CYTOLOGICAL CHANGES IN RAT'S SUBLINGUAL SALIVARY GLAND DEVELOPMENT PRODUCED BY THYROID HORMONE ADMINISTRATION DURING POSTNATAL PERIOD

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ABSTRACT

Background and Objective: Thyroid plays hormone essential role in normal growth, cytodifferentiation and proliferation and can have an influence on normal development of the salivary glands. Sublingual salivary gland is the smallest of the three major salivary glands that lies in close proximity to the submandibular gland. It has been shown that administration of T3 which is the metabolically active form of thyroid hormone can affect growth factors produced by the salivary glands, however it needs to be investigated if the exogenous administration of T3 can also cause any morphological or cytological changes in the sublingual tissue.

Methods: After obtaining ethical approval, twenty four healthy Wister rats taken at age week three and week seven were divided into two control (A1, A2) and two experimental groups (B1, B2) having six rats in each group respectively. The control and experimental animals were subcutaneously injected either normal saline or T3 at a dose of 0.5 mg/kg body weight, every alternate day for fourteen days. Animals were scarified on the fifteenth day and sublingual salivary gland was processed for macroscopic and histological analysis.

Results: No significant difference in gross appearance of sublingual salivary glands of control and experimental groups was found. The histological examination of all the four groups also revealed normal parenchymal and stromal components.

Conclusion: Thyroid hormone administration did not affect the early postnatal development of the sublingual salivary gland in low dosage.

Key words: Sublingual gland, histology, thyroid hormone, rat, development, postnatal.

INTRODUCTION

Sublingual salivary gland is the smallest of the major salivary glands and lie beneath the oral mucous membrane in the anterior part of floor of mouth under the tongue. It has a series of small ducts (eight-twenty) known as ducts of Rivinus opening along the sublingual folds[1,2] and a large duct known as Bartholin's duct opening along with submandibular duct.[3,4] Histologically, sublingual gland is a mixed mucous gland with mucous acini capped with serous demilunes.[3] Salivary glands in addition to production of saliva are also associated with production of various growth factors such as transforming growth factor-alpha (TGF-α), epidermal growth factor (EGF), hepatic growth factor (HGF) and Nerve growth factor (NGF)[4] and secretion of peptides, proteases, amylase, renin, nerve growth factor, kallikreins and glucagon.[5,6]

It has been reported that salivary glands at least in experimental animals are closely linked with various endocrine organs and disturbance of endocrine glands can affect the morphological or functional aspects of the salivary glands.[7] Thyroid hormone is produced by the follicular cells of the thyroid gland through iodination of thyrosine residues in the glycoprotein thyroglobulin.[8] 3, 3, 5- triiodo-L-thyronine (T3) is the metabolically active form of the thyroid hormone.This hormone plays important roles in normal growth and development, neural differentiation, inflammation, proliferation and metabolic regulation in mammals.[9] It is involved in regulation of many physiological processes that can differ between tissues or developmental stages or in response to environmental ques. Thyroid hormone can thus play different signaling roles depending on specific physiological context.[10] Previous reports have shown the effects of administration of thyroid hormone on EGF levels and expression in postnatal mouse submandibular and sublingual gland development[11-15] and only one study describes changes in EGF levels in postnatal rat submandibular gland following T3 administration.[16] However, it is not known what

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may be possible effects of T3 administration on histology of the rat sublingual salivary gland during postnatal development.

ANIMALS AND METHODS

It was an experimental study that was carried out after approval by the Institutional Ethical committee of the University of Health Sciences Lahore and all the instructions and guidelines set by the ethical committee were strictly followed. A total of twenty four healthy male Wistar rats of age three and seven weeks and weighing 50 grams and 201 – 225 grams respectively were used in this study. They were carefully examined and weighed to exclude any evidence of the disease before start of experimentation. Female and pregnant rats were not included. The rats were housed under controlled environmental conditions with temperature of 23 ± 0.3˚C and humidity kept at 55 ± 5%. Constant light and dark cycles of twelve hours each were maintained to provide a stable biological rhythm. Normal rat chow and water *ad libitum* was provided to all the animals.

Grouping of Animals and Dose Administration

The animals were divided into two control (A1, A2) and two experimental groups (B1, B2) with six rats in each group. The metabolically active form of thyroid hormone that is T3 (Sigma chemicals) was used in this study. The adjusted dose of T3 dose for rat was found to be 0.5 mg per kg body weight.[17] It was prepared fresh before use, at 0.3 mg/ml in 0.005N NaOH in 0.9% NaCl[18] and was given through subcutaneous injection on every alternate day for fourteen days. The control animals were given normal saline. Group (A1, B1) were given normal saline and T3 at week three and were sacrificed at week five whereas group (A2, B2) had normal saline and T3 at week seven and were sacrificed at week nine.

Sample Collection and Histological Analysis

The sublingual glands were collected within the capsule that also contained the submandibular gland. These were washed with distilled water and visualized for macroscopic analysis. The gland was then fixed with neutral buffer formal saline for 24 hours. The tissues were then processed in automatic tissue (Microm STP-120) and then dehydrated in graded series of ethanol at 70%, 90% and 100% and paraffin embedded blocks were prepared through tissue embedder (Tissue Tek TEC™, Sakura). Sections of 4-6µm thickness were obtained using rotary microtome (Leica RM 2125RT) and placed on slides for Hematoxylin and eosin staining. The stained sections were visualized under Olympus microscope (BX51TF) with camera (Infinity-1) under 10X and 40x magnification to observe any histological changes in response to administration of T3.

RESULTS

Macroscopic Analysis of the Sublingual Glands

The gross examination showed that sublingual salivary glands were enclosed in a connective tissue capsule. Sublingual glands of both control (A1, A2) and experimental groups (B1, B2) were pink in color, smooth in texture and roughly round in shape. They were smaller in size as compared to the submandibular salivary glands. There was no gross abnormality observed in any of the groups.

Histological Examination

The histological analysis of sublingual salivary glands of both the control (A1, A2) and experimental (B1, B2) groups was done at week 5 and week 9. It was found that both experimental groups showed structure similar to control groups and had a normal structure hence details of all the glands is described together with respect to parenchymal and stromal components.

Parenchymal Components of Sublingual Gland of Control and Experimental Groups at Week 5 and 9

The parenchymal components included lobule architecture, nature of acini and its nucleus, ductal epithelium and their nuclei and the myoepithelial cells. The lobes of the SLG of both the control and experimental group were well defined. All lobes were surrounded by connective tissue capsule. The connective tissue invaginated into lobes further dividing them into lobules. Each lobule contained many acini (Fig. 1).The lobes of the sublingual salivary gland contained acini. The acini of the sublingual salivary glands were mucous in nat-

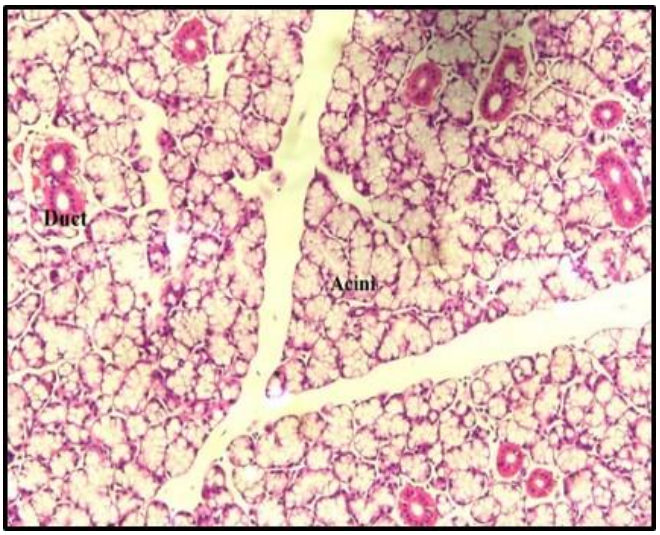

Fig. 1: *Photomicrograph of Hematoxylin & Eosin stained histological section of rat SLG illustrating presserved architecture of SLG lobules. Each lobule contains numerous mucous acini and many ducts under 10 × magnification.*

ure having a tubular pattern. The acinar cells gave an empty cell appearance. They also appeared similar in both control and experimental groups. In both the control and experimental groups, the nuclei of mucous acini were ovoid in shape having normal amount of chromatin material. The nuclei were observed to be pushed towards the basal end (Fig. 2). Numerous striated and excretory ducts were observed within the parenchymal and stromal components of the sublingual gland. They could be distinguished on the basis of type of epithelium. The striated ducts had simple columnar epithelium while the excretory ducts had the pseudostratified columnar epithelium with goblet cells. No significant difference was found between control and experimental groups. Nuclei of the ducts of both the control and experimental groups were round or ovoid with normal amount of chromatin material (Fig. 2 and 3). The myoepithelial cells were present with the acinar cells. They were identified in both control and experimental groups on the basis of their flattened nuclei (Fig. 4).

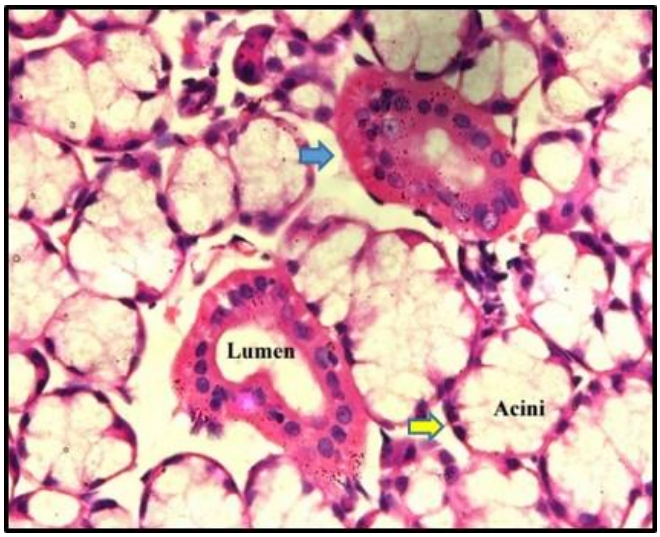

Fig. 2: *Photomicrograph of histological section of SLG showing empty looking mucous cells with basal nuclei (yellow arrow), darkly stained striated duct having simple columnar epithelium and normochromatic nuclei (blue arrow) under 40 × magnification.*

Stromal Components of Sublingual Glands of Control and Experimental Groups at Week 5 and 9

The stromal components consisted of connective tissue, adipose cells and the blood vessels. The inter-lobular and intra-lobular connective tissue of sublingual glands of both the control and experimental groups was found to be normal. The connective tissue contained nuclei of fibroblasts and no inflammatory changes were observed in any of the groups (Fig. 3). The intra-lobular and inter-lobular blood vessels were present near the ducts within and outside the lobules. They were normal lined by single layer of endothelium containing blood cells. No significant difference was found in the number of blood vessels containing blood cells between control and experimental groups (Fig. 3). Adipose cells presented as empty fat cells having signet ring appearance having a nucleus pushed to one side of the cell were also observed. They were found to be similar in both control and experimental groups.

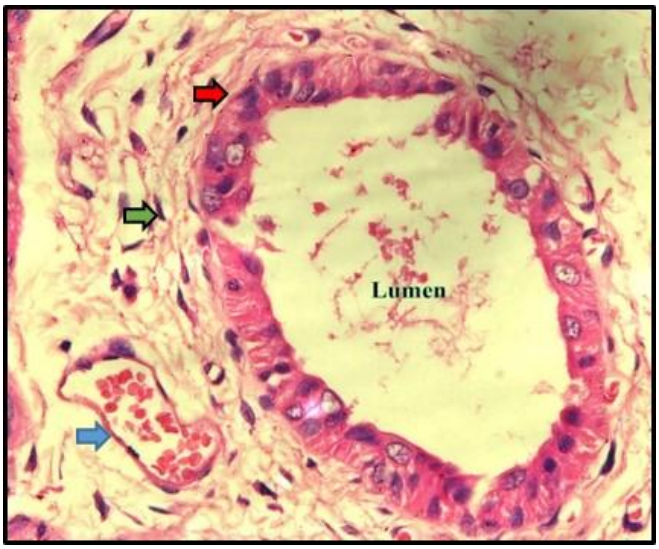

Fig. 3: *Photomicrograph of histological stained section of SLG showing excretory duct having large lumen with pseudostratified columnar epithelium (red arrow), Intralobular connective tissue with fibroblasts (green arrow), Blood vessel with blood cells (blue arrow) under 40x magnification.*

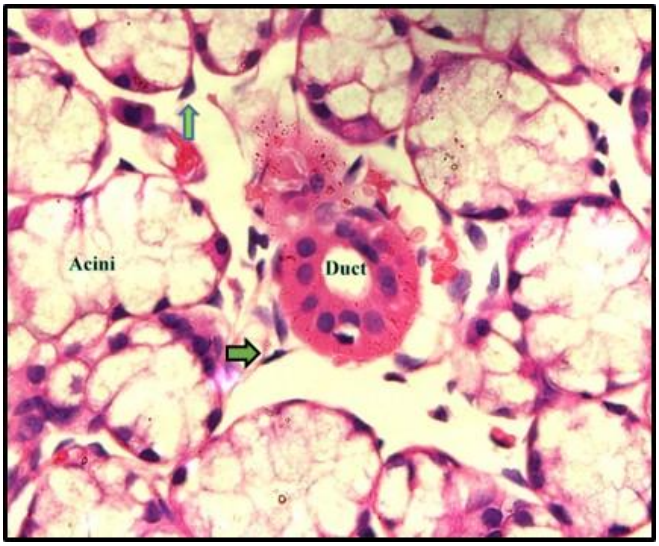

Fig. 4: *Photomicrograph of histological section of SLG showing myoepithelial cells identified on the basis of flattened nuclei and associated with mucous acini (green arrows).*

DISCUSSION

The development of the sublingual salivary gland proceed a day after the submandibular salivary gland development, however not much is known regarding mechanisms that regulate prenatal or postnatal sublingual gland development. It is believed that salivary glands of the rats are not fully functional at the time of birth and complete their morphological development by 7 – 10 weeks of age.[19-21] This period can be of a critical importance as changes in endocrine hormones as thyroid hormone, growth hormone and androgens can affect the normal development of the salivary glands.[22] As normal circulatory levels of T3 are already present in the rat[23] therefore we administrated an exogenous dose of T3 so as to observe the overall effects of this dose combined with circulatory T3 in rat. We then observe if any changes occurred in the gross or histological picture at ages week five and week nine. However, the macroscopic and histological examination of the sublingual gland showed that experimental tissues had a similar appearance with respect to the control tissues. It has been reported that mild doses of thyroid hormone had no effects on the parenchymal and stromal elements of both control and experimental groups in parotid gland tissue[24]. It may be possible that T3 was given as still a much lower dose and it will be informative to observe changes in histology following a higher dose of thyroid hormone. The salivary glands in addition to production of saliva are also associated with production of various growth factors through the granular convoluted tubules such as transforming growth factor-alpha (TGF-α), epidermal growth factor (EGF), hepatic growth factor (HGF) and Nerve growth factor (NGF)[4] and secretion of peptides, proteases, amylase, renin, nerve growth factor, kallikreins, glucagon.[5,6] GCTS are found in the rat submandibular gland but sublingual gland lies in close relation to the submandibular gland and buds off from the submandibular gland tissue early during development.[2,5] It may be possible that after T3 administration, compensatory mechanism through EGF, NGF or HGF signaling can exert their effects on the postnatal sublingual salivary gland tissue leading to a formation of a normal structure in the experimental groups similar to that of control group.[25]

It is ***concluded*** that the rat sublingual salivary gland development is not affected by T3 hormone at lower dose levels. Further studies are needed to investigate its role at much higher doses or in animal models of hyperthyroidism.

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Author's Contribution

SB: Conceived, designed, did acquisition of the published data, performed experiments and did manuscript writing. SG: Conceived, designed, provided critical revisions through intellectual output, did manuscript writing and final approval of the manuscript. NN: Provided critical analysis and interpretation of data through intellectual output.

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