The Degree of Pineal Gland Calcification in the Aged People is Associated with Changes in the Internal Structure

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Abstract

The question of the staged accumulation of brain sand in different parts of the pineal gland and the associated changes in cell composition and blood vessels in the elderly and senile people is not definitively studied. Our pilot study included 16 samples of the human pineal glands of the elderly and senile that were divided according to the degree of calcification into 2 groups. In order to objectify the data obtained on micropreparations in each group the morphometric parameters were determined. The degree of calcification of the pineal gland in the elderly and senile is associated with the topography of the inclusions of the brain sand and changes in blood vessels. Increased degree of calcification is accompanied by changes in internal structure of pineal gland which indicate involutional processes.

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Introduction

The pineal gland (PG) is a gland of the endocrine system, also known as the epiphysis. This is an unpaired gland located behind the third ventricle of the brain[1]. The main function of the PG is the synthesis and production of the hormone melatonin, as a signaling molecule of the natural photoperiodic system, which controls circadian rhythms of sleep and wakefulness, plays a significant role in the overall cell defense system and affects sleep and immunity[2,3].

The histological organization of human PG in general is similar to that in experimental animals, and includes a connective tissue capsule and parenchyma, which contains pinealocytes, gliocytes and blood microvessels[4].

The internal structure of the PG also describes specific formations, which consist of salts of calcium and magnesium and are called “brain sand” or “acervuli”. Typical for brain sand are single concentric ring structures surrounded by connective tissue, multiple concentric structures or silky lobed structures[5].

The inclusion of “brain sand” in the PGs of mammals belongs to the meningeal type and can also be observed in the surrounding tissues. These structures are defined in many species of animals and have long been considered a purely physiological manifestation of aging. Brain sand formation is likely to be associated with age-related involutional changes as its aggregates increase with age. In general, the process of calcification is interpreted as an age-related change due to degeneration of pinealocytes[6].

However, the question of the staged accumulation of brain sand in different parts of the PG and the associated changes in cell composition and blood vessels in the elderly and senile people is not definitively studied.

That is why the aim of the study was to study the characteristics of the cell composition and structure of blood vessels of the elderly in the elderly and senile people depending on the degree of accumulation of brain sand.

Materials and methods

We conducted a pilot case-control study, which was approved by Bioethical Committee of

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Ukrainian Medical Stomatological Academy. It included 16 samples of the PGs of deceased males and females of the elderly and senile. Inclusion criteria were: age from 61 to 90 years old, informed consent of relatives of patients. Exclusion criteria: age under 61 or over 90 years old, history of cancer, psychiatric or neurodegenerative diseases, use of melatonin as a drug in the last month.

The obtained samples were divided according to the degree of calcification of the PG, which was evaluated by the proportion of inclusions of “brain sand” in the internal structure, into 2 groups;

Group 1: mild calcification – the total amount of brain sand occupied less than 20% of the volume of PG (n = 9);

Group 2: moderate calcification – the total amount of brain sand occupied more than 20% of the volume of PG (n = 7).

After fixation in 10% neutral formalin, the materials were dehydrated and embedded in paraffin according to conventional methods. Histological sections were made from paraffin blocks on a rotary microtome, which were stained with hematoxylin and eosin[7]. The study of micropreparations and morphometric studies were performed using an Olympus BX-41 light microscope with a set of appropriate licensing programs.

In order to objectify the data obtained on micropreparations in each group the following morphometric parameters were determined:

1) specific gravity of cell elements (50,000 μm2) – light and dark pinealocytes;
2) the ratio of dark/light pinealocytes in the internal structure of the PG;
3) the ratio between the volumes occupied in the inner structure of the PG by light pinealocytes, dark pinealocytes, neuroglyocytes, blood vessels, brain sand and stromal component;
4) outer and inner diameters of arterial vessels of the PG, the thickness of the vascular wall (μm).
5) Kernogan’s index (the ratio of the outer diameter of the blood vessel to the diameter of its lumen).

Statistical processing of the obtained results was performed using the program IBM SPSS Statistics 26.0. The normality of the distribution of the obtained data was evaluated by the Shapiro-Wilk test. Data with normal distribution are presented as mean (M) and standard deviation (SD) and with non-normal as median (Me) and interquartile (25% -75%) amplitude (Q1-Q3) with Tukey folds.

The Mann-Whitney test was used to assess statistical differences between the study groups. To compare qualitative indicators the risk ratio and their 95% confidence interval were calculated. Spearman's rank correlation criterion was used to assess the degree of correlation between the two quantities.

Results

According to the analysis the mean age of individuals in the first experimental group was 70.4±10.3 years and in the second group – 70.2±7.2 years, which had no statistically significant differences (P = 0.826).

At the optical level in the inner space of the PG of the elderly and senile people cellular elements, blood microvessels and stromal (connective tissue) component are determined. The last which is represented by intralobular interstitium, interlobular connective tissue membranes and areas of focal growth of connective tissue (sclerosis) occupies about 80% of the internal space.

Also in the internal structure of the PG are defined homogeneous eosinophilic structures, known as ‘brain sand”, which are presented in the form of polymorphic intensely colored inclusions, which are located in the connective tissue skeleton and directly in the lobes of the gland that is shown on the figure1.

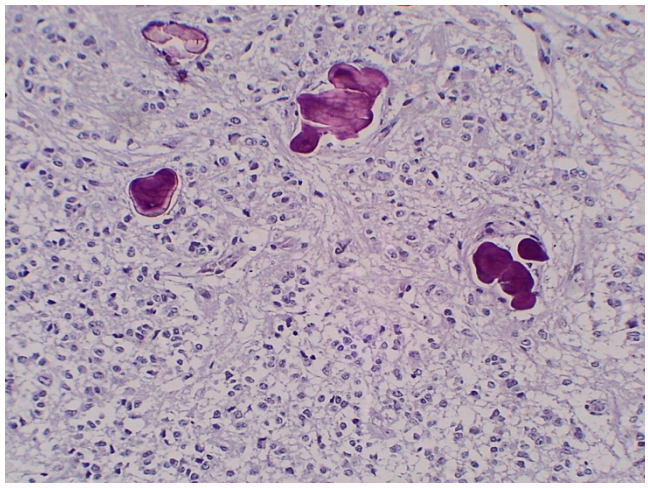

Figure 1. Brain sand deposits in the pineal glands of elderly man. Hematoxylin and eosin stain: 100×magnification.

Statistical analysis of the risk ratio did not reveal a significant difference between the degree of calcification and the topography of the deposits of "brain sand". It was visualized both in the capsule (RR = 1.1 (95% CI 0.9-1.4), P = 0.327) and directly in the particles (RR = 1.1 (95% CI 0.7- 1.8), P = 0.182). However, an increased risk of deposition of brain sand inclusions in the interparticle trabeculae was found in the group with a moderate calcification (RR = 3.9 (95% CI 1.1-13.6), P = 0.026).

The specific gravity of light pinealocytes in the group 1 was 81.7 (65.5-91.5) per 50,000 μm2, and in the group 2 – 88.7 (80.0-90.0) per 50,000 μm2. Thus, no significant differences between groups on this indicator were found.

The specific gravity of dark pinealocytes in the group 1 was 2.9 (2.7-4.0) per 50,000 μm2, and in the group 2 – 2.3 (1.6-2.6) per 50,000 μm2 respectively, which was significantly lower compared with the group 1 (P = 0.021). The average ratio of dark and light cellular elements in the PG of the elderly and senile is 1:40 in both groups.

In the group 1 light pinealocytes occupied 16.2% (13.9-17.4%) of the internal volume of the gland, dark pinealocytes – 0.9% (0.3-1.1%), gliocytes – 0.5% (0.3-1.5%), blood vessels – 2.4% (1.1-3.3%) and the share of the stromal component accounted for 69.3% (45.7-75.2%) respectively.

In the group 2 light pinealocytes occupied 16.4% (12.7-16.8) of the internal volume, dark pinealocytes – 0.8% (0.5-0.9%), gliocytes – 0.5% (0.4-0.5%), blood vessels – 1.75% (1.7-2.25%) and the share of the stromal component accounted for 74.5% (59.7-77.5%) accordingly.

Thus, statistically significant differences in the relationship between the individual structural components of the PG of elderly and senile people were not detected.

The outer diameter of the arterial blood vessels was significantly smaller (P = 0.034) in the group 1 and was 46.2 (43.4-69.6) μm vs. 54.6 (47.6-58.4) μm in the group 2, in while the diameter of their lumen had no statistically significant differences 23.2 (21.9-26.2) μm vs. 26.3 (24.7-29.7) μm respectively (P = 0.531). The average thickness of the vascular wall in the group 1 was 13.9 (11.2-14.4) μm and in the group 2 – 14.2 (14.1-14.9) μm, which was a statistically higher value (P = 0.012).

The Kernogan's index in the group 1 was 2.05 (1.9-2.3), in the group 2 – 2.4 (2.3-3.01). This indicator was statistically significantly higher in the group 2 (P=0.011).

The statistical analysis revealed a direct moderate correlation of the specific gravity of brain sand with the specific gravity of dark pinealocytes (R = 0.632; P = 0.043) and the value of the Kernogan's index (R = 0.456; P = 0.017).

Discussion

It has been established that the size and morphological structure of the pineal gland varies depending on the geographical region, due to physiological changes in the body and under the influence of various pathological factors[8]. The issue on the primary deposition of corpora arenacea in the pineal body, the structure of this gland and their relationship with various diseases and pathological processes that occur as a result of changes in the gland itself and indirectly due to physiological processes, regulated by it, remains debatable[9-12].

Elderly people are dominated by light pinealocytes, the number of which does not change depending on the amount of corpora arenacea[13,14]. However, with increasing degree of calcification of the PG decreases the number of dark pinealocytes, which is most likely the result of involutive processes[15]. The exact mechanisms of PG calcification and its association with involutive changes have not been fully determined. The appearance of corpora arenacea is associated primarily with aging and pathological conditions. It is hypothesized that its formation is an active process associated with function, since elevated melatonin contributes to calcification of the pineal body. In turn, calcification of PG inhibits the production of melatonin, which leads to various nervous disorders. The anatomical structure and function of the pineal gland play a leading role in the process of calcification[8].

Our studies have established that in the group with a significant degree of calcification of the PG, the outer diameter of arterial vessels and the thickness of the vascular wall are increased relative to the group with a mild degree with the same diameter of their internal lumen. Accordingly, in the group with a moderate degree of calcification there is an increase in the value of the Kernogan's index, which allows determining

the dependence of the degree of calcification in the PG of the elderly and senile on the condition of arterial blood vessels. As the degree of calcification of the PG increases in the elderly and senile the risk of accumulation of corpora arenacea deposits increases not only in the outer capsule, but also in the interparticle connective tissue trabeculae.

However, despite the resulting data which reveal only a scant part of the pineal gland, it is necessary to consider other factors that may affect the rate of calcification and changes in the pineal gland, such as fluoride, systemic inflammatory response, changes in sleep pattern and other external factors and the internal environment[16-18].

The pineal gland remains a mysterious organ that needs to be thoroughly studied, as the use of melatonin in the treatment and prevention of various mental disorders and diseases is promising[9,13,19]. In addition, understanding the function of the pineal body and its product of incretory activity will elucidate the key points of protective and adaptive mechanisms of our body.

Conclusions

1. The degree of calcification of the PG in the elderly and senile is associated with the topography of the inclusions of the brain sand, which may indicate the stage of its accumulation in different structures of the gland.
2. Increased degree of calcification is accompanied by changes in blood vessels, which are manifested in an increase in the outer diameter and wall thickness of arterioles and small arteries.
3. In PG there is an direct correlation between the degree of calcification and the number of dark pinealocytes, which indicate involutive processes.

Compliance with Ethics Requirements

The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law.

Declaration of Interest

The authors declare no conflict of interest regarding this article.

References

1. Ibanez MP, Noctor SC, Munoz EM. Cellular Basis of Pineal Gland Development: Emerging Role of Microglia as Phenotype Regulator. PLoS One, 2016, 11(11):63-64.
2. Sapède D, Cau E. The pineal gland from development to function. Curr Top Dev Biol, 2013, 106:171-215.
3. Gheban BA, Rosca IA, Crisan M. The morphological and functional characteristics of the pineal gland. Med Pharm Rep, 2019, 92(3):226-234.
4. Patel S, Rahmani B, Gandhi J, Seyam O, Joshi G, Reid I, et al. Revisiting the pineal gland: a review of calcification, masses, precocious puberty, and melatonin functions. International Journal of Neuroscience. 2019; 130(5): 464-475
5. Kim J, Kim HW, Chang S et al. Growth patterns for acervuli in human pineal gland. Sci Rep, 2012, 2:984.
6. Aljarba N, Abdulrahman AA. Pineal gland calcification within Saudi Arabian populations. Journal of the Anatomical Society of India. 2017; 66(1):43-47.
7. Avwioro G. Histochemical uses of haematoxylin - a review. JPCS, 2011, 1:24-34
8. Tan DX, Xu B, Zhou X et al. Pineal Calcification, Melatonin Production, Aging, Associated Health Consequences and Rejuvenation of the Pineal Gland. Molecules, 2018, 23(2):301.
9. Skrypnikov AM, Kydon PV. The role of melatonin in the pathogenesis and therapy of depressive disofers. Arkhiv Psihiatrii, 2019, 98(3):147-154
10. Grosshans, M.; Vollmert, C.; Vollstaedt-Klein, S.; Nolte, I.; Schwarz, E.; Wagner, X.; Leweke, M.; Mutschler, J.; Kiefer, F.; Bumb, J.M. The association of pineal gland volume and body mass in obese and normal weight individuals: A pilot study. Psychiatr. Danub. 2016, 28, 220–224.
11. Song, J. Pineal gland dysfunction in Alzheimer's disease: relationship with the immune-pineal axis, sleep disturbance, and neurogenesis. Mol Neurodegeneration. 2019;14:28. https://doi.org/10.1186/s13024-019-0330-8
12. Bastos JrMAV, Oliveira Bastos PRH, Portella RB, Soares LFG, Conde RB, Rodrigues PMF, et al. Pineal gland and schizophrenia: A systematic review and meta-analysis. Psychoneuroendocrinology. 2019; 104: 100-14.
13. Yaremii IM, Kushnir OYu, Khlus KM. Melatonin improves mitochondrial function and decreases oxidative stress in gums of diabetic rats. Aktualni problemy suchasnoi medycyny, 2018, 63(3):201-204.
14. Rabia A, Tahir M, Munir B et al. Quantitative and qualitative analysis of cadaveric human pinealocytes in various age groups. J Coll Physicians Surg Pak, 2011, 21(7):389-92.
15. Walaa Mohamed Sayed. Changes in Pinealocytes type I and II of Adult Albino Rat Exposed to Different Wavelengths of Light or Confined to Constant Darkness: A Morphological and Electron Microscopic Study. Egyptian Journal of Anatomy, 2017, 40(1):54-74.
16. Chlubek D, Sikora M. Fluoride and Pineal Gland. Appl. Sci. 2020;10:2885. doi:10.3390/app10082885/
17. Pham L, Baiocchi L, Kennedy L, Sato K, Meadows V, Meng F, et al. The interplay between mast cells, pineal gland, and circadian rhythm: Links between histamine, melatonin, and inflammatory mediators. JPR. 2021; 70(2): e12699. https://doi.org/10.1111/jpi.12699.
18. McCully KS. Communication: Melatonin, Hyperhomocysteinemia, Thioretinaco Ozonide, Adenosylmethionine and Mitochondrial Dysfunction in Aging and Dementia. Annals of Clinical & Laboratory Science. 2018; 48(1): 126-31.
19. Cakır ZU, Demirel C, Ugurlu P, Saruhan BG, Ketani Sennur, Seker U, Zincircioglu SB. Radio-protective effects of melatonin on the skin histology in the rat. J Int Dent Med Res. 2014;7(3):73-77.