

EFFECT OF EXPERIMENTALLY PRODUCED HYPOTHYROIDISM ON MORPHOLOGY OF ADRENAL GLAND IN IMMATURE ALBINO RAT

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ABSTRACT

Adrenal glands are important endocrine glands responsible for the synthesis of some important hormones, which control different essential metabolic functions in the body.

This experimental study was designed to observe the effects of experimentally produced hypothyroidism on the morphology of this important gland. The study was carried out at Basic Medical Sciences institute of Jinnah Post Graduate Medical Centre Karachi.

Eighteen immature rats, 2 weeks of age, were obtained from the animal house of B.M.S.I. and grouped into three groups A, B and C. Group A animals behaved as controls. Group B animals received carbimazole (to produce hypothyroidism) and group C animals received carbimazole plus thyroxine for six weeks. At the end, all animals were sacrificed and their adrenals removed. The organs were fixed, sectioned and stained with H&E and Sudan black.

Zona fasciculatae of the cortices of adrenals from group B animals (carbimazole treated) were found to be shrunken with decreased number of cells indicating an adverse effect of hypothyroidism on rat adrenal morphology.

INTRODUCTION

Thyroid gland, previously known as laryngeal gland was named as thyroid in 1656 by Thomas Wharton because of its shield like shape (Werner, 1971). Galen, a Greek physician and teacher (129 to 200 AD) was the first to describe the gland in his famous book "De Voc". The details of thyroid gland were also described by Vesalius in 1543.

It produces two idoaminoacid hormones T3 and T4 which regulate gene expression, tissue differentiation and general development through a nuclear mechanism with the help of specific high affinity receptors in the target cell nucleus. Thyroid gland thus through its hormones T3 and T4, exerts important effects on growth and differentiation of different cells in mammalian species.

The deficiency of thyroid hormones, either primary or secondary, results in decreased basal metabolic rate, slow heart rate, sluggish behavior, sleepiness, increased tiredness, a dry skin, weight gain, an increased sensitivity to cold, a sallow complexion and changes in voice (Franklyn, 1993). Hypothyroid patient may also present with unexplained pericardial-effusion or ascites, psychiatric symptoms (Gold, 1981), muscle disease (Klein, 1981) and rheumatic manifestations. Deficiency of thyroid hormones during pregnancy is accompanied by increased risk of foetal and maternal morbidity.

Though the effects of decreased level of thyroid hormones on the skeletal, nervous and reproductive tissues are well established, it does affect the adrenals also.

The purpose of present study therefore was to investigate (i) the effects of carbimazole on the morphology of 2 weeks immature rat adrenals, and (ii) effects of simultaneous administration of thyroxine (thyroid hormone analogue) with carbimazole on the morphology of rat adrenals.

MATERIALS AND METHODS

Drugs used in the Study

Carbimazole (Neomercazole)

This is an anti-thyroid drug which belongs to thioamide group of anti-thyroid drugs. It acts by inhibiting the enzyme thyroid peroxidase which blocks the iodination of tyrosine; hence prevent the formation of T3 and T4 (Gold, 1981).

Thyroid Preparation

Thyroxine (levothyroxine sodium) is the hormone of choice for thyroid hormone replacement.

For this study, 18 immature animals of two weeks age were obtained from the Animal House of Basic Medical Sciences institute (BMSI), Jinnah Postgraduate Medical Centre (JPMC), Karachi, and were divided in three groups; A, B, C, each comprising of six animals.

Group-A served as control group and received 0.5 cc (volume equivalent to thyroxine) of normal saline subcutaneously for six weeks.

Group-B received carbimazole as an anti-thyroid agent dissolved in 0.9% NaCl subcutaneously at a dose of 6 µgm/gm of body weight daily for six weeks (Haynes et al., 1980).

Group-C received carbimazole in 0.9% Normal Saline (as group B) and thyroxine in 0.01M NaOH solution in the dose of 5 µgm (0.5 cc) subcutaneously daily for six weeks (Inauwa and Williams, 1995).

Diet and water supplied ad libitum to all

animals. The animals were weighed at the end of each week.

At the end of six weeks treatment, the animals were sacrificed by an over-dosage of ether anaesthesia. Through the midline abdominal incision, abdominal cavity was opened, kidneys were identified and both the adrenals separated from the superior pole of each kidney, weighed and fixed in 10% formalin.

The tissue was processed; blocks were made and sectioned at 5µm thickness. H&E staining was done to see the morphology of adrenal cortex.

Micrometry was done by calibrating through ocular micrometer with reticule under high power and oil immersion objectives. Statistical analysis of data was carried out (Bland, 1987).

The statistical significance of difference between two means of various parameters in different groups was evaluated by student's 't' test (Hill, 1977) and P value was calculated. The difference was regarded as highly significant if P value was less than 0.01, statistically significant if P value was less than 0.05, and nonsignificant if P value was greater than 0.05.

OBSERVATIONS AND RESULTS

Controls

Hematoxylin and eosin stained sections were thoroughly examined for morphological changes. In this group adrenal capsule appeared thin and moderately cellular with infiltration of fibroblasts (Fig. 1).

The arrangement of cells in zona glomerulosa was ovoid with rich network of blood vessels. The cells in zona fasciculata were arranged in parallel cords running radially towards the medulla and were cuboidal in shape. The zona reticularis was composed of network of cells.

COLOR PICTURE ON CD

Fig. 1: Section of Adrenal cortex in two weeks control immature rat showing capsule (C), zona glomerulosa (G), zona fasciculate (F), zona reticularis (R), and adrenal medulla (M). Paraffin section, H&E stain. Photomicrograph x 217.

COLOR PICTURE ON CD

Fig. 2: Section of Adrenal in two weeks Carbimazole treated immature rat showing fat globules in zona fasciculate (F) (against arrow). Paraffin section, H&E stain. Photomicrograph x 405.

The mean width of adrenal cortex was $597.00 \pm 6.25 \mu\text{m}$. The mean widths of zona glomerulosa, fasciculata, and reticularis were $68.00 \pm 14.05 \mu\text{m}$, $318.00 \pm 10.62 \mu\text{m}$, and $210.00 \pm 16.89 \mu\text{m}$ respectively (Table-1). The

total number of cells per unit area in zona glomerulosa, fasciculate, and reticularis was 57.33 ± 3.06 , 41.66 ± 3.31 , and 69.66 ± 3.88 respectively (Table-2).

COLOR PICTURE ON CD

Fig. 3: Section of Adrenal cortex in two weeks Carbimazole treated immature rat showing congestion (against arrow) in zona fasciculata (F). Paraffin section, H&E stain. Photomicrograph x 405.

Carbimazole Treated Group

In Hematoxylin and eosin stained sections, a marked change in the morphology of rat adrenals was observed. The capsule was thickened with increased infiltration (Figs. 2 & 3) of fibroblasts. The cells in all the three zones of adrenal cortex were hypertrophied and vacuolated showing increased congestion, especially in zona fasciculata, as shown in Figs. 2 & 3.

The mean width of cortex was $558.00 \pm 5.13 \mu\text{m}$ which showed a highly significant decrease ($P < 0.001$) when compared with the controls as shown in Table-1.

The mean width of zona glomerulosa was $64.00 \pm 3.99 \mu\text{m}$ which showed a non-significant decrease ($P > 0.78$) when compared with the controls as shown in Table-1.

The mean width of zona fasciculata was $252.66 \pm 6.88 \mu\text{m}$ after carbimazole treatment. A highly significant decrease ($P < 0.001$) was noticed when compared with the control animals as shown in Table-1.

The mean width of zona reticularis was $236.00 \pm 7.37 \mu\text{m}$ which showed a non-significant increase ($P > 0.7$) when compared

with the controls as shown in Table-1.

The mean number of cells per unit area ($5.625 \times 10^{-3} \text{ mm}^2$) of zona glomerulosa was 69.33 ± 4.46 which showed a significant increase ($P < 0.05$) when compared with controls as shown in Table-2.

The mean number of cells per unit area ($5.625 \times 10^{-3} \text{ mm}^2$) of zona fasciculata was 22.16 ± 2.45 which showed a significant decrease ($P < 0.05$) when compared with the controls as shown in Table-2.

The mean number of cells per unit area ($5.625 \times 10^{-3} \text{ mm}^2$) of zona reticularis was 84.00 ± 2.54 which appeared to have increased in number statistically significant when compared with controls ($P < 0.01$).

Carbimazole plus Thyroxine treated group

The mean width of cortex was $590.00 \pm 3.68 \mu\text{m}$ which showed statistically highly significant increase ($P < 0.001$) when compared with the Carbimazole treated group but no significant change was observed when compared with the controls as shown in Table-1.

Table-1
Mean width of Cortex and Zones (μm) Mean $\pm$ S.E.M.) in 2 weeks immature albino rats

Groups	Cortex	Total width of		
		Zona Glomerulosa	Zona Fasciculata	Zona Reticularis
C (A) n=6	597.00 $\pm$ 6.25	68.00 $\pm$ 14.08	318.00 $\pm$ 10.62	210.00 $\pm$ 16.89
Ca (B) n=6	558.00 $\pm$ 2.02	64.00 $\pm$ 3.99	252.66 $\pm$ 6.88	236.00 $\pm$ 7.37
Ca+Th (C) n=6	590.00 $\pm$ 3.68	72.00 $\pm$ 6.19	286.00 $\pm$ 5.72	231.33 $\pm$ 9.20

Statistical analysis of difference in mean width of Cortex and its different Zones

Groups		Cortex	Total width of		
			Zona Glomerulosa	Zona Fasciculata	Zona Reticularis
C (A) n=6	AvB	P<0.001 HS	P>0.78 NS	P<0.001 HS	P>0.16 NS
Ca (B) n=6	BvC	P<0.001 HS	P>0.30 NS	P<0.05 Sig	P>0.70 NS
Ca+Th (C) n=6	AvC	P>0.34 NS	P>0.79 NS	P<0.01 Sig	P>0.29 NS

Key C= Control; Ca = Carbimazole; Th = Thyroxine HS = Highly Significant; Sig = Significant; NS = Non-significant.

The mean width of zona glomerulosa was $72.00 \pm 8.19 \mu\text{m}$ which showed a statistically non-significant increase ($P>0.30$) when compared with the Carbimazole treated group. A statistically non-significant increase ($P>0.79$) was also observed when compared with the controls as shown in Table-1.

The mean width of zona fasciculata was $286.00 \pm 5.72 \mu\text{m}$ which showed a highly significant increase ($P<0.05$) when compared with the Carbimazole treated group. It was decreased significantly ($P<0.01$) when compared with controls.

The mean width of zona reticularis was $231.33 \pm 9.20 \mu\text{m}$. A decrease was observed when compared with Carbimazole treated group, however it was not significant statistically ($P>0.70$). Similarly a non-significant increase ($P>0.29$) was observed when compared with the controls as shown in

Table-1.

The number of cells per unit area ($5.625 \times 10^{-3} \text{ mm}^2$) of zona glomerulosa was 51.667 ± 2.96 which showed a statistically significant decrease ($P<0.05$) when compared with Carbimazole treated group but a statistically non-significant decrease ($P>0.55$) was observed when compared with controls as shown in Table-2.

The mean number of cells per unit area ($5.625 \times 10^{-3} \text{ mm}^2$) of zona fasciculata was 30.833 ± 2.82 which showed a significant increase ($P<0.05$) when compared with the Carbimazole treated group and significantly decreased when compared with controls ($P<0.05$).

The mean number of cells per ($5.625 \times 10^{-3} \text{ mm}^2$) unit area of zona reticularis was 62.16 ± 2.76 , showed a highly significant

Table-2
Mean number of Cells in different zones of Cortex (per area of reticule)
in 2 weeks immature albino rats

Groups	Zona Glomerulosa	Zona Fasciculata	Zona Reticularis
C (A) n=6	57.33 ± 3.06	41.667 ± 3.31	69.66 ± 3.88
Ca (B) n=6	69.33 ± 4.46	22.16 ± 2.45	84.00 ± 2.54
Ca+Th (C) n=6	51.667 ± 2.96	30.833 ± 2.82	62.16 ± 2.76

Statistical analysis of difference in mean number of Cells in different Zones of Cortex

Groups		Zona Glomerulosa	Zona Fasciculata	Zona Reticularis
C (A) n=6	AvB	P>0.01 Sig	P<0.001 HS	P>0.01 Sig
Ca (B) n=6	BvC	P>0.01 Sig	P<0.05 Sig	P>0.001 HS
Ca+Th (C) n=6	AvC	P>0.55 NS	P<0.05 Sig	P>0.14 NS

Key C= Control; Ca = Carbimazole; Th = Thyroxine HS = Highly Significant; Sig = Significant; NS = Non-significant.

decrease ($P<0.01$) when compared with Carbimazole treated group but non-significant decrease ($P>0.14$) was noticed when compared with control group, as shown in Table-2.

DISCUSSION

The present study was undertaken to investigate the effects of carbimazole induced hypothyroidism on the morphology of immature (2 week) rat adrenals. Carbimazole blocks the synthesis of thyroid hormones by blocking the iodination of tyrosine. It also inhibits the coupling of di-iodo and mono-iodotyrosine (Greenspan and Dong, 1995).

A marked reduction was observed in the total width of adrenal cortex in carbimazole treated animals whereas there was a lesser shrinkage of adrenal cortex in carbimazole plus thyroxine treated animals.

This shrinkage of cortex and particularly that of zona fasciculata in adrenals of carbimazole treated animals is in accordance

with the study of Dean and Greep (1947) who had also observed adrenal atrophy following hypothyroidism.

CONCLUSION

It can be concluded on the basis of results of present study that hypothyroidism may cause adrenal atrophy and therefore its insufficiency.

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