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Histological and Immunocytochemical Studies on the Buccal Minor Salivary Glands in Rabbits

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Abstract

Buccal minor salivary glands are continuously produced saliva ,which is important to lubricate the buccal mucosa.Buccal glands were collected from thirty adult male rabbits. Wall of cheek were removed from anesthetized rabbits at the place of buccal glands. Paraffin sections stained of the glands were stained by H and E stain, PAS reaction and alcian blue (pH 2.5) stain. Immunocytochemistry to stain actin myofilaments of myoepithelial cell were applied. It's were mixed glands, but predominantly mucous in rabbit. The serous cells were represented by demilunes capping mucous tubules together with small number of serous acini. The myoepithelial cells (MECs) were seen around the mucous secretory units and around the intercalated ducts and striated ducts .The MECs were positively stained immunohistochemically for actin myofilaments. The cells secreotory mucous acini estimation of number and diamstter of ducts and thickest of connective tissue as well as number of acini mucous, serous acini and intercalated ducts and describe of the MECs.

Keywords: ducal gland; minor salivary glands and myoepithelial cells.

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1. Introduction

The buccal glands are part of ″minor salivary glands″ which are considered one of the moister component of the buccal cavity. They are not surrounded by capsule and their secretions directly bathe the tissues. These glands are exocrine type which distributed below the submucosal layer of buccal mucosa and between the strands of muscle fibers of the wall of oral cavity in mammals. The minor salivary glands number is ranging from 800 to 1000 glands [1, 2]. Their contribution to the total saliva is about 8%, but they are continuously produce saliva throughout the day and after retirement of the person to sleep, thus they play very important role in maintenance of the oral health The secretory end-pieces are composed of serous acini, mucous acini with or without serous demilunes cells capping. Highly glycosylated mucins with anti-microbial proteins which secreted [3]. by minor glands for lubrication of the tissue surface and prevent collection of bacteria [4]. The minor salivary glands secretion plays an important contribution to the local defenses mechanisms [4]. Contribution to the taste sensation and lipase synthesis during neonatal life are well-established functions for the vonEbner's minor salivary glands associated with the circumvallate and foliate lingual papillae [5]. The focus of the present study on the buccal minor salivary glands was intended to explore their nature of secretion, which is important for the physiology and health of the mouth cavity, and also for the notion that the buccal glands were considered as a starting point for a metastasizing adenocarcinoma [6]. Myoepithelial cells were included because these cells were overlooked in the previous study on these glands.

2. Material and methods

Thirty adult male rabbits weighing 1 to 1.5 kilo each were used. Earlier step of the experimentation the rabbits were kept in the animal household of the college of Sciences, university of Kufa for two weeks, as a matter of environmental adaptation. Pieces from the cheek contain the buccal glands were harvested to be fixed for 48 hours in the formalin buffer. The samples were dehydration by alcohol gradients, next step where putting in xylene after that to be put in paraffin. Six µm sections were cut to be stained in the general stain haemotoxylin and eosin, histochemical stains (alcian blue pH 2.5 and periodic acid Schiff reagent (PAS) ,and immunocytochemistry to detect actin myofilaments of the myoepithelial cells using actin (smooth muscle) M085 of Dako [7, 8].

3. Results

3.1 General stain (haemotoxylin and eosin stain)

The buccal mucosa is thick stratified squamous non-keratinized epithelium. Rabbit`s buccal glands found in submucosa as a groups of glands in-between the strands of buccinators muscle fibers, with no separate connective capsule (Fig. 1 a, b) the mucous acini were predominantly and arcs by demilune cells that seem in section of tissue were stained by H&E (Fig. 2 a, b).

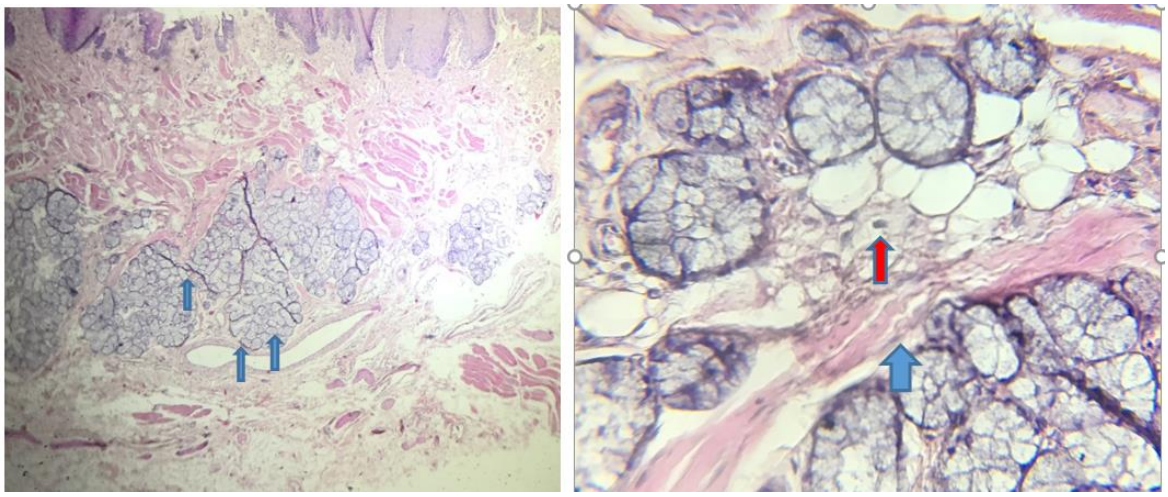


Figure 1: Sectional stained by Hematoxylin and eosin stain of buccal gland. A and B) The buccal glands distributed under the cheek mucosal epithelium and in between the strands of buccinators muscle fibers (blue arrow) the collagen fibers of the CT, show continuous to epimysium of the muscle (red arrow).A: X40. B: X400.

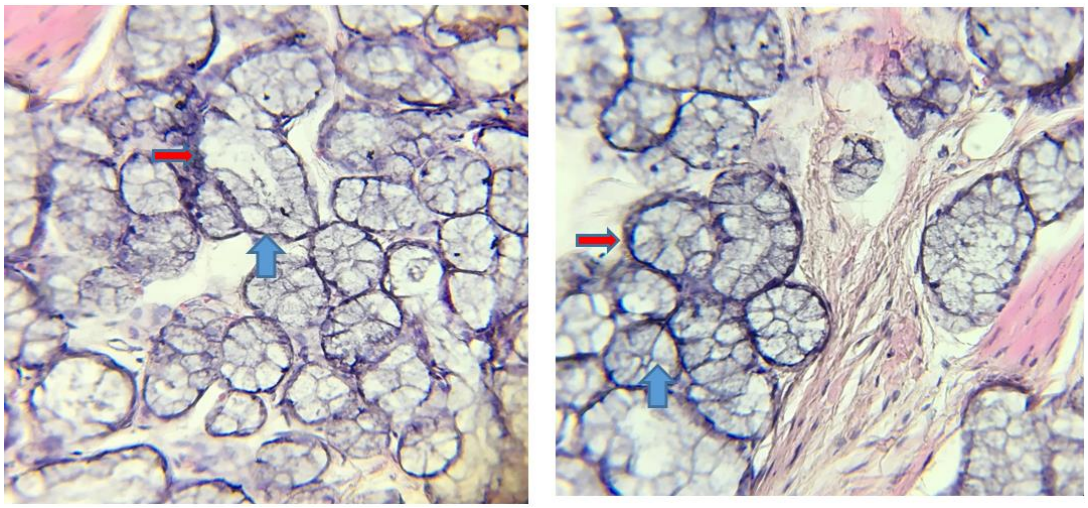


Figure 2: Sectional stained by Hematoxylin and eosin stain of buccal gland. A and B) The buccal glands secretory units are having mucous acini (blue head arrow) with serous demilunes (red head arrow). A: X40. B: X400.

3.2 Special stain

3.2.1 The periodic Acid-Schiff (PAS) stain

The buccal mucous acini were positive stained with PAS. They exhibit foamy appearance (figure (3a, b). The serous demilunes were intensity stained with PAS, they have taken magenta coloration (figure 4b). The intercalated ducts were weakly stained (Figure 5a). The striated ducts were intensely stained, they have taken magenta coloration (figure 5b).

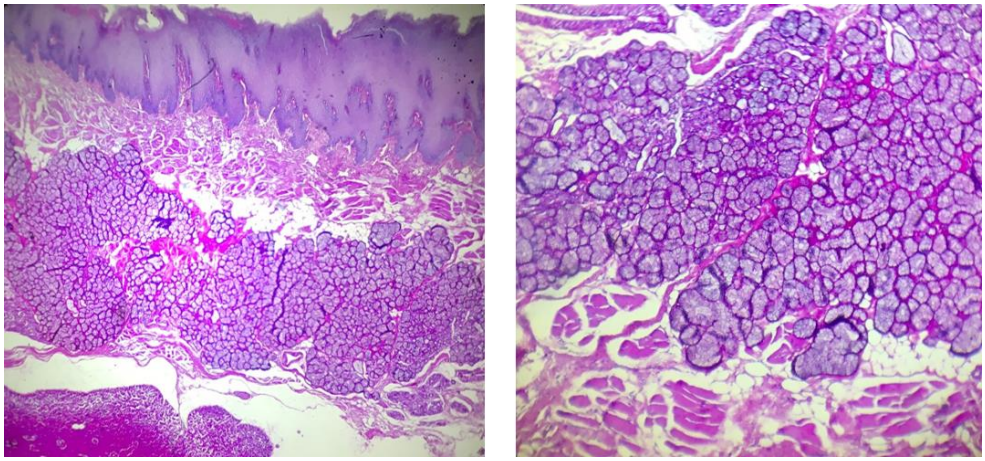


Figure 3: Histological sections of buccal glands stained with PAS. A) The buccal glands are distributed under the thick epithelium of the cheek mucosa. X40 B) The mucous acini of the buccal glands are expressing negative reaction with PAS. X100.

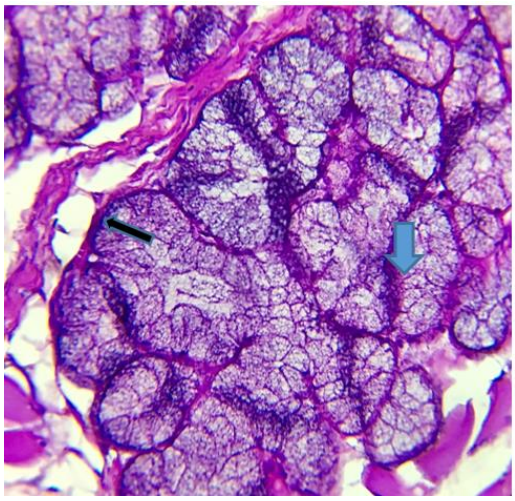


Figure 4: Higher magnification to the same buccal glands in figure 3 showing the negatively stained glands and the foamy appearance inside their acinar cells. The serous Demilunes (arrow) that cap the mucous acini were intensity stained with PAS reagent taken magenta color X400.

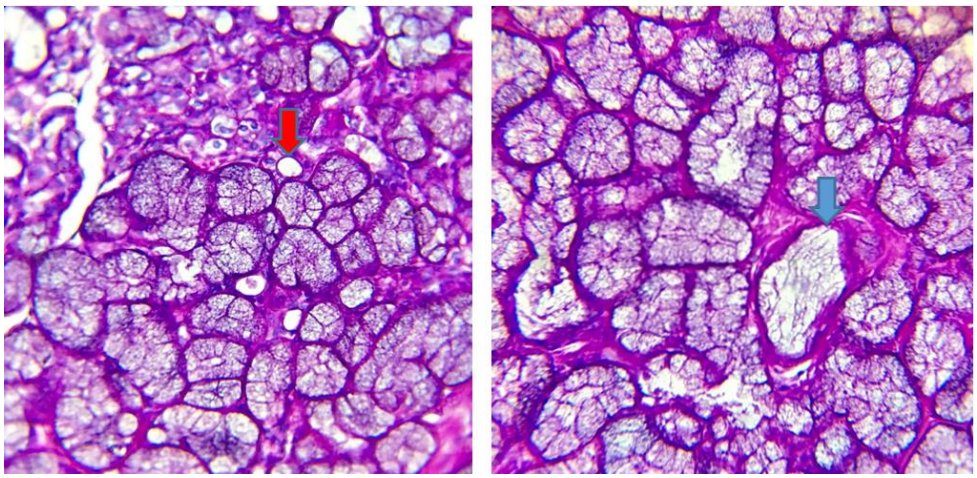


Figure 5: The micrograph is showing sections of buccal glands. A) The intercalated ducts(ID) were weakly stained with PAS reagent (red arrow). X400. B) Striated ducts (SD) were intensity stained with PAS reagent taken magenta color (blue arrow). X400.

3.2.2 Alcian blue stain (AB)

The buccal mucous acini were intensely tainted by ″alcian blue (pH 2.5)″ stain. They have taken blue coloration suggested a positive reaction for acidic mucin with this stain. They exhibit foamy appearance (figure 6)

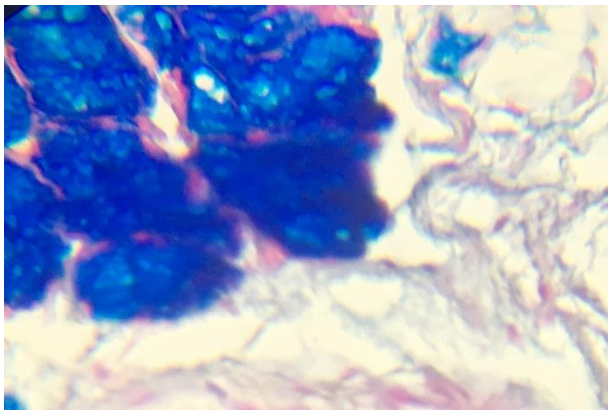
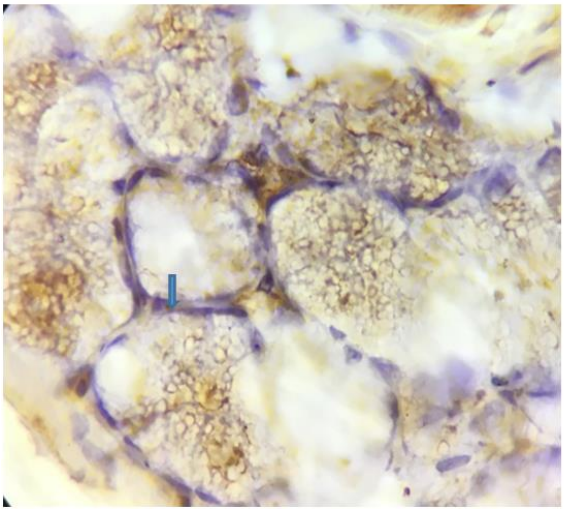


Figure 6: The histological section of buccal glands showing intense blue stained mucous acini with alcian blue stain pH 2.5. X400.

3.3 Immunocytochemistry actin stain

After using the method of immunohistochemistry. The shape of MECs around the mucous secretory units of the buccal glands were having rounded cell body with 2 to 3 primary cytoplasmic processes projecting from each one of them. These processes were embracing the secretory units of the glands (Fig. 7a and b). The MECs were also found around intercalated ducts (Fig.8a), but not around striated ducts(Fig.8b). Immunoreactivity for actin filaments showed strong staining in the distal end of a mucous glandular endpiece (demilune cells) especially under the cell membrane of the acinar cells. The intralobular ducts can be shown in (Fig. 7).



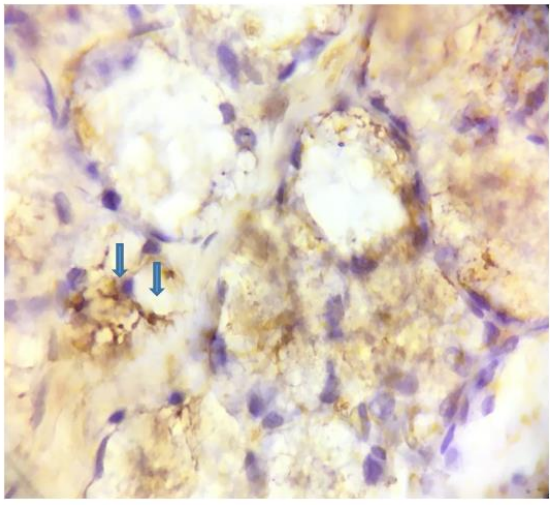


Figure (7 a and b) : The immunohistological sections of buccal glands stained for actin filaments inside the MECs (Arrowed). X 1000

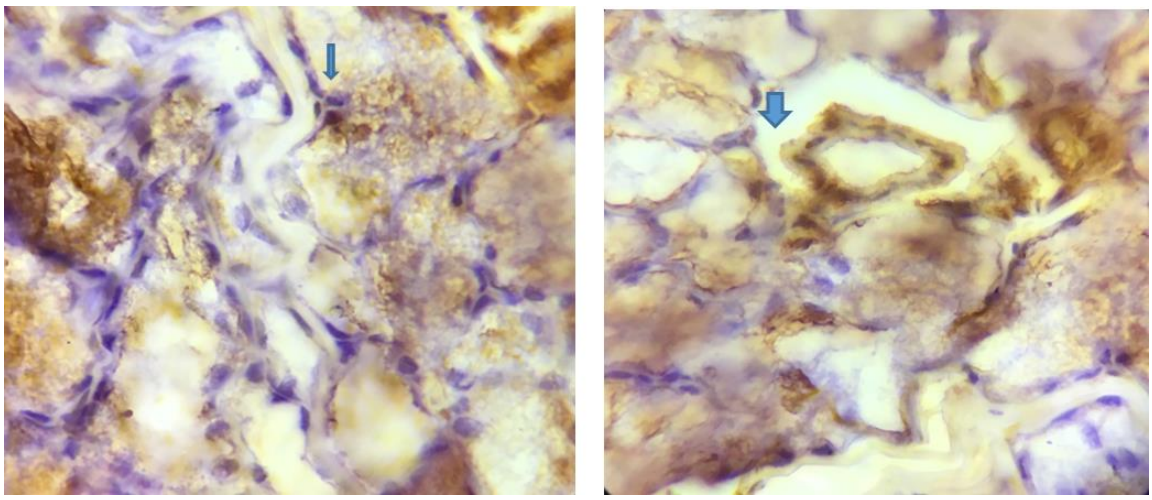


Figure 8: A section through the buccal glands showing the glands *(G)*, and the appearance of myoepithelial cells(blue arrows)around the intercalated ducts(a) and the striated duct(b). Immunohistochemical stain for actin filaments X 1000.

4. Discussion

Buccal glands as a part of moisty agent in the mouth that provide saliva and keep health of the oral cavity. The constant predominantly mucous nature of the saliva into the mouth vestibule has great impact on the lubrication of the buccal mucosa which prevents its friction with the teeth. The foremost acini in buccal glands their mucous acini which consildate the function of the mucous salivary ring seen at the oropharynx [9]. It is the mucin secretion from mucus acini from buccal gland for lubrication of dry food to facilities swallowing. The position buccal glands in each side of oral cavity that play major role for the faced any foreign antigen by means of chunk of ″the innate immune system″. The secretory end-pieces of the buccal gland were predominantly mucous acini with the capping by serous demilunes. Similar observation has reached by [10] in sublingual glands of the same animal. The mucous acini were weakly stained with PAS reagent. Instead their cells expressed foamy like appearance. The serous demilunes were intensity stained with PAS reagent indicating the seromucous nature of their secretion. The term Seromucous was coined to the serous cells of the parotid gland [11]. The intercalated ducts were weakly stained with PAS. The striated ducts were intensity stained taken magenta color. The researchers [12] performed when study on Weber's gland in rabbit the shown the mucous acini were positive stain with PAS reaction. In present study clarify the buccal mucous acini were intensity stained with ″alcian blue (pH 2.5)″ stain taken blue color suggestive acidic nature for the mucin. Similar observations have reached in the Weber's gland of rabbit [13]. In present study the positioning of the MECs and their abundance around the buccal glands secretory units suggested the great need of these cells to accelerate the predominantly mucous secretion . Our present observations regarding the myoepithelial cells were in accordance with [5] who has pointed out that more myoepithelial cells were needed around the mucous secretory end pieces than the serous end pieces due to the viscous nature of the mucin secreted by these glands. Moreover the actin filaments of the MECs seen in the buccal glands have significant effect on the on the active secretory machinery of the buccal glands. Furthermore, previous

observations reached by [14] and on the actin filaments in the labial minor salivary gland and [15] on the MECs of the major salivary glands have mentioned important contribution of these filaments to expel the secretory vesicles from the apical part of the acinar cells [16]. As in the current study which clarify that the MECs were found with many processes, the other exocrine gland have shown the MECs with many processes around the secretory units ,as in lacrimal glands, ″minor and major salivary glands″. In the major salivary glands the MECs had shown noticeable stare shape, regardless of the organ or species [17]. The researchers [18] that use SEM shown the MEC that have four different shapes and correlated with the number of primary cytoplasmic processes in major salivary glands. MECs characters were hybrid between epithelial origin and mesenchymal phenotype. The MECs contain many contractile proteins, myofilaments arranged in parallel such as myosin, calponin, and caldesmon, αSMA as well as vimentin [19]. In current study shown MECs around intercalated duct that shown in figure (7a), but not clearly around striated ducts that shown in figure (7b). Also in other study expound MECs are not shown around striated ducts in parotid, submandibular and sublingual in humane, bat and rabbit [10,20,21].

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