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Impact of Aging on the Morphology of the Adrenal Gland in Rats

Impacto del Envejecimiento en la Morfología de la Glándula Suprarrenal en Ratas

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SUMMARY: Aging is an inevitable biological process that affects the function of all organs, including the adrenal gland, which is essential for producing steroid hormones that regulate metabolism, stress response, and immune activation. Understanding how aging affects the morphology of this gland is crucial to developing interventions to mitigate its adverse effects. Thus, this study aimed to describe the morphoquantitative alterations of the adrenal gland in senescent Sprague Dawley rats compared to adult rats. Twelve male rats were divided into 6 adult rats aged 6 months (group A) and 6 senescent rats aged 36 months (group S). Histopathological studies, quantification of collagen fibers types I and III, and stereological analysis were performed to determine the volume density (Vv), surface area (Sv), and number (Nv) of the nuclei of the zona fasciculata cells. Adrenal gland tissue from group S presented dysplasia, metaplasia, intracellular fat accumulation, fibrosis, blood vessel dilation, and increased presence of apoptotic cells. Capsule thickening and increased collagen type I were also observed. There was a significant decrease in Vv, Sv, and Nv of zona fasciculata nuclei in group S compared to group A. The results indicate that aging induces significant morphoquantitative changes in the adrenal gland, which could contribute to the decrease in glucocorticoid production and alterations in aldosterone and cortisol secretion observed in senescence. Understanding these alterations is crucial to developing interventions that mitigate the adverse effects of aging on the endocrine system.

KEYWORDS: Adrenal gland; Senescence; Morphological changes.

INTRODUCTION

Aging is an inevitable biological process that entails a gradual decline in physiological function, affecting all organs, including the adrenal gland. This gland is crucial for producing essential steroid hormones, such as mineralocorticoids, glucocorticoids, and androgens, which regulate metabolism, stress response, and immune activation (Chahal & Drake, 2007; Warde *et al*., 2023a). Understanding how aging impacts adrenal gland morphology is vital to better understanding senescence-related physiological changes and their implications for overall health.

Studies have investigated the effects of aging on the adrenal gland, revealing significant changes in its structure and function. Recent research indicates a reduction in the size of the adrenal cortex, changes in its zonation, and a rise in the infiltration of myeloid immune cells. These effects are observed in both males and females, but are more noticeable in males (Jin *et al*., 2023; Warde *et al*., 2023b). Other studies have identified that the zona reticulata region, responsible for the production of dehydroepiandrosterone sulfate (DHEA-S), is particularly susceptible to aging, showing signs of senescence, exhaustion and altered hormonal production (Wang *et al*., 2024).

Furthermore, a decrease in the stress response has been observed due to changes in adrenal morphology, resulting in a reduced capacity to manage physiological and metabolic stress (Yiallouris *et al*., 2024). These changes include a reduction in glucocorticoid production and an alteration in aldosterone and cortisol secretion, which has pathological implications such as a compromised immune response, decreased bone health, and age-related diseases (Goncharova & Lapin, 2002; Moffat *et al*., 2020).

Despite these findings, there are significant gaps in knowledge about specific morphoquantitative alterations in

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the adrenal gland in senescent rats. Most studies have focused on functional and hormonal aspects, leaving a gap in the detailed characterization of quantitative structural changes. This study is essential in order to mitigate these gaps and offer a deeper understanding of the impact of aging on the adrenal gland.

The clinical and biomedical implications of this knowledge are vast. Understanding how aging affects adrenal gland morphology could help develop interventions to mitigate the adverse effects of aging on the endocrine system. This is particularly relevant given the increasing life expectancy and the growing proportion of the population reaching advanced ages (United Nations, 2019). Novel anti-aging interventions offer a promising avenue to mitigate adrenal aging and alleviate age-associated pathologies, including adrenal tumors (Warde *et al*., 2023a).

Based on the above, this study aimed to describe the morphoquantitative alterations of the adrenal gland in senescent rats compared to adult rats. We hope to provide a solid foundation for future research and potential clinical applications by analyzing the structural changes in detail.

MATERIAL AND METHOD

Animals. The experimental procedures were performed according to the Guide for the Care and Use of Laboratory Animals (2011), and the experimental protocol was approved by the Scientific Ethics Committee of the Universidad de La Frontera, Chile (File Number 041_24). Twelve male Sprague Dawley rats were used, divided into two groups: 6 adult male rats aged 6 months (group A) and 6 senescent male rats aged 36 months (group S). All rats were obtained from the vivarium in the Center of Excellence in Morphological and Surgical Studies (CEMyQ) of the Universidad de La Frontera, Chile. The rats were kept under controlled environmental conditions, including temperature, ambient noise, and a 12-hour light/12-hour dark cycle. In addition, they received food and water *ad libitum*. The hormonal differences between sexes can affect the physiological and morphological response of the adrenal gland, which justifies the use of male rats in studies on the morphology of this gland. Studies have shown significant differences in the morphology and function of the adrenal gland between male and female rats, which could confound the results if both sexes are used without adequate control. In addition, using a single sex eliminates an additional variable, allowing a more accurate assessment of the effects of age on adrenal gland alterations (Neirijnck & Schedl, 2022)
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Sample size determination was based on the bioethical principles of Russell and Burch's 3 Rs (Russell & Burch, 1959) for animal experimentation: replacement, reduction, and refinement, using the minimum number needed to achieve a significant difference (Cruz Orive & Weibel, 1990).

Euthanasia was performed by an overdose of 160/20 mg/kg ketamine/xylazine (Canadian Council on Animal Care, 1998). Subsequently, one adrenal gland was randomly selected from each animal and immersed in fixative (1.27 mol/L formaldehyde in 0.1 M phosphate buffer pH 7.2) for 24 h at room temperature.

Histological processing and staining. Once the samples were fixed, they were dehydrated and embedded in Paraplast Plus (Sigma-Aldrich Co., St. Louis, MO, USA). Once the blocks were obtained, 5 µm thick sections were made on a microtome (Leica RM 2255). Five sections were made from each block, stained with hematoxylin and eosin (H&E) for histopathological and stereological analysis, and with Sirius Red to detect and quantify collagen fibers types I and III. The histological images were obtained using a Leica® DM750 microscope with a Leica® ICC50 HD camera and projected on a View Sonic® LCD.

Histopathological analysis. A descriptive analysis of the adrenal gland was performed in both groups. Various morphological alterations were documented in the cells of the glandular tissue, including fibrosis, nodule formation, modifications in tissue structure, accumulation of intracellular pigment, and infiltration of adipose tissue, among other aspects (Warde *et al*., 2023b)

Quantification of collagen fibers. For the detection of collagen fibers types I and III in the adrenal gland, the histological sections were stained with Sirius Red F3BA 0.1 % w/v (Sigma- Aldrich Co., St. Louis, MO, USA) for 1 h in a saturated aqueous solution of picric acid (Merck, Darmstadt, Germany). Subsequently, they were rinsed in 0.01 N hydrochloric acid (Merck, Darmstadt, Germany) for 2 min, washed in distilled water, stained with Harris hematoxylin (Merck, Darmstadt, Germany) for 2 min, and washed in running water. Finally, the sections were dehydrated in ascending alcohols, cleared in xylene (Merck, Darmstadt, Germany), and mounted with Entellan® (Merck, Darmstadt, Germany). Histological images were obtained using a Leica® DM750 microscope with a Leica® ICC50 HD camera and projected on a View Sonic® LCD. The total area (μm^2) of collagen fibers, types I and III, was measured using Image-Pro Premier 9.1 Software (Media Cybernetics, Warrendale, PA, USA).

Stereology of the adrenal gland. For the stereological analysis of the adrenal gland, 5 random microscopic fields were observed for each histological section, and 60 fields in total were analyzed (30 in each group) (Mandarim-de-

Lacerda & del Sol, 2027). The slides were viewed under a Leica® DM2000 LED stereological microscope and photographed with a Leica® MC170 HD digital camera. The 36-point test system designed by STEPanizer® was used, and the following parameters were determined: volume density (Vv), surface density (Sv), and number density (Nv) of the zona fasciculata nuclei. The volume density was estimated using the following formula: $Vv = P_p/P_T$ (100 %), where P_p is the number of points that touch the structure of interest, and P_T is the total number of points in the system (36 points). Surface density was assessed using the equation $Sv = 2 \times I/L_T$, where I is the number of intersections touching the structure of interest and L_T is the total line length of the 36-point test system. Number density was quantified according to the equation $Nv = Q\text{-}/(A_T \times t)$, where Q- is the number of observations of the structures of interest counted in a given area considering the forbidden lines and the forbidden plane, A_T is the total area of the test system, and (t) is the thickness of the dissector. Slides were viewed under a Leica® DM2000 LED stereo microscope and photographed with a Leica® MC170 HD digital camera.

Statistical analysis. The Kolmogorov-Smirnov test was used to analyze the quantitative data collected from the measurement of collagen fibers for normality; Levene's test was used to confirm the homogeneity of variances; and the Mann-Whitney U test for type I collagen fibers and the Student's t-test for type III collagen fibers were used to compare medians. The Kolmogorov-Smirnov test was used to analyze the stereological data for normality; Levene's test was used to determine homoscedasticity; and the univariate analysis of variance test of general linear models (univariate ANOVA) was used to compare the means between the groups. A $p < 0.05$ was considered statistically significant. The IBM SPSS statistics program, version 21, was used.

RESULTS

Histopathological analysis of the adrenal gland in adult and senescent rats: key findings and significant differences.

The rat adrenal gland is a small, encapsulated organ located at the upper pole of each kidney. As in humans, it is composed of two distinct regions: the cortex, which occupies the outer part of the gland, and the medulla, which is located in the center. The adrenal cortex is subdivided into three histological zones: the zona glomerulosa (ZG), the zona fasciculata (ZF), and the zona reticularis (ZR), while the medulla is predominantly composed of chromaffin cells (Fig. 1).

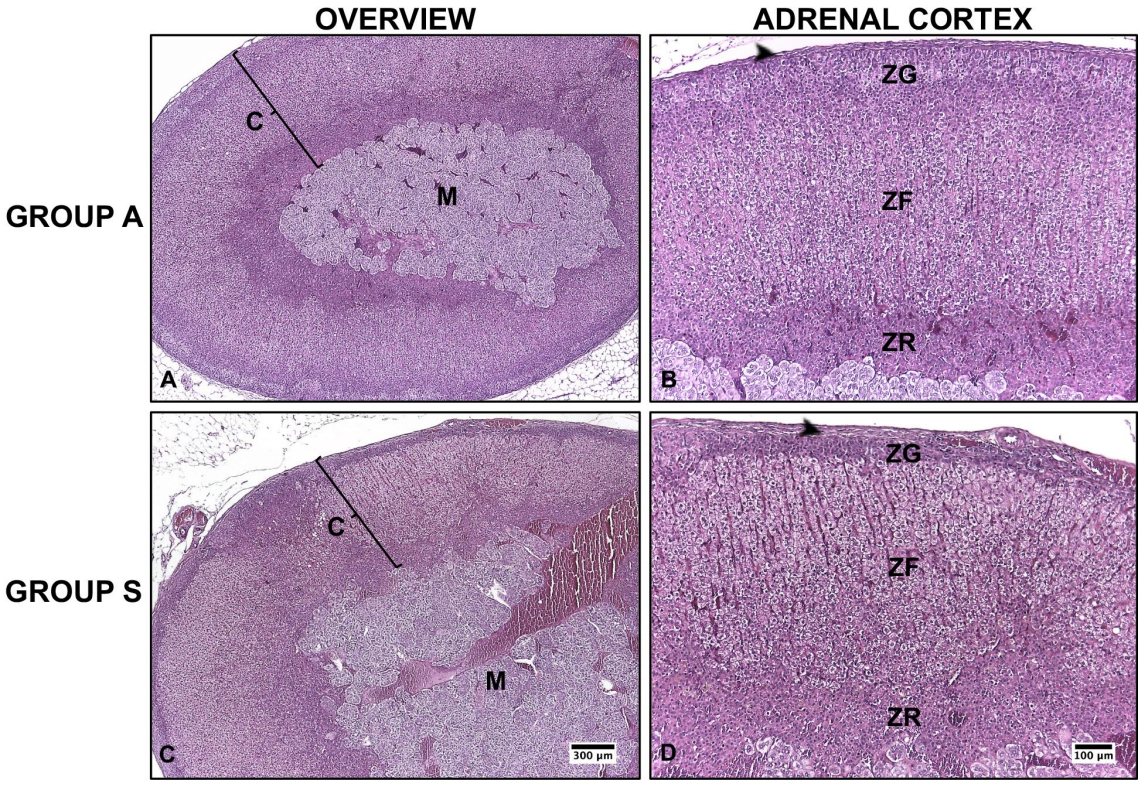

Fig. 1. Adrenal gland of adult (group A) *versus* senescent (group S) Sprague Dawley rats. C: cortex, M: medulla, ZG: zona glomerulosa, ZF: zona fasciculata, ZR: zona reticularis, arrowhead: capsule. H&E stain.

In adult rats in group A, the ZG, which is closest to the capsule, was composed of small, compact cells organized in nests or glomeruli, typical of this zone. The cells had spherical nuclei and slightly basophilic cytoplasm. Immediately below the ZG, the ZF was the thickest in the cortex. It is comprised of large cells arranged in radial cords and separated by sinusoidal capillaries. The fasciculated cells (spongy corticosterocytes) had eosinophilic cytoplasm filled with lipid droplets and central nuclei. The ZR, the innermost of the cortex, was composed of smaller cells arranged in an irregular or reticular network. The reticular cells had dense nuclei and eosinophilic cytoplasm, with fewer lipids than the ZF. The adrenal medulla, located in the center of the gland, was predominantly composed of chromaffin cells and connective tissue. Chromaffin cells clustered around blood vessels. These cells showed granular cytoplasm due to secretory granules stained intensely with H&E (Fig. 2 A-D).

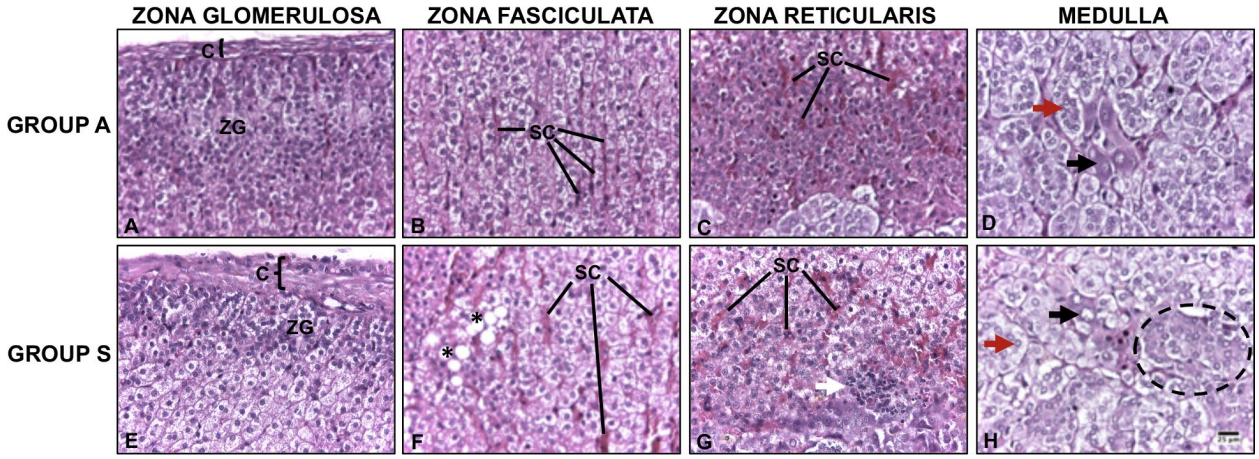

Fig. 2. Adrenal gland of adult (group A) *versus* senescent (group S) Sprague Dawley rats. C: cortex, ZG: zona glomerulosa, SC: sinusoidal capillaries, red arrow: medullary chromaffin cell, black arrow: multipolar autonomic ganglion cell, white arrow: inflammatory infiltration, asterisk: fatty infiltration, dashed circle: nest-like cellular arrangement. H&E staining.

Dysplastic and metaplastic changes were observed in more than 50 % of cases. Dysplasia was characterized by alterations in cell size, shape, and organization, including increased mitoses and nuclear pleomorphism. In contrast, metaplasia manifested as atypical changes in cell morphology inconsistent with the tissue of origin. In 66.7 % of cases, marked dilation of blood vessels was evident, resulting in extensive areas of ischemia and infarction in the senescent glandular tissue (Fig. 3E). Approximately one-third of cases showed intracellular fat infiltration (Fig. 3G) and pigment accumulation in glandular cells consistent with lipofuscin, a recognized marker of cellular aging and chronic oxidative stress (Fig. 3H), being most noticeable in the adrenal cortex. Additionally, apoptotic cells were identified in 16.6 % of cases in senescent tissues. These alterations, taken together, suggest a significant deterioration in the structure and function of the adrenal gland in senescent rats compared to adults.

In senescent rats, the ZG was less thick, the cells had less basophilic cytoplasm, and the nuclei showed signs of pyknosis, indicating cellular degeneration. Focal areas of subcapsular hyperplasia were observed in some samples (Fig. 3A and B). In the ZF, the spongy corticosterocytes had smaller nuclei, and the cytoplasm showed signs of vacuolization and degeneration, indicative of cellular stress and metabolic alterations. In addition, an accumulation of intracellular pigments was detected. The stroma of the ZF was also affected, with a notable increase in interstitial fibrosis between the cell cords. The ZR exhibited similar changes but with some peculiarities. The cells in this zone showed vacuolated cytoplasm and condensed nuclei, suggesting apoptosis and cellular degeneration processes. As in the ZF, pigment accumulation was observed. Compared with adult rats, the ZR of senescent animals showed inflammatory infiltration (Fig. 2G) and a less structured organization of the cellular network (Fig. 3H).

In the adrenal medulla of rats from group S, an atypical proliferation of cells infiltrating the cortical region was observed; this metaplasia of medullary cells was mainly observed in the ZF and ZR (Fig. 3D). Additionally, morphological abnormalities were detected in medullary cells. A decrease in the cellularity of chromaffin cells and an increase in stromal fibrosis were observed. The cells showed a reduction in the secretory granules, indicating a lower synthesis activity and release of catecholamines. Dysplastic and pleomorphic nuclei and cells with atypical mitotic processes were observed (Figs. 2F and 3I). The adrenal medulla of an individual from group S showed an altered arrangement in its histological architecture, compatible with pheochromocytoma (Fig. 3C).

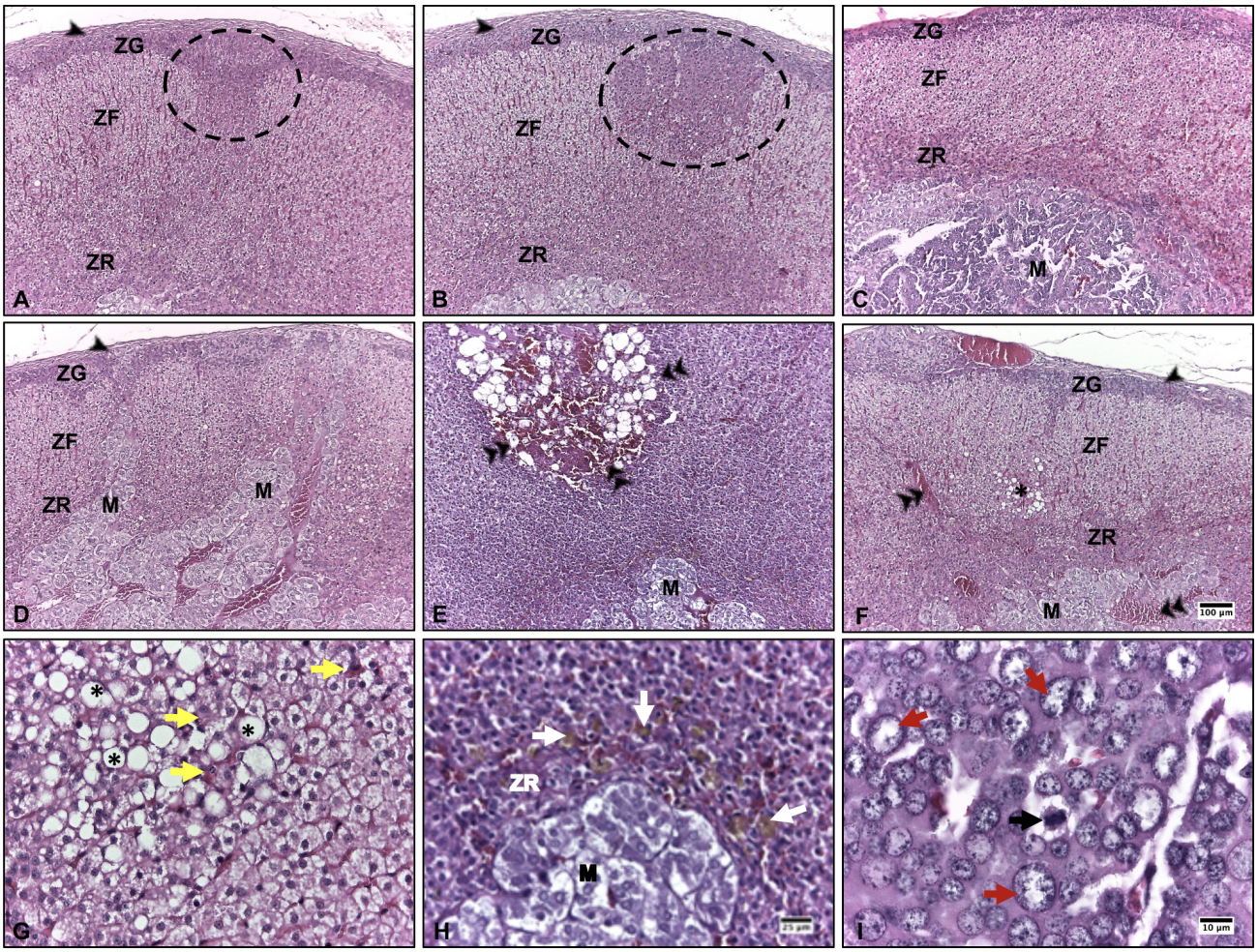

Fig. 3. Adrenal gland of senescent Sprague Dawley rats. M: medulla, ZG: zona glomerulosa, ZF: zona fasciculata, ZR: zona reticularis, arrowhead: capsule, dashed circle: focal areas of subcapsular hyperplasia, double arrowhead: vessel dilation and areas of ischemia in the glandular tissue, asterisk: fatty infiltration, yellow arrow: apoptotic cells, white arrow: intracellular pigment, red arrow: dysplastic and pleomorphic nuclei, black arrow: mitotic processes. H&E staining.

Fibrosis was identified in half of the cases studied, with a more noticeable presence in the glandular medulla stroma compared to the cortical zone. Additionally, thickening of the adrenal gland capsule was observed, accompanied by an increase in the content of type I collagen (Fig. 4) (Table I).

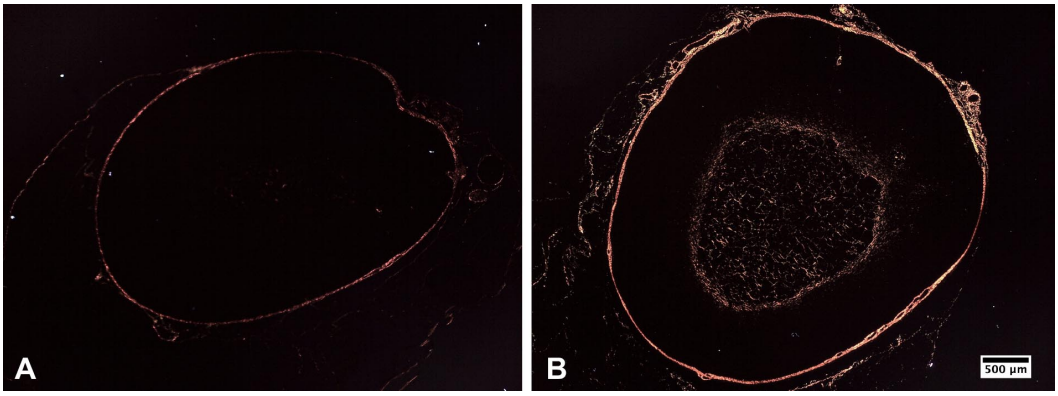

Fig. 4. Presence of collagen type I and III fibers in the adrenal gland tissue of adult (A) *versus* senescent (S) Sprague Dawley rats. Collagen type I (red and yellow) and III (green) fibers. Sirius Red staining.

Table I. Quantifying collagen content in adrenal gland tissue from adult (A) *versus* senescent (S) Sprague Dawley rats using Image Pro Premier.

	Median ± SD		
Collagen	**A**	**S**	**p**
Type I (μm^2)	47.03 ± 7.94	84.49 ± 20.92	0.001*
Type III (μm^2)	16.69 ± 2.7	18.56 ± 2.17	0.005*

*Significant differences (<0.05).

Stereological analysis revealed that during senescence, there is a reduction in the size and number of nuclei in the zona fasciculata.

Stereological analysis showed statistically significant differences in the parameters of volume density (Vv), surface density (Sv), and number density (Nv) of the ZF nuclei between the senescent group (S) and the adult group (A), Table II.

DISCUSSION

This study revealed significant morphoquantitative alterations in the adrenal gland of senescent rats compared to adult rats, highlighting both structural and functional changes. These findings are consistent with previous research into the impact of aging on the adrenal gland and its physiological implications.

More than 50 S% of the samples from the senescent (S) group presented alterations in the normal tissue arrangement, including dysplasia and metaplasia. This agrees with what was reported by Grabek *et al*. (2019), who stated that aging constitutes a risk factor for neoformations due to changes in the tissue microenvironment, such as variations in the availability of oxygen and nutrients, infiltration of inflammatory immune cells and increased fibrotic tissue in the adrenal stroma. These factors, together with the accumulation of mutations and the activation of oncogenes, can alter the DNA structure, causing unusual and uncontrolled cell growth characterized by nodules and clusters of encapsulated cells within the adrenal cortex (Gao *et al*., 2021a; Warde *et al*., 2023a).

On the other hand, changes in blood vessels, such as dilation and zones of ischemia observed in senescent rats, are consistent with vascular aging and can be explained by several interrelated mechanisms. Aging is associated with endothelial dysfunction characterized by reduced nitric oxide production and increased oxidative stress, leading to alterations in vasodilation (Donato *et al*., 2015). In addition, with age, vascular remodeling occurs, which involves thickening of the wall and increased arterial stiffness, which can result in abnormal dilations and compromised blood flow (Xu *et al*., 2017). Aging also affects angiogenesis processes, which can lead to inadequate vascularization and contribute to the formation of ischemic zones (Lähteenvuo & Rosenzweig, 2012).

Our study revealed significant alterations in the structure of the adrenal gland during senescence, affecting both the cortex and the medulla. We observed changes in tissue architecture, including intracellular lipid accumulation, inflammatory infiltration, and increased fibrous tissue. However, because our analysis was limited to a group of 36-month-old rats, we could not determine the temporal sequence of these changes. In this context, Jin *et al*. (2023) reported that, in mice, senescence-associated alterations manifested first in the adrenal cortex before affecting the medulla. For their part, Huber *et al*. (2024) observed in senescent female baboons that the ZF ages more rapidly than the ZG, attributing this phenomenon to a decrease in mitochondrial function in the production of proteins and hormones in this region. It is plausible that a similar pattern occurs in rats, although further studies are required to confirm this.

Regarding the alterations observed in the adrenal cortex during senescence, several studies have revealed significant structural and functional changes due to hormonal and cellular factors. In senescent mice, Jin *et al*. (2023) reported a reduction in the width of the ZG, a finding that we corroborated in our study. This structural decrease seems to be part of a broader phenomenon of age-related changes in adrenal function. Nanba *et al*. (2018) provide crucial context to understand these changes. According to these authors, aged adrenal glands exhibit altered aldosterone synthase (CYP11B2) expression in the ZG, along with an increase in the formation of aldosterone-producing cell clusters (APCC) in this zone. These histological changes suggest a progressive transition from a continuous expression of CYP11B2 in the ZG to a predominance of APCC with advancing age. Although the exact mechanisms behind these changes are not fully clarified, the authors propose that genomic, epigenetic, and environmental factors could be involved. Significantly, Nanba *et al*. (2018) hypothesize that these structural changes could transition from normal physiological regulation of aldosterone to a more autonomous and renin-

Table II. Stereological analysis of adult (A) *versus* senescent (S) Sprague Dawley rats.

	Mean ± SD		
Zona Fasciculata	**A**	**S**	**p**
Vv (%)	10.93 ± 2.03	6.93 ± 2.00	0.001*
Sv (mm^{-1})	41.97 ± 9.48	22.44 ± 9.00	0.001*
Nv (mm^{-3})	300000.00 ± 61339.56	114285.71 ± 43824.79	0.001*

*Significant differences (<0.05).

independent aldosterone secretion in senescence. This alteration in aldosterone production could have important clinical implications, potentially increasing the risk of autonomous aldosteronism and associated cardiovascular diseases in older individuals. Our observations, including both reduced ZG width and the presence of focal areas of subcapsular hyperplasia in this region, align with this broader framework of age-related alterations in adrenal function, underscoring the importance of considering these structural findings in the context of their potential functional and clinical implications.

Along with the above, this differential aging process is significantly influenced by the decrease in androgens, a characteristic phenomenon of senescence that plays a crucial role in the progression of the changes observed in the adrenal gland. Warde *et al*. (2023b) and Grabek *et al*. (2019) point out that this hormonal reduction affects both the recruitment and proliferation of adrenal stem cells, resulting in less cell renewal in the cortex. This partially explains the significant differences observed in the volume density, surface area, and number of cell nuclei in the ZF between the S and A groups, as detailed in Table II.

ZF atrophy during senescence has significant functional implications, particularly in cortisol secretion. A gradual decrease, with lower levels in the afternoon and evening, follows increased daytime secretion. These alterations affect the functioning of key organs, especially the brain, including the hippocampus, amygdala, and prefrontal cortex. As a consequence, mental deterioration, dementia, depression, anxiety, memory, and sleep cycle disorders, as well as an inadequate immune response, may occur (Stamou *et al*., 2023; Yiallouris *et al*., 2019). Our study corroborates these findings, evidencing atrophy, nodules, and neoformations in the ZF, changes that can alter circulating cortisol levels and the number of glucocorticoid receptors (Aiba & Fujibayashi, 2011; Gao *et al*., 2021b; Jin *et al*., 2023).

It is important to note that the findings in humans show some variability. While Nonaka *et al*. (2020) reported a lower number of cells in the ZF in senescent men, consistent with our observations, Tezuka *et al*. (2021) found a greater development of the ZF in senescent humans. The latter attributed their findings to alterations in the hypothalamic-pituitary-adrenal axis, although these results are inconclusive. This variability underlines the complexity of senescence-associated changes in the adrenal gland and the need for further research to understand these processes fully.

Our observations in the ZR of senescent rats align with age-dependent changes described in the literature, including the findings of Hornsby (2002, 2004). Cells with vacuolated cytoplasm, condensed nuclei, pigment accumulation, and a less structured cellular organization reflect the degenerative processes characteristic of adrenal aging. Hornsby (2002) highlights the complexity of these changes, pointing out that aging of the adrenal cortex involves interactions between alterations in growth, differentiation, apoptosis, and cellular senescence. In line with these observations, our study revealed an increase in the production of apoptotic cells in the senescent adrenal gland. Although apoptosis is a programmed cell death mechanism that typically maintains tissue homeostasis, we observed an abnormal accumulation of apoptotic cells in senescence. This phenomenon is attributed to irreversible cell cycle arrest and senescence-associated secretory factor (SASP). Han *et al*. (2021) and Fraser *et al*. (2022) point out that this accumulation of apoptotic cells constitutes a risk factor for developing cancer and other senescence-related diseases. Our findings of inflammatory infiltration and cellular network disorganization in the ZR could be manifestations of these interactive processes between senescence, apoptosis, and tissue alterations. Furthermore, a particularly intriguing aspect Hornsby (2004) mentions is the loss of dehydroepiandrosterone (DHEA) and its sulfate biosynthesis in the human adrenal cortex during aging. Although our study in rats did not directly measure DHEA levels, the structural changes observed in the ZR could be related to this phenomenon. The involution of the zona reticularis, which Hornsby points out as an enigma in humans, seems to have parallels in our rat model, as evidenced by condensed nuclei, pigment accumulation, and tissue disorganization. These findings underline the complexity of structural and functional changes in the adrenal cortex during aging and their potential impact on hormone production. The interaction between the processes of senescence, apoptosis, and alterations in tissue architecture appears to create a microenvironment conducive to glandular dysfunction and, potentially, to the development of aging-associated pathologies.

Our study revealed significant alterations associated with senescence in the adrenal medulla. A decreased chromaffin cell density and increased medullary stromal fibrosis were noted. Furthermore, the chromaffin cells present showed a reduction in the number of secretory granules, suggesting a decrease in the synthesis and release of catecholamines. These findings are consistent with previous observations by Jin *et al*. (2023) in senescent mice, reinforcing that these structural and functional changes in the adrenal medulla are characteristic of aging.

A particularly intriguing finding in rats from group S was the atypical proliferation of medullary cells that infiltrated the cortical region. This metaplasia was mainly observed in the ZF and ZR. Furthermore, in one individual from group S, an altered arrangement in the histological

architecture of the adrenal medulla was found to be compatible with pheochromocytoma. This finding, although isolated, underscores the potential progression of degenerative changes toward neoplastic conditions in aged adrenal tissue. These structural changes and tissue disorganization could represent stages of aging-associated alterations, ranging from degenerative changes to forming benign and even malignant tumors. This is in line with the observations of Hornsby (2002), who highlights the frequent appearance of nodules and adenomas in the aged adrenal cortex. However, he notes that carcinomas are uncommon in this context. These alterations in both the medulla and cortex underscore the complexity of aging-associated changes in the adrenal gland. The infiltration of medullary cells into the cortex and the possible formation of neoplasms, such as the observed pheochromocytoma, could have significant implications for glandular function, potentially altering hormone production and organismal homeostasis. These findings suggest that the aging process in the adrenal gland involves not only degenerative changes but also alterations in cell organization and differentiation that may lead to tumor formation. This conclusion warrants further and detailed investigation.

Despite the observed findings, one limitation of this study is the sample size, which may not be sufficient to generalize the results to all senescent rats. Furthermore, only male rats were used, which limits the possibility of extrapolating the findings to females. Another limitation is the lack of complementary functional analyses that could better understand the physiological consequences of the observed morphological alterations.

Future studies should consider a larger sample size and the inclusion of female rats to explore sex differences in adrenal gland aging. Furthermore, performing additional functional and molecular analyses to better understand the mechanisms underlying the observed morphoquantitative alterations would be beneficial. It is also important to investigate possible interventions that can mitigate the adverse effects of aging on the adrenal gland.

CONCLUSIONS

This study provides strong evidence that aging induces significant morphoquantitative changes in the adrenal gland of rats, affecting both its structure and function. The findings highlight the importance of continuing to investigate the implications of aging on the endocrine system to develop interventions that can mitigate its adverse effects. Moreover, stereological approaches are a significant tool for precisely characterizing the intricate aging-associated morphological changes.

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RESUMEN: El envejecimiento es un proceso biológico inevitable que afecta la función de todos los órganos, incluida la glándula suprarrenal, fundamental para la producción de hormonas esteroides que regulan el metabolismo, la respuesta al estrés y la activación inmunológica. Comprender cómo el envejecimiento afecta la morfología de esta glándula es crucial para desarrollar intervenciones que mitiguen sus efectos adversos. Así, el objetivo de este estudio fue describir las alteraciones morfocuantitativas de la glándula suprarrenal en ratas Sprague Dawley senescentes comparadas con ratas adultas. Se utilizaron 12 ratas macho, divididas en dos grupos: 6 ratas adultas de 6 meses de edad (grupo A) y 6 ratas senescentes de 36 meses de edad (grupo S). Se realizaron estudios histopatológicos, cuantificación de fibras de colágeno tipos I y III y análisis estereológicos para determinar la densidad de volumen (Vv), de superficie (Sv) y de número (Nv) de los núcleos de las células de la zona fasciculada. El tejido de la glándula suprarrenal del grupo S presentó displasia, metaplasia, acumulación de grasa intracelular, fibrosis, dilatación de vasos sanguíneos y mayor presencia de células apoptósicas. También se observó un engrosamiento de la cápsula y un incremento del colágeno tipo I. Hubo una disminución significativa en Vv, Sv y Nv de los núcleos de la zona fasciculada en el grupo S en comparación con el grupo A. Los resultados indican que el envejecimiento induce cambios morfocuantitativos significativos en la glándula suprarrenal, lo que podría contribuir a la disminución en la producción de glucocorticoides y alteraciones en la secreción de aldosterona y cortisol observadas en la senescencia. Comprender estas alteraciones es crucial para desarrollar intervenciones que mitiguen los efectos adversos del envejecimiento en el sistema endocrino.

PALABRAS CLAVE: Glándula suprarrenal; Senescencia; Cambios morfológicos.

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