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Original Article

Human pineal gland: Histomorphological study in different age groups and different causes of death

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ABSTRACT

Introduction: In human, the age dependent changes in the micro-anatomy and histology of different organs are most prevalent along with causes of death. As pineal is a neuro-endocrine gland and is target of various hormones of physiological importance hence, the effect of age and different unnatural causes of death may affect its histology. This aspect of histomorphology has never been studied previously. Therefore, aim of the present study was to evaluate the changes in morphology and histology of human pineal gland in different age groups in relation to different causes of death.
Methods: We collected human samples from young (5–20 yrs), middle aged (21–55 yrs) and old aged (56–95 yrs) individuals under different causes of death like septicemia (S), poisoning (P), hanging (H), burning (B), injury (I) and other reasons which are regarded as control (C) and were processed for morphometric and histological observations.
Results: Anatomically significant decrease in pineal weight, length, breadth was noted in an age dependent manner. Histological observations suggest a significant increase in numbers as well as size in pineal concretions with increasing age. Further, huge degeneration of pinealocytes and neuronal glial cells was observed upon poisoning and burning cases in comparison to control. But in case of hanging, septicemia and injury there was no significant difference in comparison to control.
Discussion: It may be concluded that not only the age but also the different causes of death may severely affect the basic micro-architecture of human pineal gland.

1. Introduction

The pineal organ is markedly different from the peripheral endocrine glands, representing a retina-like nervous tissue that is composed of cone-like pinealocytes, secondary neurons and glial cells. The pinealocyte are actually neuronal sensory cells which have both neural and hormonal efferentation. Histologically, pineal organs are composed of ependymal glial cells and pinealocytes. Pineal gland contains calcified concretions (corpora arenacea, acervuli or brain sand) measuring from some microns to several millimeters in diameters. The larger ones are easily identifiable on X-ray, CT scan and MR

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pictures. Pineal acervuli are also present in several mammals and in some birds. The calcified concretions of the human pineal gland have been known for many years. It has been generally said that calcified deposits in human pineal glands, increase in number with volume and with aging[1] or that their amount is increased by degenerative changes of the pineal. In addition, some[2] reported that calcium (Ca) deposits correlated positively with aging. Some scientists[3] also suggested that the amount of pineal concretions were increased by aging on the basis of the number of concentric rings and the calcium (Ca) and phosphorous (P) content in the pineal concretions. However, there are two studies showing a number of pineal concretions in younger subjects, as of those aged 11–15 years[4] and 7–14 years.[5] Some scientists[6] reported that the amount of pineal concretions did not appear to be age related. Recently, they suggested that there was no correlation between the amount of pineal concretions and aging when investigating the concentric rings and Ca and P content of various-sized pineal concretions. Pineal concretions are in large numbers in older patients but are seen in children too.[7] In addition some workers[8,9] reported that there may be a positive correlation between increased pineal concretions and decreased pineal melatonin bio synthesis in humans. Some workers[2] also suggested that night-time Ca levels were negatively correlated with the melatonin content in human pineal glands.

Further, in human cadavers' samples no report exists till date regarding the causes of unnatural death and histological changes in pineal micro-anatomy and micro-architecture. It may be speculated that the causes of death due to poisoning, hanging, septicemia, injury and burning may be of higher stressors than those any other causes. Thus, they may cause more changes in the histology and micro-anatomy of pineal gland and brain tissues than any other natural causes of death.

Therefore, the present study was undertaken to determine, the anatomical and histological changes in micro-anatomy of pineal gland in age dependent manner correlating it with different causes of death.

2. Material and methods

2.1. *Materials*

The study was carried out on a group of 73 human cadavers (aged 3 months to 91 years, mean 40.81 ± 13.98; 52 male and 21 female). Pineal glands were taken from consecutive autopsy cases at the Department of Forensic, Institute of Medical Sciences, Banaras Hindu University, Varanasi after the informed consent of the guardian and next to kin was taken. Donors did not suffer from any chronic disease. Age, weight and height of each of the subjects were taken before autopsies. The ethical committee of the Institute of Medical sciences approved the experimental protocols (No. Dean/2009-10/548).

2.2. *Collection of pineal gland*

Pineal gland lay just above superior colliculi of the midbrain. The pineal was carefully dissected out from the vessels and cut at the habenular and the posterior commissure. The pineal was freed from the surrounding meninges and veins since the pineal was strongly adhered to the surrounding tissue. At times a tag of meninges was left as the pineal tissue was breeched in the removal process of the adherent meninges.

2.3. *Tissue processing and sectioning*

The pineal glands were processed as published elsewhere.[10] In brief the tissues were collected and fixed in 10% Neutral Buffered Formalin (10% NBF). Further tissues were dehydrated in graded alcohols. After that tissues were embedded in paraffin (with three changes) and paraffin blocks were made. 7 μm sections were cut (Leica, RM 2245) and spread on gelatin coated slides. Further, the sections were de-paraffinized by xylene, hydrated by grades of alcohols, stained by Ehrlich's Hematoxylin, dehydrated by grades of alcohol, counter stained by eosin (1% wt/vol), cleared in xylene and mounted in DPX. After one day the slides were observed under microscope (Nikon, E200, Japan).

2.4. *Morphometric analysis*

The collected pineal glands were weighed on micro-balance (Sartorius, Germany). The pineal length, width, and diameters were measured by Vernier calipers with further illustrations in pineal density and volumes in different age groups and different autopsy cases.

2.5. *Statistical analysis*

All the parameters were statistically analyzed by Graph Pad prism (6.0 version, 2012). Analysis of variance and least square regression analysis were employed in order to determine the age dependent change in the pineal gland weight, length, breadth, height.

3. Results

3.1. *Effect of age on general histology of human pineal gland*

Histological examinations of all age group reveal that the gland can be divided into well demarcated cortex and medulla. But with the increasing age the fibrosis and the glisosis increased, with a significant increase in the connective tissue content of the pineal gland after the age of 50. Most of the pineal gland lost the cytoarchitectural morphology; the lobular pattern of the young pineal. Sheets for the connective tissue consisting of the glial cells and fibroblast cells seemed to replace the pinealocytes with increase in age especially after 30 years but this did not demonstrate a constant pattern. Cyst was a uniform finding in all pineal glands. The number and the sizes varied with the increase in age. The older the pineal gland the more cysts were present which were also larger than those found in young human pineal subjects. As a whole, the frequency of pineal calcification increased with age throughout life. The severity reached its peak in the 40–49 year and old age group, in males, while in females the peak was noted in the age group 21–30 also (Royana Singh Ph. D. thesis; unpublished data).

3.2. *Effect of autopsy cases in general histology of human pineal gland*

The general autopsy cases were designated as control groups and no significant changes were observed in different age groups as described previously. But, the autopsy cases of burning and poisoning by "Celphos" caused a huge loss in the pinealocytes and neuronal glial cells as depicted in the panel photo-micrographs. But, there was no significant variations observed in the cases of septicemia, injury or hanging cases (Fig. 1).

3.3. *Effect of age on human pineal morphometry*

In males, the weight of the pineal showed an increase at the fourth and fifth decades and then a decrease trend. Ultimately an increase in the pineal weight was observed in the eighth decade after a decrease in the sixth and seventh decades onwards. In case of female group, however, the maximum weight was observed in the adolescent age group with decrease in weights in the third as well as in the fourth decade steadily decreasing till the eighth decade (Fig. 2A). The pineal weight in between the different age group showed no significant difference. In male pineal, age related trends in the length reveal general increase in the first decade and in the middle age which persists until the age of 65 years and the declines steadily with age. The female pineal length a peak exhibit a steady increase in their length during the first two decades with a peak at 20 years and then a plateau to steadily decrease to the age of 45 years onwards. The trends in the breadth of the pineal in the normal subject showed an increase in breadth after the age of 40 years. In females, it exhibited a peak in the second decade with a steady decrease after 45 years. The thickness trend with age of male and female pineal gland shows a steady increase trend till 25 years to become almost stable in the later decades the female thickness on the other hand exhibits a steady increase in the first decade to reach its peak at the age of 17 years, steady for the next two decades and then a steady decline after the age of 40 years to reach its minimum by the age of 60 and then follow a constant path (Fig. 2B).

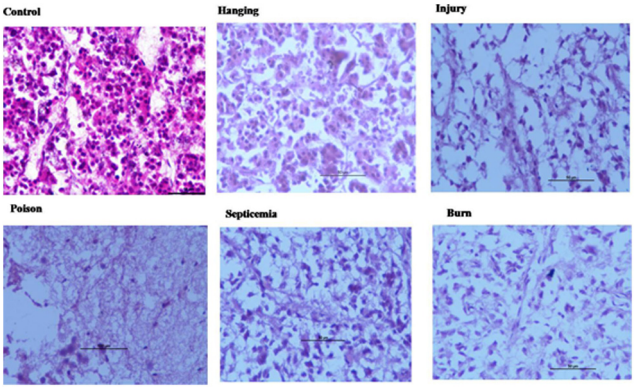

Fig. 1 – Effect of different causes of death in changes of histomorphology of human pineal micro-architecture. The panel shows there was severe loss in pinealocytes and neuronal glial cell mass in burning and poisoning cases.

3.4. *Effect of autopsy cases in pineal weight and morphometry*

An analysis of the pineal weight with reference to autopsy cases was evaluated (Fig. 2C) the weight in different groups of autopsy cases. There was a significant decrease in pineal weight in burn and poisoning cases than all other groups. The pineal length and breadth with mean of 0.8635 ± 0.27 cm and 0.42 ± 0.136 cm respectively in all autopsy cases showed no significant variation. Fig. 2D elaborates the column statistics of the volume of the pineal gland in different autopsy cases including the significant variation in density and volume of pineal gland in different autopsy cases.

4. Discussions

4.1. *General morphology of pineal gland and effect of age*

The pineal morphological features and its direct attributes to several physiological functions can be revealed if the morphological parameters can be directly correlated with age related changes. The present attempt was made to evaluate the age related morphological changes in the pineal gland so as to fill in the breech of knowledge which exists with the pineal gland. Many authors attempted to show the relationship between the weight of the pineal gland and age of the human.

Some scientists[11] described this gland to be heaviest at the fifth and the sixth decades of age. Some workers[12] suggested a correlation between the size and weight of pineal with age. However, the growth pattern was non-linear with a sharp increase in size in the fifth and sixth decades. They concluded that the pineal gland does not undergo cellular atrophy after puberty. On the contrary, some scientists[13] suggested that there was no correlation between the pineal weight and the age of the patient. Some workers[14] reported that post-pubertal atrophy of pineal gland does not occur and on an average, is larger and heavier in women.

Our study of variations in pineal length, breadth and width is in agreement with previous report,[15] reporting the high level of variations in pineal length, breadth and width in an age dependent manner. Some[16] reported a non-linear fluctuation in weight, volume and density of pineal gland in patients in an age dependent manner which is also equivocal with our study.

No sexual dimorphism existed in our study from the morphometric point of view. The non-linear trend observed in our study differ from previous report[16] indicating a slight but definite decrease from 15 to 25 years, subsequent increase till 35 years and decrease after 65 years strongly suggest the initial growth in relation of the general body growth but the subsequent growth in length can be attributed to gliosis and calcification. However they[16] did not provide any explanation. It is remarkable to note that no two studies have shown similar trend in the length, breadth and width with age. While the width and the breath displays two peaks in the previous study,[16] our study exhibits a steady plateau to decrease after 75 years. The breadth also

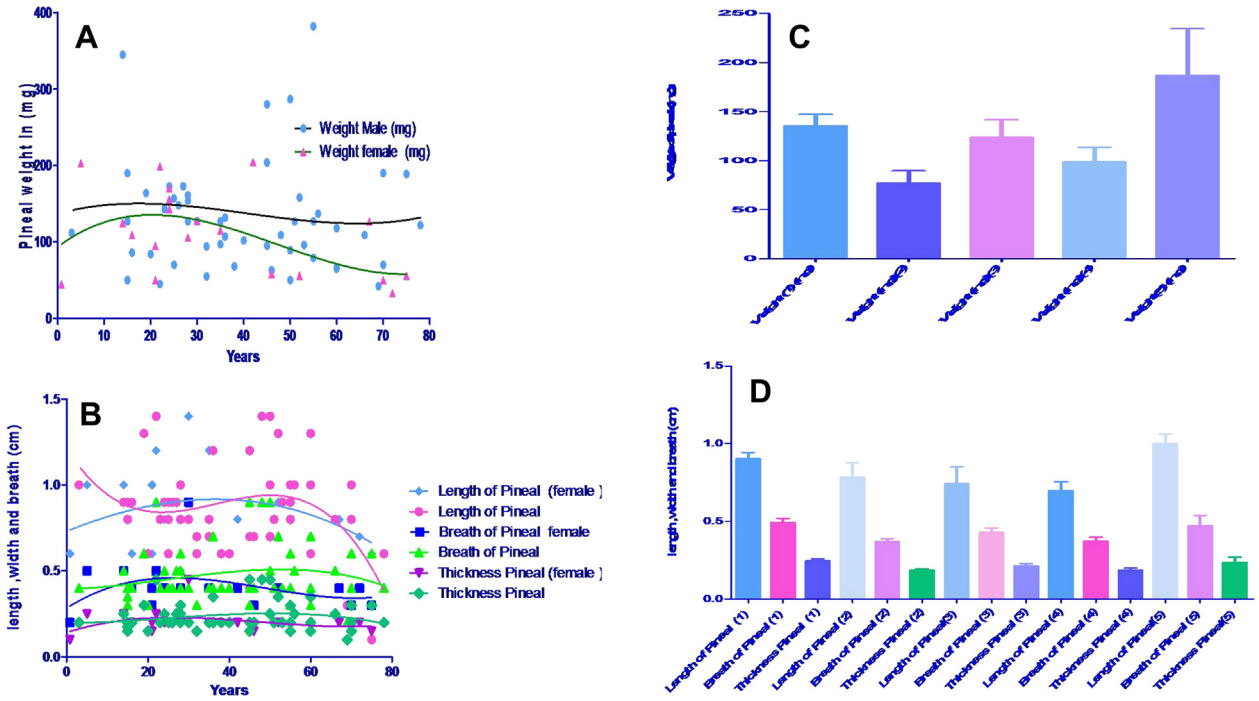

Fig. 2 – A: Pineal weights of male and female in accordance to age and their trends. The increase in weight of the pineal glands was more during the adolescence and the second decade in both males and females and in the age range between 45 and 60 years. B: Age trend in the pineal parameters length, breadth, and width with age of female versus male. Note the maximum length during the 45–60 years of age in males while in female maximum length and width is at adolescent to 40 years. C: Bar column to demonstrate the difference in the weight of different autopsy cases. 1. Accident; 2. Burn; 3. Hanging; 4. Poisoning; 5. Septicemia. D: Bar column to illustrate the different parameters of the pineal gland (length, thickness and breath) of different autopsy cases. 1. Accident; 2. Burn; 3. Hanging; 4. Poisoning; 5. Septicemia.

demonstrated a nonlinear correlation with age, but in our case the breath was definitely smaller. The width of pineal follows an increase during 31–40 years but remains within the range of 4 mm but in our study the width has increased in some cases as to 1 cm in the later years. The cause may be the spherical shape mostly observed in the sixth decade onwards. The breath and the width once attained by 30 years seem to maintain the same constant feature till 75 years suggesting that the pineal gland atrophy does not influence its breath width or length.

The findings suggest a strong relation with the reproductive cycle. The increase during adolescence and reproductive phase can be correlated with the functional physiology of the body than the peaks in the weight of the pineal initially goes with that of the generalized growth of the body in the initial lead and then with the growth spurt seen during adolescence. The post menopausal decrease can be well related and thereafter during senility. However, the increase in weight after the age of 45 years as suggested previously[12] may be related with gliosis and calcium deposition. But, the same reason cannot be extrapolated to the increase in weight during the later decade after 65 years. The increase in the calcium deposits is also related with the increase in the cyst number during this period so the increase in weight yet smaller than that of seen during the sexual spurt.

The present study provides a strong suggestion that the pineal gland does not undergo atrophy after puberty which is also supported by some study[17] which shows clearly that the hormone level of melatonin and serotonin, N-methyltransferase all remain in high concentration during adult life. This further confirms that maintenance of morphological as well as the biochemical nature of the pineal is probably to maintain the continuing activity of pineal throughout life.

4.2. *Effect of different autopsy cases*

The morphological parameters of the pineal in different autopsy cases seemed to be unaffected by the cause of death. The significant increase in the brain weight in the poisoning cases by aluminum phosphide (Celphos) in comparison to accidental cases could be due to the significant edema of the brain soon after the poison ingestion (data not shown). The brain weight in the male and female group of different autopsy cases also showed significant difference thus exhibiting sexual dimorphism. In poisoning, alteration in the length was also observed. The pineal length was shortest and significantly low than any other. The breath and width however did not display any alteration. It may be suggested the pineal is a circum-ventricular organ deprived of the Blood

Brain Barrier (BBB) in comparison to the brain, therefore the poisoning could have affected the brain and bring about alteration in the brain weight as a whole and should have resulted in the increase in the pineal length but resulted in the decrease. The pineal gland of the accidental cases on the other hand show linear correlation between the brain weight and that of the pineal i.e. the accident causes edema of the parts of the brain including the pineal. A definite yet not significant decrease in the brain weight along with corresponding decrease in the pineal weight could be explained cause of two reasons. Firstly the burn cases brain were mostly in females recovered after domestic violence or accident, secondly, the decrease in the pineal weight can be attributed to the loss of the water content from the brain as resultant overheat. In the septicemia cases there is a definite increase in the pineal weight which may be suggested due to the edema of pineal along with the overall edema of the brain. These results need more elaborative studies so as to evaluate the effect of different cause of death on the morphological parameters of the pineal gland and come to a definite conclusion especially so far the effect of burn and poisoning on the pineal. Histologically, there were no significant differences occur in the septicemia, hanging and injury cases in comparison to the normal causes of death suggesting that there may not be effect of severe stressors on these cases on pineal gland histology. However, poisoning (particularly by Celphos) and burning cases caused severe effects on the pinealocytes and neuronal glial cells in terms of total morphological degeneration of those cells. These results are important in the sense that particularly poisoning and burning cause huge changes in corticosterone and cortisol ratio of the body (Royana Singh; unpublished data) and thus affecting circulating level of melatonin. These abnormal changes in physiological melatonin level and physiological stress may in turn cause a severe change in pineal cytology.

5. Conclusion

It may be concluded that not only the effect of age but also different causes of death affect severely the morphometry and histology of pineal gland in age dependent manner.

Conflicts of interest

All authors have none to declare.

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REFERENCES

1. Winkler P, Helmke K. Age-related incidence of pineal gland calcification in children: a roentgenological study of 1,044 skull films and a review of the literature. *J Pineal Res*. 1987;4:247–252.
2. Schmid HA, Requintina PJ, Oxenkrug GF, et al. Calcium, calcification, and melatonin biosynthesis in the human pineal gland: a postmortem study into age-related factors. *J Pineal Res*. 1994:178–183.
3. Savaskan E. Melatonin in aging and neurodegeneration. *Drug Dev Res*. 2002;56:482–490.
4. Scharenberg K, Liss L. The histologic structure of the human pineal body. *Prog Brain Res*. 1965;10:193–217.
5. Tapp E, Huxley M. The weight and degree of calcification of the pineal gland. *J Pathol*. 1971;105:31–39.
6. Sener RN. The pineal gland: a comparative MR imaging study in children and adults with respect to normal anatomical variations and pineal cysts. *Pediatr Radiol*. 1995;25:245–248.
7. Mori R, Kodaka T, Sano T. Preliminary report on the correlations among pineal concretions, prostatic calculi and age in human adult males. *Anat Sci Int*. 2003;78:181–184.
8. Schmid HA. Decreased melatonin biosynthesis, calcium flux, pineal gland calcification and aging: a hypothetical framework. *Gerontology*. 1993;39:189–199.
9. Kunz D, Schmitz S, Mahlberg R, et al. A new concept for melatonin deficit: on pineal calcification and melatonin excretion. *Neuropsychopharmacology*. 1999;6:765–772.
10. Silva MJME, Singh R, Haldar C, et al. Peripheral autonomic nerves of human pineal organ terminate on vessels, their supposed role in periodic secretion of pineal melatonin. *Acta Pathol Microbiol Immunol Scand*. 2012;120:628–634.
11. Khelimsky AM. Meditsina. *Epiphysis (Pineal Gland)*. 1969.
12. Rodin AE, Overall J. Statistical relationships of weight of the human pineal to age and malignancy. *Cancer*. 1967;20:1203–1214.
13. Hasegawa Y, Inagaki T, Sawada M, et al. Impaired cytokine production by peripheral blood mononuclear cells and monocytes/macrophages in Parkinson's disease. *Acta Neurol Scand*. 2000;101:159–164.
14. Gusek W. Histology of the pineal gland in the elderly human. *Aktuelle Gerontol*. 1983;13:111–114.
15. Sumida M, Barkovich AJ, Newton TH. Development of the pineal gland: with MR. *Am J Neuroradiol*. 1996;17:233–236.
16. Golan J, Torres K, Staśkiewicz GJ, et al. Morphometric parameters of the human pineal gland in relation to age, body weight and height. *Folia Morphol*. 2002;61:111–113.
17. Arendt J, Skene DJ. Melatonin as a chronobiotic molecule. *Sleep Med Rev*. 2005;9:25–39.