

Original article

doi:10.5633/amm.2025.0317

UDC:

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

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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-LH and corticotropic-ACTH) and acidophilic (somatotropic-GH and mammatropic-PRL) cells of male cadavers during ageing. In the research, there were 15 male cadavers of different ages (44 and 89 years), which were divided into III 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 and using the appropriate antibodies: LH (β LH1:100), 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 ($p > 0.05$) during aging, while the length the gland showed statistically significant

changes between groups ($p < 0.05$). The length of the pituitary gland was statistically 29
significantly ($p < 0.05$) greater in age groups II and III, compared to group I. In conclusion, the 30
results of the examined macromorphometric parameters showed that only the length of the 31
pituitary gland was significantly changed during ageing. 32

Keywords: ageing, men, macromorphometric parameters, immunoreactive pituitary cells 33
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Cilj ove studije bio je da ispita makromorfometrijske parametre, uključujući težinu,	50
visinu, širinu, zapreminu i dužinu hipofize, i histološke karakteristike ćelija koje proizvode	51
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visina, težina i zapremina hipofize nisu značajno menjale ($p > 0,05$) u toku starenja. S druge	60
strane, uočena je statistički značajna promena dužina žlezde pri poređenju kadavera iz	61
pomenutih grupa ($p < 0,05$). Dužina hipofize je bila statistički značajno ($p < 0,05$) veća u	62
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Ključne reči: starenje, muškarci, makromorfometrijski parametri, imunoreaktivne ćelije hipofize

Introduction

Ageing is a physiological process and is the 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 (LH), thyrotropic (TSH), adrenocorticotropic (ACTH), somatotropic (GH), prolactin (PRL) cells, as well as folliculostellate (FS) cells, which are non-endocrine cells (4). With aging, 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 luteinizing hormone (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, which leads 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 are living longer and that by 2030 every sixth person in the world will be 60 years old or older, and that by 2050 this 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 work 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 in this study 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 are classified into three age groups based on their age: Group I - from 30 to 49 years old, Group II - from 50 to 69 and Group III - 70 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 mm³, 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 (β LH 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 statistically significantly ($p < 0.05$) greater in age groups II and III by 15.4% and 14.6%, respectively, compared to age group I.

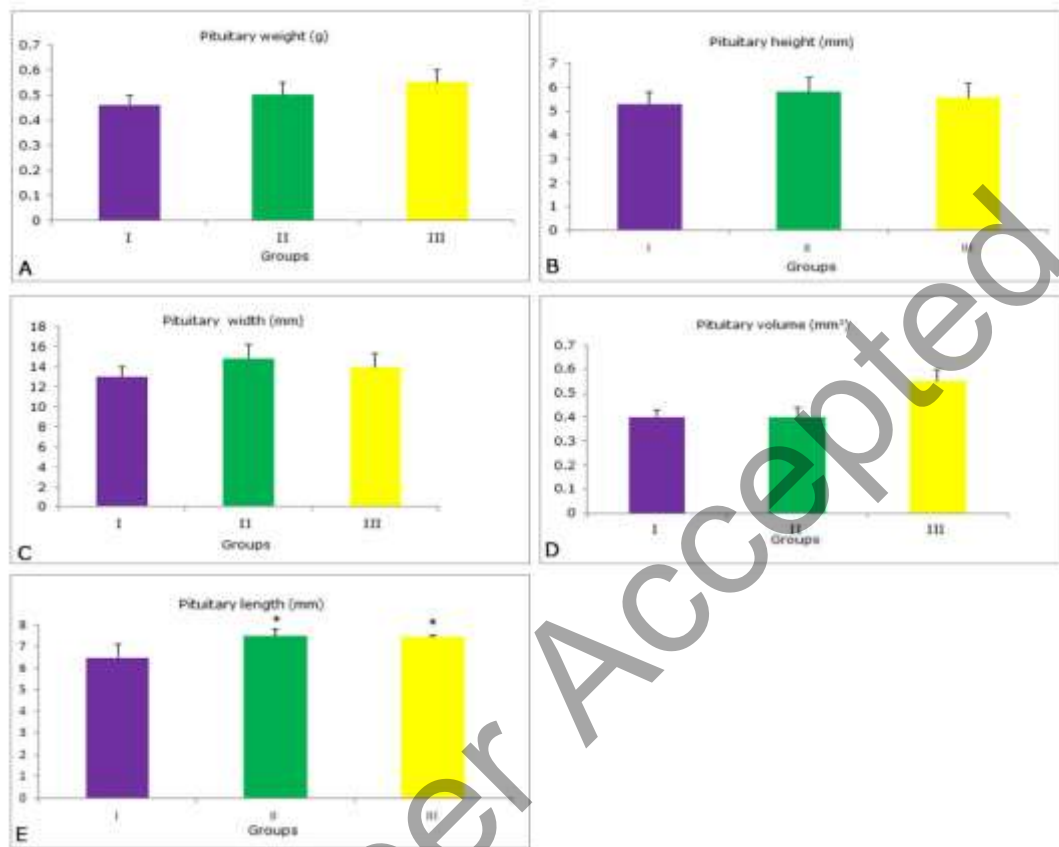


Figure 1 A-E. Macromorphometric parameters of pituitary gland in men cadavers in I (30 to 49 years), II (50 to 69 years) and III (70 years and older) groups. All values are provided as the mean ± SD; ^ap < 0.05, II vs. I groups; ^bp < 0.05, III vs. I groups.

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 are 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 cases of age group III, GH cells were rarer, larger, and had
somewhat stronger immunoreactivity with sporadic clear cytoplasmic vacuoles observed (Figure
2F). *PRL* cells of younger cadavers are spherical or irregularly polygonal in shape, with
acidophilic granules in the cytoplasm. They can be single or in smaller groups (Figure 2G). In
older cadavers, *PRL* cells show no difference in shape and distribution compared to young
cadavers, but darker colored granules are visible (Figure 2H).

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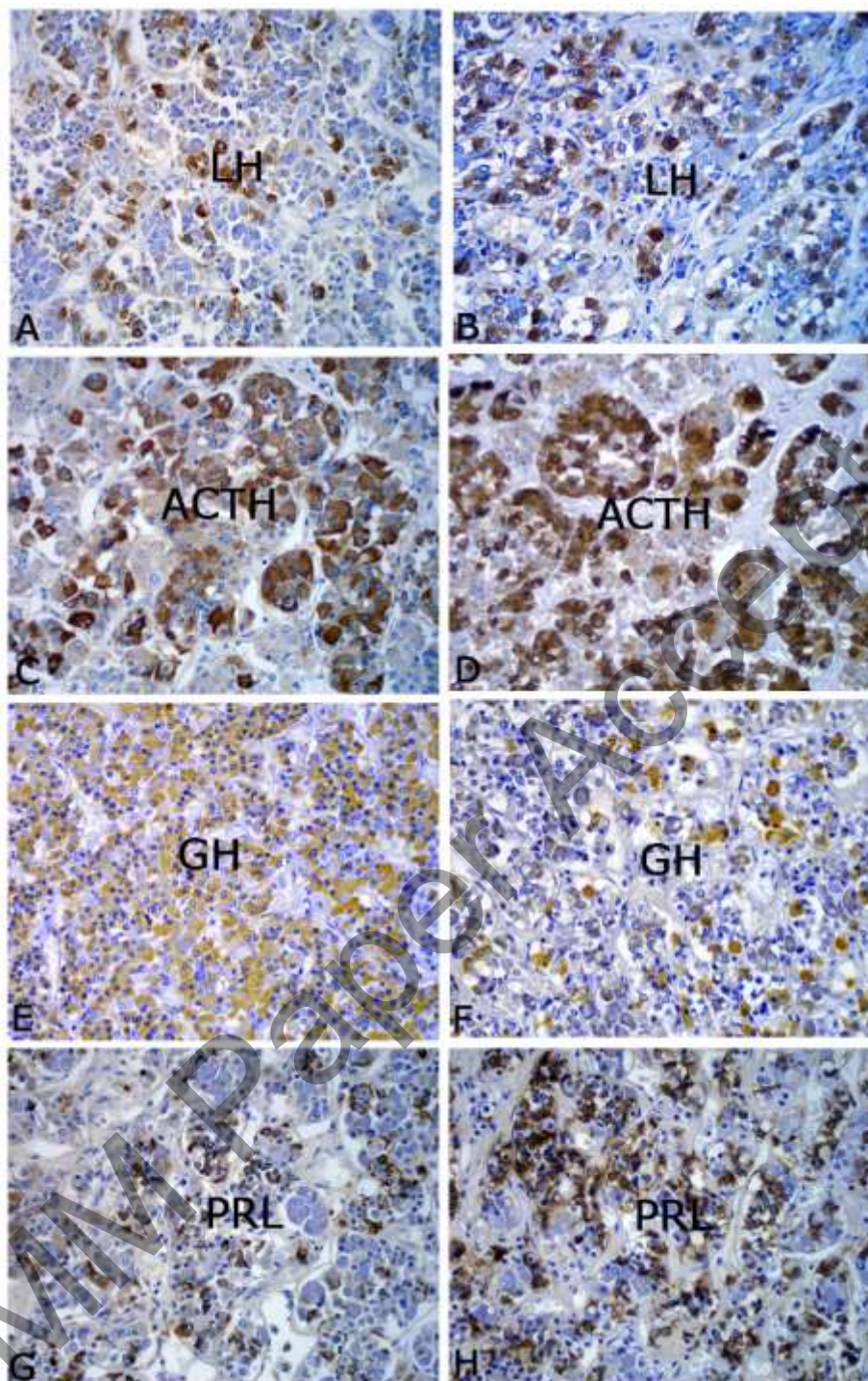


Figure 2 A-H. Representative micrographs of immunopositive pituitary cells in man cadavers. LH cells in I group (A), large immunopositive LH cells with small eccentric hyperchromatic nuclei in III group (B), ACTH I group (C), in III group (D); GH cells in: 41 years old man (E), 87 years old man (F), PRL cells in 41 years old man (G) and 87 years old (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 a person's life, which can be extremely important for the diagnosis and possible treatment of diseases affecting this gland (8).

In our study, the *weight* of the pituitary gland did not change statistically significantly with age. Pituitary weight of cadavers of both sexes in Asia (24) was equal to $(0.5 \pm 0.1 \text{ g})$ in the area of Japan (25), Chicago US (26) and India (27), which indicates similarity with 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 can be statistically significantly different between the sexes, while Ibinaiyé et al. (31) found no statistically significant differences. The *width* of the pituitary gland is the largest in the II age group; however, there are no statistically significant differences between the groups, as previously shown in the population of north-western Indians (32). In our research, no statistically significant difference was found in the *volume* of the pituitary gland between age groups. The results of the study by Ibinaiyé et al. (31) agree 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 statistically 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 the first group. 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 differs significantly between the sexes in India (30), while this difference was not found in Nigeria (31).

Histological changes occurring on basophilic *gonadotrops 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 corpses: they are numerous, 209
 oval, polygonal or star-shaped with extensive cytoplasm, without visible changes in older cases, 210
 in agreement with earlier works (8, 10). Acidophilic GH cells in younger corpses were numerous, 211
 polygonal, with acidophilic granules and an eccentric euchromatic nucleus. In older cases, they 212
 were fewer, larger, and exhibited somewhat stronger immunoreactivity, with sporadic clear 213
 cytoplasmic vacuoles observed. Similar histological characteristics of younger and older 214
 cadavers were recorded in earlier works (17, 19–21). Immunopositive acidophilic *PRL* cells of 215
 younger and older corpses are spherical or irregularly polygonal in shape, with acidophilic 216
 granules in the cytoplasm. They can be single or in small groups. Similar properties of *PRL* cells 217
 were described in earlier works (34). 218

In order to live long and with as few health problems as possible in old age, one should 219
 apply, as much as possible, the way of life of centenarians in the "blue zones" (Okinawa, Japan; 220
 Sardinia, Italy; Ikaria, Greece; Nicoya, Costa Rica; and Loma Linda, California, USA) (35). A 221
 proper and healthy diet based on the highest possible intake of polyphenols (fruits, vegetables, 222
 legumes, two glasses of red wine), socializing, physical activity, spiritual fulfilment and a stress- 223
 free life are the basis of recommendations for healthy ageing and living over 100 years (36). 224

Conclusion 226

Given that we determined in the work that there were changes in some of the examined 227
 macromorphometric parameters and histological characteristics of the examined pituitary cells, 228
 further and more extensive research is necessary to confirm with certainty whether the 229
 examined parameters undergo irreversible processes during ageing. 230

Study limitations 232

In this research, we focused on assessing macromorphometric parameters and their association 233
 with histological changes in hormone-producing (LH, ACTH, GH and *PRL*) cells of the pituitary 234
 gland during the ageing process. The limitation of this research was the small number of cases 235
 used a total of 15, divided into 3 groups depending on age. Future studies should include a 236

much larger number of cases and use immunofluorescence staining of cells for histological analysis, which would then be analyzed under a confocal microscope.

Acknowledgements

The authors would like to thank the Ministry of Education, Science and Technological Development of Republic of Serbia (Grants No: 451-03-137/2025-03/200113) for financial support.

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