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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).