ABSTRACT

Background: The present work was based on the evaluation of histological, histochemical, and quantitative study on the adrenal medulla of the white albino rat in the different postnatal age period.

Material and methods: Sixty male albino rats were used in this study. The rats were classified to 4 main groups as follows:
- Group one: One week old albino rats.
- Group two: One month old albino rats.
- Group three: Three months old albino rats.
- Group four: Senile rats.

Three main parameters were performed in this study, the first was the study of the morphological changes in the adrenal medulla in the different postnatal age groups. The second was concerned with the histochemical studies while the last parameter was the quantitative studies on the gland volume as well as its cellular count. These three parameters were performed by using different staining techniques.

Results: The results showed that medullary cells in the early age groups were arranged in non-differentiated groups and become more differentiated in the older age groups. Both reticular and elastic fibers in the older age groups showed a definite increase especially at the region of corticomedullary zone. The different types of chromaffin cells were more observed at the old age groups. The concentration of ascorbic acid granules was more marked in the senile group. The quantitative changes were in the form of increased medullary volume especially in the old age.

The number of chromaffin cells as well as the concentration of ascorbic acid contents was more noticed in the old age group.

Conclusions: The differentiation of both divisions of the adrenal gland was not noticed in the early age groups. Cellular and fibrous differentiations were more seen in older age groups which may reflects an idea about the degree of gland maturation.

Keywords: Adrenal medulla, chromaffin cells, ascorbic acid.

INTRODUCTION

The adrenal medulla represents one of the most important structures in the human body that needs extensive research study. Catecholamines secreted by the medullary Chromaffin cells have many important roles in both newly born and adults.

The adrenal gland was first described by Eustachius in 1563 and its importance was later recognized by the works of (Anderson and Axel, 1997).

The adrenal glands are embedded in adipose tissues at the cranial pole of each kidney (Don and Ronald, 2002). Their weight and size vary with age and physiological condition of the individual (Junqueira et al., 2003). Fresh sections of the adrenal gland show its covering capsule which is formed of collagenous connective tissue, the cortical tissue is yellow and peripherally situated while the medulla is reddish and vascular in the central location of the gland (Neville and Hare, 1982).

The adrenal gland is composed of two tissues with different ontogenetic origin, the cortex and the medulla. The cortex develops from the mesoderm but the medulla of neural crest lineage origin (Keith and Persaud, 1993). During the fetal life, a layer of fetal or provisional
cortex is located between the medulla and the thin permanent cortex. The function of such fetal cortex is the secretion of sulfate conjugates of androgens, which are converted in the placenta to active androgens and estrogens that enter the maternal circulation. Such fetal cortex undergoes involution after birth (Goldman, 2000).

The adrenaline-containing cells are termed chromaffin cells, there is a great histological difference between the developing Chromaffin tissue and the adult Chromaffin cells (Anderson and Axel, 1997).

Carmiceal 1993, described the medullary cells as large epithelial cells arranged in rounded clusters or short cords that are in intimate relation to the blood capillaries.

Parenchymal cells of the adrenal medulla can be regarded as modified sympathetic postganglionic neurons that lost their axons and dendrites during their embryonic development and become a secretory cells (Junqueira et al., 2003).

The adrenal medullary cells have been considered as a substitute neurons in the human brains, parts of such gland were transplanted in the brain of patient with severe Parkinsonian disease (Backlund et al., 2003), more recently such technique may be one of the effective new therapy for this common disease (Madrazo et al., 1998).

Although many studies were carried out on that organ, few literatures were available concerning the age associated structural variations. Hence, the aim of this work is to identify the different cellular changes in the adrenal medulla in different postnatal ages.

MATERIAL AND METHODS

The animals:
Sixty male albino rats were used in this study. The rats were divided to four main groups 15 rat /group as follows
Group 1: Albino rats aged seven days.
Group 2: Albino rats aged one month.
Group 3: Albino rats aged three months.
Group 4: Albino rats aged one and half year (Senile).

Methods:
The study was planned to evaluate the following items:

1- Morphological study: It was performed by using paraffin sections which were stained by hematoxylin and eosin stain (Kiernan 2001). The reticular fibers were examined by staining the sections with silver stain while the elastic fibers were stained with orcein stain (Bancroft and Gamble 2002).

2- Histochemical study, for the following:
I- Staining of both types of chromaffin cells by Wood technique (Wood 1963).
II- Staining the ascorbic acid (vitamin c) contents in the chromaffin cells using silver nitrate technique (Bancroft and Gamble 2002).

3- Quantitative study, for recording changes in the following:
I- Changes in the mean volume of the adrenal medulla in the different postnatal age groups. An eye piece micrometer “PZO” calibrated against stage micrometer was used to estimate the volume of the whole gland and that of the medulla according to the following formula:

\[ V = \frac{4}{3} \pi L^3 \]

Where \( L = \frac{1}{2} \) the mean diameter and \( \pi = \frac{22}{7} \)

II- Changes in the number of both types of chromaffin cells in the different postnatal age groups.

III- Changes in the cellular contents of ascorbic acid in the different postnatal age groups.

Computer imaging system was used for counting cell number as well as estimating the optical density of the ascorbic acid.

4- The obtained data were analyzed using student (t) test, significant differences between the means in the different groups were considered at \( p < 0.05 \) (Sokal and Rohif, 1981).

RESULTS:

I- Morphological changes:
The gland of the one week old group showed ill differentiation between cortex
and medulla (figure 1). The medullary cells arranged either in groups or in cords separated by blood sinusoid.

In second group of one month (figure 2), the gland become more larger in size and there is well differentiation between cortex and medulla. The medullary cells arranged in smaller areas of the medulla separated by wide fenestrated blood sinusoid, the medullary cells become larger in size with large nuclei and deep basophilic cytoplasm.

In third group of three months (figure 3) and in fourth senile group (figure 4) the adrenal gland become larger in size and there is sharp differentiation between cortex and the medulla, the medullary cells were arranged mainly in anastomosing cords separated by wide fenestrated blood sinusoids. The medullary cells become larger in size and increase in number with large nuclei and deeply basophilic.

Changes in reticular fibers were variable in the different groups of the study (figures 5-8). In silver stained sections of adrenal gland of three months old and senile group rats there was an increase in the network of reticular fibers at corticomедullary junction but there was a decrease in both one week and one month old aged albino rats.

In orcein stained sections (figures 9-12) of adrenal gland, the elastic fibers showed less distribution in both one week and one month while the distribution of these fibers showed observable increase in both of three months and senile groups rats.

II- Histochemical changes:
Woods stain (figures 13-16) was the suitable stain for differentiation of both types of chromaffin cells.

The adrenaline secreting cells were stained purple in color with large purple nucleus and non granular cytoplasm. The noradrenaline secreting cells were yellowish orange in color with small purple nucleus and granular cytoplasm. The differentiation between the two types of cells was more noticed in older age groups.

Sections of adrenal medulla stained with silver nitrate for localization of sites of ascorbic acids (figures 17-20).

In sections of adrenal gland of one week old age the reaction appear as weak black dots in the medulla while in sections of one month and three months the reaction was. In senile age the reaction was increased.

III- Quantitative changes: (Tables 1-4 & Figs. 21-24)
Changes in the volume of the adrenal medulla: The volume of the medulla showed gradual increase with age. The difference between the mean volume of the medulla of the adult rat and those of the other age groups showed statistical significant changes.

Changes in the mean number of medullary cells: The two types of chromaffin cells were difficult to be differentiated in young ages but the degree of differentiation was much more better in older age groups, it showed statistical significant increase in the number especially in old aged groups.

Changes in the concentration of ascorbic acid in the medulla: There was a significant increase in the optical density of ascorbic acid content with the advance in the age groups when compared to those of the adult age.
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(Fig. 1) Photomicrograph of a section in the adrenal medulla of one week old age, showing cells collected in groups without differentiation. (Hx.&E. stain X 500)

(Fig. 2) Photomicrograph of a section in the adrenal medulla of one month old age, showing differentiated cells collected in groups. (Hx.&E. stain X 500)

(Fig. 3) Photomicrograph of a section in the adrenal medulla of three months old age, showing differentiated cells collected in groups. (Hx.&E. stain X 500)

(Fig. 4) Photomicrograph of a section in the senile adrenal medulla, showing more differentiated cells collected in groups. (Hx.&E. stain X 500).
(Fig. 5) Photomicrograph of a section in the adrenal medulla of one week old age, showing the distribution of reticular fibers. (Silver stain × 500).

(Fig. 6) Photomicrograph of a section in the adrenal medulla of one month old age, showing the distribution of reticular fibers. (Silver stain × 500).

(Fig. 7) Photomicrograph of a section in the adrenal medulla of three months old age, showing the distribution of reticular fibers. (Silver stain × 500).

(Fig. 8) Photomicrograph of a section in the senile adrenal medulla, showing the distribution of reticular fibers. (Silver stain × 500).
(Fig. 9) Photomicrograph of a section in the adrenal medulla of one week old age, showing the distribution of elastic fibers. (Orcein stain  X 500).

(Fig. 10) Photomicrograph of a section in the adrenal medulla of one month old age, showing the distribution of elastic fibers. (Orcein stain X 500).

(Fig. 11) Photomicrograph of a section in the adrenal medulla of three months old age, showing the distribution of elastic fibers. (Orcein stain X 500).

(Fig. 12) Photomicrograph of a section in the senile adrenal medulla, showing the distribution of elastic fibers. (Orcein stain X 500).
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