

Effect of Hypothyroidism on the Histology of Sublingual Salivary Gland in Adult Albino Rats

Nida Qasim Hayat¹, Shahnaila Nadir², Fauzia Qureshi³

1. Department of Anatomy, Poonch Medical College, Rawalakot; 2. Department of Oral and Maxillofacial Surgery, Pakistan Institute of Medical Sciences, Islamabad; 3. Department of Anatomy, Women Medical College, Abbottabad

Abstract

Background: To investigate histological changes in the sublingual salivary gland of hypothyroid albino rat.

Methods: Twenty male albino rats were divided into two groups; control group (A) and an experimental group (B), each containing 10 albino rats. Group B was rendered hypothyroid by giving 0.02% solution of methimazole (MMI) daily in drinking water for 3-weeks. Sublingual glands were removed, fixed in Bouin's solution and processed for light microscopy. H&E stain was used for histological analysis. Serum T₃, T₄ and TSH levels were determined by enzyme immunoassay to confirm hypothyroid state of the animal.

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. The microanatomy of sublingual gland revealed normal connective tissue elements of sublingual salivary gland in 40% cases. Increased connective tissue architecture was seen in 7(35%) cases of experimental group B (p<.025). Hence in a total of 20 cases, 45% showed increased connective tissue component (p<0.007). Increased lipid tissue was evident in 50%, out of which 10% belonged to group A and the remaining 40% to group B. Dilated lumen of mucous tubules was evident in 15% cases in control group whereas 35% showed dilated lumen of mucous tubules in group B. The changes were non evident in a total of 10(50%) cases where 7(35%) cases were in group A and 3(15%) cases were in group B.

Conclusion: There is a remarkable difference in the histology of sublingual gland in hypothyroid state. This change can be visualized in the form of increased connective tissue content and lipid deposition. There is also marked dilatation of lumen of mucous tubules.

Key Words: Sublingual gland, hypothyroidism Albino rat.

Introduction

Major salivary glands in humans as well as rats are composed of three pairs of macroscopic glandular organs: parotid, sublingual and submandibular.^{1, 2} These glands arise from the epithelial lining of the oral cavity as buds. Buds grow and form an extension into underlying mesenchymal tissue and after multiple branching they develop lumina at the end.³ The surrounding mesenchyme divides the glands into lobules which forms capsule by enveloping the gland.³ The sublingual glands arise between the tongue and the mandibular arch in the ninth week.³

In humans, the sublingual gland is located against the sublingual depression on the lingual aspect of the mandible.⁴ Whereas each gland in the rat lies in close vicinity to the anterolateral surface of the submandibular gland but are paler in colour and have independent ducts which open into the mouth cavity.⁵ It is separated from the genioglossus muscle by the Wharton's duct medially which extends from submandibular gland and the sublingual nerve.⁴

Sublingual glands are mixed glands consisting of mucous acini with scattered serous demilunes.^{6,7} The mucous cells are flattened with basal nuclei and spongy cytoplasm. They occupy a larger area than serous demilunes. Acinar cells are large with irregular acinar lumen.⁶ Owing to this abundant number, they are also known as mixed tubuloacinar glands.⁸ The secretory granules of mucous acinar cells arises from Golgi vesicles.⁵

In 1989, it was observed that enlarged salivary glands were seen in patients with hypothyroidism or myxoedema. It had been suggested that parotid, submandibular and in particular the sublingual gland were enlarged and served as an indication of hypothyroidism. The gland enlargement regressed after replacement with thyroid.⁹

Regarding their cytology, histochemistry and morphological structure, the salivary glands of rats

had been the subject of tremendous attraction for researchers.¹⁰ The possibility of 5'D in the salivary gland was mentioned in subsequent reviews; and it was concluded to be insignificant because the activity per mg protein would be far lower than that in the liver. However, in 1993, the level of 5'D in the sublingual gland was reported to be approximately 80% of that in the liver with a specific gravity almost comparable to that in liver. Sublingual 5'D was likely to be of type I isozyme in nature, because it was sensitive to PTU inhibition and had higher affinity for rT₃ than T₄.¹¹ Selective localization and abundance of 5'D in salivary gland suggested a previously unrecognized thyroid-sublingual relationship.¹¹ Type II (5'D-II) with a higher affinity for T₄ than rT₃, was rather resistant to PTU inhibition and existed in brain, anterior and neural lobes of the pituitary gland, brown adipose tissue, placenta and pineal gland.¹¹

Sublingual gland is the one of the richest sources of glycoprotein and activity of glucosamine -6-phosphate synthetase is high in the gland. This implies that glycoprotein biosynthesis in sublingual glands may be regulated by thyroid hormones.¹²⁻¹⁴

After thyroidectomy in 2004; in addition to cytoplasmic vacuolization of the epithelial cells, enlargement and dilatation of lumina in most of the tubules of sublingual gland.¹³ This is probably due to severe hypothyroidism, increased mucous secretion, lipid tissue mass surrounding the parenchyma, mononuclear cell infiltration and significantly higher numbers of mast cell are also reported.¹³

Thyroid hormones not only normalize the rate of metabolism but also affect the growth and rate of function of many other systems of the body such as neuromuscular, gastrointestinal and cardiovascular system.¹⁵⁻¹⁸

The hypothyroid state is a complex hormonal disorder.¹⁹⁻²² There are many implications as apart from general metabolic disturbance, it also causes impairment of thyroid hormone production which causes serious intellectual and behavioural abnormalities that may affect patient's daily functioning resulting in additional stress and depression. Hypothyroid state led to increased levels of total cholesterol, low-density lipoproteins and apolipoprotein B.^{23,24} It had been previously observed that thyroid hormones enhance the synthesis and mobilization of triglyceraldehydes stored in adipose tissue and lipoprotein-lipase activity.^{16,28}

Hyposalivation is caused by systemic diseases such as Sjogren's syndrome, Hashimoto's Thyroiditis (HT), pharmaceutical side effects, salivary stones, and

tumors as well as medical treatments including radiotherapy.²⁹⁻³² Hashimoto's Thyroiditis is one of the most common manifestation of hypothyroidism. Swallowing function is critical for nutrition and reducing the risk of aspiration, which can cause chronic lung disease as well as affecting survival and quality of life, including health and aging. Salivary dysfunction has the negative impact on the quality of patient's life. Hypothyroid state results in xerostomia. Early detection of dry mouth is critical in preserving and promoting systemic and oral health.^{33,34}

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. The rats were kept in the Research laboratory of University of Health Sciences, Lahore under controlled conditions of temperature $22 \pm 0.5^{\circ}\text{C}$, humidity $50 \pm 10\%$, 12 hours light/dark cycle; and the animals were fed on rat chow, tap water ad libitum. They were acclimatized for a period of one week. Health condition of all animals was noted during the investigation. Rats of ill health of different in weight were not included in the study. The rats were divided into two experimental groups i.e. control and hypothyroid. Each of them contained 10 animals; Group A served as control whereas Group B was kept as an experimental group. Hypothyroidism was induced by giving the rats 0.02% w/v Methimazole (MMI) for three weeks.¹⁵ Fresh solution of MMI was prepared daily and one full feeding bottle was consumed daily. Control group received distilled water only as 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 using commercially available enzyme Immunoassay test kits (procured from Bio Check, Inc 323 Vintage Park, dr. Foster City, CA 94404).²⁷ Each animal was killed under anaesthesia, the sublingual 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. It was carefully dissected and removed in one piece and fixed in Bouin's fluid.³⁶ 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 haematoxylin and eosin for routine histological study, using light microscope (Leica DM 1000). Parameters seen in sub lingual gland are dilated mucous tubules, distended mucous acini and connective tissue stroma. The Qualitative data was entered and analyzed using SPSS 21.0. Frequencies, percentages and graphs are given for qualitative variables. Pearson chi-square test was applied to observe associations between qualitative variables. A p-value < 0.05 was considered as statistically significant.

Results

In group A, serum concentration of T₃, T₄ and TSH was 12.58±3.05(ng/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(ng/ml), 1.04±0.44(µg/dl) and 1.44±0.20(µIU/ml) respectively with p<0.000, p<0.000 and p<0.000 respectively (Table 1). The sublingual glands of the control group composed of the typical mucous acini. Mucous cells were cuboidal to columnar in shape; their nuclei were oval and pushed toward the basal part of the cells (Fig 1). The mucous acini were conspicuous because of their light staining. They were not spherical structures but, rather, an elongated or tubular structure with branching outpockets. The duct system consisted of striated and excretory ducts (Fig. 1). In the stroma surrounding the parenchymal tissue, increased lipid tissue (Fig. 2) was observed. Increased connective tissue and mononuclear cell infiltration (Fig. 2) was also evident. Significant association was observed between groups and connective tissue in sublingual gland, p<0.025. Showing that out of 20 (100%) rats, increased connective tissue was observed in 9 (45%), out of which 2 (10%) belonged to group A, whereas 7(35%) to group B (Table 2). Significant association was observed between groups and connective lipid tissue in sublingual gland, P<0.007 showing that out of 20 (100%) rats increased lipid tissue was evident in 10 (50%), out of which 2 (10%) belonged to group A and the remaining 8 (40%) to group B (Table 3). Another interesting finding in the experimental group of animals was the occurrence of distended mucous acini

of the glandular tissue (Fig. 4). In the experimental group, lumina in most of the mucous tubuli were quiet dilated (Fig. 3). Significant association was observed between the groups as regards lumen of the mucous tubules in sublingual gland, p<0.003; showing that out of 20 (100%) rats, dilated lumen was evident in 10

(50%), out of which 7 (35%) rats belonged to group B and 3 (15%) to group A. Whereas it was not evident in 10 (50%), out of which 7 (35%) belonged to group A and 3 (15%) to group B. (Table.4).

Table 1: Comparison of the mean serum concentrations of T₃, T₄ and TSH in groups A & B.

Parameter	Group A Mean ± S.D n=10	Group B Mean ±S.D n=10	p-value
T ₃ (ng/ml)	12.58±3.05	2.14±1.83	<0.01*
T ₄ (µg/dl)	4.72±1.20	1.04±0.44	<0.01*
TSH(µIU/ml)	0.25±0.24	1.44±0.20	<0.01*

*p value < 0.05 is statistically significant

Table 2: Comparison of the connective tissue from sublingual gland in groups A & B.

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

*p value < 0.05 is statistically significant

Table 3: Comparison of the lipid tissue from sublingual gland in groups A & B.

	Lipid tissue		
	Normal n(%)	Increased n(%)	Total n(%)
Group A	8(40)	2(10)	10(50)
Group B	2(10)	8(40)	10(50)
Total	10(50)	10(50)	20(100)
Pearson Chi-Square Test=7.200,p<0.007*			

*p value < 0.05 is statistically significant

Table 4: Comparison of the Lumen of the mucous tubuli from sublingual gland in groups A & B.

	Dilated lumen-Mucous tubules		Total n(%)
	Group A n(%)	Group B n(%)	
Evident	3(15)	7(35)	10(50)
Not-Evident	7(35)	3(15)	10(50)

Total	10(50)	10(50)	20(100)
Pearson Chi-Square Test=11.600,p<0.003*			

*p value < 0.05 is statistically significant

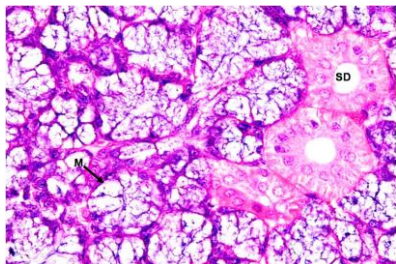


Fig. 1. Photomicrograph of sublingual gland (Group A) showing abundance of mucous acini (M) and striated ducts (SD) lined by a simple tall columnar epithelium. H&E stain. X 400.

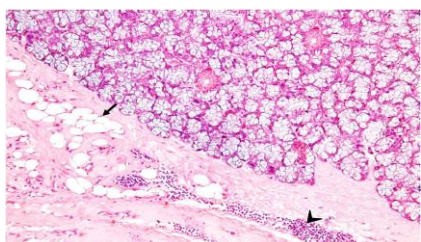


Fig. 2. Photomicrograph of sublingual gland (Group B) showing lipid tissue (arrow) and mononuclear infiltration (arrow head).

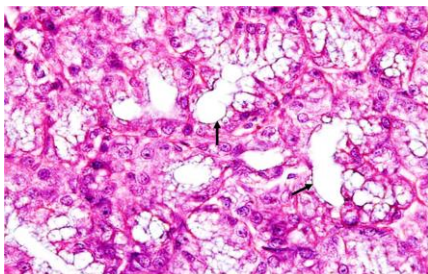


Fig. 3. Photomicrograph of sublingual gland (Group B) showing dilated lumen of mucous tubuli (arrows).

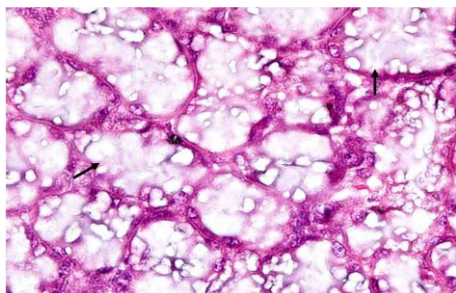


Fig. 4. Photomicrograph of sublingual gland (Group B) showing distended mucous acini (arrows)

Discussion

There have been many investigations on the physiology and biochemistry of hypothyroidism on salivary glands.^{11,12} However, the morphological aspects of these glands in hypothyroidism were not studied. This study is therefore the first to investigate the histopathological effects of hypothyroidism on the sublingual gland by an experiment. Observations of sublingual gland regarding the acini were not identical with those of parotid and submandibular salivary glands. Mucin, a major component of the mucous secreting part, is a macromolecular complex of sublingual gland glycoprotein and makes mucous viscous. Gray *et al* (2001) noted increase in the secretion of mucin and the level of MUC5AC in the cultured human middle ear epithelial cells, due to lack of T₃.³⁷ Marked luminal dilatation in most of the mucous tubuli in sublingual gland, 6-weeks after thyroidectomy, was reported by Oncu *et al* (2004).¹³ We also found luminal dilatation in the mucous tubuli of the treated group (Fig. 3); this could imply increased mucous secretion and luminal dilatation due to hypothyroidism. It is, therefore, concluded that highly thick salivation is present in hypothyroid rats.

Markitziu *et al* (1993) reported that thyroidectomy caused hypercholesterolemia and fatty degeneration of the parotid parenchyma.³⁸ Lipid cell hyperplasia in the stromal tissue surrounding the sublingual gland parenchyma was reported by Oncu *et al* (2004) in thyroidectomised rats 6-weeks after the operation.¹³ These observations are in accord with our findings showing increased connective and lipid tissue in the parenchyma of the major salivary glands.

Several workers have suggested that the thyroid hormones are important in the maintenance of normal salivary gland function and histology (Noor Afshan, 2001; Ostuni *et al.*, 2003; Onku *et al.*, 2004).^{13,14,39}

Reduction in salivary flow is not life-threatening though it results in deteriorating dental and oral health which has significant impact on the quality of life. An attempt is, therefore made in this study to show any damages resulting from hormonal imbalance on the sublingual salivary glands.

Conclusion

1. Histological alterations are produced in the glandular tissue of sublingual gland. There is an accord of thyroid and salivary glands and is mediated through thyroid hormones; mechanisms of this relationship is not exactly clear and needs further probing.

2. Thyroid hormone receptors may be playing an important role in this mechanism which should be studied at molecular level.

References

1. Amano O, Mizobe K, Bando Y, Sakiyama K. Anatomy and histology of rodent and human major salivary gland. *Acta Histochem Cytochem* 2012;45(5):241-50.
2. Amano O. The salivary gland: anatomy for surgeons and researchers. *Jpn. J. Oral Maxillofac. Surg* 2011;57:384-93.
3. Srinivasan A, Moyer JS, Mukherji SK. Unilateral submandibular gland aplasia associated with ipsilateral sublingual gland hypertrophy. *AJNR* 2006;27:2214-16.
4. Sumi M, Izumi M, Yonetsu K, Nakamura T. Sublingual Gland: MR features of normal and diseased states. *AJR* 1999;172:717-22.
5. Leeson CR and Booth WG. Histological, Histochemical and electron-microscopic observations on the postnatal development of the major sublingual gland of the rat. *J.D.Res* 1961;40(4):838-45.
6. Estecondo S, Codón SM and Casanave EB. Histological study of salivary glands in *Zaedyus pichiy* (Mammalia, Xenarthra, Dasypodidae). *Int J Morphol* 2005;23(1):19-24.
7. Smith RJ, Frommer J and Schiff R. Localization and onset of amylase activity in mouse salivary glands determined by a substrate film method. *J Histochem Cytochem* 1971;19(5):310-19.
8. Lima MC, Sottovia-Filho D, Cestari TM and Taga R. Morphometric characterization of sexual differences in the rat sublingual gland. *Braz Oral Res* 2004;18(1):53-58.
9. Fulop M. Pouting Sublinguals: Enlarged Salivary Glands in Myxoedema. *Lancet* 1989; 2:550-53.
10. Bixler D, Webster RC, Muhler JC. The effect of testosterone, thyroxine, and cortisone on the salivary glands of the hypophysectomized rat. *J D Res* 1956;36(4):566-70.
11. Tanaka K and Imura H. Iodothyronine 5'-Deiodinase is present in mouse sublingual gland. *J Endocrinol* 1993;132:1195-98.
12. Hiramatsu M, Kashimata M, Kumegawat M and Minami N. Suppression of thyroid hormones of glucosamine-6-Phosphate synthetase activity in rat sublingual glands. *Archs Oral Biol* 1984;29(10):849-51.
13. Oncu M, Kanter M, Gokcimen A, Kavakli D. Effect of thyroidectomy on the histology of rat sublingual gland. *APMIS* 2004;112:119-22.
14. Noorafshan A. Stereological study on the submandibular gland in hypothyroid rats. *APMIS* 2001;109:223-27.
15. Milosevic M, Korac A, Davidovic V. Methimazole-induced hypothyroidism in rats: Effects on body weight and histological characteristics of thyroid gland. *Jugoslav Med Biochem* 2004;23(2):143-47.
16. Sharma AK, Arya R, Mehta R, Sharma R, Sharma AK. Hypothyroidism and cardiovascular disease: factors, mechanism and future perspectives. *Curr Med Chem* 2013;20(35):4411-18.
17. Athanassiou K, Ntalles K. Hypothyroidism-new aspects of an old disease. *Hippokratia* 2010;14(2):82-87.
18. Porth CM, Gaspard K, Guven S, Kuenzi JA. Alterations in pituitary, thyroid, parathyroid and adrenal function. In: Stead L, Kogut H, Cann M, Rainey S, eds. *Essentials of pathophysiology concept of altered health status*. 6th ed. USA: Lippincott Williams and Wilkins; 2004. p. 538-59.
19. Kimura T, Furudate S. Pituitary GH and prolactin deficiency and testis enlargement in hypothyroid rats caused by goitrogen methimazole. *Exp Anim* 1996;45(4):369-75.
20. Chiao YC, Lee HY, Wang SW, Hwang JJ. Regulation of thyroid hormones on the production of testosterone in rats. *J Cell Biochem* 1999 Jun 15;73(4):554-62.
21. Antony FF, Aruldas MM, Udhayakumar RC. Inhibition of leydig cell activity in vivo and in vitro in hypothyroid rats. *J Endocrinol* 1995;144(2):293-300.
22. Duntas LH. Thyroid disease and lipids. *Thyroid* 2002;12(4):287-93.
23. Estecondo S, Codon SM, Casanave EB. Histological study of the Salivary Glands in *Zaedyus pichiy*. *Int J Morphol* 2005;23:19-24.
24. Caraccio N, Ferrannini E, Monzani F. Lipoprotein profile in subclinical hypothyroidism: Response to levothyroxine replacement, a randomized placebo-controlled study. *J Clin Endocrinol Metab* 2002;87(4):1533-38.
25. Pucci E, Chiovato L, Pinchera A. Thyroid and lipid metabolism. *Int Obes Relat Metab Disord* 2000; 24(2): 109-12.
26. Green Span FS, Dong BJ. Thyroid and anti-thyroid drugs. In: Katzung BG, editor. *Basic and clinical pharmacology*. 9thed. USA: Mc Graw Hill; 2004. 625-40.
27. Ogawa M, Oshima M, Imamura A, Sekine Y. Functional salivary gland regeneration by transplantation of a bioengineered organ germ. *Nat Commun* 2013:1-15.
28. Featherstone JD. The science and prevention of carious prevention. *J. Am. Dent. Assoc* 2000;131:887-89.
29. Vissink A, Mitchell JB, Baum BJ, Limesand KH. Clinical management of salivary gland hypofunction and xerostomia in head and neck cancer patients: successes and barriers. *Int. J. Radiat. Oncol. Biol. Phys* 2010;78:893-991.
30. Hosseini FA, Shirzad N, Moosavi MS. Evaluation of xerostomia and salivary flow rate in Hashimoto's Thyroiditis. *Med Oral Patol Oral Cir Buccal* 2016;21(1):e1-5.
31. Burlage FR, Coppes RP, Meertens H, Stokman MA, Vissink A. Parotid and submandibular/ sublingual flow during high dose radiotherapy. *Radiother. Oncol* 2001;61:271-74.
32. Fox PC. Acquired salivary dysfunction. *Ann New York Acad Sci.* 1998;842:132-37.
33. Ship JA. In "Saliva and Oral Health" 3rd, ed by M Edgar, C Dawes and D O'Mullane. British Dental Association; London: 2004. Xerostomia: aetiology, diagnosis, management and clinical implications; 41-55.
34. Saleh J, Figueiredo MAZ, Cherubini K, Salum FG. Salivary hypofunction: An update on aetiology, diagnosis and therapeutics. *Arch Oral Biol* 2015;60(2):242-55.
35. Epstein JB, Siri B. Management of hyposalivation and xerostomia: criteria for treatment strategies. *Companedium* 2015 ; 36(6): 5-9
36. Hayat NQ, Tahir M, Munir B, Sami W. Effect of methimazole – induced hypothyroidism on histological characteristics of parotid gland of albino rat. *J Ayub Med Coll* 2010;22(3):22-27.
37. Ogilvie AL. Histological findings in the salivary glands of the rat following sodium fluoride administration. *J D Res* 1951;30(5):712-27.
38. Gray T, Koo JS, Nettesheim P. Regulation of mucous differentiation and mucin gene expression in the tracheobronchial epithelium. *Toxicology* 2001; 160: 35-46.
39. Markitziu A, Lustmann J, Uzeili B, Krausz Y. Salivary and lacrimal gland involvement in a patient who had undergone a thyroidectomy and was treated with radioiodine for

- thyroid cancer. *Oral Surg Oral Med Oral Pathol* 1993; 75: 318-22.
40. Ostuni MA, Houssay AB, Tumilasci OR. Modulation by thyroid hormones of rat parotid amylase secretion stimulated by 5-hydroxytryptamine. *Eur J Oral Sci* 2003;111:492-96.