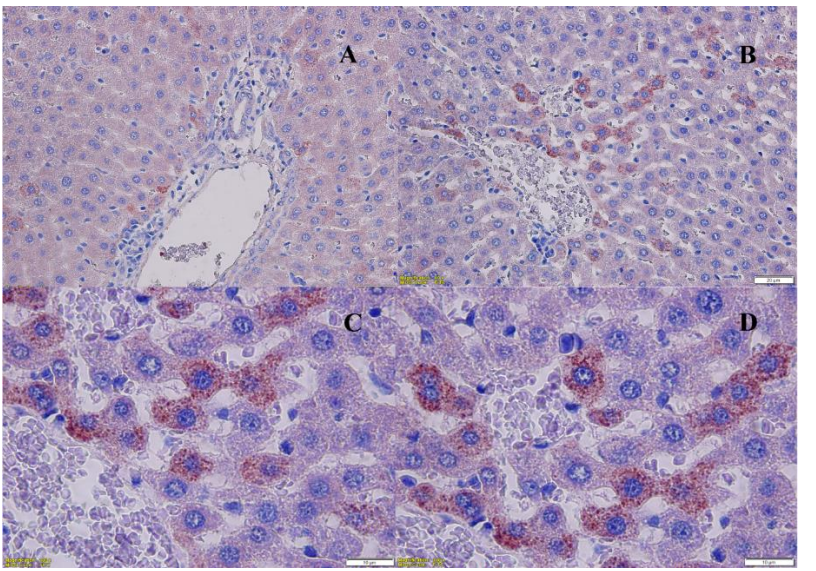
Şekil 1. *Kontrol grubu retiküler lif dağılımı. Vena centralis (➡). A) 20X, B) 20X, C) 40X, D) 40 X.*



Şekil 2. *STZ grubu retiküler lif dağılımı. Vena centralis (➡). A) 20X, B) 20X, C) 40X, D) 40 X.*



Şekil 3. *Kontrol grubu MMP-9 lokalizasyonu. A) 40X, B) 40X, C) 40X, D) 40 X.*



Şekil 4. *STZ grubu MMP-9 lokalizasyonu. A) 40X, B) 40X, C) 100X, D) 100 X.*

4. Sonuç

Diyabet kaynaklı komplikasyonların gelişmesinde hiperglisseminin indüklediği oksidatif stres önemli bir rol oynamaktadır. Kronik hiperglisemi, reaktif oksijen türlerinin aşırı üretimi sonucu vücudun redoks dengesinin değişmesine neden olarak oksidatif strese katkı sağlamaktadır (Prasath ve Subramanian, 2013:249-255). Süperoksit dismutaz, glutatyon peroksidaz ve katalaz gibi antioksidan enzimlerin ekspresyonlarının ve antioksidan kapasitenin pankreas adacık hücrelerinde, karaciğer, böbrek, iskelet kası ve adipoz doku gibi dokularda diğer dokularla karşılaştırıldığında oldukça az düzeyde olduğu bilinmektedir. Dolayısıyla oksidatif stresin ve antioksidan kapasitede meydana gelen değişikliklerin, diyabetin uzun dönemli komplikasyonlarının oluşmasıyla ilişkili olabileceğini vurgulamıştır (Ahmed vd., 2014: 1472-6882-14-243). Çalışmamızda diyabet oluşturulan grupta, elastik liflerde yoğunlaşmaya rastlanılmıştır. Yukarıda da söylendiği gibi diyabet kaynaklı oksidadif stresin, diyabet grubunda karaciğer dokusu üzerindeki etkisinden kaynaklı liflerde yoğunlaşma meydana gelmiş olabilir. Karaciğer, sentral metabolik bir organ olup diyabet sonucu meydana gelen oksidatif hasara bağlı olarak gelişen reaktif oksijen türlerine maruz kaldığı bildirilmiştir. Bu sebepten dolayı hepatositlerde ve endotelyal hücrelerde apoptozis gözlendiği belirtilmiştir (Senoner ve Dichtl, 2019:2090). STZ deney modeli ile gerçekleştirilen diyabet çalışmalarında hepatositlerde nekroz, portal alanlarda bozukluk ve sinüzoidlerde dilatasyon gibi durumlar oluşmuştur (Zhang vd., 2012). Oksidadif stres ve hiperglisemi kronik inflamasyon MMP-9 gibi matriksmetalloproteinazların ekspresyonlarını ve serum konsantrasyonlarını artırıcı yönde etki yapmaktadır. Bunun yanı sıra oksidadif stresin MMP'leri etkileyerek ekstrasellüler matriksin önemli bir düzenleyicisi olabileceği de söylenmektedir (Tacke ve Trautwein., 2015:9). Çalışmamızda da deney grubunda karaciğer örneklerinde MMP-9 ekspresyonun artış göstermesi benzer sonuç taşımaktadır. Karaciğer, diyabet ve MMP-9 lokalizasyonun aydınlatılabilmesi için tabii ki daha fazla çalışmaya ihtiyaç vardır.

Kaynakça

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