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THE EFFECT OF AGEING ON MACROMORPHOMETRIC PARAMETERS AND HISTOLOGICAL CHARACTERISTICS OF BASOPHILIC AND ACIDOPHILIC PITUITARY CELLS: ANALYSIS OF MALE CADAVERS

Jovana Čukuranović-Kokoris[1], Vesna Stojanović[1], Braca Kundalić[1], Miljana Pavlović[1], Vladimir Živković[1], Dunja Ilić[2], Rade Čukuranović[1]

The aim of this study was to examine the macromorphometric parameters including weight, height, width, volume and length of the pituitary gland, as well as the histological characteristics of the hormone-producing cells. The focus was on basophilic (gonadotropic – luteinizing hormone (LH) producing cells and adrenocorticotropic hormone (ACTH) producing cells) and acidophilic (somatotropic – growth hormone (GH) cells and mammotropic – (prolactin PRL) cells) cells in male cadavers, aiming to assess the characteristics of the pituitary gland in living individuals during ageing. The research included 15 male cadavers of different ages (44 and 89 years), which were divided into three groups. In the first group (I) there were cadavers aged 30 to 49, in the second (II) 50 to 69 years, and in the third (III) 70 years and older. The pituitary cells were immunohistochemically identified by the PAP method using the appropriate antibodies: LH (βLH 1:100), ACTH (hACTH 1:200), GH (hGH 1:200), and PRL (hPRL 1:300). Our results show that the width, height, weight and volume of the pituitary gland did not change significantly ($p > 0.05$) with ageing, while the length of the gland showed statistically significant changes between groups ($p < 0.05$). The length of the pituitary gland was significantly ($p < 0.05$) greater in age groups II and III, compared to group I. In conclusion, the results of the examined macromorphometric parameters showed that only the length of the pituitary gland changed significantly during ageing.

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Key words: *ageing, men, macromorphometric parameters, immunoreactive pituitary cells*

[1]University of Niš, Faculty of Medicine, Department of Anatomy, Niš, Serbia
[2]University of Niš, Faculty of Medicine, doctoral studies, Niš, Serbia

Contact: Jovana Čukuranović-Kokoris
81 Dr Zorana Djindjić Blvd., 18000 Niš, Serbia
E-mail: jovana.cukuranovic.kokoris@medfak.ni.ac.rs

Introduction

Ageing is a physiological process resulting from the accumulation of a wide range of molecular and cellular damage over time, which leads to a gradual decline in mental capacity, physical abilities and an increasing risk of disease (1, 2). Accumulated damage can manifest itself in health problems characteristic of advanced age, such as deterioration of urinary tract function, prostate hyperplasia, erectile dysfunction and reduced fertility, osteoporosis and general weakness (3). The pituitary gland in humans is located at the base of the brain, in a depression in the sphenoid bone called the sella turcica. It consists of hormone-producing cells that secrete hormones: gonadotropic (luteinizing hormone – LH), thyrotropic (thyroide-stimulating hormone – TSH), adrenocorticotropic (adrenocorticotropic hormone – ACTH), somatotropic (growth hormone – GH), mammotropic (prolactin – PRL) cells, as well as folliculostellate (FS) cells, which are non-endocrine cells (4). With ageing, dysregulation of the hypothalamic-pituitary-gonadal axis occurs (5), which is reflected in a decrease in the secretion of gonadotropic-releasing hormone (GnRH) from the hypothalamus, the maximum and average amplitude of LH, and a decrease in the negative feedback mechanism mediated by testosterone. This process is referred to as gonadopause or late hypogonadism (6, 7). Ageing causes functional changes in the hypothalamic-pituitary-cortical axis (8). Previous studies indicate that during ageing, the synthesis and secretion of corticotropin-releasing hormone (CRH) from the hypothalamus decreases, and the sensitivity of ACTH from the adenohypophysis and adrenal cortex increases (9), which results in the inability

to quickly terminate glucocorticoid secretion stimulated by acute stressors (10). With the ageing process, a deficiency in the secretion of growth hormone-releasing hormone (GHRH) and/or ghrelin is observed, as well as an increase in the secretion of somatostatin from the hypothalamus, both of which lead to a reduction in growth hormone secretion (11). This process is called somatopause (12) and is associated with numerous problems such as mental, metabolic and musculoskeletal (13). In people aged 70 and over, GH levels drop significantly and are approximately 1/3 of those in later puberty (14). The World Health Organization has established that people live longer and that by 2030 every sixth person in the world will be 60 years old or older, and that by 2050 the number of people over 60 will double, and the number of people over 80 will triple from 2020 to 2050 (15). Because all of these can disrupt healthy ageing, the United Nations (UN) General Assembly has declared the period 2021–2030 as the Decade of Healthy Ageing. Based on the above, this work aims to examine the macromorphometric parameters of the pituitary gland and the histological characteristics of the hormone-producing immunopositive LH, ACTH, GH, and PRL cells within the pituitary gland.

Materials and Methods

The material for this stydy was taken from 15 male cadavers, in a routine autopsy at the Centre for Forensic Medicine in Niš, Serbia, with the approval of the Ethics Committee of the University of Niš, Faculty of Medicine (Decision No. 12-2307-2/8 dated 10.03.2016 described in detail in our previous work). The cadavers used were free of previously diagnosed neurological, psychiatric or endocrine disorders during their lifetime. No visible damage to the brain or pituitary gland was observed during the autopsies. Additionally, the pathohistological evaluation of the brain and pituitary gland ruled out the presence of any hidden or misdiagnosed diseases. Cadavers were classified into three age groups: Group I - from 30 to 49 years, Group II - from 50 to 69 years, and Group III - 70 years and over.

Macromorphometric Parameters

The weight of the pituitary gland, expressed in grams, was determined using a Denver Instrument Company AA-200 DS analytical balance, the precision of which is measured to 4 decimal places. The height of the pituitary gland was the mean value of the height of the central and both lateral parts of the pituitary gland. The three listed parameters are expressed in mm and measured with a "Kennon" vernier calliper, with a precision of 1/20 (0.05 mm). The width of the pituitary gland was the largest distance between the points on the lateral parts of the pituitary gland. The volume of the pituitary gland, shown in mm^3, was determined by measuring the volume of the displaced liquid in a glass beaker with a total volume of 10 ml (16). The length or sagittal diameter represented the mean value of the same at the level of the central and two lateral parts (wings). The same person measured all five parameters.

Histological Procedure

The histological processing of material taken from cadavers was described in detail in earlier reports (17–21). For immunohistological visualisation of hormone-producing cells, primary antibodies for gonadotropic LH cells (βLH 1:100; NIH, Bethesda, Md., USA) (17, 18), ACTH (hACTH 1:200; DAKO A/S, Glostrup, Denmark) (8), GH (hGH 1:200; DAKO A/S, Glostrup, Denmark) (19, 20, 22), and for PRL (hPRL 1:300; DAKO A/S, Glostrup, Denmark) (23) are used.

Statistical Analysis

The statistical analysis of the data was performed using SPSS v. 15.0. Given that these are small samples and that the continuous variables deviate from the normal distribution (as determined by the Shapiro–Wilk test), they are presented as medians, along with the minimum and maximum values. The dependence of these variables in relation to age (belonging to an age group) was determined by the Kruskal–Wallis test, and the Mann–Whitney test determined the difference in values between individual groups. As a threshold of statistical significance in the conclusion, the level of the error of estimation lower than 5% ($p < 0.05$) was used.

Results

Macromorphometric Parameters

The values of the macromorphometric parameters of the pituitary gland of male cadavers are shown in Figure 1. Our results show that the weight, height, width and volume of the pituitary gland did not change significantly ($p > 0.05$) during ageing (Figure 1A-1D), while the length of the gland showed statistically significant changes between groups ($p < 0.05$) (Figure 1E). The length of the pituitary gland was significantly ($p < 0.05$) greater in age groups II and III by 15.4% and 14.6%, respectively, compared to age group I.

Histological Characteristics

In group I, gonadotropic LH cells were polygonal or oval in shape, either in groups or as single cells, with an eccentrically positioned nucleus (Figure 2A). In cadavers of the third group, LH cells were darker in colour, more often oval, single with an eccentric, smaller, hyperchromatic nucleus and larger in volume

compared to younger cases (Figure 2B). In younger cadavers, ACTH cells were numerous, oval, polygonal or stellate with extensive cytoplasm (Figure 2C). In cases belonging to age group III, these cells did not change shape but were observed to be distributed in larger irregular groups or in smaller oval regular groups (Figure 2D). GH cells in younger cadavers were predominantly polygonal, with an eccentric euchromatic nucleus (Figure 2E). In age group III, GH cells were less numerous, larger, and exhibited somewhat stronger immunoreactivity with sporadic clear cytoplasmic vacuoles observed (Figure 2F). PRL cells of younger cadavers were spherical or irregularly polygonal in shape, with acidophilic granules in the cytoplasm. They were either single or present in small groups (Figure 2G). In older cadavers, PRL cells showed no difference in shape and distribution compared to young cadavers, but darker colored granules were visible (Figure 2H).

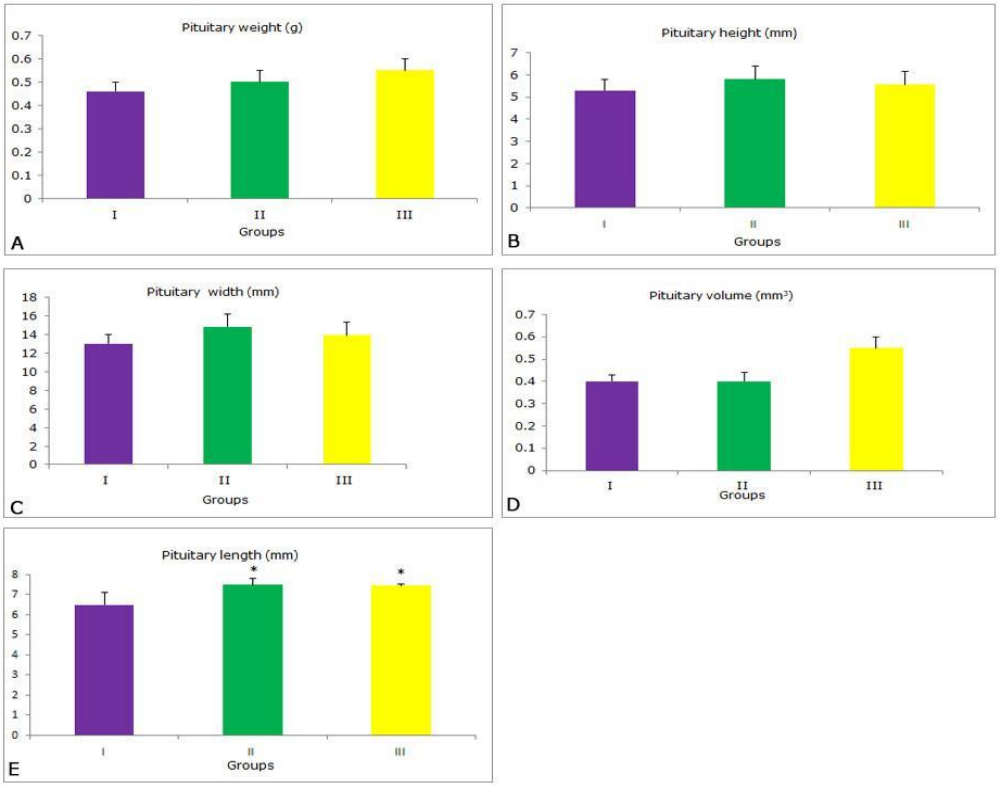

Figure 1 A–E. Macromorphometric parameters of pituitary gland in men cadavers in I (30 to 49 years), II (50 to 69 years), and III (70 years and older) groups. All values are provided as the mean ± SD; *p < 0.05, II and III vs. I group.

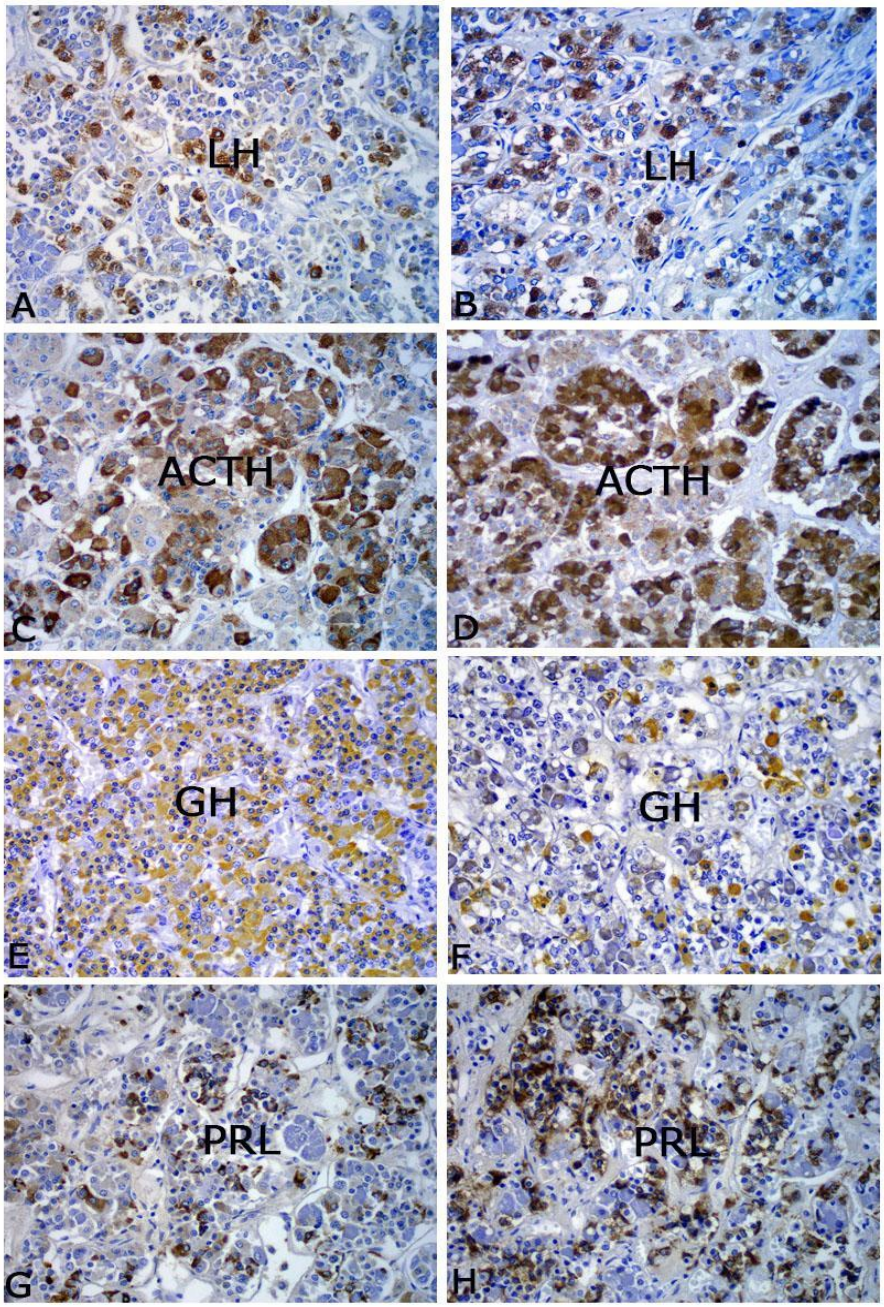

Figure 2 A–H. Representative micrographs of immunopositive pituitary cells in man cadavers. LH cells in I group (A), large immunopositive LH cells with small eccentric hyperchromatic nuclei in III group (B), ACTH I group (C), in III group (D); GH cells in: a-41-year-old man (E), an 87-year-old man (F), PRL cells in: a-41-year-old man (G) and an 87-year-old man (H). I (30 to 49 years), II (50 to 69 years) and III (70 years and older) groups. PAP,objective magnification 40x

Discussion

It has been observed that the size and shape of the pituitary gland change throughout life, which can be highly important for the diagnosis and possible treatment of diseases affecting this gland (8).

In our study, no statistically significant change in the weight of the pituitary gland was observed with age. Pituitary gland weight of cadavers of both sexes in Asia (24) was equal to (0.5 ± 0.1 g), similar to values reported in Japan (25), Chicago US (26), and India (27), which is similar to our results on male cadavers. Our results show that the peak height of the pituitary gland was recorded in the II age group, although the differences between individual age groups are not statistically significant. Similar values of hypophysis height to ours were recorded by Denk et al. (28) and Ikram et al. (29). Earlier research by Singh et al. (30) indicated that the height of the pituitary gland differed significantly between the sexes, while Ibinaiye et al. (31) found no statistically significant differences. The width of the pituitary gland was the largest in the II age group; however, there were no statistically significant differences between the groups, as previously shown in the population of north-western Indians (32). In our research, no significant difference was found in the volume of the pituitary gland between age groups. The results of the study by Ibinaiye et al. (31) are consistent with our results. Determining the volume of the pituitary gland is crucial in various pathological conditions of this gland, as traumatic brain injuries have been noted to involve the pituitary gland in both the early and chronic phases (33). The length of the pituitary gland was significantly increased in male cadavers in group II (50−69 years) by 15.4% and by 14.6% in group III (70 years and older) compared to group I. An examination of the length and peak that the pituitary gland reaches in both sexes in India was recorded in men in the fifth decade and in women after 50 years (30) which corresponds with our results. Literature data show that on magnetic resonance, the length of the pituitary gland differed significantly between the sexes in India (30), while this difference was not found in Nigeria (31).

Histological changes occurring on basophilic gonadotrope LH cells in old male cadavers compared to young cadavers agree with those described in earlier works (17, 18, 20, 21). Histological characteristics of basophilic ACTH cells in young male cadavers: cells were numerous, oval, polygonal or star-shaped with extensive cytoplasm, without visible changes in older cases, which is in agreement with earlier works (8, 10). Acidophilic GH cells in younger cadavers were numerous, polygonal, with acidophilic granules and an eccentric euchromatic nucleus. In older cases, they were fewer, larger, and exhibited somewhat stronger immunoreactivity, with sporadic clear cytoplasmic vacuoles observed. Similar histological characteristics of younger and older cadavers were recorded in earlier works (17, 19–21). Immunopositive acidophilic PRL cells of younger and older corpses are spherical or irregularly polygonal in shape, with acidophilic granules in the cytoplasm. They were either single or present in small groups. Similar properties of PRL cells were described in earlier works (34).

To live long and minimize health problems in old age, one should, as much as possible, adopt the way of life of centenarians in the "blue zones" (Okinawa, Japan; Sardinia, Italy; Ikaria, Greece; Nicoya, Costa Rica; and Loma Linda, California, USA) (35). A proper and healthy diet based on the highest possible intake of polyphenols (fruits, vegetables, legumes, two glasses of red wine), socializing, physical activity, spiritual fulfilment and a stress-free life are the basis of recommendations for healthy ageing and living over 100 years (36).

Conclusion

Given that we determined in this study that there were changes in some of the examined macromorphometric parameters and histological characteristics of the examined pituitary cells, further and more extensive research is necessary to confirm with certainty whether these parameters undergo irreversible processes during ageing.

Study limitations

In this study, we focused on assessing macromorphometric parameters and their association with histological changes in hormone-producing (LH, ACTH, GH and PRL) cells of the pituitary gland during the ageing process. The main limitation of this research was the small sample size - a total of 15 cases, divided into three age groups. Future studies should include a larger number of cases and use immunofluorescence staining of cells for histological analysis, followed by examination under a confocal microscope.

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UTICAJ STARENJA NA MORFOMETRIJSKE PARAMETRE I HISTOLOŠKE KARAKTERISTIKE BAZOFILNIH I ACIDOFILNIH ĆELIJA HIPOFIZE: ISPITIVANJE MUŠKIH KADAVERA

Jovana Čukuranović Kokoris[1], Vesna Stojanović[1], Braca Kundalić[1], Miljana Pavlović[1], Vladimir Živković[1], Dunja Ilić[2], Rade Čukuranović[1]

[1]Univerzitet u Nišu, Medicinski fakultet, Katedra za anatomiju, Niš, Srbija
[2]Univerzitet u Nišu, Medicinski fakultet, student doktorskih studija, Niš, Srbija

Kontakt: Jovana Čukuranović Kokoris
Bulevar dr Zorana Đinđića 81, 18000 Niš, Srbija
E-mail: jovana.cukuranovic.kokoris@medfak.ni.ac.rs

Cilj ove studije bio je da ispita makromorfometrijske parametre, uključujući težinu, visinu, širinu, zapreminu i dužinu hipofize, i histološke karakteristike ćelija koje proizvode hormone. Ispitivanje je bilo usmereno na bazofilne (gonadotropne ćelije koje proizvode luteinizirajući hormon (engl. *luteinizing hormone* – LH) i adrenokortikotropne ćelije koje proizvode adrenokortikotropni hormon (engl. *adrenocorticotropic hormone* – ACTH)) i acidofilne (somatotropne ćelije koje proizvode hormon rasta (engl. *growth hormone* – GH) i mamotropne ćelije koje proizvode hormon prolaktin (engl. *prolactine* – PRL)) ćelije muških kadavera i njihovu povezanost sa starenjem. Istraživanje je obuhvatilo petnaest muških kadavera različite starosti (od 44 godine do 89 godina), koji su podeljeni u tri grupe. U prvoj grupi (I) bili su kadaveri muškaraca starih od 30 do 49 godina, u drugoj (II) kadaveri muškaraca koji su imali između 50 i 69 godina, a u trećoj (III) kadaveri muškaraca starijih od 70 godina. Ćelije hipofize su imunohistohemijski identifikovane PAP metodom i korišćenjem odgovarajućih antitela: LH (βLH 1 : 100), ACTH (hACTH 1 : 200), GH (hGH 1 : 200) i PRL (hPRL 1 : 300). Rezultati su pokazali da se širina, visina, težina i zapremina hipofize nisu značajno menjale ($p > 0,05$) u toku starenja. S druge strane, uočena je statistički značajna promena dužina žlezde pri poređenju kadavera iz pomenutih grupa ($p < 0,05$). Dužina hipofize je bila statistički značajno ($p < 0,05$) veća u grupama II i III nego u grupi I. Rezultati dobijeni ispitivanjem makromorfometrijskih parametara pokazali su da se samo dužina hipofize značajno menja u toku starenja.

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***Ključne reči**: starenje, muškarci, makromorfometrijski parametri, imunoreaktivne ćelije hipofize*