

An Experimental Model Study: Histomorphological and Ultrastructural Renal Changes in 1/2-5/6 Nephrectomized and Diabetic Rats

DeneySEL Model Çalışması: 1/2-5/6 Nefrektomize ve Diyabetik Ratlarda Böbreğin Histomorfolojik ve Ultrastrüktürel Değişiklikleri

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ABSTRACT Objective: Diabetes and hypertension are often seen together and their prevalence shows large variability according to geographical, cultural, demographic, nutritional and genetic factors. These diseases affect the kidneys directly or indirectly. In this study, our aim is to compare of the effect of diabetes on kidney histomorphologically and by electron microscopic evaluation using two models of the different variations of the remnant kidney model as in surgical unilaterally nephrectomized (1/2Nx) and subtotal nephrectomized (5/6Nx) rats. **Material and Methods:** For this aim, six groups were created: Control, Diabetes mellitus (DM), 1/2 (right) nephrectomy, 5/6 nephrectomy, 1/2 (right) nephrectomy+DM and 5/6 nephrectomy+DM. Kidney tissues were analyzed using histomorphometric and ultrastructural analysis. **Results:** Renal damage has showed a very rapid progression with DM and hypertension. In 1/2 Nx group with unilateral nephrectomy, the renal damage progression is not rapid as in 5/6 Nx group, but glomerular injury increases with diabetes. Scar areas, Bowman capsule thickness, nuclei/glomerulus and glomerular diameter in DM and hypertension groups were significantly higher than control and 1/2 nephrectomy groups. 5/6 Nx+Diabetes group observed the most rapid progress overall in both light and electron microscopic measurements. **Conclusion:** Glomerular damage has showed a very rapid progression together with diabetes and hypertension. We think that our results would supply important histomorphometric and ultrastructural data and contribute to the literature especially for the model studies of hypertension and diabetes, together and separately.

Key Words: Nephrectomy; diabetes mellitus; rats, wistar; kidney; hypertension

ÖZET Amaç: Diabetes mellitus (DM) ve hipertansiyon sıklıkla birlikte gözlenir ve görülme sıklıkları coğrafik, kültürel, demografik, beslenme ve genetik faktörlere bağlı olarak geniş bir çeşitlilik gösterir. Bu hastalıkların etkileri böbreği direkt ya da indirekt olarak etkiler. Bu çalışmada, diyabet modeli ve cerrahi yolla oluşturulan iki farklı model olan unilateral nefrektomi (1/2Nf) ve subtotal nefrektomili (5/6Nf) sıçanlarda, (DM) ve iki farklı remnant böbrek modelinin birlikte böbrek üzerine etkisinin histomorfolojik ve elektron mikroskopik olarak karşılaştırılması amaçlanmıştır. **Gereç ve Yöntemler:** Çalışmada 6 grup oluşturuldu: Kontrol, Diyabet, 1/2 (sağ) Nf, 5/6 Nf, 1/2 (sağ) Nf+Diyabet ve 5/6 Nf+Diyabet. Böbrek dokuları histomorfometrik ve ultrastrüktürel olarak analiz edildi. **Bulgular:** DM ve hipertansiyonun birlikte gözlenmesiyle böbrek hasarının artış gösterdiği gözlemlendi. Unilateral 1/2 Nf grubunda böbreğin hasarlanması 5/6 Nf grubundaki kadar hızlı ilerlemedi, ancak diyabetle birlikte glomerül hasarda artış gözlemlendi. DM ve hipertansiyon gruplarında skar alanı, Bowman dış yaprak kalınlığı, çekirdek/glomerül ve glomerül çapı, kontrol ve 1/2 Nf grubuna göre anlamlı olarak artmıştı. 5/6 Nf+Diyabet grubunda ise hem ışık mikroskopik, hem de elektron mikroskopik ölçümlerde çok hızlı ilerleme olduğu saptandı. **Sonuç:** DM ve hipertansiyon birlikte gözlemlendiğinde glomerül hasar hızı çok artmaktadır. Bizim sonuçlarımızın hipertansiyon ve diyabetin birlikte ve ayrı ayrı gözlemlendiği bir model çalışması olarak, histomorfometrik ve ultrastrüktürel verilerle birlikte literatüre önemli bir katkı sağlayacağını düşünmekteyiz.

Anahtar Kelimeler: Nefrektomi; diabetes mellitus; sıçanlar, wistar; böbrek; hipertansiyon

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Hypertension is the most common cause of morbidity and mortality. It may lead to stroke, end-stage renal failure, myocardial infarction and congestive heart failure. It is a polygenic and a multifactorial disease that can develop as a result of genetic and environmental factors. Diabetes mellitus is defined as an impaired insulin production and/or development of glucose intolerance resulting in endocrine disorders. These two diseases are often seen together and their prevalence shows large variability according to geographical, cultural, demographic, nutritional and genetic factors.^{1,2} Furthermore, these diseases affect the kidneys directly or indirectly. It is still unexplained whether renal disease causes hypertension or hypertension aggravates the renal disease. Mostly, retrospective clinical studies are undertaken in order to clarify this problem. Besides, in order to explain the mechanisms between these diseases, different experimental model studies are continuing.

Experimental hypertension models include, genetic phenotype-driven (eg, spontaneously hypertensive rats, SHR) or genotype-driven (eg, null mice) models, or nongenetic (surgical intervention, as the endocrine, diet modification, partial or subtotal nephrectomy) models.³ One of the surgical models is obtained by total or subtotal nephrectomy. By this method, creation of secondary hypertension causes the renal damage.⁴

Experimental models of diabetes are applied by chemical agents [Alloxan, Streptozotocin (STZ) injection], spontaneously (genetically induced), through viruses or created through surgical procedures (eg, pancreatectomy).⁵⁻⁷ With these methods, progressive renal injury is investigated by many studies in literature.^{8,9}

In this model of hypertension, the effect of systemic hypertension on the kidney is controversial, while in diabetic rats, increased glomerulosclerosis is seen with the systemic hypertension added to diabetes. Similarly, hypertension is also a bad predictor of renal prognosis with different etiologies of renal insufficiencies.¹⁰⁻¹³ In the experimental models of diabetes and hypertension, generally spontaneously hypertensive rats or transgenic species are used. Increased renal damage is

parallel with these two diseases.¹⁴⁻¹⁶ Recently, renal damage is evaluated by addition of partial or total nephrectomy to STZ-induced diabetes models.

In this study, our aim is to compare the two models of the different variations of the remnant kidney model as in surgical unilateral nephrectomy (1/2Nx) and subtotal nephrectomy (5/6Nx) and in nephrectomised+STZ injected rats histomorphologically and by electron microscopic (EM) evaluation.

MATERIAL AND METHODS

The experiments were performed on 42 male Wistar (6–8 months old and weighing 230–260 g) rats and in accordance with the guidelines provided by the Experimental Animal Laboratory. The study is approved by the Animal Care and Use Committee of University School of Medicine. Experimental design is seen in Table 1.

EXPERIMENTAL MODELS

Diabetes mellitus (DM) model: DM was induced by administration of intraperitoneal STZ (S0130-1G, Sigma, Aldrich) (dissolved in citrate buffer of pH 4.5) at a dose of 45 mg/kg to the rats.¹⁷ On the 3rd day after STZ administration, nonfasting serum glucose levels were measured using a Medisense Optium glucometer (Roche Diagnostic, Germany). Rats with blood glucose levels higher than 250 mg/dl were considered as diabetic.

Nx models: *1/2Nx model*; subsequent to a mid-line abdominal incision, rats were 1/2 right nephrectomized. *5/6 Nx model*; was performed by

TABLE 1: Experimental design.

Group	Abbreviation	Experimental Time
Control	C	8week
Diabetes mellitus	DM	8week
1/2 (right) nephrectomy	1/2 Nx	8week
5/6 nephrectomy	5/6 Nx	8week
1/2(right) nephrectomy+ Diabetes Mellitus	1/2 Nx+DM	3 week+8week
5/6 nephrectomy+ Diabetes Mellitus	5/6 Nx+ DM	3 week+8week

right nephrectomy followed by partial infarction of approximately two-thirds of the left kidney by selective ligation of two to three of three to four extrarenal branches of the left renal artery.¹⁸ *Nx+DM model*; 1/2Nx and 5/6Nx models were applied and 3 weeks after the procedure, the single dose STZ was administered intraperitoneally and 8 weeks were awaited for Nx+DM models to be settled.

SYSTOLIC BLOOD PRESSURE MEASUREMENTS

The animals were anesthetized by 1.5 g/kg intraperitoneal urethane. Tracheal cannula was inserted via a tracheotomy and sutured. A catheter was inserted into the right carotid artery in order to monitor systemic mean blood pressure with a pressure transducer. MP30 BPT300 transducer was connected to a computerized system for data acquisition (MP Biopac system Inc., Santa Barbara, California, USA). Arterial catheter was filled with heparinized saline (250 U/ml). Blood samples were measured. Rats with blood pressure levels higher than 120 mgHg were considered as hypertensive.¹⁹

LIGHT MICROSCOPIC EXAMINATION

Left kidneys were removed and fixed in 10% formalin. After routine histological procedures, kidney specimens were blocked and 4 μ m sections were taken on poly-L lysine coated slides.

In the hematoxylin and eosin (H&E) stained sections, the number of nuclei per glomerulus, glomerular diameter (x40), in the PAS stained slides, the Bowman capsule thickness (BCT) (x100), in the Masson's trichrom stained slides, scar areas were evaluated (x20).

For each slide, five standard areas (SA) were determined by drawing lines, extending from cortex to medulla. The images obtained from the selected areas and were analyzed by using a computer assisted image analyzer system consisting of a microscope (Olympus BX-51, Tokyo, Japan) equipped with a high-resolution video camera (Olympus, DP70, Japan). Three cortical images from in each SA and totally 15 images were evaluated for each rat (Figure 1A). Measurements were made using UTHSCA Image Tool for windows version 3.0 software program.

Scar areas included, global rather than segmental sclerotic glomeruli. Areas of interstitial fibrosis were solid and deeply stained compared to normal interstitial tissue; atrophic tubules defined as tubules smaller than normal, with thickened basement membranes or tubules larger than normal, with thin epithelium, artery and arterioles which were completely occluded. In these 15 cortical images, overall value was found as the total interstitial scar area scoring for every rat. The percentage of renal scar area was determined by dividing the total interstitial scarring area to the total test area.¹⁸

ELECTRON MICROSCOPIC EXAMINATION

Tissue samples for EM (about 1 mm³ in size) were fixed in 2.5% gluteraldehyde. The specimens were washed in phosphate buffer and subsequently submerged in a solution containing equal amounts of osmium tetroxide and phosphate for 1 h. This procedure provided both staining and fixation. The specimens were then passed through a series of graded alcohol solutions and afterwards left in propylene oxide preparation for 10 min; they were then kept away from any further exposure to alcohol and prepared for final fixation: They were embedded in a fixative substance containing Araldite-CY 212 and dodecanyl succinate anhydride (DDSA) for one night. The next day they were placed in gelatin capsules that had been filled with a combination of Araldite-CY 212, DDSA and benzyl dimethylamine (BDMD), and these were incubated in an autoclave, first for 24 h at 40°C and then for 48 h at 60°C. The autoclave was shut down after this, and the tissues were left in it to cool so that polymerization was attained. The specimen lumps were cut into sections with a Reicheld LK ultra-microtome and stained with toluidine blue. These sections were examined with an Olympus BH-2 microscope, and the detection areas for inspection were marked for further slicing. The marked sections were cut into thin slices with the same ultra-microtome, and these thin slices were stained with uranyl acetate and then with lead citrate. Finally, all these tissue samples were examined in a Libra120 Carl-Zeiss transmis-

sion electron microscope, and the magnified images were photographed.

Thicknesses of the glomerular basement membranes (GBM) and parietal Bowman capsules were measured in fifteen glomerulus, for each rat. Areas of wrinkled glomerular basal membranes were excluded. The measurements were taken from electron graphs with magnifications x 10.000.

STATISTICAL ANALYSIS

Initial and final body weight, blood pressure, and blood glucose values were analyzed using the Wilcoxon signed rank test. Histomorphometric investigations (Bowman capsule thickness, scar areas, nuclei/glomerulus (n/Gl), glomerular diameter (GD) were evaluated by ANOVA variance analysis and Bonferroni test. All values were expressed as the mean \pm standard deviation. The Mann-Whitney U test was used to compare staining intensities values between groups. Spearman correlation test

was used for correlations between light and electron microscopic measurements. All statistical procedures were performed by SPSS software for Windows, Version 15.0 SPSS, Chicago, IL, USA. A value of $p < 0.05$ was considered significant.

RESULTS

Initial and final body weight measurements were evaluated for each rat. In the control (C) group, no significant difference was observed (239 ± 28.5 - 255.8 ± 21.9) [$p = 0.180$, no significant (NS)], 1/2 Nx (244.2 ± 50 - 259.6 ± 39.3) ($p = 0.345$ NS) and 5/6 Nx (228.8 ± 26.3 - 276.6 ± 34.4) ($p = 0.17$ NS). However in DM (263.8 ± 17 - 221 ± 35.1) [$p = 0.005$, significantly (S)], 1/2 Nx+DM (246 ± 21.1 - 213.5 ± 22) ($p = 0.024$, S) and 5/6 Nx+DM (256 ± 26.4 - 204.5 ± 44.5) ($p = 0.028$, S) groups, the body weights were decreased significantly at the end of the experiment (Figure 1B).

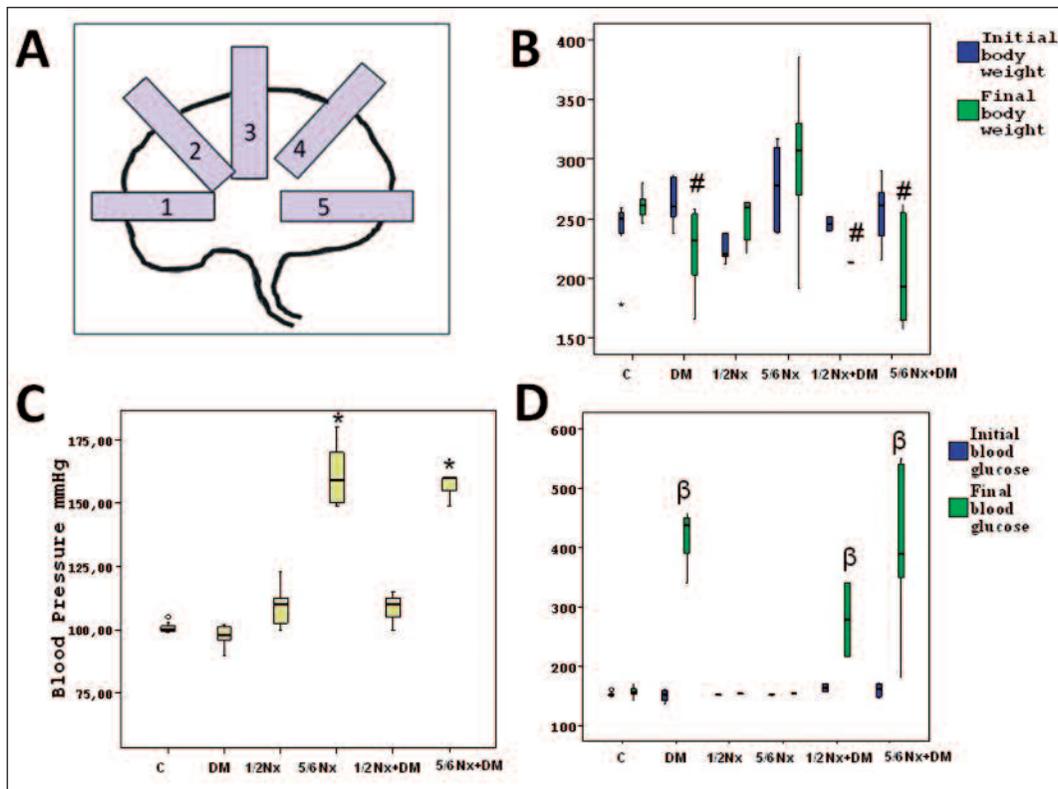


FIGURE 1: A: shows standard areas in the kidney slides for histomorphometric measurements. B: shows initial and final body weight, C: shows final blood pressure, D: shows initial and final blood glucose level measurements. #: Significant decrease vs initial body weight. *: Significantly increased vs other group, β: Significantly increased vs initial blood glucose level. $p < 0.05$ BP: blood pressure.

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Carotid artery blood pressure measurements were evaluated at the end of the study. 5/6 Nx (161.10±13.6) and 5/6 Nx+DM (159.80±10.1) groups' blood pressure measurements were significantly higher than the other groups (C: 98± 8.6, DM:97.7± 4.2, 1/2 Nx:109± 12.7, 1/2 Nx+DM:110± 8.6) ($p<0.05$, S) (Figure 1C).

When we looked at the initial and final blood glucose values; there was no significant difference observed between the C (151.8±4.1-156.6±9.5) ($p=0.176$, NS), 1/2 Nx (151± 4.2-153± 9) ($p=0.170$, NS) and 5/6 Nx (150± 4.1-157± 5) ($p=0.17$, NS) groups. On the other hand, in DM (150± 9.2-419± 42) ($p=0.005$, S), 1/2 Nx+DM (163.5± 7.1-277.5± 68) ($p=0.024$, S) and 5/6 Nx+DM (159.6± 11.4-399.8± 136) ($p=0.028$, S) groups, the initial and final blood glucose values showed a significant increase at the end of the experiment (Figure 1D) (Table 2).

LIGHT MICROSCOPIC EVALUATION

Light microscopic evaluation of the renal cortex revealed that, the glomeruli had normal histomorphology, the epithelium was normal in proximal and in distal tubules, proximal tubules had intact brush border, the connective tissue between tubules and glomeruli had normal structure in H&E staining in the C group. In the 1/2 Nx group, interstitial fibrosis, dilatation of tubules and debris, expansion in glomerular matrix were seen. In DM group, glomerular sclerotic structures, interstitial fibrosis, tubular dilatation and debris were observed as seen in diabetic nephropathy. In 5/6 Nx group, severe glomerular sclerosis, tubular hyalinization, debris and degradation of the tubular structures were seen. In 5/6 Nx+DM and 1/2 Nx+DM groups, combinations of all findings in diabetes and nephrectomy groups were found (Figure 2).

HISTOMORPHOMETRIC ASSESSMENT

Scar Areas: Scar areas of all groups were significantly higher than the C group ($p<0.001$, S). There was no significant difference between DM and 1/2 Nx groups ($p=1.00$, NS). 5/6Nx group's scar area was significantly larger ($p=0.02$, S) than DM group. In 1/2 Nx group, the scar area was significantly smaller ($p=0.002$, S) than 5/6 Nx group. Be-

TABLE 2: Blood glucose values of groups (mg/dL).

Group	Blood Glucose	
	Initial (mean±SD)	Final (mean±SD)
C	151.8±4.1	156.6±9.5
DM	150± 9.2	419± 42
5/6 Nx	150±4.1	157±5.0
1/2 Nx	151.0± 4.2	153± 9.0
1/2Nx+DM	163.5±7.1	277.5±68
5/6NX+DM	159.6±11.4	399.8±136

DM: Diabetes mellitus, Nx: Nephrectomy.

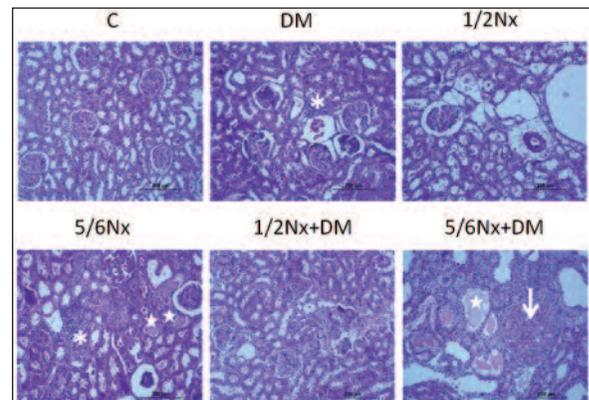


FIGURE 2: H&E staining of all groups. Normal histological structure was seen in renal cortex in C group. Histological abnormalities were seen in other groups. (*): interstitial inflammation, (star): accumulation of hyaline material in tubules, (Arrow): sclerotic glomerulus. Scale bars: 200 µm.

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sides, the scar area was significantly smaller in 1/2 Nx+DM group compared to 5/6 Nx+DM group ($p<0.001$, S). (Table 3). There was no significant difference between 1/2 Nx -1/2 Nx+DM groups and 5/6 Nx -5/6 Nx+DM groups ($p=1.000$, NS). However, scar areas were increased in 1/2 Nx+DM and 5/6 Nx+DM compared to 1/2 Nx and 5/6 Nx groups.

Bowman's Capsule Thickness (BCT): BCT in the C group showed an increase when compared to 1/2 Nx group, but the difference was not significant. BCT measurements in the other groups were significantly higher than the C group ($p<0.001$, S). BCT was significantly higher ($p<0.001$, S) in DM group compared to 1/2 Nx group. However, BCT in 5/6 Nx and 5/6 Nx+DM groups were significantly higher ($p=0.008$, S) than DM and 1/2 Nx+DM groups ($p<0.001$, S) (Table 3). BCT was significantly

TABLE 3: Histomorphometric measurements of groups.

Group	Scar area(μm^2)	BCT (μm)	n/GI	GD (μm)	GBM thickness (nm)
	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD
C	0.0104 \pm 0.01	1.50 \pm 0.45	61.35 \pm 11.8	59.49 \pm 10.53	160,65 \pm 19,6 ^d
DM	0.1111 \pm 0.05	3.85 \pm 1.31	80.73 \pm 12.7	71.06 \pm 9.21	339,42 \pm 42,0
1/2Nx	0.0783 \pm 0.05	1.98 \pm 0.69	71.76 \pm 11.3	64.62 \pm 9.57	159,81 \pm 13,5 ^d
5/6Nx	0.2703 \pm 0.20 ^a	4.49 \pm 1.63 ^c	93.36 \pm 16.8 ^c	75.69 \pm 11.3 ^c	366,80 \pm 34,7
1/2Nx+DM	0.0983 \pm 0.05	3.69 \pm 2.55	78.86 \pm 11.9	66.81 \pm 8.57	331,22 \pm 22,4
5/6Nx+DM	0.3090 \pm 0.11 ^b	4.73 \pm 1.57 ^c	87.78 \pm 21.1 ^c	77.44 \pm 13.4 ^c	373,27 \pm 59,6
	P	P	P	P	P
C -DM	<0.001	<0.001	<0.001	<0.001	<0.001
C-1/2Nx	1.000	1.000	0.39	0.02	1.000
C-5/6Nx	<0.001	<0.001	<0.001	<0.001	<0.001
C-1/2Nx+DM	<0.001	<0.001	<0.001	<0.001	<0.001
C- 5/6Nx+DM	<0.001	<0.001	0.28	<0.001	<0.001

BCT: Bowman capsule thickness; n/GI: nuclei/glomerulus; GD: Glomerular Diameter; GBM: Glomerular basal membrane; #: Significantly increased vs DM. #: Significantly increased vs 1/2Nx+DM. #: Significantly increased vs DM and 1/2Nx+DM. #: Significantly decreased vs other group. ^{a,b,c,d}: p<0.001.

higher in 1/2 Nx+DM group compared to 1/2 Nx groups (p<0.001, S) and in 5/6 Nx+DM compared to 5/6 Nx group (p<0.001, S).

Nuclei/glomerulus/ (n/GI): The number of nuclei per glomerulus in all groups were significantly higher than the C group (p<0.001, S). There was no significant difference between DM and 1/2 Nx groups (p=0.171, NS). In 5/6 Nx group, GI/n ratio was significantly higher (p=0.02, S) than DM and 1/2 Nx groups. In 1/2 Nx+DM group, GI/n was significantly lower (p<0.001, S) than 5/6 Nx+DM group (Table 3). There was no significant difference between 1/2 Nx -1/2 Nx+DM groups and 5/6 N -5/6 Nx+DM groups (p=1.000, NS). On the other hand, n/GI was increased in 1/2 Nx+DM and 5/6 Nx+DM groups compared to 1/2 Nx and 5/6 Nx groups.

Glomerular diameter (GD): GD was significantly higher than the C group (p<0.001, S) in other groups. GD in DM group was significantly higher than 1/2 Nx group (p=0.02, S). GD in 5/6Nx group was significantly higher than 1/2 Nx group and DM group (p=0.02 S and p=0.0, S). 5/6Nx +DM group's GD was significantly higher than 1/2 Nx+DM (p=0.001, S) group (Table 3). There was no significant difference between 1/2 Nx -1/2 Nx+DM groups and 5/6 N -5/6 Nx+DM groups (p=1.000, NS). However, GD was increased in 1/2 Nx+DM

and 5/6 Nx+DM groups compared to 1/2 Nx and 5/6 Nx group.

ULTRASTRUCTURAL EVALUATION

Ultrastructural evaluation of GBM in the C group showed a regular structure, mesangial matrix was found to be normal. In all other groups, irregular GBM, subendothelial deposits, podocyte loss and foot process slimming were seen (Figure 3). GBM thickness of DM, 5/6Nx, 1/2 Nx+DM and 5/6

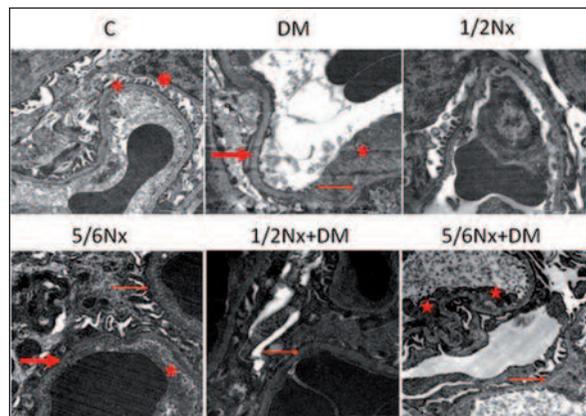


FIGURE 3: Electron microscopic micrographs. *: normal morphology of basement membrane, #: normal morphology of pedicels, thin arrow: irregularity at the glomerular basement membrane, thick arrow: pedicel loss, star: subendothelial deposits. Scale bars: 200 nm.

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Nx+DM groups were significantly increased compared to C group and 1/2 Nx group. Electron microscopic measurements of GBM are given in Table 3.

CORRELATION ANALYSIS

When all groups were analyzed, there was a statistically significant correlation between all groups: BCT and scar area ($r=0.82$, $p=0.042$, S), scar area and Gl/n ($r=0.88$, $p=0.042$, S), Gl/n and GD $r=0.88$, $p=0.019$, GBM and scar area ($r=0.94$, $p=0.005$, S) (Figure 4).

DISCUSSION

Many investigations are going on about the pathways that cause progressive damage in the kidneys by hypertension and diabetes.^{20,21} These diseases have effects on the kidneys such as glomerular hyperfiltration, increased glomerular capillary pressure, structural changes in glomeruli, increase in extracellular matrix, GBM thickening, enlargement of mesangial matrix and fibrosis.^{15,20,21}

Taking relationship between the kidney and hypertension into account, renal autoregulation is provided in essential hypertension and renal damage does not occur unless malignant hypertension develops. When the amount of kidney mass decreases and in the presence of diabetes mellitus, the pathophysiology of hypertensive renal damage changes. Preglomerular vascular dilatation and impaired renal autoregulation causes reflection of large proportion of systemic blood pressure to the renal microvascular bed. Therefore, damage is seen

in glomerular structures more than vascular structures.^{22,23} In animal experiments, Goldblatt et al. narrowed the renal vessels and formed hypertension for the first time in 1934.²⁴ By this model they brought an understanding to the pathogenesis of hypertension.

In experimental studies, loss of functional renal mass is created. As a result, glomerular hyperfiltration and glomerular hypertrophy are seen. Glomerular filtration rate remains constant in the short term, glomerular capillaries expose to high hydrostatic pressure and progressive renal damage starts. Hyperfiltration is provided with preglomerular vasodilatation and this leads more reflection of systemic blood pressure to the capillaries. In the experimental studies, 5/6 nephrectomy or remnant kidney model is a very common model for studying the progression of chronic renal failure.²⁵⁻²⁸ In this study, we examined and compared two models of the different variations of the remnant kidney model, unilateral nephrectomy (1/2Nx) and subtotal nephrectomy (5/6Nx). In the literature, Guray et al. formed unilateral and subtotal nephrectomy in rats to examine the effect of cyclosporine on renal mass loss.¹⁸ In histopathologic examination of the kidney; renal scar area, tubular damage, arterial damage scoring were used. A significant difference in the renal scar in unilateral and subtotal nephrectomy groups was observed. Renal scar area was significantly lower than in unilateral nephrectomy compared to subtotal nephrectomy.

In experimental diabetes, STZ-induced diabetic renal damage is formed.¹⁴⁻¹⁶ We have observed an increased renal damage in diabetes-induced rats both by light microscopic and electron microscopic evaluation. The effect of hypertension on renal injury alone is controversial while in rats with diabetic nephropathy, addition of systemic hypertension causes an increasing rate of glomerulosclerosis. Similarly in different etiologies of renal insufficiency, hypertension is also a bad predictor of renal prognosis.²⁹ Souza et al. induced diabetes by applying 50 mg/kg STZ to spontaneously hypertensive rats and examined GLUT-1 expression in renal cortex.¹⁵ In an

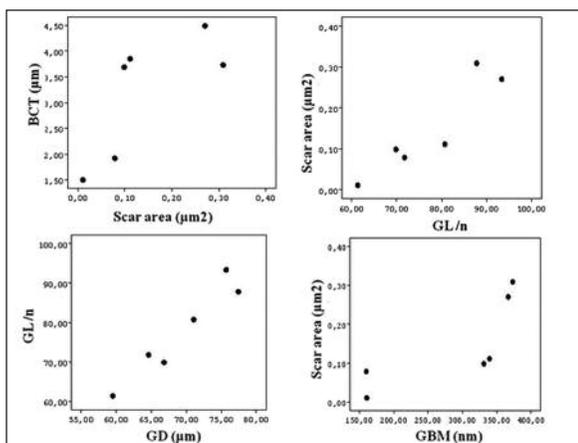


FIGURE 4: All parameters showed positive correlation.

other study, ren-2 transgenic mice with AII-dependent hypertension, diabetes was by created by applying 70 mg/kg STZ and renal injury was examined.⁸ Renal artery stenosis was evaluated in a clinical study with type II diabetic and non-diabetic hypertensive individuals.¹⁶ In addition to these studies, Clarkson et al. used uninephrectomized and 5/6 nephrectomized rat models, similar to our study.³⁰ They administered STZ and investigated the RNA extraction of the renal cortex. Chamberlin et al. observed an increase in hematopoietic growth factor inducible neurokinin (HGFIN, also known as Gpmb / Osteoactivin) in 5/6 nephrectomized and streptozotocin-induced diabetic rats and in patients with chronic kidney disease with real-time PCR.³¹ Uninephrectomized rats were made diabetic by streptozotocin by Komers et al. and in this model they examined Rho-associated kinases (ROCK) and their effects on blood pressure of the kidney.³² Similar to these models, we used a model, nephrectomy with diabetes, to examine the renal damage by histomorphometry and constituted a reference by comparing light microscopic and ultrastructural findings for advanced studies in the future. Similar results were obtained in our study also with renal damage. The renal scar area was measured as $0.0983 \pm 0.05 \mu\text{m}^2$ in 1/2Nx + DM group and as $0.1111 \pm 0.05 \mu\text{m}^2$ in DM group. The 5/6Nx + DM ($0.3090 \pm 0.11 \mu\text{m}^2$) group showed an increased renal scar area compared to all other groups.

We mainly aimed to assess the damage in kidney glomeruli in our study. We examined the light microscopic parameters such as increase in mesangial matrix, thickening of the parietal Bowman capsule and the glomerular diameter. By assessing the number of nuclei per glomerulus, we evaluated the glomerular damage. Considered together with all of these findings, glomerular injury occurs in the 1/2 nephrectomy group, but the damage is less than that observed in DM, 5/6Nx, 1/2Nx+DM and 5/6Nx+DM groups. In general, when DM and 5/6Nx groups were compared, 5/6Nx group had more rapid progression than DM group. On the other hand, the 5/6Nx + DM group showed the most rapid overall progress.

Aunapuu et al. created an experimental remnant kidney model.³³ They examined the effects of angiotensin receptor antagonist, losartan, and beta-blocking agent, atenolol in early kidney damage. They observed glomerular hypertrophy and focal segmental glomerulosclerosis in the early stages. In ultrastructural examination, Bowman's capsule was found normal or thicker than normal and the lumen was empty especially in the nephrectomized group. Besides, an increase in number of podocyte was seen. Large nuclei, electron dense granules, large vacuoles, lipid granules, increased GER and Golgi complex were found in podocytes. The number and cristae of mitochondria were increased and disorganized in the podocytes. Foot processes were wider than normal and the width of endothelial cells in glomerular capillaries were increased and their cytoplasm contained small vacuoles.³⁴ We observed similar findings in all groups except the C group, like the basement membrane irregularities, subendothelial deposits and podocyte loss in our ultrastructural examinations.

When literature findings and our results are considered, we conclude that evaluating the renal damage using both histomorphometric and ultrastructural data together will give more accurate results. There is a significant correlation between light microscopic and electron microscopic findings. The extent of renal injury/damage is increased in diabetes + 5/6 nephrectomy group, therefore all results should be interpreted as a whole.

As a result, glomerular damage has showed a very rapid progression with DM and hypertension. In 1/2 Nx group with unilateral nephrectomy, the renal damage progression is not rapid as in 5/6 Nx group, but glomerular injury increases with diabetes. We think that our results supply important histomorphometric and ultrastructural data and contribute to the literature especially for the model studies of hypertension and diabetes together and separately.

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