INTRODUCTION

Pineal gland is a small, reddish-grey organ, occupying a median depression between the superior colliculi. It is 8 to 10 mm in length and 5 mm in maximum width and its weight ranges between 50-150 mg. Its base, directed anteriorly, contains habenular and posterior commissures and is intimately related to the third ventricle. Its light-transducing ability has led some to call it the “Third Eye”; many consider it the ‘Spiritual Eye’, the inner vision.

Light and electron microscopic investigations on the human pineal gland had been undertaken earlier. The reports on light microscopic examination of the pineal gland described a well defined capsule surrounding it which sends septa into the parenchyma and divides it into lobules. It was reported to be composed of neuroglial cells and light and dark pinealocytes; neuroglial cells being predominant in the pineal stalk. Pinealocytes, the highly modified neurons, were arranged as cords or clusters within the gland with a network of fenestrated capillaries and nerve fibers ramifying among them. Ultrastructurally, human pinealocytes had been classified into light and dark cells on the basis of their staining densities. The light pinealocytes were reported to possess round or oval cell bodies with an average diameter of 9 micrometer having round or oval nuclei; their cytoplasm contained vesicles and synaptic ribbons. The dark pinealocytes were reported to be round, oval or elongated cells with an average diameter of 9.4 micrometer and large irregular nucleus; there were numerous infoldings of nuclear membrane with deep invaginations of cytoplasm within the nuclear folds, giving the appearance of nuclear pellets or “Kernkugeln”. The cytoplasm of dark pinealocytes contained pigment. Two or more cytoplasmic processes were observed to extend from cell body and ended in bulbous expansions near capillaries or on ependymal cells of the pineal recess; these expansions contained rough endoplasmic reticulum, mitochondria, and dense-cored vesicles which stored monoamines and polypeptide hormones. The light pinealocytes outnumbered the dark ones in the parenchyma. It had been reported that pineal concretions or acervuli start appearing in the dark pinealocytes. Cilia with usual microtubular pattern were noted in fetuses suggesting that the human pineal gland may maintain photoreceptor function and a high level of secretory activity at all ages. It had been reported that there was progressive degeneration of pinealocytes with advancing age whereas, a decrease in secretory activity of pinealocytes

ABSTRACT

Objective: To determine age-related quantitative and qualitative changes in human pinealocytes using cadaveric material.

Study Design: Analytical cross-sectional study.

Place and Duration of Study: The study was conducted in the Department of Anatomy, University of Health Sciences, Lahore, from January to December 2008.

Methodology: Thirty pineal glands from human cadavers ranging from 16-80 years of age were collected from mortuary of King Edward Medical University, Lahore, using purposive non-probability sampling. These were divided into three different age groups: I, II and III each between 16 to 30, 31 to 45 and 46 to 80 years of age respectively. Pinealocytes were counted; their mean diameter and that of their nuclei was calculated from a total of 30 cells per slide, using 4 µm thick H and E stained histological sections. Mean ± S.E.M. was calculated for quantitative variables. One-way ANOVA was applied to observe group mean differences among three groups.

Results: The number of pinealocytes decreased with aging but the difference was statistically insignificant when compared between groups (p=0.234). There was no change in size of pinealocyte soma and its nucleus (p=0.889 and 0.898 respectively).

Conclusion: The number and size of pinealocytes, and their nuclei remained unaltered with advancing age.

Key words: Age. Pinealocyte. Pineal gland.
had also been observed. Some workers reported an age-related decrease in the number of pinealocytes with an increase in amount of glial fibers.

In view of the above mentioned conflicting reports on pinealocytes with advancing age from other parts of the world and due to paucity of work; in this regard, in Pakistan, it was decided to investigate the effects of advancing age on pineal gland among local population, using cadaveric material.

**METHODOLOGY**

The present study was carried out in Department of Anatomy, University of Health Sciences, Lahore, from January to December, 2008. A total of thirty specimens of pineal glands were obtained from the cadavers of different age groups from the mortuary of King Edward Medical University, Lahore. Purposive, non-probability sampling were used; the cadavers were brought to cold storage within 3 hours after death and kept at a temperature of 4°C; pineal glands were removed within 24 to 48 hours after death. The study was carried out in three groups I, II and III according to their age: 16 to 30, 31 to 45 and 46 to 80 years respectively. The cadavers with history of accidental brain damage, Diabetes mellitus, hypertension, disease of central nervous system or drug intake were excluded.

Pineal gland was identified and removed along with superior colliculi to include the pineal recess in the sample and was fixed in 10% formol-saline for six to eight days. Each pineal gland was bisected; each half of sample was processed and the specimens were properly oriented in the paraffin blocks. Four \( \mu m \) thick sections were obtained and stained with Hematoxylin and Eosin (H and E) for examining them under light microscope (Leica DM 1000).

Pinealocytes were counted by superimposing the ocular graticule on the pineal gland preparation. Pinealocytes were identified by their large nuclei and prominent nucleoli and these were counted from randomly selected fields each from anterior, middle and posterior parts of the gland per section; those lying on the lower and left edges of the graticule were excluded. These were added to get the total number of cells per slide of the preparation. Two slides from the same preparation were used to calculate the mean number of pinealocytes per specimen.

The size of body of pinealocytes and their nuclei was measured with the help of ocular micrometer calibrated with the stage micrometer at X1000 in a usual way. Ten eyepiece divisions were equal to one stage division; as 1 stage division = 10 \( \mu m \); accordingly one division of eyepiece micrometer = 10/10 = 1.0 \( \mu m \).

The scale of the eyepiece micrometer was superimposed on the body of the pinealocyte which displayed the nucleus in the center and the number of divisions covering it at the maximum diameter multiplied by 1.0 was taken as actual size of body of pinealocyte in \( \mu m \). The size of pinealocyte nucleus was measured in the same manner.

The diameters of ten pinealocytes and their nuclei were measured from each of three different locations i.e.; anterior, middle and posterior parts of the gland for calculating the mean diameter of the pinealocytes and their nuclei.

The data was entered and analyzed using SPSS version 17.0. Mean ± S.E.M. is given for quantitative variables. One-way ANOVA was applied to observe group mean differences. P-value of < 0.05 was considered as statistically significant.

**RESULTS**

The pineal gland was located in the centre of depression between superior colliculi of midbrain and was attached to the roof of third ventricle by a stalk which comprised of superior and inferior laminae containing habenular and posterior commissures respectively. The stalk contained pineal recess of the third ventricle.

Light microscopic examination of pineal gland showed a thin connective tissue capsule covering it; septa of variable thickness extended into the parenchyma (Figure 1).
The parenchyma was composed of pinealocytes and neuroglial cells; former were arranged as cords or clusters and the latter as few scattered cells among them.

Pinealocyte soma varied from round to oval in shape; their pale staining nuclei were also round to oval in shape and larger than those of the neuroglial cells. Few of the pinealocytes showed infoldings of their nuclear membrane entrapping part of cytoplasm into the nuclear folds; this gave it an appearance of nuclear pellets. Nucleoli were discernable in some pinealocytes otherwise only Nissl substance was observed in the cytoplasm (Figures 2 and 3).

There was an age-related decrease in the number of pinealocytes and when compared between the three age groups; the difference was statistically insignificant, p-value being 0.234, so there was the difference in size of pinealocytes and their nuclei, when compared between the groups, p-value being 0.889 and 0.898 respectively (Table I).

### Table I: Mean number, size of pinealocytes soma and their nuclei compared in three groups.

<table>
<thead>
<tr>
<th>Pinealocytes</th>
<th>Group I (n = 12)</th>
<th>Group II (n = 11)</th>
<th>Group III (n = 7)</th>
<th>p -value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>160 ± 11.4</td>
<td>151 ± 10.5</td>
<td>122 ± 25.7</td>
<td>0.234</td>
</tr>
<tr>
<td>Soma size</td>
<td>8.43 ± 0.20</td>
<td>8.55 ± 0.19</td>
<td>8.43 ± 0.20</td>
<td>0.889</td>
</tr>
<tr>
<td>Nuclear size</td>
<td>6.29 ± 0.13</td>
<td>6.20 ± 0.16</td>
<td>6.22 ± 0.14</td>
<td>0.898</td>
</tr>
</tbody>
</table>

Nuclear size: 6.29 ± 0.13, 6.20 ± 0.16, 6.22 ± 0.14, respectively.

There was an age-related decrease in the number of pinealocytes, although difference between age groups was statistically insignificant, p-value being 0.234, so there was the difference in size of pinealocytes and their nuclei, when compared between the groups, p-value being 0.889 and 0.898 respectively (Table I).

**CONCLUSION**

Pinealocytes are reported to respond to immobilization with an increased peptidergic activity, leading to degeneration of these cells in gerbils; this could be implied that there is a deleterious action of immobilization stress on these functionally stimulated cells. Retinal damage leads to photic elimination resulting in increased density of pinealocytes. Immunocytochemical and electron-microscopic investigations of the pineal organ in adult agamid lizards, Uromastyx hardwickii showed dense-core granules in pinealocytes indicating secretory activity of these cells.

Nuclear and nucleolar size had been used to assess varying degree of activity in the pineal gland in experimental animals (quoted by Quay, 1965). It was concluded from a study on pineal gland of sheep that functional activity of gland was related to the number of pinealocytes and not with their size. An extensive number of pinealocytes with increased cytoplasmic organelles and double nucleoli in some of these cells had been reported in rats which were exposed to constant darkness, leading to their increased activity; whereas a decrease in number of these cells and their cytoplasmic organelles leading to decreased pineal activity, had been observed in rats constantly exposed to light. It had been reported that a decrease in production of melatonin led to impaired body resistance. The findings regarding pinealocytes were similar to earlier reports in which it was suggested that these cells neither showed atrophy nor hyperplasia with aging and selective senile atrophy of the pineal did not occur. However, clinical evidence suggests a decrease in the functional activity of pineal gland which is indicated by decrease in serum melatonin level with advancing age and in age-related morbidity conditions like atherosclerosis, Alzheimer's and Parkinson's disease. The possible mechanisms by which melatonin secretion declines with age may depend on some regressive changes in the structure of the pinealocytes which are not discernable with the light microscope.

It is, therefore, proposed to extend the work correlating structure with function of the pineal gland using ultra structural study and serum melatonin level with advancing age in an experimental setup after making the variables uniform.

In this study, there was no statistically significant difference between the number and size of pinealocytes, and their nuclei with advancing age.
REFERENCES


