ORIGINAL ARTICLE

EFFECT OF METHIMAZOLE-INDUCED HYPOTHYROIDISM ON HISTOLOGICAL CHARACTERISTICS OF PAROTID GLAND OF ALBINO RAT

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Background: The current study was carried out to investigate the effect of hypothyroidism on the histological structure of parotid salivary gland of the rat. **Methods:** Twenty male albino rats, weighing between 130–150 grams, were used which were divided into two groups; control group (A) and an experimental group (B), each containing 10 animals. Group B was rendered hypothyroid by giving methimazole (MMI) as 0.02% solution in drinking water daily for 3 weeks. On day 22^{nd} parotid and thyroid glands were removed, weighed and processed for light microscopy. Salivary gland was fixed in Bouin's solution, H&E and Toluidine blue stains were used for histological examination. Serum T_3, T_4 and TSH levels were determined by enzyme immunoassay. **Results:** In group A, serum concentration of T_3, T_4 and TSH was 12.58±3.05 ηg/ml, 4.72±1.20 µg/dl, and 0.25±0.24 µIU/ml respectively, where as in group B it was 2.14±1.83 ηg/ml, 1.04±0.44 µg/dl and 1.44±0.20 µIU/ml respectively. When differences between T_3, T_4 and TSH of the groups were compared, the *p*-value was <0.000, <0.000, and <0.000 respectively. Mean thyroid weight significantly increased in group B (44.1 0±1.66 mg) when compared to that in group A (33.70±1.56 mg). These findings established the occurrence of hypothyroid state in the experimental group. There was a statistically significant reduction in the parotid gland weight in the animals of the experimental group (38.30±1.15 mg) when compared to the control group (39.60±0.84 mg), ($p<0.01$). With light microscopy, group A showed a normal structure of parotid salivary gland, whereas multiple histological changes were observed in parotrid gland of the experimental group. Number of mast cells in parotid gland was also significantly higher ($p<0.017$) in group B (3.70±1.11/mm^2) than in group A (2.25±1.34/mm^2). **Conclusion:** The level of T_3, T_4 decreased and that of TSH increased in the experimental group when compared with control group; there were also changes in the histological structure of the parotid salivary gland.

Keywords: Methimazole, parotid gland, hypothyroidism, histology, rat

INTRODUCTION

Hypothyroidism is one of the common thyroid disorders in humans in which production of the thyroid hormones decreases below the normal level[1]; it can occur as a congenital or an acquired defect[2]. Hypothyroidism can result from thyroid dysfunction, from impediment in mechanisms that control formation of thyroid hormones, or may arise as a result of complication during treatment of hyperthyroidism.[1]

The hypothyroid state is a complex hormonal dysfunction rather than a single hormonal defect[3], manifested largely by a reversible slowing down of all body functions[4]. Apart from general metabolic disturbance, impairment of thyroid hormone production causes serious intellectual and behavioural abnormalities that may affect patient's daily functioning and result in additional stress and depression. Studies had indicated that it diminished gonadotropin-releasing hormone from hypothalamus and lutinizing, follicle-stimulating and growth hormones of pituitary gland.[5–7] Hypothyroid state led to increased levels of total cholesterol, low-density lipoproteins and apolipoprotein B.[8,9] It had been previously shown that thyroid hormones increased the synthesis and mobilisation of triglyceraldehydes stored in adipose tissue and lipoprotein-lipase activity.[10]

The clinical manifestations of hypothyroidism range from mild non-specific complaints associated with sub clinical hypothyroidism to those associated with overt hypothyroidism. In humans commonly documented clinical manifestations which include pale/ cool/puffy skin, dry/brittle hair, nails, drooping of eyelids in addition to periorbital oedema, large tongue, decreased appetite, ascites, muscle stiffness, decreased deep tendon reflexes and lethargy.[4] However, in 1989, it was reported that enlarged salivary glands were common in patients with hypothyroidism (myxoedema), but this finding was not widely accepted. It had been suggested that parotid, submandibular and in particular the sublingual gland were discernibly enlarged and served as a useful clue to the diagnosis of hypothyroidism.[11] Regarding their morphology, histochemistry and ultra structure, the salivary glands of rats had been the subject of immense interest for researchers. Alterations in the glandular structure, after administration of sodium fluoride[12], melatonin[13], Fluorouracil plus Leucovorin[14] and actinomycin D[15] had been reported. Effect of hypophysectomy upon the histology of salivary glands had also been documented.[16] Along with qualitative histological analysis, quantitative (stereological) analysis had received much importance. Quantitative study on the rat's parotid gland was carried out at different intervals after orchidectomy, analysing the reaction of parotid acini, duct system and the glandular connective tissue.[17] Morphological alterations in the parotid gland maintained on a liquid diet had also been studied.[18]

The histological aspects of salivary glands had not been sufficiently studied in hypothyroid state though investigations regarding its physiological and biochemical effects received sufficient attention.[19,20]

In 2003 it was reported that 5-HT stimulated parotid amylase secretion and this effect was modulated by thyroid status, as amylase release was significantly lower in the hypothyroid group and higher in the hyperthyroid rats than in control group.[21]

It had already been reported that lack of thyroid hormones modulated the 5-HT-induced amylase secretion in rat's parotid gland[21]; further, it is also well known that thyroid hormones interact with serotinergic cells in GIT.[22] It is, therefore, noteworthy that thyroid function and oral health are closely associated. A correlation had been shown between autoimmune thyroiditis and salivary gland dysfunction/Sjögren's syndrome.[23,24] Sjögren's syndrome and hypothyroidism both resulted in xerostomia.[25] Further, there are reports indicating that dental caries susceptibility increased in hypothyroidism and diminished in hyperthyroidism. The incidence of dental caries in experimental animals increased after treatment with Propylthiouracil.[26] Conversely, specific effect of hypothyroidism on the histological aspect of salivary glands had not been properly studied.

The role of saliva in maintaining the oral health and even quality of life is obvious in people who are lacking sufficient saliva. Patients experiencing reduced salivary flow (xerostomia) suffer considerable morbidity, including dental caries, mucosal infections, dysphagia, and discomfort; there are problems in eating, speaking, swallowing and frequent disturbances in taste perception[25]. Any alterations in the integrity and activity of the salivary glands can change salivary flow and its composition, thus affecting patient's nutritional intake causing additional stress and depression. Therefore, the research on the salivary glands has not only significant medical but also social implications.

Histological analysis of the salivary glands after administration of a variety of drugs and pathological conditions had been illustrated in a number of studies[12–18], but it is evident that information about the histology of salivary glands in hypothyroidism is sparse and fragmentary. Therefore, the present study stipulates to analyse the histological features of parotid salivary gland of rats after experimentally induced hypothyroidism. The data collected may be useful for a better understanding of the factors influencing the functions of the salivary gland and its interactions with thyroid hormones. Thus treating the hypothyroid patients may improve the morbid modalities, specifically relating to oral health, caused primarily due to lack of salivary flow; it may help to modify treatment and prevention programs to control oral health problems mentioned earlier.

MATERIAL AND METHODS

Twenty male Albino rats, 6–8 weeks old, weighing between 130–150 grams were procured from the National Institute of Health, Islamabad. All the animals were examined thoroughly and weighed before the commencement of the experiment. The rats were housed in the Research laboratory of University of Health Sciences, Lahore under controlled conditions of temperature 22±0.5 ºC, humidity 50±10%, 12 hours light/dark cycle, and the animals were fed on rat chow, tap water *ad libitum* and were acclimatised for a period of one week.

Body weight was recorded at the beginning and on alternate days. Health condition of all animals was noted during the investigation.

Twenty male Albino rats were divided into two groups of 10 each; Group A served as control whereas Group B was used as an experimental group. Animals were made hypothyroid by giving them 0.02% w/v Methimazole (MMI) for three weeks; one full feeding bottle was consumed daily.[1] Fresh solution of MMI was prepared daily. Control group received distilled water only.

On day 22nd the experimental animals were weighed and euthanized with chloroform, 6 ml of blood was drawn by cardiac puncture in 10 ml disposable syringe for determination of thyroid hormone concentrations in the serum. The blood sample was allowed to stand for one hour and centrifuged at a speed of 3,000 rpm for 10 minutes. The clear serum was collected with the help of a clean dropper in sterilized plastic tubes. These plastic tubes were then placed in freezer and stored at -20 °C for testing on a later date; the tubes were properly labelled.

Total serum T_3, T_4 and TSH concentrations were quantitatively determined by using commercially available enzyme Immunoassay test kits (procured from Bio Check, Inc 323 Vintage Park, dr. Foster City, CA 94404).

Each animal was killed under anaesthesia, the parotid and thyroid glands were removed through a transverse incision in the upper part of the neck. Skin was carefully reflected from one side of the face to reveal these glands. Parotid salivary gland is located on lateral side of the submandibular salivary gland below the ear, it is easily identified because it is irregular in shape and loosely organised and was carefully dissected and removed in one piece and weighed before fixing it in Bouin's fluid[12]. Thyroid gland was recognized from its position close to trachea and it was also removed and immediately weighed using an analytical scale. The gland was fixed in 10% formalin for 48 hrs. The fixed tissues were processed in automatic tissue processor. The tissue pieces were embedded in paraffin wax and 5 µm thick sections were obtained using a rotary microtome (Leica RM 2125). The slides thus prepared were stained with hematoxylin and eosin for routine histological study, using light microscope (Leica DM 1000). Toluidine blue staining technique[3] was used for the mast cells examination. Ten fields from each slide were randomly selected for counting mast cells at ×400 magnification.[27] The data was entered and analysed using SPSS 16.0. Mean±SD is given for normally distributed quantitative variables. Frequencies, percentages and graphs are given for qualitative variables. Two independent sample test was applied to observe group mean differences. Pearson chi-square and Fisher exact test was applied to observe associations between qualitative variables. A p-value <0.05 was considered as statistically significant.

RESULTS

Statistically significant difference was observed between the weight of parotid gland in group A (39.60±0.84 mg) and B (38.30±1.15 mg), p<0.010 (Table-2) Parotid gland of group A was composed of several lobes of different sizes. Each lobe comprised several lobules which were divided by an interlobular connective tissue. Excretory ducts were present within the interlobular connective tissue ducts were lined by one or two layers of cuboidal or low columnar epithelial cells depending on their dimensions and location. Lobules consisted of numerous serous acini, lying close to one other and were separated by a fine network of an interacinar connective tissue (Figure-1).

Acinar cells were pyramidal in shape with regular, round nuclei situated at the base of the cell (Figure-2) The acini drain into the intercalated ducts, composed of a single layer of cuboidal epithelium with an oval/rounded nucleus. The intercalated ducts emptied into the striated ducts showing basal striations, which were lined by a single layer of columnar epithelium with mostly round nucleus. The ducts which were located in the parenchymal lobule (intralobular ducts) were lined by cuboidal or columnar epithelium.

An increase in the interacinar, intralobar and interlobular connective tissue was observed. Animals with hypothyroid functions showed increased amount of connective tissue and less number of acini. (Figure-4). The serous acini were generally smaller and more irregular in size and arrangements, atrophic with indistinct outline. The nuclei of some cells were ill defined, irregular in shape and pyknotic. The cell cytoplasm was not uniformly stained and showed clear unstained vacuolar spaces (Figuer-4). However, the structure of the duct system did not show any discernable change when compared with the control.

Significant association was observed between serous acini of the groups (p<0.000). Showing that out of 20 (100%) rats, 12 (60%) had normal acini, out of which 10 (50%) were from group A and 2 (10%) from group B. In the remaining 8 (40%) rats from group B, the acini were atrophic (Table-3).

Significant association was observed between groups and serous acinus shape (p<0.000) showing that out of 20 (100%) rats, 12 (60%) had pyramidal acini, out of which 10 (50%) were from groups-A and 2 (10%) from B. whereas, in the remaining 8 (40%) rats from group-B, the acini seemed to be irregular in appearance (Table-3).

Significant association was observed between nuclear morphology and the serous acinus of the groups (p<0.000) showing that out of 20 (100%) rats, 6 (30%) had heterochromatic nuclei, all belonging to group B. Eleven (55%) had euchromatic nuclei, out of which 10 (50%) were of group A and only 1 (5%) was from group B. Mixed nuclei were observed in 3 (15%), all belonging to group-B (Table-4).

Significant association was observed between groups and connective tissue in the parotid gland, (p<0.025) showing that out of 20 (100%) rats, 11 (55%) had normal connective tissue, out of which 8 (40%) were of group A and 3 (15%) belonged to group B. whereas, 9 (45%) animals had increased connective tissue mass, with 2 (10%) belonging to group A and the remaining 7 (35%) were of group-B (Table-5).

Mast cells in sections stained with toluidine blue had various size and appearance. They were flat, round or oval shaped (Figure-5). Light microscope revealed homogenous cytoplasm rather than having a granular appearance. It was found that mast cells were predominant near blood vessels within the interlobular connective tissue. However, they were also found in the intralobular connective tissue around the secretory acini.

Significant difference was observed in the mean number of mast cells in parotid gland of the control (2.25±1.34/mm^2) and the experimental (3.70±1.11/mm^2) groups, (p<0.017, Table-6)

Table-1: Comparison of mean serum T_3, T_4 and TSH in group A & B (Mean±SD)

Parameter	Group A n=10	Group B n=10	p
T_3(ηg/ml)	12.58±3.05	2.14±1.83	<0.000*
T_4(µg/dl)	4.72±1.20	1.04±0.44	<0.000*
TSH(µIU/ml)	0.25±0.24	1.44±0.20	<0.000*

*p<0.05 is statistically significant

Table-2: Mean weight (mg) of Thyroid and Parotid glands in group A & B (Mean±SD)

Parameter	Group A n=10	Group B n=10	*p*
Thyroid weight (mg)	33.70±1.56	44.10±1.66	<0.000*
Thyroid/body weight ratio	12.29±0.66	22.0±0.86	<0.000*
Parotid gland weight (mg)	39.60±0.84	38.30±1.15	<0.010*

**p*<0.05 is statistically significant

Table-3: Serous acini in parotid gland in group A & B

Group	Serous acini			Serous acinus shape		
	Atrophic n (%)	Normal n (%)	Total n (%)	Irregular n (%)	Pyramidal n (%)	Total n (%)
A	0 (0)	10(50)	10 (50)	0 (0)	10 (50)	10 (50)
B	8 (40)	2 (10)	10 (50)	8 (40)	2 (10)	10 (50)
Total	8 (40)	12 (60)	20 (100)	8 (40)	12 (60)	20 (100)
	Pearson Chi-Square=13.333,			Pearson Chi-Square=13.333,		

**p*<0.05 is statistically significant

Table-4: Nuclear morphology of serous acini from parotid gland in group A & B

Nuclear Morphology	Group A n (%)	Group B n (%)	Total n (%)
Irregular/Heterochromatic/Pyknotic	0 (0)	6 (30)	6 (30)
Large/Round/Euchromatic	10 (50)	1 (5)	11 (55)
Mixed	0 (0)	3 (15)	3 (15)
Total	10 (50)	10 (50)	20 (100)
Fisher's Exact Test=16.262, *p*<0.000*			

**p*<0.05 is statistically significant

Table-5: Comparison between Connective tissue of the parotid gland in group A & B

Characteristic of Connective tissue	Group A n (%)	Group B n (%)	Total n (%)
Normal	8 (40)	3 (15)	11 (55)
Increased	2 (10)	7 (35)	9 (45)
Total	10 (50)	10 (50)	20 (100)
Pearson Chi-Square Test=5.051, *p*<0.025*			

**p*<0.05 is statistically significant

Table-6: Mean number of mast cells in parotid gland in group A & B (Mean±SD)

Parameter	Group A n=10	Group B n=10	*p*
Mast cells in Parotid gland (number/mm^2)	2.25±1.34	3.70±1.11	<0.017*

**p*<0.05 is statistically significant

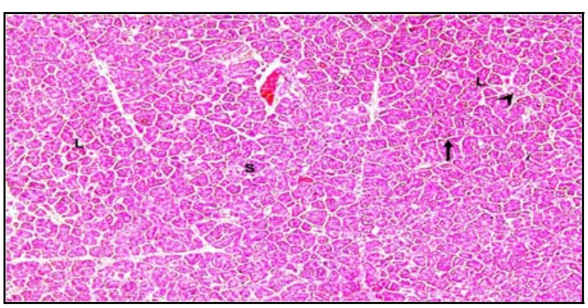

Figure-1: Photomicrograph of Parotid gland
(Group A) is divided into numerous lobules (L) by connective tissue septa (arrow). Each lobule contains many serous secretory units (S) which are darkly stained in this H&E preparation. Intralobular ducts (arrowhead) are also seen among the serous acini. H&E stain, ×50.

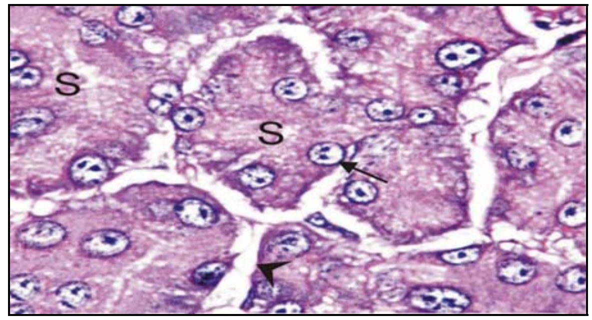

Figure-2: Photomicrograph of Parotid gland
(Group A) showing serous secretory cells (S) with strongly stained cytoplasmic granules, round euchromatic nucleus (arrow) with a prominent nucleolus and interacinar connective tissue (arrowhead). H&E stain, ×400.

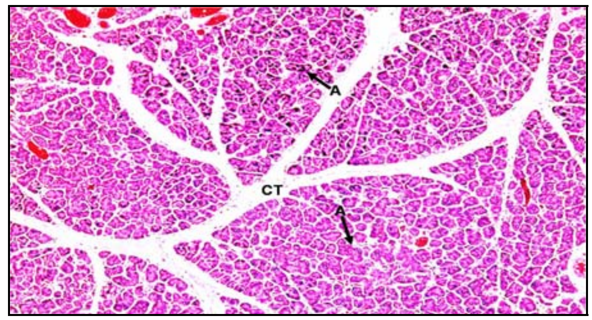

Figure-3: Photomicrograph of Parotid gland
(Group B). Several lobules composed of the acini (A) are divided by connective tissue septa (CT) which are thicker than those in the control (Figure-1). H&E stain, ×50.

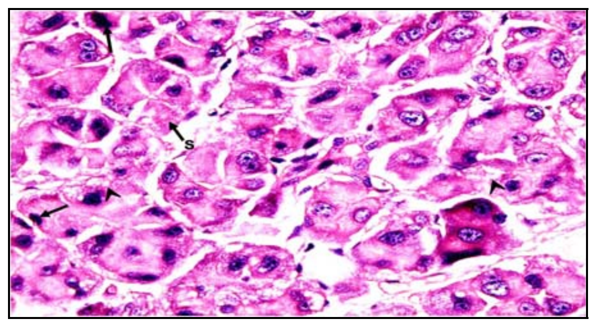

Figure-4: Photomicrograph of parotid gland
(Group B) showing atrophic serous acini (S) with irregular size and arrangement and indistinct outlines, irregularly shaped Pyknotic nuclei (arrows) and vacuoles (arrowheads) in the acinar cytoplasm. H&E stain, ×400.

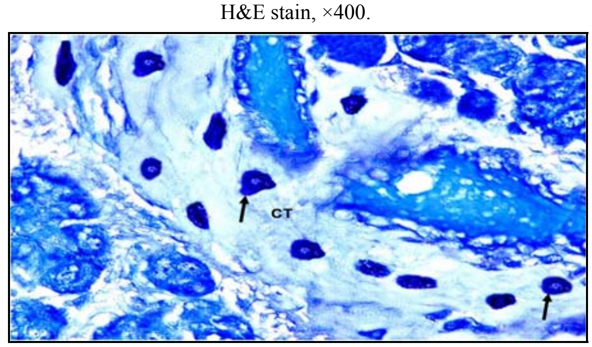

Figure-5: Photomicrograph of Parotid gland
(Group B). Mast cells (arrows) are abundantly present within the connective tissue (CT). Toluidine blue stain, ×400.

DISCUSSION

In previous experimental studies on animal models, thyroid gland was successfully rendered hypo-functional upon treating it with MMI.[1,5,28–32] The functional state of thyroid gland was established by histological changes and serum level of T_3, T_4 and TSH hormones; it was postulated that the drug acts as a false substrate for thyroid peroxidase, thus blocking the iodination of tyrosine residues within thyroglobulin.[1] In our experimental model, development of hypothyroidism was confirmed both by histological changes in the gland and T_3, T_4 and TSH serum levels. Significant decrease in T_3, T_4, and increase in TSH serum levels (Table-1) was indicative that the quantity and duration of treatment was sufficient to induce hypothyroid status in the experimental group of rats.

Our findings regarding weight loss of the parotid salivary gland in experimental group did not agree with the previous reports[21] in which it was stated that there is no change in the weight of the gland upon treating the animals with MMI. Decrease in the parotid gland weight of the experimental animals in our study could be related to the decreased cellular activity in hypothyroid state or by degenerative changes in parotid acini (Figure-4). Inuwa and Williams (1996) stated that thyroid hormone exerted its influence on tissues by facilitating the transcription of DNA, resulting in new protein synthesis.[32]

Cellular changes in the glands may be found to stem from the adverse effects of hypothyroidism upon metabolic systems within the cell. Glandular tissues have a secretory function which is possible only by the exceptional metabolic activity of the cells. Serous cells of the parotid gland are specifically affected by hypothyroidism. There is reason to believe that the enzymatic contents of the saliva alter as a result of histological changes in the glands upon treating the animals with MMI. Our results, therefore, could imply the existence of functional relationship between salivary and thyroid glands.

Our study showed that the nuclei of parotid gland were large/round/euchromatic with prominent nucleoli in the serous acini of the control group; these were, however, heterochromatic and occupied most of the nucleus with little or no euchromatin in the experimental animals. Ashour (1998) reported that the amount of euchromatin associated with a large nucleolus (nucleoli) was active in RNA synthesis and was used as an indicator of the metabolic activity of cells; conversely a high proportion of heterochromatin indicates a cell with low metabolic activity.[13]

Heterogenicity of mast cells in a variety of organs had been reported earlier.[33,34] Saglam *et al* (2005) stated that mast cell recruitment was the result of inflammation. Additionally, it contributes to the process through production of histamine, heparin and tryptase. Bischoff and Sellge (2002) associated the increase of mast cells with different pathological conditions, such as chronic inflammatory processes, fibrotic disorders, wound healing and neoplastic tissue transformation, but the functional significance of the accumulation of mast cells in these processes is mostly unknown. Oncu *et al*[3] reported significantly increased number of mast cells, 6 weeks after thyroidectomy in the sublingual gland of rat. Our findings corroborate those of Oncu *et al*[3] as there was a statistically significant increase in the number of mast cells in parotid gland of the experimental group when compared with that in the control group ($p<0.017$).

Ostuni *et al* (2003) reported the effect of thyroid status upon 5-HT stimulated amylase secretion in parotid glands.[21] Results of our study showed that baseline amylase activity was significantly higher in the hyperthyroid group ($p<0.01$) and lower in the hypothyroid group than in control group. Atrophy of glands had been observed in many studies.[12,13,18] There exists a consensus in literature concerning the decrease of the size and weight of salivary glands when function is reduced by eliminating the need for mastication. As there is a decrease in secretion from parotid gland in hypothyroidism, which could be implied to conduce the acinar atrophy due to decreased in functioning of the gland. These findings, however, cannot be considered conclusive; we suggest more morphometric studies to draw definite conclusions.

CONCLUSIONS

Hypothyroidism produces histological alterations in glandular tissue of parotid gland indicating that thyroid hormones are essential for its normal functioning. The results of our experiment support the idea expressed by other investigators that the thyroid-salivary gland relationship exists and is mediated through thyroid hormones; mechanisms of this relationship is not clear and warrants further investigations. Thyroid hormone receptors of salivary glands might be playing major role in this mechanism. The receptors have not hitherto been reported; further work is needed to go in search of these.

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