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Morphometric Study of Connective Tissue in the Human Pituitary during Aging Process

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SUMMARY

The aim of the research was to quantify the connective tissue and blood vessels in the aging human pituitary, using the morphometric methods.

The material contained 29 pituitary tissues from cadavers of both sexes (14 females and 15 males), aged 33-95 years. The pituitary tissue was processed by standard histological procedure and stained by Mallory trichrome method. Histological samples were analyzed using a light microscope with magnification of 400x. Morphometric analysis was performed on digital pictures with the resolution of 1.3 megapixels, at 40 pituitary visual fields of each case examined. Fovea Pro 4 system was used for the analysis. Above the digital images of each field of view, there was inserted a simple network test system. Using this programme, there were determined bulk densities of connective tissue and blood vessels in the examined cases. The results were statistically analyzed using the package NCSS-PASS 2007.

Correlation analysis showed that the bulk density of connective tissue statistically significantly increased during aging. The average bulk density of blood vessels in the pituitary manifested statistically significant decrease during the aging process. These results were confirmed by the linear regression analysis. The average bulk density of the connective tissue, as well as the average bulk density of blood vessels in the pituitary cases, did not differ significantly among males and females.

The process of aging is followed by the proliferation of the connective tissue and fibrosis of the pituitary, which is accompanied by the reduction of its vascular network.

Key words: **human pituitary, connective tissue, morphometry, aging**

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INTRODUCTION

Aging is a universal process that results in a gradual loss of function of most of the organs. Endocrine tissues, as well as other tissues, change during aging. That leads to corresponding changes in secretion of hormones. The pituitary gland, with that respect, is not an exception. It suffers aging in functional decline mainly reflected in its secretory activity, which was confirmed by the results of some studies that detect lower levels of certain hormones in the blood, such as growth hormone, prolactin, thyrotropin and gonadotropin (1-3).

Morphologically, the adenohypophysis is the front part of the pituitary gland, which consists of three parts: pars distalis or anterior lobe, pars intermedia (small portion which is located between the anterior and posterior lobe - in humans it is mostly scrubby) and pars tuberalis (the part that surrounds the infundibulum). Parenchymal cells of the anterior lobe present the endocrine structural component of the pituitary gland. By the affinity to staining, cells are divided into two cell types: chromophils and chromophobes. Chromophils secrete hormones that regulate growth, metabolism and reproduction. Based on their responses to color, chromophils can be divided into acidophils and basophils. Chromophobes do not secrete hormones and their function is not fully understood (4, 5).

Connective tissue forms a fibrous capsule on the surface of the pituitary, which stretches inside the tissue as the interstitial fibrous connective tissue that forms layers to acini of parenchymal cells and sinusoidal capillaries. Parenchymal reduction with reducing the number of cells and atrophy of residual hormone-producing cells with the concomitant proliferation of connective tissue, specifically collagen, is a direct consequence of aging (6).

Despite the fact that the results of some studies indicate the presence of morphological changes in the human pituitary gland during the aging process, we could not find the data of their quantification in the available literature.

AIM

Based on all the foregoing, the aim of the research was to quantify the connective tissue and blood vessels in the aging human pituitary, using the morphometric methods.

MATERIAL AND METHODS

The material contained 29 pituitary tissues from cadavers of both sexes (14 females, 15 males), aged 33 -95 years. Tissues were obtained during routine autopsies performed at the Institute of Forensic Medicine in Niš. In the accompanying medical documents of cadavers whose pituitary tissues were sampled by the dissection method, there were no data of previously diagnosed neurological, endocrine, or any other systemic disorder. Also, the autopsy did not show any visible damage of the brain that could be the cause of death of cadavers. Then, the pituitary tissue was fixed in 10% buffered formalin for no longer than 24 hours, and subsequently embedded in paraffin. After that, the sections 5 μm thick were made and then processed by standard histological procedures and subsequently stained by the Mallory trichrome method. The obtained histological samples were analyzed using a light microscope with magnification of 400x. Morphometric analysis was performed on digital pictures with the resolution of 1.3 megapixels, at 40 pituitary visual fields of each of the 29 cases examined. Fovea Pro 4 system (http://reindeergraphics.com/) was used for the analysis and processing the digital images. Above the digital images of each field of view, there was inserted a simple network test system, generated by the software (Pt=144, At= 31958.40 μm^2, a=220.93 μm^2, d=14.90 μm). Using this programme, volume density of the connective tissue (V_{Vct}) was measured according to the method by Kališnik and Russ (7, 8), while volume density of anterior pituitary's blood vessels (V_{Vbv}) was determined by method of Manoonkitiwongsa et al. (9) examined cases, which were calculated using the formula. The results were statistically analyzed using the package NCSS-PASS 2007 (http://www.ncss.com/). To investigate the relations between the age of the analyzed cases and the values of morphometric parameters, the correlation analysis and linear regression analysis were used. The significance of differences of values of morphological parameters between the cases of males and females was tested by the Student t - test for two independent samples.

RESULTS

Morphological analysis

At the histological preparations of the human pituitary in younger cases that were stained by the Mallory trichrome staining (Figure 1A), in the *pars distalis*, many chromophils grouped in the form of strips or acini were observed. Acidophils were characterized by abundant, uniformly orange to bright red colored cytoplasm and centrally placed, round, dark blue colored euchromatic nucleus. There were slightly less basophils than acidophils, with gray blue colored cytoplasm and vacuoles observed inside. Vacuoles suppressed the dark blue colored euchromatic nucleus to the opposite pole of the cell in these cases. Distribution of acidophils and basophils of the pars distalis was not a homogeneous and it was characterized by the presence of numerous basophils in the middle part of the gland tissue, directly in front of the posterior lobe (pars intermedia), whereas acidophils were significantly greater in extent in the lateral parts of the pars distalis (pars lateralis). Between the chromophils, there were noticed rare chromophobes with poorly visible uncolored cytoplasm and large, round, dark-blue

colored euchromatic nucleus. Green colored connective tissue formed a capsule on the surface of the pituitary. Connective tissue inside the gland provided to the thin partition connective tissue bands between the acini of glandular cells. The aforementioned connective tissue compartments consisted predominantly of the connective tissue fibers between which there were rare, dark-blue colored, elliptical euchromatic fibroblast nuclei and sinusoidal capillaries with thin walls and wide lumens filled with orange painted erythrocytes.

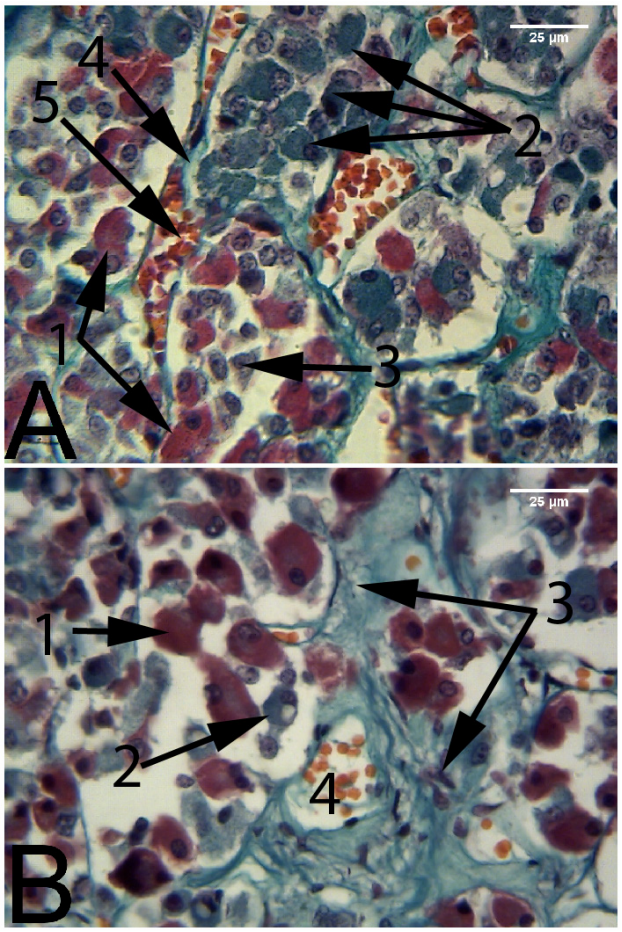

Figure 1. ***(A)*** *Anterior pituitary lobe of a 44-year-old male, 1 - acidophils, 2 - basophils, 3 - chromophobes, 4 - thin connective tissue compartments, 5 - Blood vessels filled with red blood cells,* ***(B)*** *Anterior pituitary lobe of an 89-year-old male; 1 - acidophils, 2 - basophils with vacuole present in the cytoplasm and eccentrically placed nucleus, 3 - thickened connective tissue; 4 - blood vessel; Mallory trichrome staining, 400x.*

Anterior pituitary lobe in older cases (Figure 1B) was characterized by a striking proliferation of the connective tissue that has formed much thicker connective tissue compartments, composed of much more numerous fibers of the connective tissue and by a lesser extent elliptical, dark-blue colored fibroblast nuclei, compared to younger cases. Within these compartments there were scarce blood vessels whose lumens were much smaller. Connective tissue compartments surrounded rare and, to some extent, larger and more irregular distributed chromophils. In older cases, there has been noted a slight increase in the presence of basophils in which cytoplasm the large vacuoles were more commonly observed, resulting in more frequent eccentric position of the dark-blue colored, euchromatic nuclei of these cells. Between the chromophils, there is a noticeable presence of dark-blue colored, euchromatic nuclei of the chromophobes.

Morphometric analysis

Results of the morphometric analysis are shown in Table 1. The correlation analysis showed that the bulk density of the connective tissue statistically significantly increased during aging ($R=0.76$, $n=29$, $p< 0.0001$) in the analyzed cases. Additionally, a linear regression analysis confirmed that the age of the cases represent a statistically significant predictor of average bulk density of connective tissue of the pituitary ($F(1,27)=37.88$, $p<0.0001$).

Table 1. *The values of bulk densities of connective tissue (V_{Vct}) and blood vessels (V_{Vbv}) of the pituitary, age and sex of the analyzed cases*

Case	Age	Sex	V_{Vct} (%)	V_{Vbv} (%)
1	33	Male	11.31	5.25
2	35	Male	12.47	4.76
3	44	Male	13.98	4.95
4	45	Male	11.92	4.07
5	45	Female	12.00	2.62
6	46	Female	11.66	4.97
7	51	Female	14.62	5.53
8	52	Male	14.60	3.22
9	55	Male	15.18	5.87
10	56	Female	15.23	4.44
11	64	Male	19.53	5.30
12	66	Male	18.87	4.03
13	68	Female	19.65	3.94
14	70	Male	17.03	1.93
15	71	Female	14.61	3.02
16	73	Male	16.02	3.87
17	73	Female	17.63	4.30
18	75	Male	18.35	4.38
19	75	Male	19.56	0.14
20	75	Male	18.14	4.94
21	76	Female	22.60	4.97
22	78	Female	16.77	4.96
23	78	Female	17.09	4.92
24	80	Male	16.38	3.99
25	80	Female	15.36	2.83
26	81	Female	19.49	3.66
27	83	Female	17.04	1.42
28	89	Male	17.31	2.92
29	95	Female	20.05	2.40

According to the identified model (V_{Vct}=7.52+ 0.13 x age of the tested cases), age of the above-mentioned cases explained 57% of the total variance of this parameter (adjusted R^2=0.57), which is a very large effect size (Figure 2A). In contrast to the average bulk density of the connective tissue, the average bulk density of blood vessels in the pituitary manifested statistically significant decrease (R=-0.42, n=29, p=0.024) during the aging process, which is further confirmed by the linear regression analysis (F(1.27)=5.76, p= 0.024). In fact, age is a significant predictor of average bulk density of blood vessels in the pituitary, which can be identified by the following model: V_{Vbv}=6:17-0:03 x age of the tested cases. However, the age of the cases explained only 15% of the total variance of this parameter (adjusted R^2=0.15), which is a lower parameter compared to the previous one, but still a moderate effect size (Figure 2B). Student's t - test for two independent samples showed that the average bulk density of the connective tissue, as well as the average bulk density of blood vessels in the pituitary cases, males (16.04 ±2.73%) and females (16.70±3.09%) did not differ significantly (p>0.05).

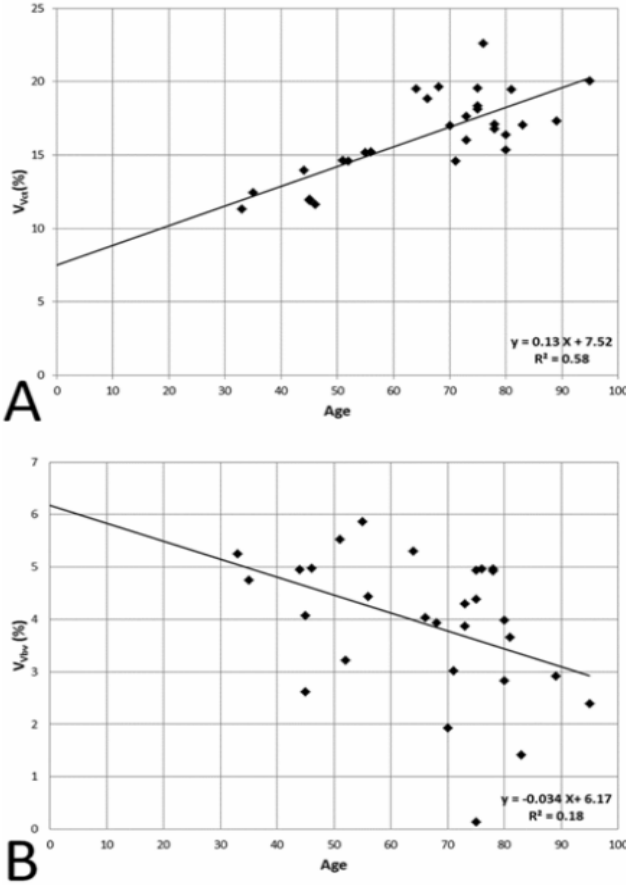

Figure 2. ***(A)*** *Scatter diagram of correlation between the bulk density of connective tissue of pituitary and age of the examined cases,* ***(B)*** *Scatter diagram of correlation between the bulk density of blood vessels and age of the examined cases*

DISCUSSION

Aging is a complex, multifactorial process which causes a reduction in ability to respond to stress, disruption of homeostasis and increased incidence of pathological processes, which finally can have a lethal outcome. According to the System-based theories of aging, it is the consequence of weakening of organs necessary for the control and maintenance of other organ systems, as well as the body's ability to communicate with the environment in which it lives and to which it adapts. The nervous, endocrine and immune systems play a crucial role in these processes. Changes that occur during aging affect not only the neurons and hormones that regulate important functions such as reproduction, growth and development, but also those that regulate survival through adaptation to stress. One of the System-based theories is the Neuroendocrine theory which gives special importance to the hypothalamic-pituitary-adrenal (HPA) axis as the main regulator of the physiological adaptation required for creating and maintaining homeostasis. Chronic exposure to physical, biological or emotional stress during life can lead to exhaustion or weakening of the ability of adaptation and occurrence of "diseases of adaptation" or death (10).

Morphological basis for such physiological changes during life are aging changes that can be observed at the elements of the HPA axis. This time, the subject of the research was the connective tissue of the pituitary.

Changes occurring during the aging process in pituitary are observed both in the parenchyma and in the stroma. Pituitary is covered by a fibrous capsule. Parenchyma is composed of epithelial secretory cells arranged in the cords or follicles, incompletely separated by a basement membrane, and surrounded by reticular fibers and a dense anastomosing network of fenestrated capillaries. Traditionally, the main function of the stroma is mechanical and their main cellular components are fibroblasts. According to the literature (11), the main components of the pericapillar stroma are pericapillary and adventitial cells which provide the cell support and also produce the extracellular matrix supporting the spatial organization of secretory cell populations in this way. The recent studies have shown that perivascular stromal cells may have other important functions, such as the creation of the pluripotent mesenchymal tissue progenitors with extensive tissue differentiation ability. During the aging process, it leads to fibrosis of the gland, which is reflected in the increase in intercelular and perivascular connective tissue. Histochemically and immunohistochemically processed preparations of the pituitary tissue showed that it is mainly made of the collagen fibers. Thus Ayisi et al. (6) in their biochemical study demonstrated a significant increase in the level of hydroxyproline, an integral element of collagen, which indicates the fibrosis of the gland with aging, with a further finding of the pronounced increase in connective tissue in

the adenohypophysis compared to the neurohypophysis. The results of the analysis of a three-dimensional structure of collagen of the goat's pituitary by Nishimura et al. (12) have shown that the collagen fibers form a nest structures containing groups of parenchimal cells, as in other endocrine glands. He also noted the thickening of the basement membranes, especially in the sinusoidal capillaries of the capillary network, which also occurs as a result of the proliferation of collagen fibers, especially in the blood vessels of large caliber and, less often, in the blood vessels of smaller caliber or sinusoidal capillaries (12). Ayisi et al. (6) and Bailey et al. (13) suggest that when the proliferation of connective tissue occurs in aging it mostly contains the collagen type IV, which is the most important structural component of the basement membrane. A significant increase in the bulk density of the connective tissue accompanied by a simultaneous decrease of bulk density of blood vessels during the pituitary aging found during our research agrees with aforementioned literature. According to Bailey et al. (13), this increase is the result of the proliferation and thickening of collagen fibers due to cross-linking of its molecules and consequent polymerization. Physical properties of collagen fibers modify by change. They lose their elasticity, and the tissue containing these fibers becomes firmer. Changes in the collagen of basement membranes of blood vessels lead to disorder of their permeability and reduce the fixing ability of the endothelium, so the proliferation of connective tissue, in addition to reduction of pituitary parenchyma, entails a disorder in functions of its blood vessels (13).

CONCLUSION

Based on the foregoing, it can be concluded that the process of aging is followed by the proliferation of the connective tissue and fibrosis of the pituitary, which is accompanied by the reduction of its vascular network, which can indirectly demonstrate the change of its function during the aging process.

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MORFOMETRIJSKA STUDIJA VEZIVNOG TKIVA HUMANE HIPOFIZE TOKOM PROCESA STARENJA

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Sažetak

Cilj istraživanja bio je da se morfometrijskom metodom kvantifikuje prisustvo vezivnog tkiva i prokrvljenost adenohipofize tokom starenja čoveka.

Materijal je tkivo adenohipofize 29 kadavera oba pola (14 ženskih i 15 muških), starosti 33-95 godina. Tkivo hipofize obrađeno je standardnom histološkom procedurom i bojeno Mallory trihromnom metodom. Dobijeni histološki preparati analizirani su pomoću svetlosnog mikroskopa pod uvećanjem 400x. Morfometrijska analiza je obavljena na digitalnim fotografijama rezolucije 1.3 mega piksela, po 40 vidnih polja adenohipofize svakog od ispitanih slučajeva, uz pomoć Fovea Pro 4 sistema za analizu. Preko digitalne slike svakog vidnog polja superponiran je prosti mrežni testni sistem. Pomoću njega su određivane zapreminska gustina vezivnog tkiva (V_{vt}) i krvnih sudova (V_{vks}) ispitanih slučajeva, koje su izračunavane pomoću formule Kališnika (1985). Dobijeni rezultati su statistički analizirani korišćenjem paketa NCSS-PASS 2007.

Korelaciona analiza je ukazala da zapreminska gustina vezivnog tkiva analiziranih slučajeva statistički značajno raste tokom procesa starenja. Prosečna zapreminska gustina krvnih sudova adenohipofize ispoljavala je statistički značajan pad tokom procesa starenja. Ovi rezultati su potvrđeni linearnom regresionom analizom. Prosečna zapreminska gustina vezivnog tkiva, kao i prosečna zapreminska gustina krvnih sudova adenohipofiza kod muškog i ženskog pola ne razlikuju se statistički značajno.

Proces starenja praćen je proliferacijom vezivnog tkiva i fibrozom adenohipofize koja je praćena redukcijom njene vaskularne mreže.

Ključne reči: **adenohipofiza, vezivno tkivo, morfometrija, starenje**