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Ultrastructural changes in the adrenal gland in terms of age and gender-related

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ABSTRACT

Objective: The adrenal glands are endocrine organs that synthesize hormones with crucial functions in the body. This irreplaceable structure performs multiple functions, from the metabolism of carbohydrates, fats, and proteins to sex development. Any disorder concerning the adrenal glands can give rise to life-threatening conditions. The aging process inevitably affects the adrenal glands. The effects of aging are marked by a rise in cortisol secretion in the zona fasciculata and a decrease in androgen secretion in the zona reticularis. Elevated cortisol levels in the blood disturb most bodily systems in favour of catabolism, amplifying the cellular decline and destruction that accompanies aging. The deterioration of the adrenal glands relates not just to spongiocytes and chromaffin cells, but also to the endothelium, which enables circulation. It is clear that a decline in vascular function will have a negative impact on endocrine activities. Our study aims to explore the influence of aging on the adrenal glands in rats, analyzing sex-specific ultrastructural scales.

Materials and Methods: A total of 28 Sprague-Dawley rats, 14 males, and 14 females, were planned to be used in the study. Of these 28 rats, 4 females and 4 males constitute the control group. The rats in the control group at approximately 10 weeks old and the rats in the experimental group at approximately 19 weeks old were sacrificed. All animals in the study have been anaesthetized and then sacrificed by removal of the heart. The right and left and adrenal glands were removed from the sacrificed animals. Removed and adrenal glands prepared for TEM examination. For each animal, at least four TEM images were taken from four different sections of the same block.

Results: Our findings demonstrate that the aging process not only affects spongiocytes within the adrenal gland but also contributes to the deterioration of endothelial cells. As anticipated, our results indicate the presence of senescence and apoptosis in endothelial cells. The observed vascular separations and ruptures are due to endothelial deterioration. Hypertrophy has been observed in the spongiocytes to compensate for the functional deficiencies following a decrease in their number due to aging. Elevated levels of lipofuscin, lipid droplets, and lysosomes were discovered in spongiocytes. Impaired endothelium potentially contributes to certain changes in spongiocytes.

Conclusions: While aging-related changes appear similar in both genders, males tend to be more impacted.

Keywords: *Adrenal gland, Endothelium, Ultrastructural, Endocrine, Gender-dependent difference*

INTRODUCTION

Adrenal (suprarenal) glands are cap-like endocrine glands located above the kidneys. Adrenal glands are retroperitoneal organs embedded in adipose tissue. Adrenal glands have a rich vascular support such as endocrine organs. The inferior phrenic artery, the abdominal aorta, and the renal artery supply the adrenal glands by giving rise to the superior, middle, and inferior suprarenal arteries respectively. The adrenal glands consist of two parts, the cortex (80-90%), which synthesizes

steroid hormones, and the medulla (10-20%), which synthesizes catecholamines (Hansen, 2021).

Adrenal cortex consists of zona glomerulosa (ZG), zona fasciculata (ZF) and zona reticularis (ZR). These cell layers have different properties from each other. The zona glomerulosa makes up about 15% of the cortex and is responsible for the synthesis of mineralocorticoid aldosterone. The zona fasciculata makes up 80% of the cortex and is responsible for the synthesis of glucocorticoids, especially cortisol. The zona reticularis is the smallest part of the cortex (5-6%) and is responsible for the synthesis of androgens called dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEA-S). Unlike the cortex, the adrenal medulla is composed of chromaffin cells of neural crest origin. The adrenal medulla is not stimulated by hormones from the hypothalamic-pituitary-adrenal axis like the cortex but by nerves from the sympathetic ganglia. There are two types of chromaffin cells, norepinephrine-secreting and epinephrine-secreting. About 80% of the secretion from the adrenal medulla is epinephrine, 20% norepinephrine and a small amount of dopamine. The blood supply to the adrenal glands is provided by a system of the three vessels mentioned above, which enter the capsule. Capsular capillaries supply the capsule, vessels enter the cortex and form the fenestrated cortical sinusoidal capillaries, medullary arterioles pass through the trabeculae in the cortex, reach the medulla and bring arterial blood to the medullary capillary sinusoids. The fenestrated cortical sinusoidal capillaries supplying the cortex bring venous blood to the medullary capillary sinusoids. The medulla is supplied by two bloodstreams. Venous blood from venules originating in the cortical and medullary sinusoids empties into the adrenomedullary collecting veins and finally leaves the adrenal gland via the central adrenomedullary vein (Kierszenbaum and Tres, 2019; Mescher, 2013).

Since endocrine organs release their secretions into the blood, any disruption in their circulation will have undesirable effects on the body. So much so that endocrine organs are the places in the body where the most blood per gram is seen. The adrenal gland is one of the most blood-rich tissues and, as mentioned above, it has sinusoidal capillaries. Sinusoids are densely located in organs and tissues where the transport of large molecules is intense and where substance exchange is high (Augustin and Koh, 2017; Schaeffer et al., 2011). Disruption of the sinusoids prevents the adrenal gland from performing its full function.

The secretions of the adrenal glands play an important role in maintaining homeostasis. Corticotropin-releasing hormone (CRH) secreted by the hypothalamus stimulates the secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary. ACTH stimulates the adrenal cortex to produce steroid hormones, particularly cortisol. The rising levels of cortisol and other glucocorticoids in the circulation control hormone secretion through negative feedback on the hypothalamus and pituitary. The main stimulus for aldosterone synthesis from the cortex is the renin-angiotensin system. Synthesis and secretion of androgens are controlled by CRH-ACTH, as are glucocorticoids. Glucocorticoids secreted from the adrenal cortex mainly affect carbohydrate, protein, and lipid metabolism, but also the immune system, hematopoiesis, vascular action of catecholamines, bone reabsorption, glomerular filtration, gastrointestinal system, and central nervous system. The main action of aldosterone is to ensure the retention of sodium ions and associated water from the kidney. Aldosterone increases potassium and hydrogen ion excretion while increasing sodium retention. Androgens have effects on protein anabolism and growth promotion as well as the development of secondary sex characteristics during puberty (Jameson et al., 2016; Hall and Hall, 2020; Costanzo, 2021).

The functions of the adrenal glands change with age. The zona reticularis decreases in activity with age, while hypertrophy of the zona fasciculata and zona glomerulosa has been reported. There are several theories as to why adrenal function may deviate from physiological limits, one of which is that the blood supply to the adrenal glands decreases with age. In addition, the effect of aging on the adrenal glands differs between men and women. Men are said to be more affected by adrenal aging (Parker et al., 1997; Warde et al., 2023). In our study, we tried to understand the effects of aging on the adrenal glands in men and women by examining the adrenal glands ultrastructurally.

MATERIALS and METHODS

A total of 28 Sprague-Dawley rats, 14 males, and 14 females, were used in the study. Of these 28 rats, 4 females and 4 males constituted the control group. The rats in the control group were

approximately 10 weeks old, and the rats in the experimental group, which represented the aged group, were 19 weeks old. All animals in the study were anaesthetized with 60 mg/kg ketamine hydrochloride and 10 mg/kg xylazine hydrochloride and then sacrificed by removing the hearts and the right and left suprarenal glands were removed from the sacrificed animals.

The removed glands were immediately placed in a 0.1 M phosphate buffered container containing 2.5% glutaraldehyde for 24 hours. The next morning the tissues were postfixed in 1% osmium tetraoxide (OsO4) in 0.1 M phosphate buffer for 1 hour and dehydrated by graded alcohols (25-100%). After passing through propylene oxide, the samples were embedded in Araldyte CY 212, DDSA (2-dodecenylsuccinic anhydride), BDMA (benzyldimethylamine), and dibutylphthalate. Semi-thin sections (1µm) of suprarenal glands were stained with toluidine blue and examined by light microscopy. Ultrathin sections of adrenal glands were stained with uranyl acetate and lead citrate and examined by LEO 906E transmission electron microscopy (TEM). For each animal, at least four TEM images were obtained from four different sections obtained from the same block.

Ethical approval

The study was approved by the Ethical Committee of the Faculty of Medicine of Baskent University. The ethics approval number: is DA09/16.

RESULTS

In the control group, male rats of vessels lumen surrounded by endothelial cytoplasm, and the nucleus was characterized by a rich euchromatin content. The vessel was surrounded by actively secreting cells that were rich in mitochondria (Figure 1).

On the other hand, in the control female rat vessels, the endothelium is seamlessly connected to the basal lamina. Notably, vesicles located in both the apical and basolateral regions of the endothelium play a crucial role in the transit and transport of necessary substances to the adjacent secretory cell (Figure 1).

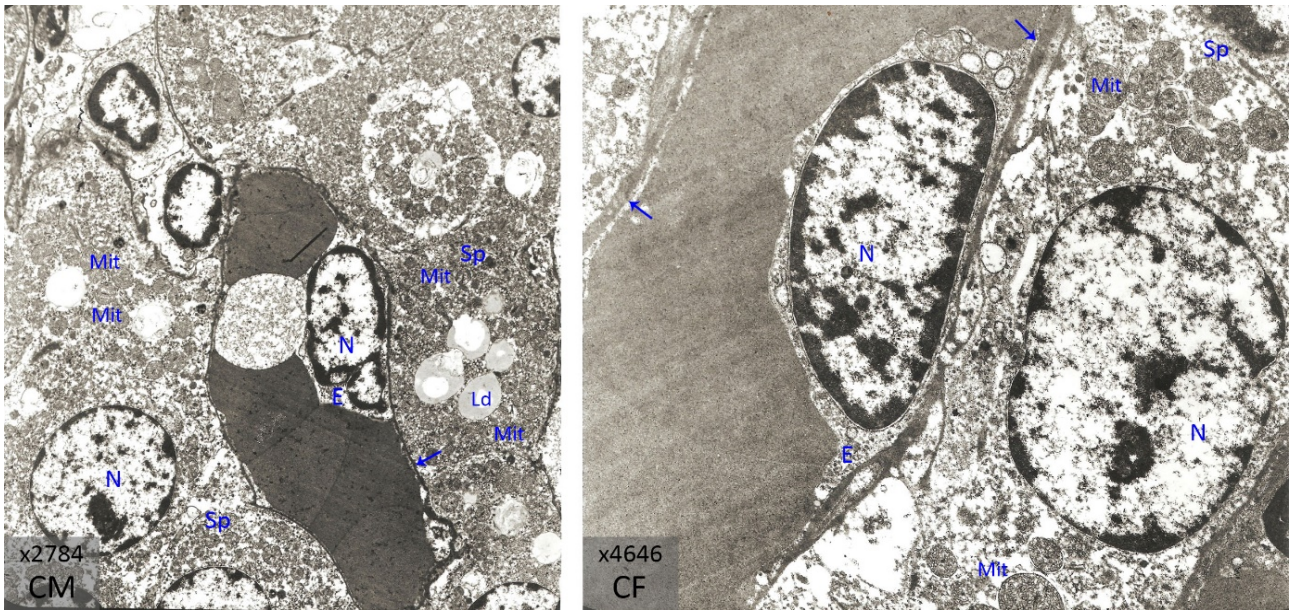

Figure 1. Control group electromicrographs (male on the left [CM], female on the right [CF]). E: endothelial cell, Sp: spongiocyte, Ld: lipid droplets, N: nucleus, Mit: mitochondrion, →: refers to basement membrane

The observations revealed various structural alterations within the endothelial cells and their microenvironment. Signs of degeneration were evident in specific regions of the endothelial cells and some spongiocyte mitochondria (Figure 2 OF-1). Vacuolization was noted in the endothelial part of the basal lamina and there were folds on its surface facing the spongiocytes. White spaces within spongiocytes were recognized as residual lipid droplet remnants following lipid removal, while an observed thickening occurred in the basal lamina. Mitochondrial degeneration in both endothelial and secretory cells was notable, alongside increased heterochromatin regions within cell nuclei. In addition, an increase in lipofuscin was also observed due to degenerated mitochondria, lysosomes, and lipid droplets increasing in spongiocytes (Figure 2 OF-2, OF-3).

Membrane folding was observed in the basal lamina and at regions of cell contact. Vacuolization at the border of the basal lamina and bubble-like separations within it were also reported. Details included dilated cristae in endothelial cell mitochondria, heterochromatin-rich regions in cell nuclei, and thickening of the basal lamina (Figure 2 OF-4, OF-5). These comprehensive observations highlight a range of structural changes and cellular variations within the endothelial cells and their immediate surroundings (Figure 2 OF-6).

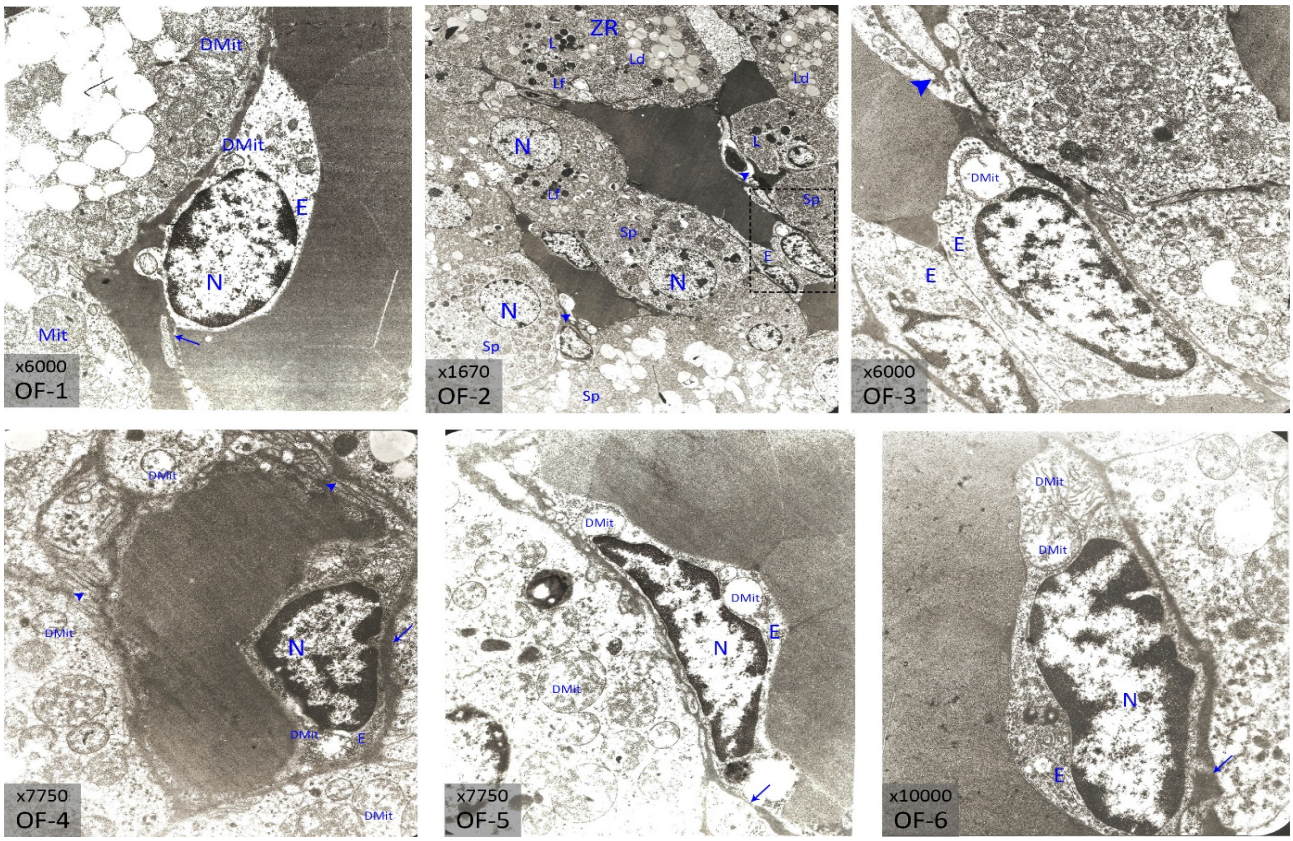

Figure 2. Electromicrographs of the old female group E: endothelial cell, Sp: spongiocyte, ZR: zona reticularis, L: lysosome, Lf: lipofuscin, Ld: lipid droplets, N: nucleus, Mit: mitochondrion, DMit: degenerated mitochondrion, →: refers to basement membrane

Darkening of the endothelial cell nucleus, mitochondrial degeneration, cytoplasmic vacuolization, lipofuscin, cell growth, separation, thickening of the basal lamina, and mitochondrial degeneration in the spongiocyte were observed (Figure 3 OM-1). Vacuolization of endothelial cells increased, and the lumen was covered with endothelial cells. It was observed that the integrity of the basal lamina was disrupted and some mitochondria in the spongiocytes were disrupted. Extravasation and stasis formation were observed due to deterioration of the vascular wall (Figure 3 OM-2, OM-3). There was GER dilatation in the endothelium and dilatation in the perinuclear space (Figure 3 OM-4). The endothelial nucleus was completely filled with heterochromatin, and there was dilation of the perinuclear space and thickening of the basal lamina between the endothelial cell and spongiocyte. Numerous detachments, degenerated mitochondria, and lipofuscin accumulations were observed in spongiocytes (Figure 3 OM-5, OM-6). There were many spaces within and between cells, and debris in some of the larger spaces has been thought to remain from degenerated cells (Figure 3 OM-7). An excessive increase in endothelial cytoplasm and detachment around the basal lamina were observed. There was a rupture between the spongiocyte and the vessel wall. In the sample, the erythrocyte extravasated abnormally into the endothelial cell line (Figure 3 OM-8.). Overgrowth of endothelial cells caused the artery to narrow, leading to blockage. Increased mitochondria were observed around the heterochromatin-rich nucleus in the endothelium, and lipofuscin formation was observed in the endothelium and spongiocytes. Additionally, cell contents were seen to leak into the arterial lumen (Figure 3 OM-9).

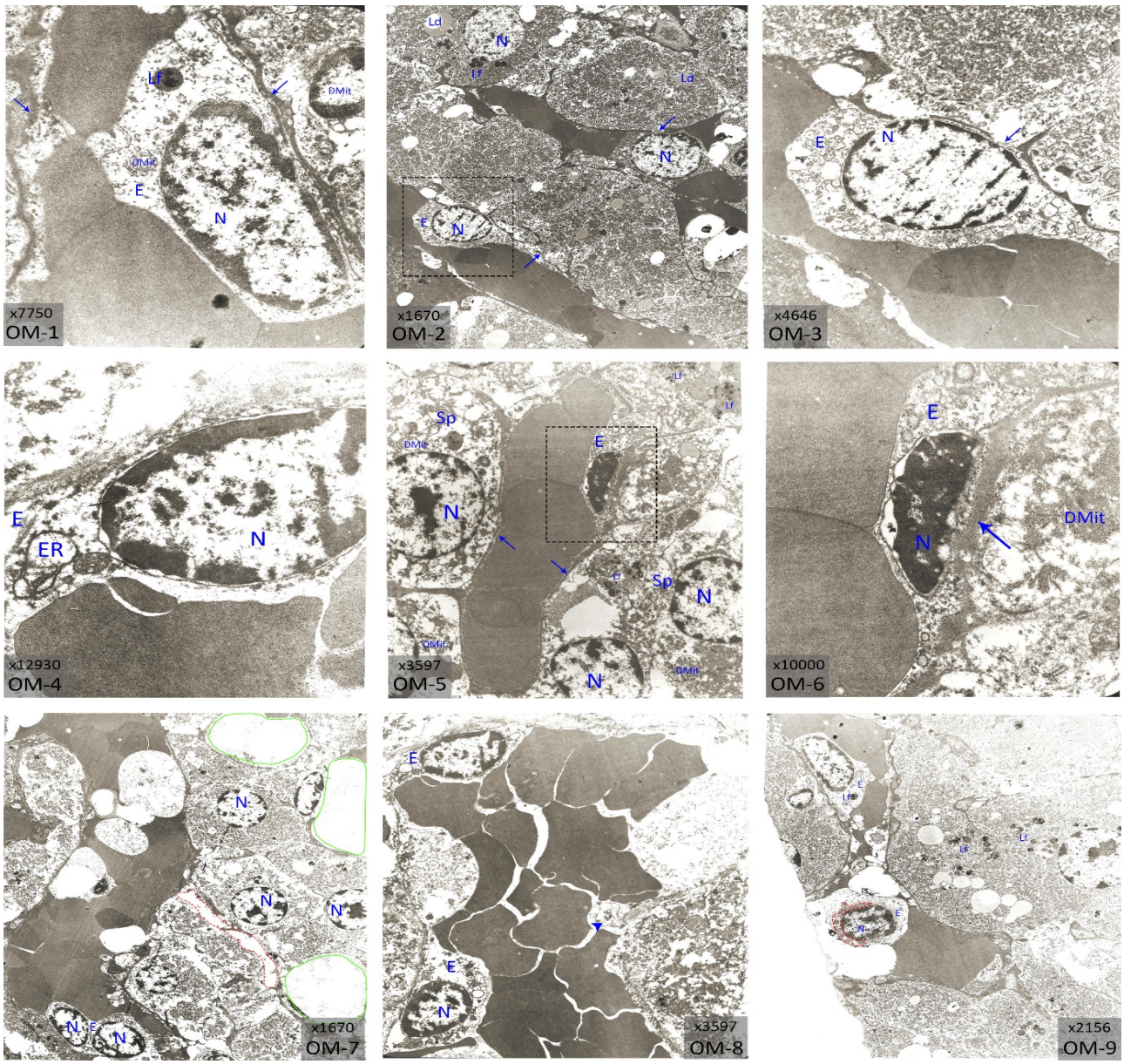

Figure 3. Electromicrographs of the elderly male group E: endothelial cell, Sp: spongiocyte, ZR: zona reticularis, L: lysosome, Lf: lipofuscin, Ld: lipid droplets, N: nucleus, Mit: mitochondrion, ER: endoplasmic reticulum, DMit: degenerated mitochondrion, →: basement membrane

DISCUSSION

Age-related changes in the endothelial cells of the adrenal gland have been suggested to play an important role in transcapillary transport under extreme conditions. These changes in the endothelium reflect the deterioration in energy production and protein synthesis in old animals. The effect of these changes has been the facilitation of the progression of pathological formations in the glands in old animals (Stupina and Shaposhnikov, 1981). One study suggested that the loss of function of the adrenal cortex in humans has been associated with senescence and apoptosis. The causes that may be responsible for the development of senescence and apoptosis are the development of ischaemia-reperfusion injury and its consequences as a result of impaired circulation. As a result of ischaemia-reperfusion injury, increased ROS, endothelial leakage, extravasation, and inflammation are observed. It is thought that such damage, which recurs throughout life, leads to the loss of adrenocortical cells (Hornsby, 2002). A similar study reported that transient ischaemia can cause deterioration of the adrenal cortex over the years. In addition, selective apoptosis of ZR was thought to be related to increased expression of MHC-II and Fas (CD95) ligands with age, and cellular aging in general was thought to be the result of telomere shortening (Hornsby, 2004). In our study, spongiocyte degeneration and vessel wall separation were

observed. Disorders in the vessel wall disrupted the permeability of the endothelium and led to extravasation. In addition, stasis formations were also present in our study. It is possible that the endothelial cells lining the lumen in our study may contribute to circulatory disturbances by narrowing the vessel.

A study analyzing the ultrastructural structures of secretory cells in aging endocrine glands reported fibrillar formations in the nuclei of adrenal spongiocytes and mitochondrial cristae in both normal and reduced amounts (Shaposhnikov, 1985). In our study, darkening in the form of streaks was observed in the nuclei of some cells. Mitochondria in spongiocytes had both normal and degenerated appearance. In a study conducted in rats, it was reported that dead parenchymal cells protruded into the vessel lumen as a result of endothelial damage in adrenal glands (Chen-Pan et al., 1996). In our study, similar ruptures in the vessel wall and cell contents in the lumen were observed.

In a study in rats, hypertrophy was observed in ZF and ZR, which increased with age. Hypertrophy in ZF and ZR was associated with the proliferation of mitochondria and smooth ER involved in steroid hormone synthesis. Increased lipofuscin accumulation in the ZR with age and markedly increased lipid droplets in old ZF and ZR cells were reported (Rebuffat et al., 1992). A study conducted on males reported that ZR occupies a larger area in the adrenal cortex in young than in older animals. The ratio of ZF and ZG to the zona reticularis is lower in young than in old (Parker et al., 1997). We also observed the accumulation of lipofuscin and lysosomes in ZR cells. The increase in the number of mitochondria and lipid droplets in spongiocytes with age is in accordance with previous studies. We can conclude that the adrenal gland tries to compensate for the decreasing number of cells due to aging through cellular hypertrophy. Lipofuscin increase is an indicator of cellular aging in spongiocytes. As the spongiocytes respond to the decreasing number of cells by hypertrophy, they prolong cell life more than expected and the number of lipofuscin-like pigments also increases. The decrease in volume of the ZR may be due to its hypersensitivity to the decrease in blood supply due to endothelial damage. Because, as mentioned above, the vessels that come to the ZR come after passing through the ZG and ZF in the capsule part, a previous disturbance in the blood supply affects the ZR the most. Also, there are studies showing that changes in the endothelium with age impair blood supply (Scioli et al., 2014).

Decreased mitochondrial function with age leads to an increase in reactive oxygen species (ROS) and triggers vascular inflammation in endothelial cells (Ungvari et al., 2008). We also observed degeneration and cristae lysis of mitochondria in endothelial cells, some of which were accompanied by heterochromatin-rich regions in the nucleus and disruption of the perinuclear space. We hypothesized that all of this caused dysfunction in the endothelial cells, leading to circulatory disturbances. It can be concluded that circulatory disturbance leads to ischaemia-induced damage to adrenal cells and disruption of endocrine functions.

It has been described that age-related increases in cortisol and decreases in DHEA-S may be associated with the onset and progression of neurodegenerative diseases (Ferrari et al., 2001). It has been reported that aging affects glucocorticoid metabolism in a similar way to chronic stress, leading to neurological and cognitive changes, osteopenia, diabetes mellitus, visceral obesity, altered immune competence, and other disorders through cortisol (Yiallouris et al., 2019). We have mentioned that blood supply deteriorates with age. The effect of impaired circulation on the hypothalamus-pituitary-adrenal cortex axis may explain the changing hormone levels. In our study, we observed thickening of the basal lamina, deterioration of the endothelial cells, tears and separations in the vessel wall, and separations between the basal lamina and the intercellular spaces. These are all changes that can disrupt the exchange of substances between the spongiocytes and the circulation.

We did not find any gender differences in the changes that occur with the aging of the adrenal glands. However, we concluded that the degeneration of endothelial cells and vessels is relatively more severe in males. Although the reason for the difference between women and men is not well understood, it is known that the risk factor for estrogen-dependent cardiovascular disease is lower in women. However, we did not look at this in our study.

CONCLUSION

It is a fact that aging causes undesirable changes in the adrenal glands. It is known that some of these

changes are caused by cellular senescence, apoptosis, and telomere shortening that accompany aging. However, we can now say that changes occurring in the circulation as a result of endothelial aging are also responsible for these changes. Since the signals received by endocrine organs come from the blood and are secreted into the blood, they are directly affected by a circulatory disorder. In order to prevent age-related deterioration of the adrenal glands, blood flow must be fully guaranteed. In order to fully understand the effects of aging on the adrenal gland, designing and conducting a study to prevent endothelial damage will shed more light on the missing aspects of the subject.

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