ORIGINAL ARTICLE

A HISTOLOGIC STUDY OF CARBIMAZOLE-INDUCED HYPERPLASIA OF ADENOHYPOPHYSIS WITH PROTECTIVE ROLE OF THYROXINE IN MALE ALBINO RATS

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ABSTRACT

Objective : To assess the frequency and degree of pituitary hyperplasia in albino rats made hypothyroid by Carbimazole, and the association of severity of pituitary enlargement, and the response to treatment with throxine. To determine the microscopic changes occurring in Beta basophil cells (Thyroid stimulating hormone & Adreno corticotrophin hormone producing cells) of Anterior Pituitary gland by giving an anti-thyroid drug, Carbimazole and Carbimazol plus Thyroxin on Anterior pituitary glands of male albino rats with increasing time period.

Design: Experimental study

Place: Anatomy Department, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre Karachi.

Methods: Forty five healthy, young adult male albino rats were selected for the study. They were distributed into 3 main groups of 15 rats each. Group A served as control while Group B received injection Carbimazole 6 µgm/G body weight subcutaneously daily. Group-C were treated with injection Carbimazole 6 µgm/G body weight subcutaneously plus injection Thyroxin 5 µgm intraperitoneally daily for their respective period of treatment. Each group was further subdivided into three sub-groups according to the period of treatment they received i.e. 2, 4 & 6 weeks at the end of which animals were sacrificed. The Pituitary glands were dissected out after processing and staining (Wilson-Ezrin method). The tissues were subjected to detailed micrometric examination.

Result: The results are based on changes in morphometric study of number and diameter of Beta Basophil cells in anterior Pituitary gland. Mean value of number of Basophils were increased significantly ($P < 0.001$) in group B (Carbimazole treated) i.e 151.0 ± 3.38 than group A (control) i.e 82.2 ± 3.48. While in group C (Carbimazole plus Thyroxin treated) the number of cells were decreased i.e 117.6 ± 3.83 than group B but were more than group A, Beta Basophil cell size (diameter) was also increased significantly ($P < 0.001$) in group B i.e 17.64 ± 1.06 than group A 14.45 ± 3.28.

In group C Beta Basophil cell size was16.16 ± 2.02, which was more than group A but less than group C. The number and size of Beta Basophils in group C was significant ($P<0.05$), when compared with corresponding controls.

Conclusion:. In conclusion these results strongly suggest that Carbimazole-induced hyperplasia and hypertrophy of Anterior pituitary gland may be prevented by simultaneous treatment with Thyroxin. Long standing treatment with Carbimazole in Hyperthyroid patients as in Graves disease should accompany, small doses of Thyroxin as well, to avoid the enlargement of Anterior Pituitary gland during their treatment.

Keywords:. Pituitary, Adenohypophysis, Carbimazole, Hyperplasia, Thyroxin.

INTRODUCTION

Pituitary hyperplasia has been known for a long period of time and yet remains poorly understood.[1]

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Pituitary hyperplasia due to primary hypothyroidism and presents growth arrest causing mass on MRI can regress with thyroxine therapy and symptoms be resolved.[2]

Regulation of the thyroid gland by the hypothalamo-pituitary axis depends on feedback loops. The thyroid gland secretes triiodothyronine (T3) and thyroxine (T4). Hypothyroidism arises through iodine

deficiency, autoimmunity or surgery: treatment is by replacement of T3 and T4. Hyperthyroidism is also an autoimmune disease, commonly Graves' disease. Treatment is by selective destruction of thyroid cells by radioiodine. Alternatively thioureylenes (carbimazole) are used to inhibit iodination of thyroglobulin, thereby reducing T3 and T4 synthesis.[3]

Many cases of Thyroid hyperplasia resulting from prolonged primary hypothyroidism have been reported.[4,5] Thyrotroph hyperplasia is usually reversible with the institution of thyroxine replacement therapy. Thyrotroph releasing hormone is likely to be a major player initiating thyrotroph proliferation.[1,4]

Pituitary hyperplasia is defined as a non neoplastic increase in one or more functionally distinct types of pituitary cells. The acini though expanded, remain intact.[5,6]

The anterior pituitary gland integrates the repertoire of hormonal signals controlling thyroid, adrenal, reproductive, and growth functions. The pituitary gland responds to complex central and peripheral signals by two mechanisms. First trophic hormone secretion is exquisitely controlled to regulate homeostasis. Second, developmental or acquired pituitary signals may elicit plastic pituitary growth responces, consisting of either hypoplasia, hyperplasia or adenoma formation.[7]

Any cell population within the pituitary gland can undergo hyperplasia and when prolonged may progress to adenoma formation.[8,9] Pituitary enlargement occurs in primary hypothyroidism due to pituitary thyrotroph hyperplasia.[10] Untreated primary hypothyroidism has long been recognized as causing enlargement of the pituitary gland and sella turcica.[11] The pituitary enlargement, representing hyperplasia or adenomatous transformation of pituitary thyrotropic cells in response to primary thyroid deficiency.[12] Patients with longstanding primary hypothyroidism may have pituitary enlargement visible on MRI or computed tomography.[13] Thiomides-methimazole- and propyl thiouracil are major drugs for the treatment of thyrotoxicosis. In the United Kingdom Carbimazole which is converted to methimazole in vivo is widely used. Methimazole is about 10 times more active than propyl thiouracil.[14]

The principal hormones of the thyroid gland are the iodine containing amino-acid derivative of thyroxine (T4 and T3). Patients with hypothyroidism are usually treated with thyroxine (levothyroxine) only, although both thyroxine and tri-iodothyronine are secreted by normal thyroid gland.[15] In any patient with pituitary gland enlargement, primary hypothyroidism must be excluded. Circulating thyroxine normally serves as a negative feedback on release of thyrotropin-releasing hormone by the hypothalamus. If the thyroid gland secretes insufficient quantities of thyroxine, levels of thyrotropin-releasing hormone will increase. This, in turn, results in thyrotroph hyperplasia and pituitary gland enlargement.[16,17]

MATERIALS AND METHODS

This study was conducted in Department of Anatomy, BMSI, JPMC, Karachi during the period 2004--2005. The animals used for this experimental study were adult male albino rats. A total of 45 animals of 190-250 G were selected for this experimental study and maintained on balanced laboratory diet.

All the animals were divided into three groups A, B and C each group comprising 15 animals. Each group is further divided with three sub-groups based on the period of treatment 2, 4 & 6 weeks respectively, each sub-group comprising of five animals.

The animals of group-A served as normal control received injections of normal saline 1c.c daily for 2, 4 and 6 weeks respectively, and animals of group-B were treated with injection Carbimazole 6 µgm/G body weight subcutaneously daily for 2, 4 and 6 weeks respectively.

The animals in group-C were treated with injection Carbimazole plus injection Thyroxine 5 µgm in 0.9% NaCl intraperitoneally daily for their respective period of time.

They were sacrificed according to time period of treatment under ether anaesthesia.

Pituitary glands were fixed in Zenker formal (Helly's solution) for 4-6 hours. After tissues processing, Five micron thick sections were cut on rotatory microtome and mounted on glass slides. These were stained with Wilson-Ezrin method.

In Wilson-Ezrin method (PAS, Orange G and

Methylene blue) beta basophil cells of anterior pituitary were counted with the help of ocular counting reticule. The size of beta basophil cells was measured with the help of ocular micrometer scale and counting reticules.

The statistical significance of differences of various quantitative changes between Carbimazole and Carbimazole plus Thyroxine treated and control rats were evaluated by student 't' test.

OBSERVATIONS AND RESULTS

The present study was designed to observe the effects of Carbimazole and Carbimazole plus Thyroxine on the number and size of Basophils particularly Beta Basophils in anterior pituitary gland at variable time intervals.

The observation and results of the present study demonstrated that Carbimazole is effective in producing hyperplasia in the anterior pituitary gland and reversion of hyperplasia by giving thyroxine in experimental animals.

Mean values of Basophil numbers were recorded and shown in table-1. When comparing group B with group A there was statistically significant increase ($P < 0.001$) in numbers of Basophils ,as seen in Fig-1 (control) , Fig-2 (treated with Carbimazole) and Fig-3 (treated with Carbimazole and Thyroxine).

Table 1: Mean* Basophil Cell Count of Animals in Different Groups at Variable Time Period

Group	No. of	Basophil Counts		
		Durations		
	Subject	2 Week	4 Week	6 Week
Control (A)	n=5	80.8 ± 4.16	80.8 ± 5.0	82.2 ± 3.48
Carbimazole (B)	n=5	129.6 ± 9.69	184.8 ± 9.86	151.0 ± 3.38
Carbimazole + Thyroxine (C)	n=5	116.2 ± 3.02	142.8 ± 4.93	117.6 ± 3.83

*Mean ± Standard Error

P value = 0.001 means statistically highly significant.

Note: Microscopy was performed under high power i.e 40x objective and 8x occular with the help of ocular micrometer scale and counting reticule.

A stage micrometer was used for the calibration of the ocular micrometer scale and ocular counting reticule. The stage micrometer used in the study had a scale of 1mm length divided into 100 parts. So each division measured 10 µm. The ocular micrometer used in this study had 100 divisions. The reticule used had five squares along both the 'x' and 'y' axes. The ocular micrometer was placed in right eyepiece of microscope and reticule in the left eyepiece.

Table 2: Mean* Beta Basophil Cell Count of Animals in Different Groups at Variable Time Period

Group	No. of Subjects	Beta Counts		
		Durations		
	Subject	2 Week	4 Week	6 Week
Control (A)	n=5	28.6 ± 169	30.6 ± 2.29	34.6± 3.34
Carbimazole (B)	n=5	80.4 ± 9.04	110.06 ± 10.42	91.0± 2.21
Carbimazole + Thyroxine (C)	n=5	55.4 ± 9.84	79.2 ± 2.08	67.0 ± 1.52

*Mean ± Standard Error

Mean values of Beta Basophil numbers were recorded and shown in table 2. There was significant increase ($P < 0.05$) in Beta cell count while comparing group A with group C. The highly significant increase ($P < 0.001$) in Beta cell count when comparing group A with groups B as shown in Graph-1.

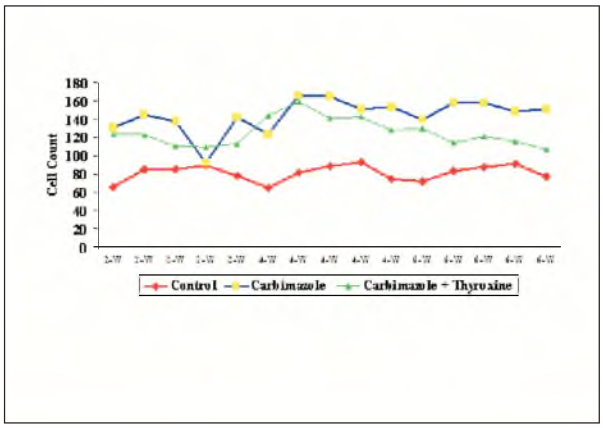
Table 3: Mean* Basophils Size (microns) Count of Animals in Different Groups at Variable Time Period

Group	No. of	Basophil Counts		
		Durations		
	Subject	2 Week	4 Week	6 Week
Control (A)	n=5	13.78 ± 2.90	14.04 ± 2.36	14.45 ± 3.28
Carbimazole (B)	n=5	16.94 ± 5.77	17.5 ± 6.04	17.64 ± 1.06
Carbimazole + Thyroxine (C)	n=5	13.22± 3.84	14.91 ± 0.44	16.16 ± 2.02

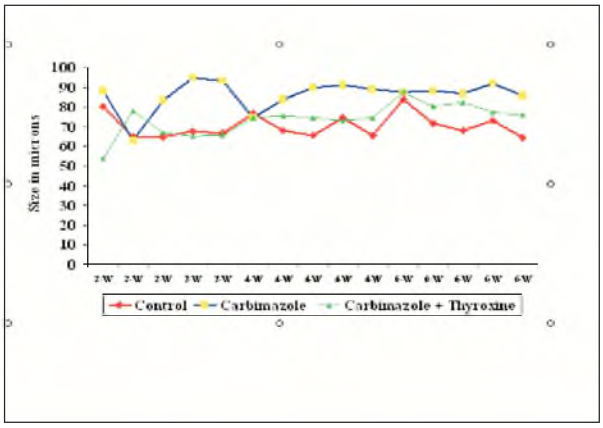
*Mean ± Standard Error

P value = 0.001 means statistically highly significant.

Mean values of Beta Basophil Size were recorded and shown in table-3 when comparing group A with group B there was statistically highly significant increase ($P < 0.001$) in size of Beta Basophils. Significant increase ($P<0,05$) in size of cells comparing group C with group A . When comparing group C with group B, highly significant increase ($P < 0.001$) in size of cells were noted as shown in Graph-2.



Graph – 1 : Beta Basophil Cell Count in Control , Carbimazole and Carbimazole + Thyroxine Groups



Graph – 2 : Size of Basophils (microns) in Control, Carbimazole and Carbimazole + Thyroxine Groups

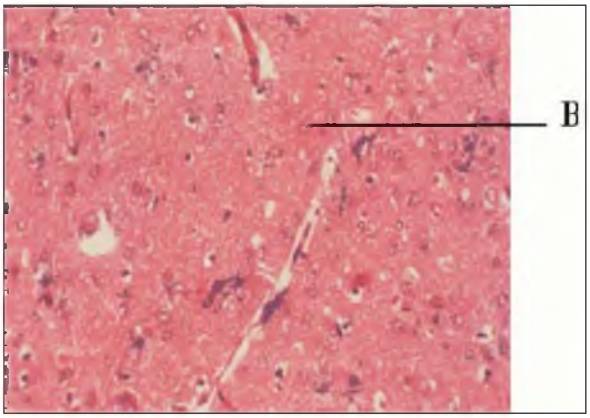


Figure – 1 : PAS-Orange-G, Methylene Blue (Wilson Ezrin Method) stained 5 µm thick section of the pituitary gland showing basophils (B) in control rat. (Photomicrograph under low power x 40)

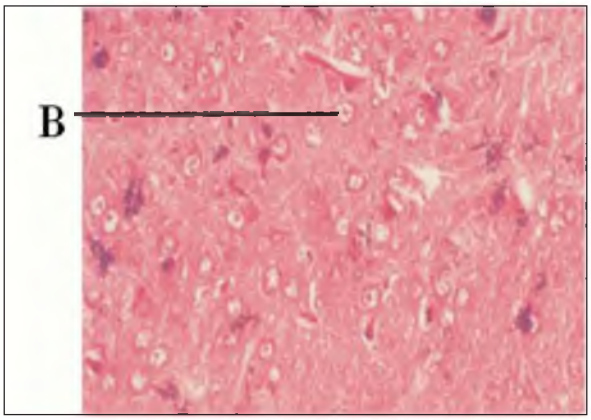


Figure – 2 : PAS-Orange-G, Methylene Blue (Wilson Ezrin Method) stained 5 µm thick section of pituitary gland showing increased number of basophils (B), after four weeks Carbimazole treatment in rat. (Photomicrograph under high power x 40)

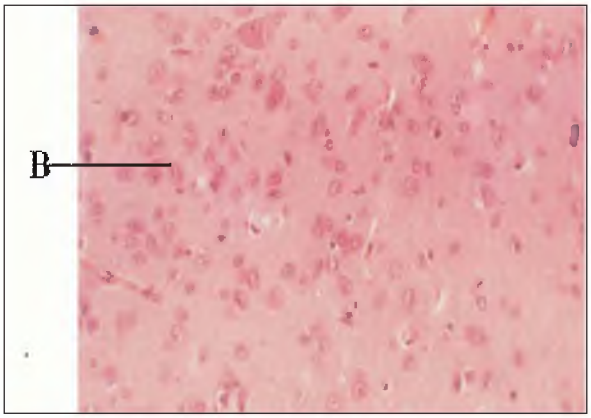


Figure – 3 : PAS-Orange-G, Methylene Blue (Wilson Ezrin Method) stained 5 µm thick sections of pituitary gland showing increased number of basophils (B) after six weeks Carbimazole plus Thyroxine treatment in rat. (Photomicrograph under high power x 40)

DISCUSSION

This study was designed according to Inauwa and Williams in 1995 who produced hypothyroidism in rates by administering Carbimazole and then treated tham with Thyroxine.[18] They observed the effects of these drugs on the uterine horns. In this study the effects of two drugs (Carbimazole and Thyroxine) were observed on the adenohypophysis of male albino rats. The present study demonstrates the acute reversal of pituitary hyperplasia after Thyroxine therapy. Total number of Basophil cells in Wilson Ezrin method and high magnification, increased markedly in group-B (Carbimazole treated) as compared to

age matched controls, but they were less increased after the combination of Carbimazole with Thyroxine in group-C. However these cells were more than control group.

The effect on pituitary basophils of introducing thyroxine daily injection of 5 µg of L-thyroxine shows regressive changes in the central group of basophil cells. Thyroxine is the treatment of choice in hypothyroidism. Thyroxine was first isolated in the crystalline form, from a hydrolysate of thyroid by Kendall in 1915. The principal hormones of the thyroid gland are the iodine containing aminoacid derivative of thyronine (T4 and T3).

Dose of thyroxine is 1.7µg/kg/day with requirements falling to 1 µg/ kg/day in the elderly (Hueston, 2001).[19]

This study matches with the study (Pioro EP et al 1988). Primary hypothyroidism may also produce pituitary enlargement secondary to thyrotroph hyperplasia and present with a sellar mass.[20] Although laboratory and radiologic abnormalities of pituitary enlargement may resolve after corrective thyroid therapy. Histologic examination revealed thyrotroph hyperplasia. Thyrotroph hyperplasia probably results from lack of negative feedback of thyroid hormone upon the anterior pituitary, which is probably due to hypothalamic release of thyrotropin-releasing hormone (TRH). Pituitary hyperplasia is characterized by increased proliferation of a single cell type, which may be focal, nodular, or diffuse. There is an absolute increase in numbers of specific cells, with pituitary enlargement visible on MRI. Pituitary hyperplasia may range from modest cell type increases to large glandular expansion with grossly altered tissue architecture and morphology.[21] Specifically, corticotroph hyperplasia may be associated with Crooke's hyaline changes, and thyrotroph hyperplasia, with periodic acid Schiff–positive lysosomes. Rarely, pituitary hyperplasia may be of primary origin, and is usually secondary to extrinsic signals. Normal pituitary height as assessed by MRI is up to 9 mm in healthy subjects, while adolescent females tend to have larger pituitary glands.[22]

This study also correlates with the study performed by (Shlomo Melmed 2003), who states that target hormones (sex, adrenal steroids, and thyroid hormones) exert powerful negative feedback inhibition of their respective trophic hormone gene transcription and hormone secretion, as well as suppression of pituitory growth. Failure of target glands (thyroid, adrenal, and gonads) leads to loss of negative feedback inhibition and resultant compensatory hyperplasia of the respective pituitary trophic hormone cells. Thus, longstanding primary hypothyroidism, hypogonadism, or hypoadrenalism may be associated with a clinically enlarged pituitary gland visible on MRI, with involution of the gland occurring after appropriate target hormone replacement and restoration of negative feedback.[7]

There was a marked increase in beta cell count in group-B, same was also increased in group-C which was more than controls but less marked than group-B. So we conclude that increase in Basophil count was due to Beta basophils which include Corticotrophs and Thyrotrophs. This increase in Beta count was due to positive feedback produced by lower level of circulating thyroid hormones as a result of Carbimazole treatment. This feedback stimulates thyrotrophs for more production of TSH by throtrophs. The increase was more in Carbimazole treated group, than in Carbimazole plus Thyroxine treated group but increase was more than control. In conclusion these results strongly suggest that Carbimazole-induced hyperplasia and hypertrophy of pituitary gland may be prevented by simultaneous treatment with Thyroxine. Thyroxin along with Carbimazole is more effective, to make euthyroid and to prevent the hyperplasia of anterior pituitary gland..

The Electron microscope study should be perfomed for the cellular details for their more and clear differentiation which were not performed because of its non-availability.

CONCLUSION

The present study supports the view that the animals were made hypothyroid with Carbimazole treatment, and that Thyroxin in support to the treatment reversed the changes in adenohypophysis.

In conclusion, these results strongly suggest that Carbimazole-induced hyperplasia and hypertrophy of Anterior pituitary gland may be prevented by simultaneous treatment with Thyroxin. Long standing treatment with Carbimazole in Hyperthyroid patients as in Graves disease should accompany, small doses

of Thyroxin as well to avoid the enlargement of Anterior Pituitary gland during their treatment.The association between pituitary gland enlargement and hypothyroidism should be kept in mind when pituitary hyperplasia is detected on MRI, before unwarranted and drastic interventions are initiated.

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