

COMPARISON OF BODY WEIGHT, ABSOLUTE AND RELATIVE WEIGHT OF PITUITARY GLAND IN CARBIMAZOLE AND CARBIMAZOLE PLUS THYROXIN TREATED MALE ALBINO RATS

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ABSTRACT:

Overt hypothyroidism and hyperthyroidism may be associated with weight gain and loss. The present study was designed to determine the effects of Carbimazole, an antithyroid drug (ATD) used in the treatment of hyperthyroidism and Grave's disease, on body weight, weight of Pituitary gland and relative weight of male albino rats and compared it with the effect of Carbimazole plus Thyroxin on body weight, absolute and relative weight of Pituitary gland of male albino rats. An Experimental study conducted in Anatomy Department, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi

Keywords: Hyperthyroidism, hypothyroidism, anterior pituitary gland (adenohypophysis), thyroid hormones, absolute weight, relative weight, albino rats.

INTRODUCTION

Hypothyroidism is one of the commonest chronic disorders in Western populations. In the United Kingdom, the annual incidence of primary hypothyroidism in women is 3.5 per 1000 and in men 0.6 per 1000 (Bijay *et al.*, 2008). It is well known that overt hypothyroidism is associated with weight gain and overt hyperthyroidism with weight loss and hyperthyroid individuals lose weight despite increased appetite and food intake and that weight is regained with treatment (Hoogwerf and Nuttall, 1984). The relations between overt thyroid dysfunction and body weight are well established. Experimental Studies have shown that overt hyperthyroidism is associated with weight reduction and that overt hypothyroidism is related to weight gain (Gopinath *et al.*, 2008). Thyroid dysfunction is well recognized as a cause of weight change. Weight loss is a frequent manifestation of hyperthyroidism, and hyperthyroid patients who are treated adequately gain nearly 4 kg/y.

Conversely, weight gain is a common complaint in patients with hypothyroidism, and treatment with thyroid hormone is associated with modest weight loss (Fox *et al.*, 2008). All patients treated for thyrotoxicosis gain body weight, irrespective of the treatment used (Pears *et al.*, 1990). Hypothyroidism is divided in primary, caused by failure of thyroid function and secondary (central) due to the failure of adequate thyroid-stimulating hormone (TSH) secretion from the pituitary gland or thyrotrophin-releasing hormone (TRH) from the hypothalamus (Athassiou, and Ntalles, 2010). ATD are widely used in the therapy of hyperthyroidism (Abraham *et al.*, 2010). The Carbimazole is commonly used thionamide an antithyroid drug. This drug inhibits iodination of tyrosine in thyroglobulin. Thionamides may also have immune suppressive effects (Cooper, 2005). Carbimazole is an orally administered thionamide which is a prodrug and is converted to methimazole after absorption. Carbimazole is a synthesis inhibitor, affects

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iodination of thyroglobulin and also inhibits the coupling reaction (Chiranjib *et al.*, 2010). Hypertrophy is an increase in the size of cells resulting in increase in the size of the organ. In contrast, hyperplasia is characterized by an increase in cell number. Hypertrophy and hyperplasia can also occur together, and obviously both result in an enlarged (hypertrophic) organ (Kumar *et al.*, 2007). The practitioners evaluating overweight persons often request thyroid tests and prescribe levothyroxine for mild hypothyroidism in hope of assisting with weight loss (Clarke, 2008).

Pituitary hyperplasia secondary to primary hypothyroidism has been well described (Joshi, 2005). It results from the loss of thyroxin feedback inhibition and the subsequent overproduction of the thyroid-stimulating hormone (TSH). Pituitary hyperplasia resolves after thyroid hormone replacement therapy. Regression of the pituitary enlargement after treatment with levothyroxine confirmed the pituitary hyperplasia secondary to primary hypothyroidism (Koller *et al.*, 1987).

MATERIALS AND METHODS

This study was conducted in Department of Anatomy, Basic Medical Sciences Institute (BMSI), Jinnah Post Graduate Medical Centre (JPMC), Karachi. Thirty adult male albino rats of Whistar strain weighing between 190-252 grams were selected for this study. Animals were put under observation for assessment to see their health. General condition on the basis of weight gain or loss.

The animals were divided into three groups, A, B and C. Each group was comprised of fifteen animals, and was further divided into three sub groups, based on period of treatment i.e. two, four and six weeks.

- Group-A: A1, A2, A3
- Group-B: B1, B2, B3
- Group-C: C1, C2, C3

Group A served as normal control, received injection of normal saline 1c.c subcutaneously daily for their respective period of time. Group B rats received injection Carbimazole (Neomercazole), used as an anti-thyroid agent in a dose of 6 µgms/Gms body weight subcutaneously daily for two, four and six weeks. The animals in group-C were treated with injection Carbimazole plus injection Thyroxin 5 µgm in 0.9% NaCl intraperitoneally daily for their respective period of time.

Animals were weighed with the help of triple beam balance at the start of treatment and then at the time of sacrifice. On the day of completion of treatment, animals were anaesthetized with ether. The heads were separated from body, pituitary fossae were exposed by removing the skull bones, and pituitary glands were dissected out, examined for their gross appearance, weighed and fixed. Absolute weights of pituitary glands were recorded with the help of Sartorius electro balance.

The relative weight of pituitary gland was calculated with the help of formula:

$$\text{The relative weight of pituitary gland} = \frac{\text{Mean weight of Pituitary Gland (mgs)}}{\text{Mean Final weight of the animal (gms)}} \times 100$$

STATISTICAL ANALYSIS

The data was entered and analyzed on SPSS version 16.0. Mean \pm standard deviation of mean were computed for the quantitative variables like weight of animals, weight of pituitary glands (Absolute weight) and Relative weight.. Two sample *t*-tests were used to calculate P-values in comparison of population means.

OBSERVATIONS AND RESULTS

The present study was designed to observe changes in body weight of animals and Absolute & Relative of weight of Pituitary

gland both in Carbimazole, Carbimazole plus Thyroxine treated and control groups of Albino rats. The observations and results on body weights, and weights of Pituitary gland were observed and compared with the control animals and with the previous studies.

During the experimental study, the animals from normal control group-A and treated groups-B and C were remained alive for their respective periods for treatment. The animals in group-A were active and healthy. The animals in group-B, those were received Carbimazole, appeared ill-looking, decreased response to external stimuli, the loss of appetite and showed increased body weight.

The mean body weights of animals were recorded at the beginning of study and at the time of sacrifice. The changes in weights of

animals in different groups at different time intervals were recorded as shown in Table-1. The Final body weights of animals in group B were increased significantly ($P<0.001$) as compare to group A and when group B was compared to group C the weight of animals were also increased, but not to the extent as seen in group B ($P<0.000$). There was also significant increase in weight gain when group A and C were compared as shown in Table-1.

The mean absolute weights of Pituitary gland were recorded at different time intervals in different groups as shown in Table-2. The mean values of Absolute weights of Pituitary gland in Group B were increased significantly ($P<0.001$) as compare to group A and when group B was compared to group C the weight of Pituitary gland were also increased, but not to the extent as seen in B ($P<0.000$) i.e. highly

Table-1
Mean* Body Weight (G) of Male Rats in Different Groups at Variable Time Period

Groups	Subgroups	Body Weights (G)			
		Initial Weights	Final Weights at Variable Time Interval		
			2 nd Week	4 th Week	6 th Week
A (n=15)	A1 (n=5)	220.6 ±13.145	243.0 ±11.640	--	--
	A2 (n=5)	204.4 ±18.582	--	254.2±14.132	--
	A3 (n=5)	205.2 ±20.167	--	--	264.4±12.219
B (n=15)	B1 (n=5)	227.6 ±10.644	288.6± 06.804	--	--
	B2 (n=5)	214.8 ±13.989	--	296.2±07.791	--
	B3 (n=5)	214.4 ±11.845	--	--	310.4±09.236
C (n=15)	C1 (n=5)	217.2 ±14.043	264.2 ± 6.686	--	--
	C2 (n=5)	221.6 ±19.756	--	272.2±08.468	--
	C3 (n=5)	223.6 ±13.107	--	--	276.4±08.933

*Mean ± Standard Deviation (SD)

Statistical analysis of the difference in mean body weight between groups

Group comparison between A1 and B1 (Control and Treated) at 2 nd week	$P<0.000$ ***
Group comparison between A2 and B2 (Control and Treated) at 4 th week	$P<0.000$ ****
Group comparison between A3 and B3 (Control and Treated) at 6 th week	$P<0.001$ ***
Group comparison between A1 and C1 (Control and Treated) at 2 nd week	$P<0.000$ ****
Group comparison between A2 and C2 (Control and Treated) at 4 th week	$P<0.001$ ***
Group comparison between A3 and C3 (Control and Treated) at 6 th week	$P<0.000$ ****
Group comparison between B1 and C1 (Control and Treated) at 2 nd week	$P<0.000$ ****
Group comparison between B2 and C2 (Control and Treated) at 4 th week	$P<0.000$ ****
Group comparison between B2 and C3 (Control and Treated) at 6 th week	$P<0.000$ ****

Key: ***Highly Significant

Table-2
Mean* Absolute Weight of Pituitary Gland (mg) of Animals in
Different Groups at Variable Time Period

Groups	No. of Subjects	Weight of Pituitary Gland (mg)		
		Durations and N=5 in each group		
Control (A)	n= 15	2 weeks (A1)	4 weeks (A2)	6 weeks(A3)
		3.36 ± 0.207	3.10 ± 0.682	3.30 ± 0.235
Carbimazole treated group (B)	n=15	2 weeks (B1)	4 weeks(B2)	6 weeks(B3)
		4.94 ± 0.207	4.96 ± 0.182	5.0 ± 0.332
Carbimazole plus thyroxin treated (C)	n=15	2 weeks (C1)	4 weeks (C2)	6 weeks(C3)
		4.060 ± 0.114	4.180± 0.1924	4.160± 0.1140

*Mean ± Standard Deviation (SD)

Statistical analysis of the differences in mean pituitary weight between Different groups

Statistical Comparison	P-value Significant / Insignificant		
	2 weeks	4 weeks	6 weeks
A vs B	P < 0.000***	P < 0.000***	P < 0.000***
B VS C	P < 0.000***	P < 0.000***	P < 0.001***
A VS C	P < 0.000***	P < 0.009***	P < 0.000***

Key: ***Highly Significant

significant. There was also significant increase in weight of Pituitary gland when group A and C were compared as shown in Table-2.

The mean relative weights of Pituitary gland were recorded at different time intervals in different groups as shown in Table-3. The mean values of Relative weights of Pituitary gland in Group B were increased significantly (P<0.001) as compare to group A and when group B was compared to group C the relative weights of Pituitary gland were also increased, but not to the extent as seen in group B (P<0.000) i.e. highly significant. There was also significant increase in relative weight when group A and C were compared as shown in Table-3.

Comparison between mean final body weight (G), mean absolute weight, and mean relative weight of Pituitary gland (mg) of Animals in different groups at variable time period were also made and were found significant.as shown in Table-4.

DISCUSSION

Carbimazole is an anti-thyroid agent belonging to the group Thio-mides-methimazole and propylthiouracil are major drugs for the treatment of thyrotoxicosis. Anti-thyroid drugs inhibit the formation of thyroid hormones by interfering with the incorporation of iodine into tyrosyl residues of thyroglobulin. They also inhibit the coupling of these iodotyrosyl residues to form iodothyronines. This implies that they interfere with the oxidation of iodide ion and iodotyrosyl group by inhibiting peroxidase enzymes (Taurog., 2000). Thyroxin is a treatment of choice in hypothyroidism. Dose of thyroxin is 1.7 µg/kg/day with requirements falling to 1 µg/kg/day in the elderly (Hueston, 2001).

Synthetic levothyroxin is the preparation of choice for thyroid replacement and suppression therapy with once daily administration (Katzung, 2001).

Table-3
*Mean Relative Weights (mg) /100 of body weight) of Pituitary Glands of Animals in Different Groups at Variable Time Intervals

Groups	No. of Subjects	Durations and number of animals in each group = 5		
		2 weeks	4 weeks	6 weeks
Control (A)	n= 15	1.4307± 0.1006	1.3147± 0.2973	1.4264±0.12142
Carbimazole (B)	n= 15	1.7117±0.06117	1.6366±0.07632	1.5798 ±0.07506
Carbimazole + Thyroxine (C)	n= 15	1.5369±0.03520	1.5950± 0.07797	1.5621± 0.03500
P value A Vs B	n= 15	0.0001	0.0001	0.0001
B Vs C	n= 15	0.0001	0.0001	0.006
A Vs C	n= 15	0.000	0.000	0.006

Mean ± Standard Deviation (SD)

P value ≤ 0.05 means statistically significant.

Table-4
Mean* Final Body Weight (G), Mean *Absolute Weight, and Mean* Relative Weight of Pituitary Gland (mg) of Animals in Different Groups at Variable Time Period

Groups	Final Body Weight	Abousult Body weigh	Relative Body Weight
Control (A) N=15	257.20± 17.869	3.253± 0.4172	1.3906±0.18827
Carbimazole treated group (B) N=15	302.73± 14.330	4.967± 0.2320	1.6427±0.0862
Carbimazole plus thyroxin treated group (C) N=15	267.8± 16.150	4.133± 0.1447	1.5646±0.05520
P value A Vs B	0.0001	0.0001	0.0001
B Vs C	0.0001	0.0001	0.006
A Vs C	0.000	0.000	0.006

Mean ± Standard Deviation (SD), P value ≤ 0.05 means statistically significant.

This study was designed to observe the effects of two drugs (Carbimazole and Thyroxin) on the adenohypophysis of male albino rats.

Several investigators produced hyperplasia i.e. an increase in the number of cells of an organ or tissue in response to a stimulus. Any cell population within the pituitary gland can undergo hyperplasia. The process can be physiological or pathological (Asa, 1998 and Horvath, 1988) and when prolonged may progress to adenoma formation (Zimmerman *et al.*, 1993). Thyrotroph hyperplasia develops in patients with prolonged primary hypothyroidism (Khalil *et al.*, 1984). Before

recording the findings on pituitary, the effects of Carbimazole on the behaviour of animals, body weight and weight of pituitary were noted.

The animals treated with Carbimazole in group-B appeared ill looking with increase in weight gain; animals appeared slow and had sluggish behaviour while the animals in group-C, i.e. treated with Carbimazole plus Thyroxin appeared healthy and active throughout the period of experimental study and their general condition was comparable to group-A (control). It is concluded from this study that there is marked weight gain after treatment with (Carbimazole). Weight continued to rise

with time. Patients who became hypothyroid, despite Thyroxin replacement, also gained weight, but not to the extent when only Carbimazole was used in experimental male albino rats.

Simultaneous treatment with Carbimazole and Thyroxin resulted in the increased weight gain but did not reach the values observed in Carbimazole treated control animals.

However, it was moderately increased in Carbimazole plus Thyroxin treated animals when compared with age matched Control and Carbimazole treated albino rats.

The weight of the pituitary gland was increased in group-B as compared to the control while weight in group-C was also increased but gain in weight of pituitary gland was more in group-B than in group-C and this increase in pituitary weight is due to hyperplasia and enlargement of pituitary gland because of positive feedback from hypothyroidism. The gross observation on external surface did not reveal any abnormality or change in colour or consistency. No area of hemorrhage or necrosis seen.

In the present study we, demonstrated a marked increase in body weight of animals with ATD treatment. We observed continuing gain in weight with duration of time. This study matches with the studies which show that weight gain after treatment with ATD reflects predominantly an increase in muscle and bone mass (Bayley *et al.*, 1980; De La Rosa *et al.*, 1997). Once hypothyroidism is achieved, the metabolic rate reduces in association with decrease in serum thyroid hormone concentrations (Abid *et al.*, 1999).

Weight gain following treatment with ATD is primarily due to reduced concentrations of thyroid hormones and presumably in metabolic rate associated with these hormones. Reduced metabolic rate, is implied by consistent weight gain Clearly without increased food intake.

CONCLUSION

It is concluded from this study that there is marked increase in weight gain after treatment with Antithyroid drug (Carbimazole). Weight continued to rise with time Enlargement of pituitary gland due to its hyperplasia results in increase in absolute weight. The increase in generalized body weight results in increase of relative weight of organ. Patients who became hypothyroid, despite Thyroxin replacement, also gained weight, but not to the extent when only Carbimazole was used in experimental male albino rats.

Poor control of thyroid function with replacement therapy is a significant factor contributing to weight gain Thyroid hormone replacement therapy can cause reduction in weight gain, regression of the enlarged pituitary, thus avoiding unnecessary surgery, and decrease in relative weight of the organ.

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