

HISTOCHEMICAL STUDY OF MUCOSUBSTANCES OF NORMAL HUMAN PAROTID SALIVARY GLANDRohini R. Karambelkar¹, Avinash D. Shewale², Rajesh A. Karambelkar³, B. N. Umarji⁴**HOW TO CITE THIS ARTICLE:**

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ABSTRACT: INTRODUCTION: The oral cavity is one of the important routes for infections and infestations. Majority of the infections occur through food and air. The oral cavity is said to be even worst than anal canal due to its direct exposure to atmosphere and the moist condition, which facilitate the bacterial growth. Plenty of lymphoid tissue and glandular secretions protects the mucosa of oral cavity. Glandular secretions are serous and mucous in nature and are rich in Mucosubstances which protect the oral mucosa from bacteria and viruses. They help to lubricate the oral mucosa. In unstimulated state major bulk of secretion is from parotid salivary gland. **METHODS:** In the present study Mucosubstances present in parotid salivary gland are studied. 30 normal human parotid salivary glands were collected and fixed in 10% formalin with 2% Ca acetate and pinch of phosphotungstic acid. 4 to 5 micron thick sections were cut and blocks were prepared. The sections were stained with periodic acid Schiff (PAS), alcian blue (AB) at different pH and Aldehyde fuchsin (AF) individually and in combinations. Confirmatory tests were carried out. **RESULTS:** the normal parotid salivary gland shows presence of neutral Mucosubstances with very trace amount of sialomucins and sulphomucins. Presence of neutral mucins indicates that the secretions of parotid salivary gland are rich in enzymes.

KEYWORDS: Parotid, Mucosubstances, PAS, AB, salivary mucins.

INTRODUCTION: The parotid salivary gland is the largest salivary gland which communicates the oral cavity through vestibule of mouth. It is situated upon the side of the face, immediately below and in front of the external ear.

It is pyramidal in shape with apex directed downwards. It is drained by a duct which opens in the vestibule of mouth opposite the crown of upper second molar tooth. Histologically it is a compound tubule-alveolar gland with lobes and lobules separated by connective tissue septa. The parotid gland contains 95% serous alveoli and 5% mucous alveoli.

These alveoli secrete Mucosubstances which performs a wide variety of functions like lubrication, protection against acids, maintenance of hydration etc. They also contain immunoglobulin's mainly IgA type, lactoferrins which chelate the iron necessary for growth of some bacteria and lysozymes which destroy some of bacteria. The mucins play an important role defense against bacteria. Sulphomucins are acting as antiulcerogenic as they coat and protect mucosal surface while neutral mucins help for secretion of enzymes.¹⁻⁴

Many workers like Dr. Ganga (2004),⁵ Gad,^{6,7} Filpe and Fenger⁸ have studied the Mucosubstances of GIT but very few have studied the salivary gland mucins. So the present study has been undertaken to study the mucins in the parotid gland in human and correlate it with previous studies.

MATERIAL AND METHODS: Thirty normal human parotid salivary glands were obtained during autopsies and routine dissection. The tissue was fixed in 10% formalin with 2% Ca acetate. By routine procedure paraffin blocks were prepared and 4-5 micron thick sections were cut. They were stained by.⁹⁻¹¹

1. Haematoxyline and Eosin (H&E).
2. Periodic Acid- Schiff (PAS).
3. Periodic Acid- Schiff with diastase digestion (PAS-D).
4. Periodic Acid- Schiff with phenyl hydrazine (PAS-PH).
5. Alcian blue 2.5 pH (AB 2.5 pH).
6. Alcian blue 1 pH (AB 1 pH).
7. Combined AB-PAS.
8. Aldehyde fuschin (AF).
9. Combined AF-AB.

The slides were photographed using digital camera. The histochemical data staining methods employed in the present work are recorded according to visually estimated intensity of staining and shades with four plus representing strongest activity. Nomenclature applied to the mucosubstances is taken from the discussion of a proposed general terminology of histochemically recognized material.^{12,13} histochemical results requiring further description and consideration are presented here along with their interpretations.

OBSERVATION AND RESULTS:

- i. Numerous lobes and lobules separated by connective tissue septa are seen. Each lobule contains the secretory acini of parotid salivary gland. They are mainly seromucus. Majority are serous acini with very few mucus acini are seen. The cells in the serous acini are roughly pyramidal. Nuclei are more spherical and less basally placed. The cytoplasm is darkly stained showing basal basophilia suggesting the presence of the rER. Secretory granules are seen near apical part of cells, showing supranuclear secretional zone. Plenty of intercalated ducts, striated and excretory ducts are seen scattered. The cells lining the intercalated ducts are flat or low cuboidal. The cells lining the striated duct are simple cuboidal. All these acini and ducts are packed with a cushion of adipose tissue. (Figure No. 1).
- ii. When the sections of parotid gland are stained with PAS stain, it was found that the acinar cells are deeply stained in SN region with magenta showing the presence of PAS positive substances like carbohydrate and neutral mucins. (Figure No. 2).
- iii. With diastase digestion the magenta colour intensity was reduced indicating the presence of non-mucinous carbohydrates like glycogen. (Figure No. 3)
- iv. Reduction of colour intensity with PAS-PH indicates the presence of neutral mucins in the acinar cells. Few cells show presence of acidic mucins also. (Figure No. 4).
- v. With AB 2.5 pH the acini are stained dark blue which confirms the presences of both types of acidic mucins. (Figure No. 5).
- vi. When stained with AB 1 pH very few acini are stained showing presence of very few sulfomucins. (Figure No. 6).

ORIGINAL ARTICLE

- vii. When the sections are stained with AF, it shows very low colour intensity, so the presence of trace amounts of sulfomucins is confirmed. (Figure No. 7).
- viii. With combined AB-PAS staining the parotid acini show varied colour intensity. Many acini are intensely stained with magenta colour with few blue acini. It indicates the presence of combination of acidic and neutral mucins. (Figure No. 8).
- ix. With AF-AB staining few acini are stained blue showing presence of sialomucins while very few are stained purple confirming the presence of very few sulfomucins. (Figure No. 9)

The results are tabulated in table no. 1. From the table no. 1 we can say that the parotid gland shows presence of both neutral and acidic mucins in SN region. In that also neutral are more and in acidic sialomucins are more than sulfomucins.

DISCUSSION: The parotid gland is the largest salivary gland. It is composed of multiple lobules separated by interlobular fascial septa and is nearly a pure serous secreting gland. In resting state the gland contributes about 20% of saliva but in stimulated state it contributes about 50% of salivary secretion.

The mucins are complex molecules in the sense that they contain both proteins and polysaccharide components linked together by strong covalent chemical bonds. The mucosubstances are conveniently divided into glycoprotein and proteoglycans on the basis of structural characteristics.

The glycoproteins contain one or many carbohydrate side chains of relatively small size and gross analysis shows that polypeptide chain to be the major component of the molecule. The side chains are often branched and contain sugar units with neutral charge. There is an important group of glycoprotein which have residues of strongly acidic sugars, called sialic acid, at free ends of their carbohydrate side chains so that molecules as a whole acquire a strong negative charge.¹⁵

The mucins may be present as a mixture of different types. Epithelial mucins have been classified into neutral and acidic types, the later being subdivided into sulphomucin and sialomucins.¹⁶ It is therefore not sufficient to apply a single stain for their identification. So the mucins are studied by many methods specific for different chemical components.

Mucin barrier is formed of the mucin blanket covering the luminal surface of the epithelium of the mucosa. Mucins create a medium in which macromolecules and large particles are insoluble. PH gradient exists across mucin barrier and protects underlying mucosa. Mucins also play an antibacterial activity^{17,18} and lubrication.⁷ Sulphomucins are antiulcerogenic.¹⁹ Sialomucins and other components of glycocalyx are responsible for cell recognition, contact inhibition and various immunological phenomena.

The mucins are the main components of the pre epithelial protective layer of the mucosa not only represent a mechanical barrier but also a dynamic structure modeling the oral cavity environment. As a result of reduction in mucin concentration in saliva of patients, the mucosa may be more susceptible to the effect of damaging factors which, with long term exposure may lead to initiation, promotion & progression of the carcinogenic process.¹⁹

Mucins are altered in normal and pathological states so it is of ever increasing importance in the investigation of normal and disease process. Using special stains like PAS, AB, AF, they are categorized into acidic, both sulphomucin and sialomucins and neutral mucins.

ORIGINAL ARTICLE

In normal parotid salivary gland, the acini are positively stained with PAS indicating the presence of PAS positive substances like carbohydrates and neutral mucins. Few of the acini are negative for PAS staining which may contain enzymes or some of the sulfomucins which are PAS negative. While little reduction in the magenta color intensity after diastase digestion shows the presence of non-mucinous carbohydrates also. In PAS-Ph many acini are non-reactive indicating the presence of large amount of neutral mucins.

The serous acini show strong positivity for AB 2.5 but negativity to AB 1 pH and AF. Occasional AF and AB1 pH positive acini are also seen. It shows presence of both types of acidic mucins but in that sulfomucins are present in very much trace amounts. These results are in accordance with the study of S. Naag¹⁴ on salivary gland mucins.

The combined AB-PAS and AF-AB also showed the acini positive for AB and PAS individually indicating the presence of both neutral and acidic mucins in equal amounts.

The sialomucins are important for their antibacterial and antiviral property. Also they have secretory IgA while the neutral mucins are important in the secretion of various digestive enzymes.¹¹ Priya 1985²⁰ conducted a similar study of histochemical analysis of normal salivary glands in mammals by using H&E, PAS, mucicarmine, AB & AF. The parotid gland of guinea pigs and sheep showed neutral mucins and that of the cat and dogs showed sulfomucins.

CONCLUSION: The parotid gland is a predominantly serous gland having 5% of mucus acinii. The serous cells in parotid show presence of neutral mucins with very trace amounts of sialomucins and sulfated mucins. Acidic mucins may be due to heterogeneity i.e. having mucous cells and acinii spread amongst serous acinii. This shows parotid has watery secretion which is rich in enzymes. Any change in its nature may induce disease process.

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ORIGINAL ARTICLE

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No.	Stain	Intensity	Inference
1	H&E	-	Serous acini present with ducts and adipose tissue
2	PAS	++++	Presence of PAS positive substances i.e. carbohydrates and neutral mucins.
3	PAS-D	++	Presence of glycogen.
4	PAS-PH	+ / -	Presence of large amount of neutral mucins.
5	AB 2.5	+++	Presence of acidic mucins.
6	AB 1	+	Presence of sulfomucins in trace amount.
7	AF	+	Confirms presence of sulfomucins.
8	AB-PAS	Magenta +++	Presence of neutral and acidic mucins.
		Blue ++	
9	AF-AB	Blue ++	Confirms presence of sialomucins with trace amounts of sulfomucins.
		Purple +	

Table No. 1 showing results of parotid staining

ORIGINAL ARTICLE

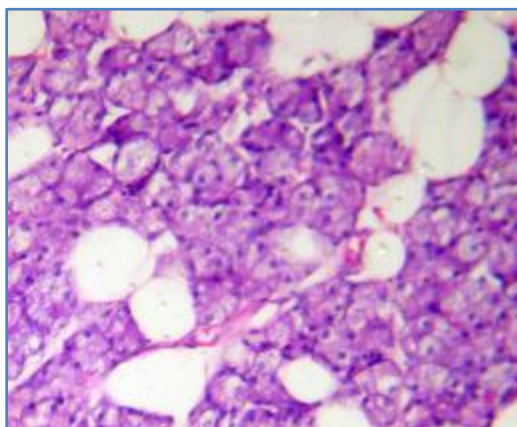


Fig. 1: Parotid section stained with H & E stain (400 x)

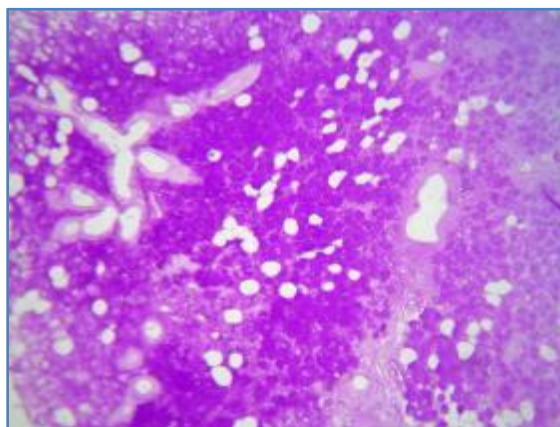


Fig. 2: Parotid section stained with PAS stain (100 x)

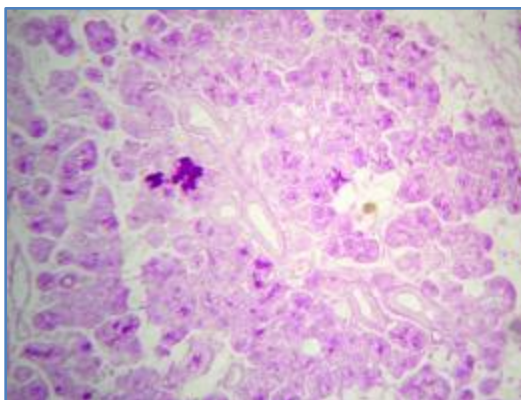


Fig. 3: Parotid section stained with PAS-D stain (100 x)

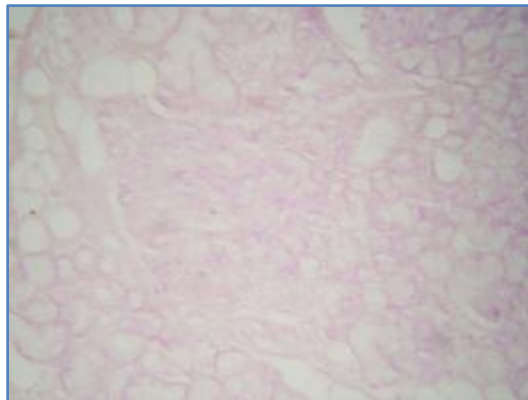


Fig. 4: Parotid section stained with PAS-PH stain (100 x)

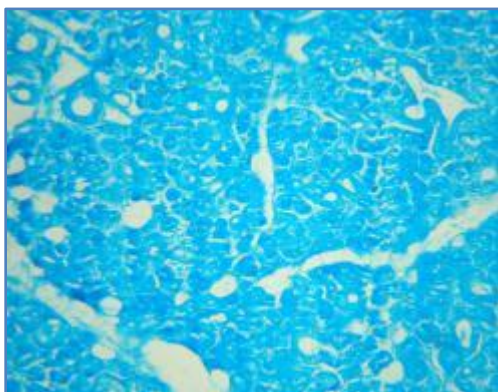


Fig. 5: Parotid section stained with AB 2.5 pH stain (100 x)

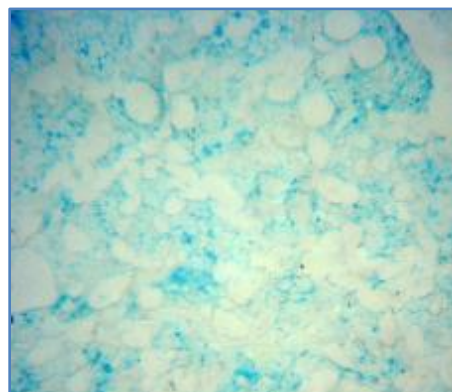


Fig. 6: Parotid section stained with AB 1 pH stain (100 x)

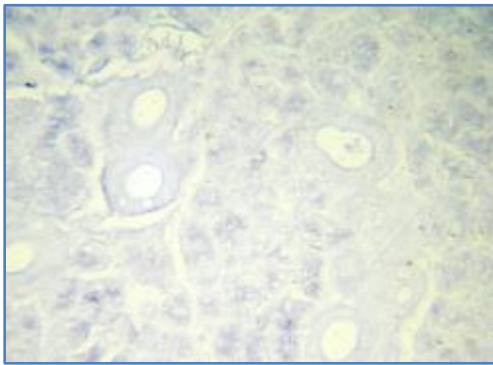


Fig. 7: Parotid section stained with AF stain (100 x)

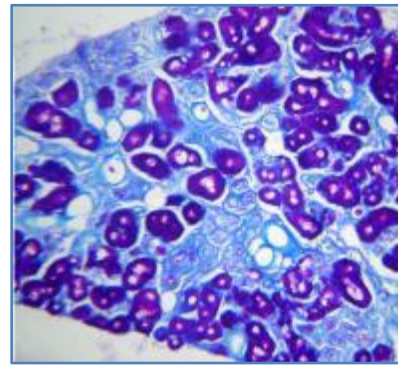


Fig. 8: Parotid section stained with AB-PAS stain (100 x)

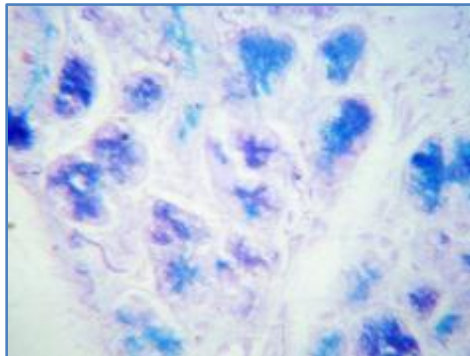


Fig. 9: Parotid section stained with AF-AB stain (400 x)

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