Histological Characteristics of Submandibular Gland after Induction of Hypothyroidism in Adult Albino Rat

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Abstract

Background: To investigate the histological changes in the submandibular salivary gland of the rat after inducing hypothyroidism by methimazole.

Methods: The study included twenty male albino rats, weighing between 130-150 grams. They were divided into two groups. Group A (n=10) as the control group and Group B (n=10) was labeled as the experimental group. Group A was given normal feed and water. Whereas, group B was given methimazole (MMI) as 0.02% solution in drinking water daily for 3-weeks. Submandibular glands were excised, weighed and processed for light microscopy on 22nd day. It was fixed in Bouin’s fluid. For histological analysis H&E stain was used. For the confirmation of hypothyroid state of albino rat serum T₃, T₄ and TSH levels were done by enzyme immunoassay.

Results: Statistically significant decrease in the concentrations of T₃, T₄ and a statistically significant increase in the serum concentration of TSH was observed when the experimental group was compared to the control. Results showed no significant difference in combined weight of submandibular and sublingual salivary glands between group A and B (p=0.397). Histological analysis of submandibular gland was done under light microscope. Group A showed normal morphology. Whereas, Pearson Chi-Square test showed atrophic changes in serous acini of group B. Fishers Exact Test showed the presence of heterochromatic nuclei in the submandibular gland of the same group. Increased connective tissue was also observed in group B. No significant difference was observed in the mean number of vacuoles present in the submandibular gland of the control and the experimental group (p=0.535). Significant difference was observed in the mean diameter of the striated ducts of the submandibular gland of the control and the experimental (p<0.007).

Conclusion: Histology of the submandibular salivary gland is affected by hypothyroidism resulting in changes in the histological features of the gland.

Key Words: Submandibular gland, Methimazole, Hypothyroidism.

Introduction

Hypothyroidism is caused by reduced function of the thyroid gland which is attributed to defects in secretion of thyroid hormones triiodothyronine (T3) and tetra-iodothyronine or thyroxine (T4) below normal level. Acquired hypothyroidism develops later in life because of primary disease of the thyroid gland or secondary to disorders of hypothalamic or pituitary origin. According to the level of endocrine dysfunction in primary and secondary or central and according to severity in severe or clinical and mild or subclinical types. Hypothyroidism can result from thyroid dysfunction, from impediment in mechanisms that control synthesis of thyroid hormones or may arise as a result of complication during treatment of hyperthyroidism.

The hypothyroid state is a complex hormonal dysfunction rather than a single hormonal defect, manifested largely by a reversible slowing down of all body functions. Thyroid hormones are known to regulate the rate of metabolism also affecting the growth and rate of function of many other systems of the body such as neuromuscular, gastrointestinal and cardiovascular system. Impaired thyroid hormone production causes serious intellectual and behavioural abnormalities.

The clinical manifestations of hypothyroidism range from mild non-specific complaints associated with sub clinical hypothyroidism to those associated with overt hypothyroidism. Thyroid dysfunction affects salivary gland functioning as well and subjects with hyposalivation should have thyroid function assessment done. 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.
Submandibular glands were also a target organ for thyroid hormones. In 2001, morphometric methods were used to study the effects of hypothyroidism on submandibular glandular structure, serous and mucous acini, major intralobular ducts (granular and striated ducts), interlobular connective tissue and excretory duct lying in the connective tissue. Occurrence of significant alterations in the submandibular structure of the rat in state of hypothyroidism; these occurred basically in two major morphologically and biochemically distinct exocrine compartments of the gland, the serous acinus and the granular duct. Decrease in the volume density of the granular ducts and the mean volume of the serous acini were seen.

A decrease in soluble protein concentration of the rat’s submandibular gland in hypothyroidism had been reported and it was suggested that decrease in volume of granular ducts could be a direct effect of decreased thyroid hormone level. It had already been reported that lack of thyroid hormones provoked physiological and histological changes in the submandibular, sublingual and parotid glands respectively.

Regarding their morphology, histochemistry and ultrastructure, the salivary glands of rats had been the subject of immense interest for researchers. Alterations in the glandular structure, after administration of sodium fluoride, melatonin, fluorouracil plus leucovorin and actinomycin D had been reported. Effect of hypophysectomy upon the histology of salivary glands had also been documented. Along with qualitative histological analysis, quantitative (stereological) analysis had been the subject of interest. The profound influence of thyroid hormones on physiological and biochemical effects of salivary glands received sufficient attention. Whereas, their histological aspects had not been extensively studied in the hypothyroid state.

Thyroid function and oral health are closely linked. Diseases such as Sjögren's syndrome with sicca symptoms or endocrine conditions such as hypothyroidism can result in xerostomia. A correlation had been shown between autoimmune thyroiditis and salivary gland dysfunction / Sjögren's syndrome. 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.

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 used in the study. The rats were housed in the Research laboratory of University of Health Sciences, Lahore under controlled conditions of temperature 22 ± 0.5°C, humidity 50 ± 10%, 12 hours light/dark cycle; and the animals were fed on rat chow, tap water ad libitum and were acclimatized for a period of one week. Twenty male Albino rats were divided into two groups of 10 each; Group A served as control, whereas Group B was used as the experimental group. Animals were rendered hypothyroid by giving them 0.02% w/v Methimazole (MMI) for three weeks; one full feeding bottle was consumed daily. Fresh solution of MMI was prepared daily. Control group received distilled water only as a placebo. On day 22nd the experimental animals were euthanized with chloroform. The blood sample was taken from the rat for determination of thyroid hormone concentrations in the serum obtained in a usual way from 6 ml of blood taken in 10 ml disposable syringe by cardiac puncture. Total serum T3, T4 and TSH concentrations were quantitatively determined by Eliza technique.

Each animal was killed under anaesthesia, the submandibular glands were removed. Through a transverse incision in the upper part of the neck skin was carefully reflected in the neck and one side of the face to reveal these glands. After removal they were fixed in Bouin’s solution. The fixed tissues were processed in automatic tissue processor. The tissue pieces were embedded in paraffin wax and 5μm thick sections were obtained using a rotary microtome (Leica RM 2125). The slides thus prepared were stained with haematoxylin and eosin for routine histological study, using light microscope (Leica DM 1000). Submandibular gland was evaluated for weight (g),normal and atrophic serous acini, nuclear morphology, connective tissue stroma, number of vacuoles and diameter of striated duct. The diameter of the striated duct of the submandibular gland was measured at X40 objective using a calibrated micrometre. The largest diameter of transversely sectioned ducts was measured and a second measurement was repeated at right angle to it, to determine the mean. Five ducts from different locations randomly selected in the preparation were used for the calculation. Mean diameter of the ducts
was calculated and these results subjected to a statistical analysis. A stage micrometer having 1mm scale divided into 100 equal divisions engraved on a 3 x 1 inch glass slide was placed on the microscope stage and viewed with X 40 objective to calibrate the linear ocular micrometer.A disc shaped linear ocular micrometer, having an engraved scale of 100 divisions, was placed inside the right eyepiece of the microscope. For calibrating the ocular micrometer X 10 eyepiece and X 40 objective were used and the stage micrometer was brought under focus.Number of divisions of eyepiece micrometer scale equal to an exact number of divisions of stage micrometer scale was determined.15 eyepiece divisions were equal to 4 stage divisions;100 stage divisions =1mm = 1000μm;1 stage division=10μm;15 divisions of eyepiece micrometer=4 stage divisions(4x10) =40μm;1 division of eyepiece micrometer =40/15=1 division of eyepiece micrometer= 2.7μm.The scale of the eye piece micrometer was superimposed on the striated duct of the submandibular gland; its diameter in “μm” was calculated by counting the number of divisions of the micrometer covering the duct, multiplied by 2.7. For counting the number of vacuoles in submandibular gland,vacuoles of all sizes were counted using H&E stained sections of submandibular gland; ocular graticule was calibrated in the same way as already described. A 20 x 20 squares (area = 1mm²) eyepiece graticule was used.Counting was done in 5 randomly selected areas from the slide using X40 objective and avoiding any overlapping of the areas counted. Total estimated area per section was calculated to be 0.0625 x 5= 0.31mm². Vacuoles of submandibular gland were counted using squares of the ocular graticule which were superimposed on the area to be used for counting; the vacuoles lying on the upper and right sides of the squares were excluded to avoid counting for more than once.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 found between two groups (Table 1).No significant difference was observed in combined weight of sublingual and submandibular salivary glands between group A and B (p=0.397) (Table. 2).Histological observation of the submandibular glands from the animals of the control group showed normal morphology. The secretory portion contained both serous and mucous acini. Serous acini predominated with a very few scattered mucous acini within the main serous secretory portion of the gland; this feature was common to both the groups. The serous acinar cell was regular in shape and composed of round, euchromatic nuclei (Figs. 1). Excretory, striated, and intercalated ducts were evident. Ducts were abundantly present and were separated by crowded acini (Figs. 1 and 2).

Certain concrete differences distinguished the glandular tissue of the experimental animals from that of the control group. Group B revealed loss of the normal architecture of most of the acini and degenerative changes in the acinar cells, including the cytoplasm and the nuclei which were of variable sizes and exhibited different degree of staining. The acini were generally smaller and irregular in size and arrangement resulting in acinar atrophy. The cytoplasm of some cells lacked uniformity of staining (Figs. 3-5). Some nuclei were deeply stained and others were lightly stained (Figs. 3 and 5). Increased connective tissue was also observed (Fig. 4).Significant association was observed between groups and serous acini, p <0.000. Showing that out of 20 (100%) rats, 11 (55%) had normal acini; out of which 10 (50%) were from group A and 1 (05%) from group B. whereas, in the remaining 9 (45%) rats from group B, the acini were atrophic (Table 3).Significant association was observed between groups and nuclear morphology of the serous acinus, p<0.000. Showing that out of 20 (100%) rats, 7 (35%) had heterochromatic nuclei, all belonging to group B. 11 (55%) had euchromatic nuclei, out of which 10 (50%) were of group A and only 1 (5%) was from group B. Whereas, mixed nuclei were observed in 2 (10%), all belonging to group B (Table.4). Significant association was observed between groups and connective tissue in the submandibular gland, p<0.025. Showing that out of 20 (100%) rats, 9 (45%) had normal connective tissue, out of which 8 (40%) were of group A and 1 (5%) belonged to group B. whereas, 11 (55%) animals had increased connective tissue mass, with 2 (10%) belonging to group A and the remaining 9 (45%) were of group B (Table.5).Significant difference was observed in the mean diameter of striated duct of submandibular gland of the control (48.58±4.53/mm²) and the experimental (41.89±5.33/mm²) groups (Table.6).Significant difference was observed in the mean number of vacuoles in submandibular gland of the control (5.33/mm²) and the experimental (2.24±1.40/mm²) groups (Table.7).
Table 1: Mean serum concentrations of $T_3$, $T_4$ and TSH in groups A & B.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group A Mean ± S.D (n=10)</th>
<th>Group B Mean ±S.D (n=10)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_3$(ng/ml)</td>
<td>12.58±3.05</td>
<td>2.14±1.83</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>$T_4$(µg/dl)</td>
<td>4.72±1.20</td>
<td>1.04±0.44</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>TSH(µIU/ml)</td>
<td>0.25±0.24</td>
<td>1.44±0.20</td>
<td>&lt;0.01*</td>
</tr>
</tbody>
</table>

*p value < 0.05 is statistically significant

Table 2: Mean weight (mg) of combined submandibular and sublingual glands in groups A & B

<table>
<thead>
<tr>
<th>Weight(mg) Submandibular + sublingual gland Mean ± S.D n=10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A 117.025±0.10</td>
</tr>
<tr>
<td>Group B 116.98±0.12</td>
</tr>
</tbody>
</table>

p-value =0.397

*p value < 0.05 is statistically significant

Table 3: Comparison of the serous acini of submandibular gland in groups A & B

<table>
<thead>
<tr>
<th>Serous acini</th>
<th>Total No(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atrophic No(%)</td>
<td>Normal No(%)</td>
</tr>
<tr>
<td>Group A 0(0)</td>
<td>10(50)</td>
</tr>
<tr>
<td>Group B 9(45)</td>
<td>1(5)</td>
</tr>
<tr>
<td>Total 9(45)</td>
<td>11(55)</td>
</tr>
</tbody>
</table>

Pearson Chi-Square Test=16.364,p<0.01*

*p value < 0.05 is statistically significant

Fig.1. Submandibular gland (Group A) showing normal histological features. Illustrated in the section are serous acini (S), adipocytes (AD) and numerous ducts (arrows) scattered among the secretory tissue.

Fig.2. Submandibular gland (Group A) showing normal histological features. Illustrated in the section are serous acini (S), euchromatic, round nuclei (arrows) with a prominent nucleoli and ducts (D) within the connective tissue (CT).

Fig.3. Submandibular gland (Group B) showing abundance of serous cells (S). Adipocytes (AD) and ducts (arrowhead) are scattered in the secretory tissue. Within the augmented connective tissue (CT), interlobular ducts (arrow) are situated.

Fig.4. Submandibular gland (Group B) showing abundance of serous cells (S), connective tissue (CT), adipocytes (AD) and striated ducts (SD).

Fig.5. Submandibular gland (Group B) showing atrophic serous cells (S).
Table 4: Nuclear morphology of the serous acini from submandibular gland in groups A & B

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No(%)</td>
<td>No(%)</td>
<td>No(%)</td>
</tr>
<tr>
<td>Irregular/Heterochromatic/Pyknotic</td>
<td>0(0)</td>
<td>7(35)</td>
<td>7(35)</td>
</tr>
<tr>
<td>Large/Round/Euchromatic</td>
<td>10(50)</td>
<td>1(5)</td>
<td>11(55)</td>
</tr>
<tr>
<td>Mixed</td>
<td>0(0)</td>
<td>2(10)</td>
<td>2(10)</td>
</tr>
<tr>
<td>Total</td>
<td>10(50)</td>
<td>10(50)</td>
<td>20(100)</td>
</tr>
</tbody>
</table>

Fisher’s Exact Test=16.364, p<0.01*
*p value < 0.05 is statistically significant

Table 5: Comparison of the connective tissue from submandibular gland in groups A & B

<table>
<thead>
<tr>
<th>Connective tissue</th>
<th>Normal No(%)</th>
<th>Increased No(%)</th>
<th>Total No(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>8(40)</td>
<td>2(10)</td>
<td>10(50)</td>
</tr>
<tr>
<td>Group B</td>
<td>1(5)</td>
<td>9(45)</td>
<td>10(50)</td>
</tr>
<tr>
<td>Total</td>
<td>9(45)</td>
<td>11(55)</td>
<td>20(100)</td>
</tr>
</tbody>
</table>

Pearson Chi-Square Test=9.899, p<0.002*
*p value < 0.05 is statistically significant

Table 6: Mean diameter (µm) of the striated ducts of the submandibular gland in groups A & B

<table>
<thead>
<tr>
<th>Diameter of striated duct (µm)</th>
<th>Group A Mean ± S.D (n=10)</th>
<th>Group B Mean ± S.D (n=10)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Submandibular - ar gland</td>
<td>48.58±4.53</td>
<td>41.89±5.33</td>
<td>&lt;0.007*</td>
</tr>
</tbody>
</table>

*p value < 0.05 is statistically significant

Table 7: Comparison of the mean number of vacuoles (number/mm²) present in the submandibular gland in groups A & B

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control Mean ± S.D (n=10)</th>
<th>Experimental Mean ± S.D (n=10)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of vacuoles / number/mm²</td>
<td>1.84±1.41</td>
<td>2.24±1.40</td>
<td>0.535</td>
</tr>
</tbody>
</table>

*p value < 0.05 is statistically significant

Discussion

In the present study hypothyroidism was successfully produced by potent antithyroid drug methimazole (MMI) that had been frequently and preferentially used previously in experimental studies on animal models. 2,33-37 The development of hypothyroidism was evidenced by biochemical findings; the drug acts by blocking the iodination of tyrosine residues within thyroglobulin and the coupling of iodothyrosines into iodothyronines thus acting as a false substrate for thyroid peroxidase. 2 In the present study quantity and duration of treatment had sufficiently induced hypothyroidism in the experimental group of rats. These findings are in accord with those reported earlier. 16,27

The histological picture of cell derangement in the gland may be found to stem from the adverse effects of hypothyroidism upon metabolic systems within the cell. Apparently serous cells of submandibular glands are specifically injured by hypothyroidism. The enzymatic contents of the saliva alter as a result of histological changes in the glands upon treating the animals with MMI. Results, therefore, revealed functional relationship between salivary and thyroid glands.

Our study showed that the nuclei of submandibular gland were large/round/ euchromatic with prominent nucleoli and were common 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 et al (1998) reported that the amount of euchromatin associated with a large nucleolus (nucleoli) is active in RNA synthesis and is used as an indicator of the metabolic activity of cells. Conversely a high proportion of heterochromatin indicates a cell with low metabolic activity.20 Thyroid hormone had been reported to regulate many functional aspects of the submandibular gland; i.e. growth factors (Aloe and Montalcini, 1980; Walker et al., 1981), rennin (Karen and Morris, 1986), adrenergic receptor number (Medina et al., 1984) or (Na +, K+) ATPase activity20, 38, 39, 40, 41. The mouse submandibular gland contains bioactive peptides, including NGF and EGF; Production of these peptides is known to be dependent on thyroid hormones.38, 39 Several investigators had shown the relationship between the size of the submandibular gland and thyroid activity. A decrease in size of submandibular gland is observed due to hypothyroidism.17,42-45 However, no difference in gland size was obtained in the present study possibly reflecting either a strain difference in animals. However, our results were in accord with those of Morgan et al (1984) regarding the weight of the gland. Noorafshan et al (2001) used “point-sampled
intercepts” to quantify the effects of hypothyroidism on the acini volume; it was shown that mean volume of the serous acini was significantly decreased in hypothyroid rats; suggestive of altered structure of rat submandibular gland in hypothyroidism.\textsuperscript{16,17} Our results were comparable to those previously reported, atrophy of the serous acini along with changes in the nuclear morphology. This is indicative of hypofunction of submandibular gland manifested by xerostomia. As reported by Mandel and Wotman (1976), large proportion (40\%) of the total saliva secreted into the mouth is provided by submandibular gland.\textsuperscript{28,46} Saliva secretes a number of macromolecular factors directly involved in maintenance of oral health; they also stated that thyroid and the sex hormones were assumed to be responsible for maintaining the histological structure of the rat submandibular gland; they also showed that testosterone and thyroxine injections produced a hypertrophy of the intralobular duct system of the submandibular gland.

Absolute volume of the granular ducts of submandibular gland decreased as a result of diminished thyroid hormone levels, but striated and excretory ducts remained unaffected.\textsuperscript{17} Our results showed a significant decrease in the diameter of the striated ducts of submandibular gland, implying decrease in the quantum of the secretion from the granular ducts. This corroborates the findings of earlier study.\textsuperscript{17} Our finding of decrease in the diameter of the striated ducts may be indicative of their hypofunction and atrophy in thyroid deficiency. Vacuolation in the submandibular acinar cells had also been reported by other researches (Leal et al., 2003), but Leal et al. (2003) could not identify the organic compounds inside the vacuole because vacuolar content reacted negatively with specific staining techniques for glycoprotein and mucopolysaccharides.\textsuperscript{25} It was evident in this study that unstained circular areas in the acinar cytoplasm could be fatty degeneration as it was also previously shown by Ogilvie (1951) that the hypothyroid state led to increased levels of total cholesterol, low density lipoprotein and Apolipoprotein B.\textsuperscript{9,10,19} Possibly these facts and other submandibular gland findings as to the size of the individual acini and their apparently greater separation from one another due to the increased connective tissue are significantly related. They might indicate that the cells of the gland, due to lack of functioning, were never able to reach usual dimensions and the entire cells seems to be atrophic.

Thyroid hormones play an essential role in maintaining normal functioning of salivary gland as previously suggested by various authors.\textsuperscript{4,17,47} Hypo salivation is not life-threatening, although it indirectly affects quality of life resulting from deterioration in oral health.

**Conclusion**

1. Thyroid hormones are essential for normal function of the submandibular salivary gland.
2. Hypothyroidism leads to histological alterations in submandibular glandular tissue. Thyroid-salivary gland relationship exists and is mediated through thyroid hormones.
3. Elucidation of the mechanisms involved must await further experimentation. Thyroid hormone receptors might be playing major role in this mechanism which is unclear and warrants further investigations.

**Acknowledgement**

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**References**


