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MORPHOLOGY AND HISTOCHEMICAL CHARACTERISTICS HUMAN PINEAL GLAND ACERVULI DURING THE AGING

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Summary. *Pineal acervuli are calcificated structures, present in gland's parenchyma as well as in its leptomeningeal capsula.. The aim of our research was to determine the morphological and histochemical characteristics of pineal acervuli during the aging process. During our research, 30 corpses pineal glands were analyzed with light microscope. Their age ranged from 20 to 82, and they were classified into three age groups. Samples were processed by standard histological method, and thereafter stained with HE stain, PAS and AB PAS stain, modified aldehyde fuchsine stain, resorcin fuchsin stain, Gordon Sweet method and Mallory's trichrome connetctive tissue stain. Acervuli stained blue on HEstained sections. They were PAS and AB PAS positive. On aldehyde fuchsin stained sections they were violet and on resorcin fuchsine stained sections they were red colored. Acervuli had blue periphery and red core on Mallory's trichrome connective tissue stained sections. With Gordon Sweet's method they had yellow colored core and black colored periphery. The first age group acervuli were small with regular round or oval shape. They were localized at gland's periphery. Their size increased during the aging, and their shape became more irregular. Finally, large mulberry like acervuli were formed in the third age group probably as result of the conglomeration process. Hence, pineal acervuli are the structures which are consisted from inorganic and organic substances. During the aging they become larger with more irregular shape, which finally lead to mulberry like composite pineal acervuli formation which dominate in the pineal parenchyma.*

Key words: *Pineal gland, acervuli, morphology, histochemistry*

Introduction

Pineal acervuli (brain sand, pineal sand, corpora arenacea) are the structures which can be find not only in human, but also in other mammals pineal gland (1). They were described for the first time by Giovanni Batista Morgagni (1682-1771) in the 18th century, and from then they have been the subject of many research workers investigations (2). However, in spite of large number of investigations that were carried out, the exact mechanism of their formation process still has remained unknown. Different authors have different position about the connection between acervuli formation and aging process. Two major groups of theories, which try to epxlain acervuli origin differetiated with time. The first group includes older theories. These theories consider that acervuli formation is the result of the pineal gland regular neurosecretory activity. They also deny the connection between acevuli formation and aging process (3). More modern theories are included in the second group. They consider acervuli formation as the result of pinealocytes degeneration which progress during the human aging (4,5,6). Nevertheless, in spite of latter facts, pineal acervuli way of formation, their influence on pineal function, still remain mistery and probably that is the reason why they still attract many research workers attention.

The aim of our research was to determine chemical composition of pineal acervuli with histochemistry method application, while histology analysis should reveal morphological characteristics of acervuli, dynamicly, in correlation with aging.

Materials and methods

Material was 30 corpses pineal glands tissue, obtained from autopsies at the Institute for forensic medicine of Medical faculty in Niš. All the samples were obtained within 24 hours post – mortem. The cause of death was accident without brain damage, and there weren't any data of corpses previously diagnosed nervous system disease. Corpses were classified into three age groups: I (20-44) with mean age of 37.75 years, II (45-69) with mean age of 55.67 years and III (70 and older) with mean age of 77.40 years. We approuched to the pineal region after one to two cm long sagital section of corpus callosum splenium was made. After that, leptomeningies and blood vessels of the vellum interpositum were noticed. Pineal gland, with adjacent epithalamus tissue, was separated from the rest of the diencephalon with semicircular cut. Next, tissue was fixed in 10% neutral formaldehyde. Afterwards, samples were histologically processed. They were embedded in paraffin, and then cut into sections 10 μm thick

which were stained with HE, PAS, AB PAS, modified aldehyde fuchsine stain, resorcin fuchsin stain, Gordon Sweet method (7) and Mallory's trichrome connective tissue staining (8). Next, sections were analyzed with "Reichart Visopan" light microscope with projection screen. Characteristic forms of acervuli were verified with digital imaging system "Olympus BX 50".

Results

Two types of pineal acervuli were detected by histologic analysis. The first one was localised inside the pineal parenchyma (intrapineal type), while the second type was localised in the pineal capsula (extrapineal type). These two types of pineal acervuli differentiated not only in their localisation, but also in their morphology and structure. Extrapineal acervuli had predominantly regular round or oval shape and distinct concetric laminate structure (Fig. 1A). Intrapineal acervuli had predominantly homogeneous structure and more irregular shape, especially in the second and the third age group (Fig. 1B). Lamellas, which were noticed inside them, were visible only with higher magnification and had not so concentric disposition like in extrapineal acervuli (Fig. 1C). The intrapineal acervuli number, distribution and shape depended on age of corpse. They showed different affinity for the different stains, after different histochemical methods application. On HE stained sections they were dark blue colored (Fig. 1B). After PAS method application they were stained violet (Fig. 1D), while after AB PAS method application, they showed unequeal coloring of their layers. Central parts of acervuli were stained blue, while peripheral parts were stained violet, which was especially noticeable in larger one (Fig. 1E). On resorcin fuchsin stained sections acervuli were red colored (Fig. 1F), while on modified aldehyde fuchsine stained sections they were violet colored (Fig. 2A). After Gordon-Sweets method application, the central part of the acervuli was yellow colored, while the narrow peripheral part was black colored (Fig. 2B). In extrapineal acervuli after the application of this method, both lamellas in the central part and lamellas in the peripheral part, were stained black (Fig. 2C). On Mallory's trichrome connective tissue stained sections, the core of the acervuli was stained red and the periphery of the acervuli was stained blue (Fig. 1C).

Follicular structures were detected in two cases, one in the first and the second in the second age group. These structures dominated in the pineal parenchyma and in that cases the number of pineal acervuli was negligible. Structures, which were more basophilic than the adjacent pineal parenchyma, with the spiral disposed pinealocytes were detected in their vicinity (Fig. 2D).

Morphologic characteristics of acervuli depended on age group of the corpse, especially in intrapineal acervuli. Extrapineal acervuli showed the increase of size and insignificant change of shape. Sometimes, their conglomeration was detected, when the structures, which most frequently consisted of two smaller acervuli surrounded with common peripheral lamella, were created (Fig. 1A). Intrapineal acervuli of the first age group were small, with regular round or oval shape, blue colored. They formed groups of several small pineal acervuli. These groups were mostly located at pineal glands periphery. The most frequently, these groups consisted of several small, single or complex acervuli which were composed of two to three subunits (Fig. 2E). Some of acervuli were localised adjacent to connective tissue septa (Fig. 2F). In that case they were localised next to the blood vessels.

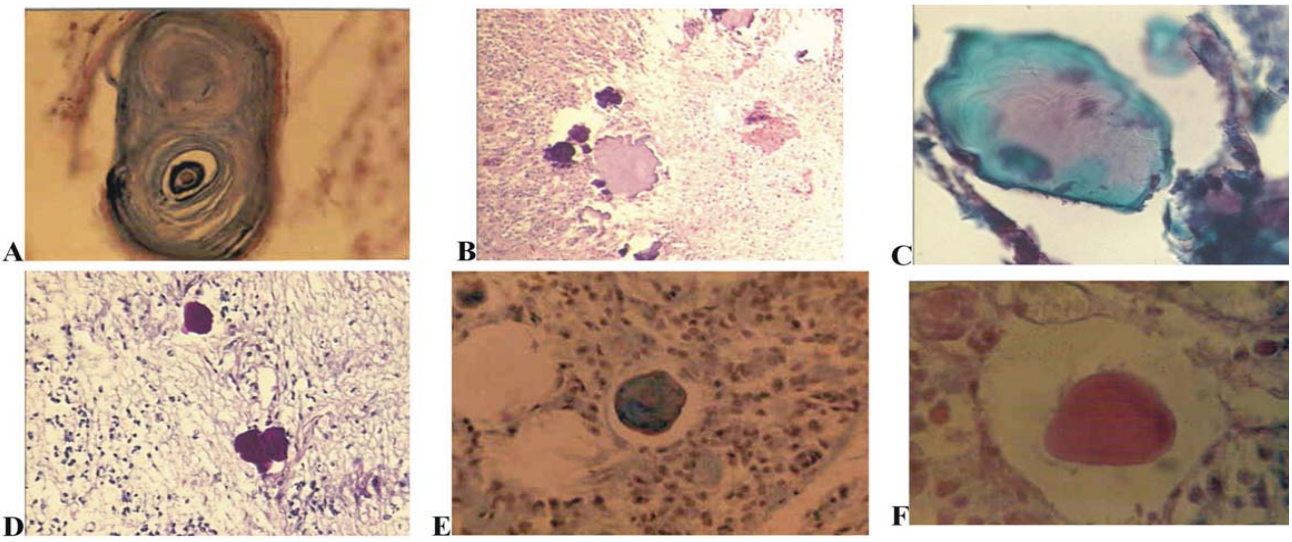

Fig. 1. A-Extrapineal acervuli; AB PAS; 180 ×; B-Intrapineal acervuli with irregular shape; HE; 40 ×; C-Intrapineal acervuli; Mallory's trichrome connective tissue staining; 400 ×; D-Intrapineal acervuli; PAS; 200 ×; E- Intrapineal acervuli; AB PAS; 180 ×; F- Intrapineal acervuli; resorcin fuchsin; 150 ×.

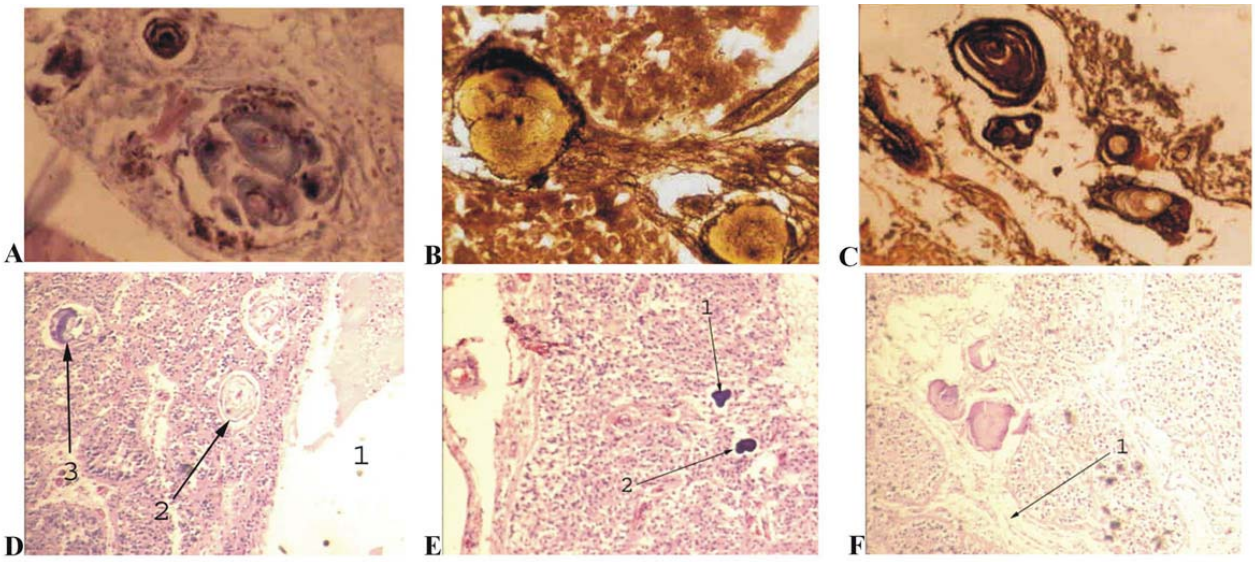

Fig. 2. A-Group of intrapineal acervuli; modified aldehyde fuchsine; 120 ×; B-Two intrapineal acervuli; Gordon-Sweet method; 150 ×; Extrapineal acervuli; Gordon-Sweet method; 100 ×; D-1-Follicule in the pineal parenchyma; 2-structure with spiral disposition of pinealocytes; 3-similar structure which is more basophilic than adjacent pineal parenhima; HE; 100 ×; E-Two acervuli at pineal glands periphery; 1-acervuli which consists from thre subunits; 2-acervuli which consists of two subunits; HE; 100 ×; F-Three intrapineal acervuli; 1-connective tissue septa; HE; 40 ×.

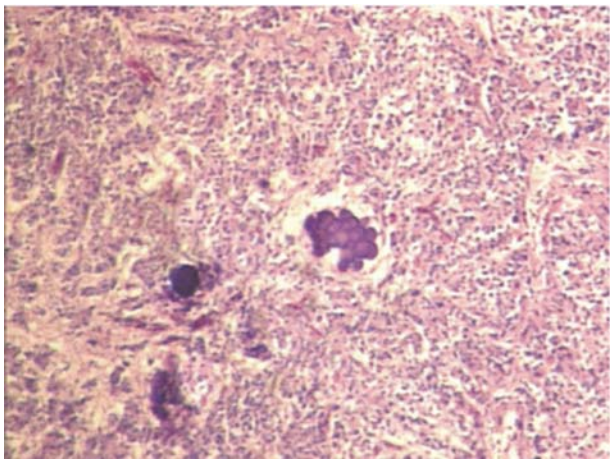

Fig. 3. Mulberry like intrapineal acervuli; HE; 40 ×.

Acervuli of the second age group were larger and with more irregular shape. They had more vrinkled, more uneven surface than latter age group acervuli and were localised not only in glands periphery but also in its core, mostly intralobularly. One larger mulberry like acervuli, which consisted of larger number of subunits, was surrounded with several small ones with more regular mostly round or oval shape, similar to the shape of the first age group acervuli (Fig. 1B).

Acervuli of the third age group had the largest dimensions. They didn't have connections with connective tissue septa. We detected smaller number of much larger acervuli in this age group than in previous two age groups. They had more irregular shape than latter age roups acervuli, with distinct wrinkled and uneven surface, because of large number of subunits which they were composed of. Smaller, acervuli, which also had mulberry like shape, were detected in the vicinity of these structures. Only, those acervuli were composed of lesser number of subunits opposite to the central acervuli (Fig 3). These structures pushed out the pineal parenchyma at the glands periphery, reducing it to narrow zone beneath the leptomeningeal capsula, which surrounded centraly localised acervuli.

Discussion

There were a large number of manuscripts about ultrastructure of pineal acervuli. However, the exact mechanism of their formation remain mystery for the majority of the authors. Lukaszyk and Reiter (3) associated the acervuli genesis with pineal glands regular neurosecretory activity and denied the connection of their formation with aging process. Recently, Humbert and Pevet (4,6) during their research on Wistar rats established two populations of pinealocytes which differentiate during the aging process in the pineal gland: light pinealocytes which present normal, functional pinealocytes and dark pinealocytes, which present pinealocytes in different stages of degeneration. They detected, after application of lanthanum nitrate, which is solely extracellular cation, that it was present inside the cytoplasm of dark pinealocytes. That ponted to the breakdown of dark pinealocytes plasma membrane. Later, after the potassium pyroantimonate application, they detected the presence Ca-pyroantimonate deposits in the dark pinealocytes cytoplasm, which probably presented the cytoplasmatic microacervuli. So, in that way they proved the participation of degeneration

process in the acervuli formation. This process progresses during the human aging. During our research we detected the structures in the vicinity of the follicles that had spiral disposition of the pinealocytes and were more basophilic on HE stained sections than adjacent pineal parenchyma (Fig. 2E). These structures could be the places of pinealocytes degeneration and therefore acervuli formation.

Application of X-ray microanalysis showed that acervuli of Wistar rats were composed mostly of hydroxyapatite crystals, more uncommon of ß-tricalciumphosphate (9). Small amounts of S, Na, Mg and Sr were also detected in their structure besides Ca and P (6,9,10). Magnesium amount increased from the core to the acervuli periphery and it has inhibitory effect on the acervuli growth (11). Glycogen, glycoproteins, proteoglycans and other neurosecretory material presence was detected by PAS and AB PAS application method. Galliani et al. (9) and Galliani and Mongiorgi (11) considered that these substances compose the core of the pineal acervuli, while hydroxyapatite crystals precipitate on it. Red color of the acervuli after the resorcin fuchsin stain application (Fig. 1F) and violet color after modified aldehyde fuchsine application (Fig. 2A) pointed to the elastic fibres participation in the acervuli structure. Gordon-Sweets method showed black colored narrow peripheral zone and yellow colored core of the acervuli (Fig. 2B) which pointed to the larger presnce of reticular fibres in acervuli periphery (12). Reticular fibres presence in acervuli periphery confirmed the finding of Krstić (10) who detected fibrous baskets arround the pineal acervuli which connected them mutualy and with adjacent pineal parenchyma. Extrapineal acervuli showed black colored concentric lamellas in the core and periphery of the acervuli which pointed to the larger participation of connective tissue in their structure. They were localised in the leptomeningeal pineal capsula. Their localisation, shape and structure pointed to the conclusion that they are, by character, very similar to psammoma bodies of the choroid plexus. Mallory's trichrome connective tissue method showed red colored core and blue colored periphery of the acervuli. This finding pointed to the possible fibrin presence in the core of acervuli and possible inflammatory process participation in the acervuli formation (13). Blue stained periphery of acervuli is in accordance to the black stained periphery finding after the Gordon-Sweets method application (Fig. 1C) which pointed to the larger presence of connective tissue in pineal acervuli periphery (12). Hence, acervuli formation process is probably the combination of the neurodegeneration and neurosecretory processes (12,13). Neurodegeneration probably presents initial process, while further growth of acervuli is the result of neurosecretory processes and conglomeration of acervuli. Pineal gland connective tissue probably participates in the conglomeration process, in such a way that it enables attachment of just formed for the surface of already present pineal acervuli (12,13).

Human pineal acervuli are detected already in children up to 3 years old in 2% of the cases. Their frequency increases with age and between the age of 10 to 18 they are present in 32% of the cases, between the age of 20 to 29 in 53% of the cases and after the age of 30 in 83% of the cases (14).Their number significantly increases after the puberty (15). They are more frequent in women than in men before the menopause (16).The number of pineal acervuli varies in different nations, probably because of different climatic conditions and different nutrition habits. The largest frequency of pineal acervuli was detected in Ugandas population, where, because of equator proximity, is the largest circannual light intensity (17). We detected that acervuli of the first age group which were small with regular form and were localised in glands cortex, were replaced with acervuli of the second and the third age group, which were larger and with more irregular form, localised more frequently in the core of the pineal gland (12,13). Duvernoy (18) in his manuscript also concluded that pineal acervuli start to appear in the cortex of the gland, mostly in its dorsal part. Older age groups acervuli show not only the increase of the size, but also considerably increase of shape irregularity. Finally, this lead to mulberry like acervuli formation in the third age group, which were composed of large number of small globular subunits (12,13). These subunits were very similar to the acervuli of the first age group. Similar structures were also detected by Krstić (10), who established that their diameter ranged from 400 μm to 3 mm. Similar structures were also detected by Galliani et al. (9,11,19) and Vigh et al. (1). These structures practicaly dominate in the pineal parenchyma. They occupy the core of the pineal gland and push the the pineal parenchyma on the glands periphery. These structures formation is probably the result of acervuli conglomeration process which occurs during the human aging. Namely, just formed acervuli probably attach for the surface of already formed acervuli without their structural mergeing. Acervuli attachment enables pineal gland connective tissue. In such way the number of acervuli doesn't change significantly during the aging, because of conglomeration process annuls the effect of new acervuli formation. Thats why we didn't detect significant increase of acervuli number during the aging in our research. Their number in the third age group remained at approximately same level in regard to the first age group. However, the third age group acervuli are larger, with more irregular form and they occupy the core of the gland. Hence, conglomeration process presents also the form of acervuli growth and it is probably the consequence of the limiting space where their formation takes place. Namely, the size of the pineal gland doesn't change during the aging and once formed acervuli doesn't disappear. Thats why the amount of the calcified pineal parenchyma increases during the aging in regard to the amount of the normal pineal parenchyma.In such way uncalcified occpys more and more narrow space in pineal gland which

forces just formed acervuli to attach for the surface of already formed acervuli. Kunz et al. (20) detected on the CT images this structures as massive calcifications in the pineal region.They measured the amount of the calcified pineal tissue. Afterwards, they calculated the amount of the normal uncalcified pineal tissue as the result of substraction of calcified pineal tissue amount from the total pineal glands volume. At the same time, they measured daily amount of 6-sulfatoxymelatonin (aMT6) which was secreted by urine. They concluded that the amount of secreted aMT6 significantly decreased during the aging. This was significantly correlated with the significant decrease of the normal pineal tissue amount during the aging. Hence, more intensive acervuli presence in older persons pineal glands is probably the result of the progressive pinealocytes degeneration, which leads to the decrease of the amount of normal uncalcified tissue and consequently the decrease of pineal gland function during the aging. This confirms the decrease of daily melatonin secretion during the human aging. So pineal acervuli don't have practical importance only as landmark during neuroimaging images analysis in radiology. They also influence on pineal gland function and through it indirectly on the global process of aging.

Conclusion

Pineal acervuli are calcified structures which can be detected in the pineal parenchyma and pineal capsula. They have laminate structure which is more distinct in the extrapineal acervuli. Acervuli have the core mostly composed of neurosecretory material and, the peripheral part which is mostly composed of connective tissue. the increase of their size and their form irregularity occurs during the human aging. Acervuli of the first age group are small, mostly localised at pineal glands periphery, sometimes near the connective tissue septa or blood vessels. They are light colored and have regular form with homogenous surface. Acervuli of the second and the third age group are larger with intralobular location in the glands core. They have mulberry shape, laminate structure and are dark blue colored because of more intensive calcification. These structure dominate in pineal parenchyma and probably are the result of their conglomeration process.

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MORFOLOGIJA I HISTOHEMIJSKE KARAKTERISTIKE ACERVULUSA PINALNE ŽLEZDE ČOVEKA TOKOM STARENJA

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Kratak sadržaj: *Acervulusi pinealne žlezde su kalcifikovane strukture, koje se uočavaju u njenom parenhimu i leptomeningealnom omotaču. Cilj našeg istraživanja bio je da se sagledaju morfološke i histohemijske karakteristike acervulusa tokom starenja čoveka. Analiza tkiva pinealne žlezde 30 kadavera starosti od 20 do 82 godine koji su klasifikovani u tri starosne grupe izvršena je uz pomoć svetlosnog mikroskopa. Tkivo je obrađivano standardnim histološkim metodom i zatim je bojeno HE, PAS, AB PAS, rezorcin fuksin, modifikovanim aldehid fuksin, Gordon Sweet-ovim metodom i trihromnim bojenjem za vezivno tkivo - modifikacija po Mallory-u. Na preparatima bojenim HE acervulusi su se bojili plavo. Oni su PAS i AB PAS pozitivne strukture. Aldehid fuksinom se boje ljubičasto, a rezorcin fuksinom crvenkasto. Primenom Gordon Sweet-ove metode središte im se boji žuto, a periferija crno. Na preparatima bojenim trihromnim bojenjem modifikacija po Mallory-u periferija im se boji plavo a centar crveno. Acervulusi prve starosne grupe su sitni pravilnog okruglog ili ovalnog oblika, lokalizovani na periferiji žlezde. Tokom starenja, dolazi do porasta veličine acervulusa pri čemu njihov oblik postaje nepravilniji što na kraju u trećoj starosnoj grupi rezultira formiranjem krupnih kupinastih acervulusa, koji nastaju najverovatnije kao rezultat procesa konglomeracije. Prema tome, acervulusi su strukture koje se sastoje iz organskih i neorganskih materija. Tokom starenja oni postaju veći a njihov oblik nepravilniji što, na kraju dovodi do stvaranja složenih kupinastih struktura koje dominiraju parenhimom žlezde.*

Ključne reči: *Pinealna žlezda, acervulusi, morfologija, histohemija*