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ORIGINAL RESEARCH
ORJİNAL ARAŞTIRMA

Immunohistochemical Expression of Leptin (Ob-protein) in Experimentally Hypertensive Rat Kidney Tissues

Deneysel Hipertansif Sıçanların Böbrek Dokularında Leptin (Ob-pretein)'in İmmünohistokimyasal Ekspresyonu

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ÖZET

Amaç: Leptin 16 kDa (kilo dalton) molekül ağırlığında bir ob geni ürünü proteindir. Bu çalışmadaki amacımız sıçan böbrek dokularında Leptin proteini ekspresyonu ile hipertansiyon arasındaki bağlantıyı immünohistokimyasal olarak tespit etmektir.

Materyal ve Metot: Yetişin Wistar albino sıçanlar her birinde altışar adet olacak şekilde kontrol ve deney olarak iki gruba ayrıldılar. Deney grubunu L-NAME verilerek sıçanlar hipertansif hale getirildi. Tüm sıçanların böbrekleri cerrahi olarak çıkartıldı. Böbrekler rutin histolojik ve immünohistokimyasal işlemlerden geçirildikten sonra mikroskobik olarak analiz edildiler.

Bulgular: Deney grubunda kan basıncı değerlerinin kontrole göre belirgin olarak yükselmiş olduğu ve böbrek dokularında bazı belirgin histopatolojik değişikliklerin varlığı tespit edildi. Ayrıca böbrek boşaltım tübülleri hücrelerinde Leptin ekspresyonu skorları artmış idi.

Sonuç: Leptin ekspresyonunun artışı yükselen kan basıncına karşı böbrek dokularının uyum sağlaması için bazı renal fonksiyonların düzenlenmesi gibi fizyolojik olarak ya da bunun aksine patofizyolojik olarak ta artmış olabilir. Bu artışın fizyolojik veya patofizyolojik olup olmadığını belirlemek için ileri üst düzey çalışmalar yapılmalıdır.

Anahtar Kelimeler: Leptin, Hipertansiyon, İmmünohistokimya, Böbrek, L-NAM.

ABSTRACT

Objective: Leptin is an ob gene protein which has a 16 kDa weight. The aim of this study is to determine immunohistochemically the correlation between immunohistochemical expression of Leptin and hypertension in rat kidney tissues.

Material and Methods: Wistar albino adult rats were divided into control and experiment groups, with six rats in each. L-NAME was given to the experiment group. The kidneys of all rats were excised surgically. After these kidneys were subjected to routine histologic and immunohistochemical processes, microscopic analyses were performed.

Results: The blood pressure levels of the experimental group were higher than in the control, and their renal tissues had some distinctive histopathological changes. Additionally, Leptin immunostaining scores increased in the excretory tubule cells of hypertensive rats.

Conclusion: Upregulation of Leptin expression may indicate that Leptin molecules have an important physiological role such as regulation of some kidney functions to adapt high blood pressure; or, contrary to this, they may be a pathophysiological sign. Further research is necessary to determine whether this situation is physiological or pathophysiological process.

Key words: Leptin, Hypertension, Immunohistochemistry, Kidney, L-NAME.

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INTRODUCTION

Leptin is an anti-obesity hormone synthesized and excreted especially by fat cells which has a 16 kDa (kilo Dalton) weight and a glycoside polypeptide, is a product of the ob (obese) gene, and circulates in the blood according to the amount of fatty tissue (1-3). Prior to the discovery of the Leptin protein, it was estimated that a factor was produced in the blood regulating nutritional intake and body weight (4). This factor was determined in 1994, and it was named Leptin, after "leptos," meaning "slim" in Greek (1). After the discovery, studies on the Leptin protein and Leptin receptor (LR) intensified and more information was obtained on Leptin (5-7). Leptin metabolism is important in insulin secretion and body weight regulation (8).

It has been suggested that the absence or deficiency of Leptin bioactivity is responsible for the occurrence of the obese phenotype, and some studies have shown that Leptin usage in obesity-hypertension treatment is considerably beneficial (1,9). Leptin is involved in the realization of cardiovascular and urinary system functions, in addition to crucial functions in sustaining homeostasis, which has roles in many important duties (such as nutrition intake, energy balance regulation, control of the start of puberty, regulation of hypothalamic-hypophyseal functions, and insulin resistance) (10-12). The Leptin molecule improves these biological functions by means of LR (10,11). Leptin passes the blood-brain barrier and activates LR located in the brain stem and hypothalamus. Leptin deficiency or LR mutations preventing normal intracellular signal activations lead to the early start of some health problems, such as obesity and obesity-related hypertension (13). There is strong proof of the necessity of the melanocortin system to carry out many functions of Leptin, such as the regulating of the renal sympathetic nervous system and blood pressure (14,15).

Hypertension, occurs as a result of chronic inhibition of Nitric oxide (NO) synthase, is a condition defined as systolic blood pressure higher than 140 mmHg and diastolic blood pressure higher than 90 mmHg (16,17). The World Health Organization International Society of Hypertension (WHO-ISH) provided a definition of hypertension as arterial pressure measurements of 140/90 mm Hg or greater (18). In combined hypertension, systolic and diastolic blood pressure are both high; however, isolated hypertension is defined as only a systolic blood pressure increase, which is primarily observed in hypertension patients of an advanced age (16,17).

Hypertension is a crucial risk factor for cardiovascular disease morbidity and mortality. In Western societies, hypertension affects about 20%-30% of the adult population (19). The prevalence of hypertension increases with advancing age to the point where 30 to 50 percent people of 40-59 years of age, more than half of the people of 60-69 year of age and approximately three fourths of those ≥70 years of age are affected. Chronic diseases are important among adult population all over the world. The prevalence of chronic diseases like hypertension, diabetes etc, is showing an upward trend (20).

Hypertension is a part of the clinical spectrum of chronic kidney disease. It is the most common complication to occur across all stages of this disease and one of the risk factors for its progression, with the prevalence depending on the exact definition of hypertension. Its prevalence and resistance to drug treatment increase with progression of renal failure (21). Basic research studies has shown that vascular inflammation could contribute to the development of hypertension. Renal injury has been proposed as the crucial step in the pathophysiological cascade of essential hypertension. In particular, arterial and systemic inflammatory

processes have been indicated as possible active mediators contributing to the increase of renal damage (22).

In the present study, we aimed to determine the histological changes and the immunohistochemical expression of Leptin protein in the renal tissues of rats which were made hypertensive with L-NAME.

MATERIAL AND METHODS

Laboratory Animals

After the animal experimental protocol was approved, adult Wistar albino rats weighing 200-210g were divided into two groups (control/normotensive, experimental/ hypertensive) of six rats each. The rats were caged and kept in a temperature-controlled environment (22-24ºC) under a 12hrs light/ dark cycle. Animals were provided standard rat chow and water ad libitum. Tap water was given to all of the animals, but L-NAME (Sigma, N-5751) ($C_7H_{15}N_5O_4$ HCL N -nitro-L-arginine methyl ester hydrochloride) was added to the water of the experimental rats for 21 days in order to make them hypertensive.

Making the Rats Hypertensive

Hypertension was formed by adding L-NAME, an NO synthase enzyme inhibitor, to the drinking water. The daily water consumption of the rats was estimated before starting the experiment. By taking into consideration the daily water consumption and rat weights, an oral dose of L-NAME of 60mg/kg/day was calculated and added to their drinking water. The drinking water with L-NAME added was changed once every two days, because L-NAME that is dissolved in water loses its activity with time, and the remaining drinking water was not reused. Therefore, each of the rats received fresh L-NAME in a daily average of 60mg/kg dosage. In the last day of the study, the blood pressure of all the animals were measured, and it was determined that the rats were hypertensive.

Blood Pressure Measurement of the Rats

Blood pressures were measured from their tail cuff arteries by a non-invasive method while the animals were under anesthesia. Measurement values obtained by applying anesthesia were somewhat lower than the measurement results obtained by application without anesthesia (23). In order to enable the enlargement of the tail cuff arteries and easier pulsation detection, an application of heat was made before each blood pressure measurement.

The signals were received by an annular pressure probe attached to the tail and transferred to the computer by means of the MP 100A-CE data collection system and MAY-BPHR200 unit (BIOPAC Systems, CA-USA), and the measurements were made by pressure traces drawn by the Acknowledge package program (23). At the end of the material supply, the blood pressures of the control and experiment groups were measured as described, and the measurement results are shown in **Table 1.**

Table 1: Arterial blood pressure values recorded from experimental and control group rats (±: standard deviation, *: P<0.01)

	Systolic arterial blood pressure measurement values (mmHg)					
	1. rat	2. rat	3. rat	4. rat	5. rat	Mean *
Hypertensive	129.67 ± 6	129.00 ± 6	128.33 ± 5	129.50 ± 5	130.00 ± 6	129.30 ± 6
Normotensive	89.50 ± 2	89.17 ± 3	89.17 ± 3	88.50 ± 1	88.17 ± 2	88.90 ± 2

Histological Studies

The animals were sacrificed under deep anesthesia by the vascular perfusion method. The kidney tissues from the rats (n:6/group) were fixed in 4% formalin for 24 hours, embedded in paraffin, and sectioned for histological examination. Five-µm-thick sections taken with a rotary microtome were placed on slides and deparaffinized for good adhesion and staining. Slices were stained with hematoksilen-eozin, and the tissue preparations were analyzed histologically under a light microscope.

Immunohistochemical Analysis

Five-µm-thick sections, placed on slides with poly lysine, were deparaffinized in xylenes and rehydrated through a graded ethanol series and distilled water. The tissues were kept in a citric acid solution for 5 minutes in a microwave (Med High). They were cooled immediately by adding cold distilled water. The sections were kept in distilled water for approximately 10 minutes, bringing them to room temperature. Next, the tissue sections were placed in 3% hydrogen peroxide for 15 minutes at roomtemperature and washed three times with a 0.01M phosphate buffer solution (PBS) 3x5 minutes. The sections were encircled with a hydrophobic pap pen. After a 15-minute incubation in blocking serum (non-immune) at room temperature, primary antibody (anti-ob protein, A-20; sc-82, Santa Cruz) was added to the tissue sample and they were incubated in a moist environment in the dark (1:50, 40C, 18hrs); then the sample was stabilized at room temperature for 30 minutes and washed three times with PBS. The sections were then incubated for 30 minutes with the biotinylated secondary antibodies and washed three times with PBS. A streptavidin-marked secondary antibody was added to the tissue sample, incubated for 30 minutes in a humidified atmosphere in the dark, and washed three times with 0.01M PBS. The renal tissue sections were finally stained with AEC (aminoethylcarbosol) for 5 minutes, washed for 10 minutes in water, counterstained for 10 seconds with hematoksilen, and washed again for 10 minutes in water. They were mounted with an aqueous mount solution. For the negative control, PBS was dropped on the sections instead of a primary antibody, and the same procedures were applied in the other steps.

Immunohistochemical Evaluation

All the tissue sections were viewed under a microscope, to identify the Leptin reactive cells. The kidney tissues were studied separately as the cortex and medulla. A semi-quantitative evaluation system, a scoring scale, was used based on the intensity of immunostaining that occurred as a result of the antigen antibody reaction. In this scale, a total of four values, grading from one negative to three positive, were made. This meant that (-): no staining, (+): detectable weak staining, (++): medium severity staining, and (+++): severe staining. By using this scale, the results found in the cortex were as follows: glomerular (mesangial cell, endothelial cell), Bowman capsule parietal and visceral layers, kidney tubules (proximal, distal), and collective canal. For the medulla, the loop of Henle, collective canals and capillary blood vessels, and cells' immunostaining was evaluated.

Semi-quantitative H-Score Analysis

Five areas were selected randomly from the sections belonging to each animal to be examined, with the medulla and cortex inspected separately under a light microscope with 40X magnification. Categorical enumeration of the cells within these areas was made according to their immunostaining intensity. The average of the results of a blind study was taken. During these counts, both the number of the cells showing positive immunostaining and the intensity degrees of immunostaining in these cells, and all cells which were stained and not stained, were considered. H-Scores were calculated as H score = ∑ (i+l) pi, where i is the intensity score and pi is the percentage of cells showing that intensity.

Statistical Analysis

The statistical analyses for this study were performed using the SPSS-20 Windows statistical package program (IBM Co., Somers, NY, USA). Evaluation of the results between the experiment and control groups was performed by means of non-parametric Mann-Whitney-U test. Differences were considered significant at P<0.05.

RESULTS

Blood Pressure Findings

Prior to the sacrifice of all animals that were included in the study, their blood pressures were measured and recorded as described in the material and methods section. The recorded arterial blood pressure measurement values are shown in **Table 1.** There was a statistically significant difference in the comparison of the mean blood pressures of the hypertensive (129.30 mmHg) and normotensive groups (88.90 mmHg) (P<0.01); therefore, it was decided that the rats in the experimental group were hypertensive.

Histopathological Findings

In histological examinations of kidney tissues of normotensive rats, there were no considerable structural histological changes observed. Some significant structural changes were observed in microscopic examination of the kidney tissues of hypertensive rats. Among these histopathological findings, tubular dilatations were specifically observed. In some of the proximal and distal tubular cells, pyknotic nuclei and eosinophilic cytoplasm were observed. Focal mononuclear cell infiltration in the interstitial area was frequently observed. In particular, eosinophilic material accumulation in the lumen of collective tubules in the medulla was observed. Moreover, conspicuous pyknotic nuclei and glomerulosclerosis findings in some glomerular cells were observed as well **(Figure 1).**

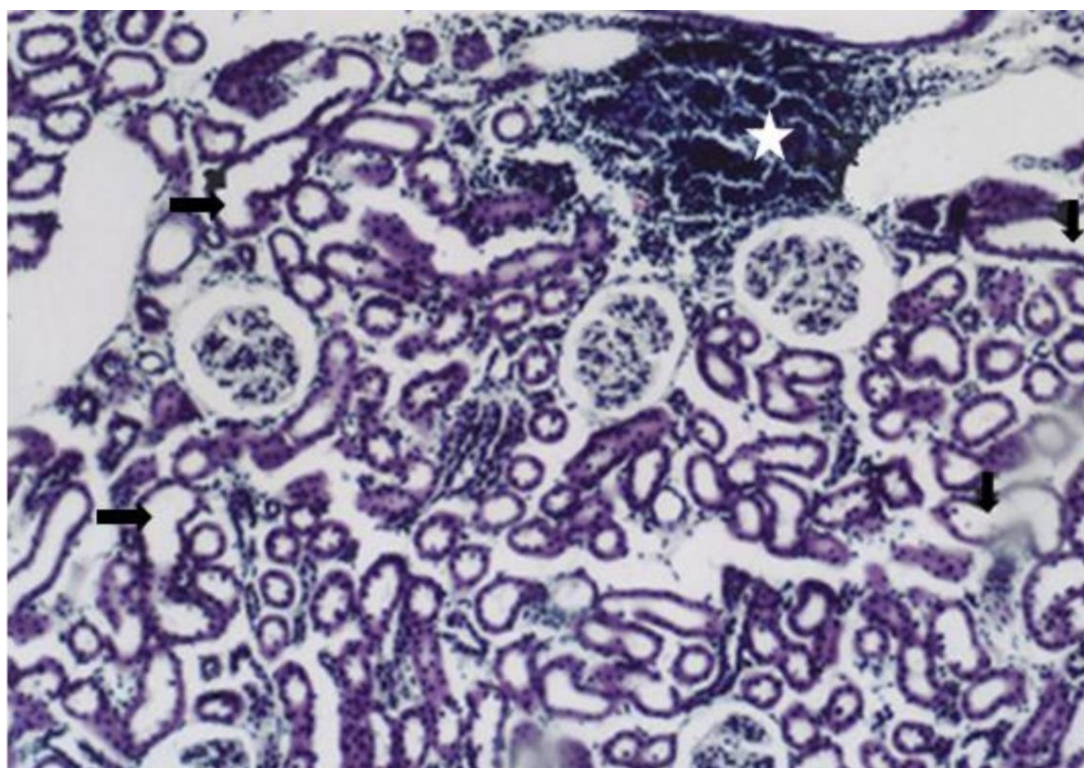

Figure 1. *The experimental group rat renal tissues' histological appearance. Focal mononuclear cell infiltration (asterisk) observed in the interstitial space. Expansion of the proximal and distal tubule cells (arrows) and increase of the Bowman's capsule space area available. Increased signs of interstitial fibrosis characterized by the connective tissue between the tubules can be seen (H&E x 10 obj).*

Immunohistochemical Findings

In the normotensive rats' renal tissues, a moderate immune reaction was observed in the cytoplasm of proximal tubular cells (mean H-Score value: 235.04), and in the distal tubules, a weak reaction (mean H-Score value: 53.08) was observed **(Figure 2).** In the hypertensive rats, strong immune reactions were observed in the cytoplasm of proximal tubule cells (mean H-Score value: 322.39) and in the distal tubules, relatively weak staining (mean H-Score value: 65.76) was determined **(Figure 3).** Additionally, some collector tubule cells were stained very weakly in rare areas in the hypertensive kidney medulla. Immunostaining was negative in the loops of Henle. In normotensives, immunostaining was observed showing similarities with the experimental group's medulla. Immunohistochemical distribution of Leptin in the kidney tissues of the control and experimental groups are shown comparatively in **Table 2**. No immunostaining could be observed in the glomerulus, Bowman capsule, and veins. Immunostaining H-Score values of the proximal and distal tubules of the experimental and control group are shown comparatively in **Figure 4**. Immunostaining intensity of the proximal and distal excretion tubules was significantly increased in the hypertensive rats ($P<0.01$, $P<0.05$, respectively). In the collector tubules of both groups, no considerable immunostaining was observed.

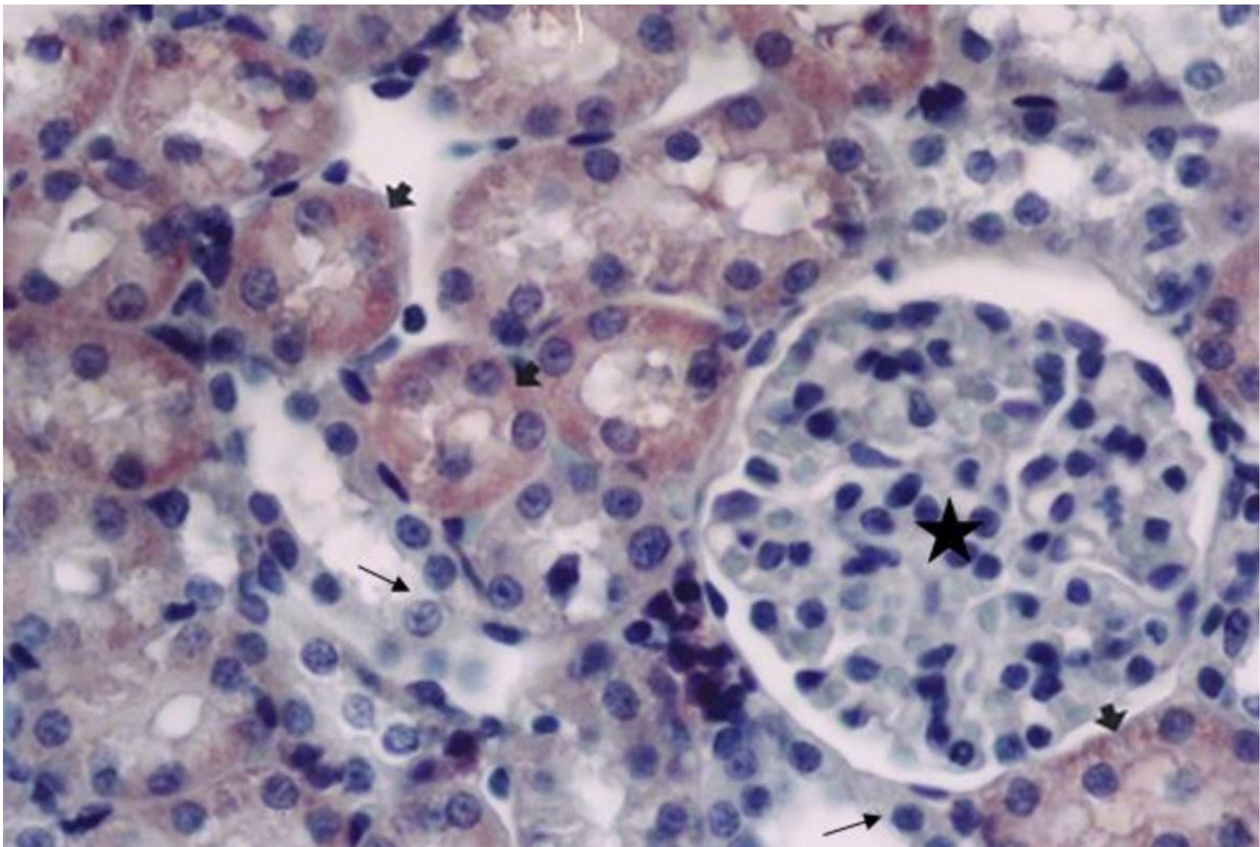

Figure 2*. The renal tissues of the normotensive rats were stained with Leptin Ab immunohistochemically. The proximal tubule cells' cytoplasm (thick arrows) show a monitored positive immune reaction for Leptin Ab but distal tubules (thin arrows) and glomeruli (asterisks) have negative immune reactions (Immunoperoxidase x 40 obj)*

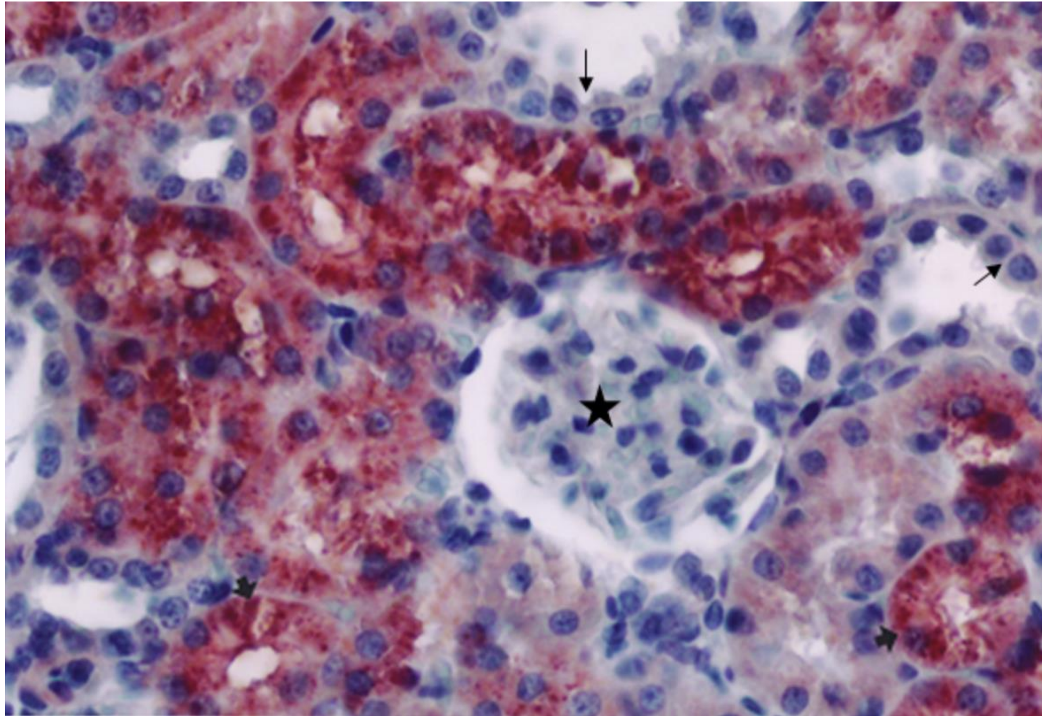

Figure 3. *The renal tissues of the hypertensive rats were stained with Leptin Ab immunohistochemically. Proximal tubule cells' cytoplasm (thick arrows) have a strongly positive immune reaction for Leptin Ab but distal tubules (thin arrows) and glomeruli (asterisks) have negative immune reactions (Immunoperoxidase x 40 obj).*

Table 2: Leptin immunostaining intensity of normotensive and hypertensive rat renal tubules

	Proximal tubule	Distal tubule	Collector tubule	Loop of Henle
Hypertensive	+++	+	+	-
Normotensive	++	+	±	-

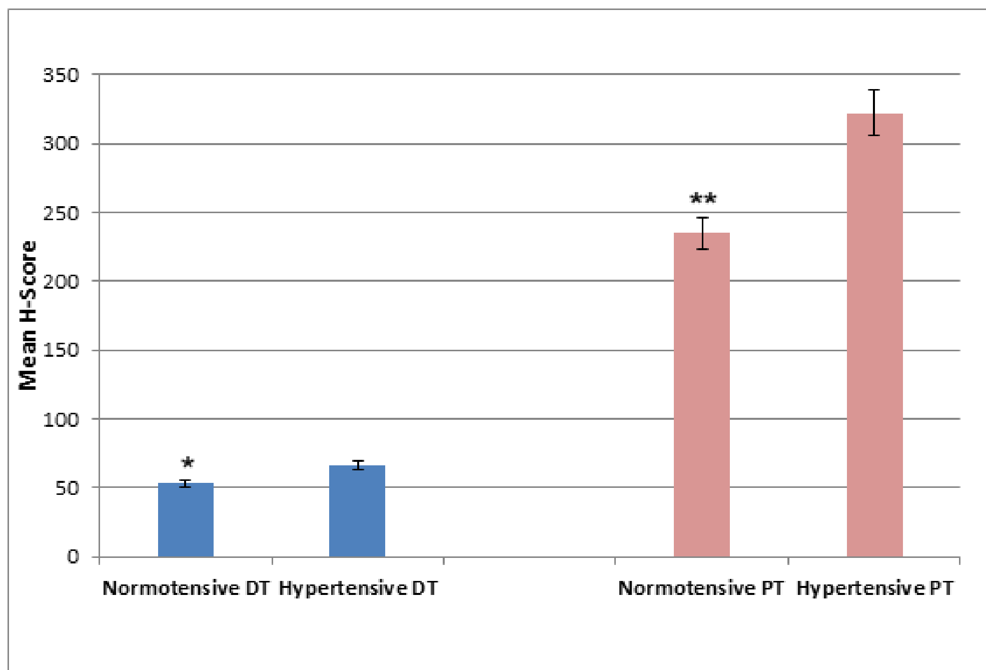

Figure 4. *Comparison of the mean H-Score values of renal tubules (DT: Distal Tubules. PT: Proximal Tubules. *: P<0.05, **: P<0.01).*

DISCUSSION

Leptin metabolism has a very important role in body weight regulation, nutritional intake, insulin secretion, and glucose hemostasis (24-26). It was determined recently that plasma Leptin levels increase in patients with arterial hypertension. This suggests that this situation could be related to the increase of Leptin secretion from adipose storage and decrease of its excretion from the kidneys (8,27,28).

Insufficiency of Leptin bioactivity is known to be responsible for the occurrence of the obese phenotype. Therefore, it has been indicated that Leptin is rather beneficial in the treatment of obesity-related hypertension (9). Obesity is one of the most important factors in the appearance of many secondary diseases, especially cardiovascular illnesses and hypertension (29,30). In this study we did not investigate hypertension resulted from obesity but our study related to experimentally chronic hypertension. Chronic hypertension can lead to renal damage and functional nephron loss. In relation to this, hemodynamic and structural adaptations have a high risk for cardiovascular and renal events (7).

In hypertension components of the innate immune system like dendritic cells, macrophages, natural killer (NK) T cells, and Toll-like receptors (TLRs) of the inflammasomes have been investigated (31). Dendritic cells are increased, infiltrating the kidney and arterial walls in hypertension models. Dendritic cells promote a differentiation of T cells toward a CD4+ IL-17 phenotype in response to aldosterone (32). A consistent finding in experimental models of hypertension is the infiltration of macrophages in the kidney and periadventitial areas in the aorta and medium-sized arteries (33). Interferon gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), interleukin 2 (IL-2), and interleukin 4 (IL-4) are all rapidly released by NK cells, and some important studies have suggested they may play a role in hypertension related inflammation. Koomans et al. (34) have shown that monocytes and NK cells present a program of reciprocal activation in hypertension. The inflammation and vascular dysfunction induced by angiotensin II are associated with the accumulation of NK cells and macrophages in the aortic wall.

In our study, tubular dilatations were observed in the microscopic examination of kidney tissues of hypertensive rats. In some of the proximal and distal tubule cells, pyknotic nuclei and eosinophilic cytoplasm were clearly observed. Focal mononuclear cell infiltrations in the interstitial area and eosinophilic material accumulations were seen in the lumen of collecting tubules in the medulla. Moreover, increased pyknotic cells and glomerulosclerosis findings were seen in some glomerular cells. These histological changes are consistent with the effect of the hypertension. In connection with these findings, we think that our current histopathological results may originate from not only increased blood pressure but also a toxic effect of exogenous L-NAME. Therefore we recommend elucidating this issue in detail. The conducted studies point out that Leptin and LR can be a regional connection between excessive weight gain and sympathetic activity, by means of a variety of interactions among other neurochemical pathways in the hypothalamus (42). Leptin has effects on the central nervous system to diminish appetite and increase energy consumption. These effects of Leptin, or abnormalities in its production, may lead to renal modifications related to the sympathetic and cardiovascular systems and obesity (43).

In literatures several studies suggested that Leptin could play a role in blood pressure regulation (44). Kuo et al. have found that Leptin increases NO secretion from the vascular endothelium, and that hyperleptinemia causes tachycardia (45).

Villarreal et al. determined that exogenous Leptin causes a significant increase in sodium excretion (natriuretic effect). However, they were not able to see its effect at the same rate in hypertensive and obese animals. As a result of this, they proposed that Leptin could be a potential salt excretion factor in normal rats, and that it can have a pathophysiological function in obesity hypertension (46).

In conclusion, we can say that Immunohistochemical expression of the Leptin is upregulated in the kidneys of hypertensive rats, especially in the excretion tubular cells. This may indicate that Leptin molecules have an important physiological role. For example, they may force the kidneys to reduce arterial tension, or prevent further increase. This also points to a possible role of Leptin molecules in the regulation of some functions of kidney tissue in hypertension. Contrary to these, Leptin was over-expressed as a pathologic marker as well. In order to clarify this, we suggest that a very high level study should be made.

REFERENCES

1. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional clonig of the mouse obese gene and its human homologue. Nature 1994; 372: 425-32.
2. Considine RV, Caro JF. Leptin and the regulation Of body weight. Int. J. Biochem Cell Biol 1997; 29: 1255-1272.
3. Tadokoro S, Ide S, Tokuyama R, Umeki H, Tatehara S, Kataoka S, Satomura K.. Leptin Promotes Wound Healing in the Skin. PLoS ONE 2015; 10: e0121242.
4. Münzberg H, Morrison CD. Structure, production and signaling of leptin. Metabolism 2015; 64: 13-23.
5. Doehner W, Anker SD. The significance of leptin in human - do we know it yet? Int J Cardiol 2000; 76: 122- 23.
6. Serradeil-Le Gal C, Raufaste D, Brossard G, Pouzed B, Marty E, Maffrand J, Fur GL. Characterization and localization of leptin receptor in rat kidney. FEBS Lett 1997; 404: 185-191.
7. Zhang R, Reisin E. Obesity-hypertension: The effects on cardiovascular and renal systems. Am J Hypertens 2000; 13: 1308-14.
8. Henriksen JH, Holst JJ, Moller S, Andersen UB, Bendtsen F, Jensen G. Elevated circulating levels in arterial hypertension. Clin Sci (Colch) 2000; 99: 527-34.
9. Prolo P, Wong ML, Licinio J. Leptin, Int. J. Biochem Cell Biol 1998; 30: 1285-90.
10. Friedman JM, Halaas JL. Leptin and their regulation of body weight in animals. Nature 1998; 392: 763-71.
11. Wıesner G, Vaz M, Collier G, Seals D, Kaye D, Jennings G, Lambert G, Wilkinson D, Esler M. Leptin is released from the human brain: İnfluence of adiposity and gender. J Clin Endocrinal Metab 1999; 84: 2270-74.
12. Goumenu AG, Matalliostakis IM, Koumantakis GE, Panidis KD. The role of leptin in fertility. Eur J Obstet Gynecol Reprod Biol 2003; 106: 118-24.
13. do Carmo JM, da Silva AA, Dubinion J, Sessums PO, Ebaady SH, Wang Z, Hall JE. Control of Metabolic and Cardiovascular Function by the Leptin–Brain Melanocortin Pathway. IUBMB Life 2013; 65: 692-98.
14. Tallam LS, Stec DE, Willis MA, da Silva AA, and Hall JE. Melanocortin-4 receptor-deficient mice are not hypertensive or salt-sensitive despite obesity hyperinsulinemia and hyperleptinemia. Hypertension 2005; 46: 326-32.
15. Tallam LS, da Silva AA, Hall JE. Melanocortin-4 receptor mediates chronic cardiovascular and metabolic actions of leptin. Hypertension 2006; 48: 58-64.
16. Li JS, Schiffrin EL. Resistance artery structure and neuroeffector mechanisms in hypertension induced by inhibition of NO synthase. Am J Hypertension 1994; l7: 998-04.
17. Kone BC, Baylis C. Biosynthesis and hemeostatic roles of NO in the normal kidney. Am J Physiol 1997; 272: 561-78.
18. Whitworth JA; World Health Organization, International Society of Hypertension Writing Group. 2003 World Health Organization (WHO)/ International Society of Hypertension (ISH) statement on management of hypertension. J Hypertens 2003; 21: 1983-92
19. Montecucco F, Pende A, Quercioli A, Mach F. Inflammation in the pathophysiology of essential hypertension. J Nephrol 2011; 24: 23-34

20. Singh R, Agarwal R, Singh S, Gupta S C. A cross-sectional study on prevalence of hypertension and its relationship with selected demographic factors in western Uttar Pradesh. Ind J Comm Health 2014; 26:10-14

21. Roszkowska-Blaim M, Skrzypczyk P. Hypertension in children with end-stage renal disease. Adv Med Sci 2015; 60: 342-8

22. Montecucco F, Pende A, Quercioli A, Mach F. Inflammation in the pathophysiology of essential hypertension. J Nephrol 2011; 24: 23-34

23. Crofton JT, Ota M, Share L. Role of vazopressin the renin-angiotensin system and sex in Dahl salt-sensitive hypertension. J Hypertens 1993; 1: 1031-38.

24. Levi J, Huynh FK, Denroche HC, Neumann UH, Glavas MM, Covey SD, Kieffer TJ. Hepatic leptin signalling and subdiaphragmatic vagal efferents are not required for leptin-induced increases of plasma IGF binding protein-2 (IGFBP-2) in ob/ob mice. Diabetologia 2012; 55: 752-62

25. Emond M, Ladenheim EE, Schwartz GJ, Moran TH. Leptin amplifies the feeding inhibition and neural activation arising from a gastric njutrient preload. Physiol Behav 2001; 72: 123-28.

26. Attele AS, Shi ZQ, Yuan CS. Leptin gut and food intake, Biochemical Pharmacology 2002; 63: 1579-83.

27. Khokhar KK, Sidhu S, Kaur G. Correlation between leptin level and hypertension in normal and obese pre- and postmenopausal women. Eur J Endocrinol 2010; 163: 873-78.

28. Schutte R, Huisman HW, Schutte AE, Malan NT. Leptin is independently associated with systolic blood pressure pulse pressure and arterial compliance in hypertensive African women with increased adiposity: the POWIRS study. J Hum Hypertens 2005; 19: 535-41.

29. Moest H, Frei AP, Bhattacharya I, Geiger M, Wollscheid B, Wolfrum C. Malfunctioning of adipocytes in obesity is linked to quantitative surfaceome changes. Biochim Biophys Acta 2013; 1831: 1208-16.

30. Schlesinger S, Aleksandrova K, Pischon T, Fedirko V, Jenab M, Trepo E, et al. Abdominal obesity weight gain during adulthood and risk of liver and biliary tract cancer in a European Cohort. Int J Cancer 2013; 132: 645-57.

31. Rodríguez-Iturbe B, Pons H, Quiroz Y, Johnson RJ. The immunological basis of hypertension. Am J Hypertens 2014; 27: 1327-37.

32. Herrada AA, Contreras FJ, Marini NP, Amador CA, Gonzalez PA, Cortes CM, Riedel CA, Carvajal CA, Figueroa F, Michea LF, Fardella CE and Kalergis AM. Aldosterone promotes autoimmune damage by enhancing Th17-mediated immunity. J Immunol 2010; 184: 191-202.

33. Rodriguez-Iturbe B, Quiroz Y, Nava M, Bonet L, Chavez M, Herrera-Acosta J, Johnson RJ, Pons HA. Reduction of renal immune cell infiltration results in blood pressure control in genetically hypertensive rats. Am J Physiol Renal Physiol 2002; 282: 191-201.

34. Kossmann S, Schwenk M, Hausding M, Karbach SH, Schmidgen MI, Brandt M, Knorr M, Hu H, Kröller-Schön S, Schönfelder T, Grabbe S, Oelze M, Daiber A, Münzel T, Becker C, Wenzel P. Angiotensin II-induced vascular dysfunction depends on interferon-γ-driven immune cell recruitment and mutual activation of monocytes and NK-cells. Arterioscler Thromb Vasc Biol 2013; 33:1313–1319

35. Rodriguez-Iturbe B, Pons H, Quiroz Y, Lanaspa M, Johnson RJ. Autoimmunity in the pathogenesis of hypertension. Nat Rev Nephrol. 2014; 10: 56-62.

36. De Miguel C, Guo C, Lund H, Feng D, Mattson DL. Infiltrating T lymphocytes in the kidney increase oxidative stress and participate in the development of hypertension and renal disease. Am J Physiol Renal Physiol. 2011; 300: 734-42.

37. Hughson MD, Gobe GC, Hoy WE, Manning RD Jr, Douglas-Denton R, Bertram JF. Associations of glomerular number and birth weight with clinicopathological features of African Americans and whites. Am J Kidney Dis 2008; 52: 18-28.

38. Youn JC, Yu HT, Lim BJ, Koh MJ, Lee J, Chang DY, Choi YS, Lee SH, Kang SM, Jang Y, Yoo OJ, Shin EC, Park S. Immunosenescent CD8+ T cells and C-X-C chemokine receptor type 3 chemokines are increased in human hypertension. Hypertension 2013; 62: 126-33.

39. Rodriguez-Gomez I, Wangensteen R, Atucha NM, O'Valle F, Del Moral RG, Garcia-Estan J, Vargas F, Osuna A. Effects of omapatrial on blood pressure and renal injury in L-NAME and L-NAME plus DOCA-treated rats. Am J Hypertens 2003; 16: 33-38.

40. Uhlenius N, Tikkanen T, Miettinen A, Holfhofer H, Tornroth T, Eriksson A, Fyhrquist F: Renoprotektive effects of captopril in hypertension induced by NO synthase inhibition in experimentl nephritis. Nephron 1999; 81: 221-29.

41. Kashiwagi M, Shinozaki M, Hirakata H, Tamaki K, Hirano T, Tokumoto M, Goto H. Locally activated

renin-angiotensin system associated with TGF-beta 1 as a major factor for renal injury induced by chronic inhibition of NO synthase in rats, J Am Soc Nephrol 2000; 11: 616-24.

42. Gao RJ, Elmquist JK, Bjorbaek C, Ahima RS, Filer JS, Saper CB. Distributions of leptin receptor mRNA isoforms in the rat brain. J Comp Neurol 1998; 395: 535-47.

43. Haynes WG. Interaction between leptin and sympathetic nervus system in hypertension. Curr Hyppertens Rep 2000; 2: 311-18.

44. Kokot F, Wiecek A, Adamczak M, Ulamn I, Spiechowicz U, CieplokJ, Mesjasz J. Pathophysiological role of leptin in patients with chronic renal failure in kidneytransplant patiens in patients ith` essential hypertension and in pregnant omen` ith` preeclampsia. Artif rgans` 1999; 23:70-4.

45. Kuo JJ, Jones OB, Hall JE. Inhibition of NO synthesis enhances chronic cardiovascular and renal actions of leptin. Hypertension 2001; 37: 670-6

46. Villarreal D, Reams G, Freeman RH. Effects of renal denervation on the sodium excretory actions of leptin in hypertensive rats. Kisney Int 2000; 58: 989-94.