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RESEARCH ARTICLE

DISTRIBUTION OF EPITHELIAL MUCIN IN DEVELOPING NASAL CAVITY OF HUMAN FETUS, ADULTS AND IN CARCINOMA - A COMPARATIVE HISTOCHEMICAL STUDY

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ABSTRACT

Material and Methods: Tissue sections were stained by the following stains to demonstrate different mucins: H&E, Periodic acid-Schiff's (PAS), PAS-D, PAS-Ph AB ph 2.5 and 1, AF, combined AB-PAS, and combined AB 2.5–AF.

Results: In group 1 - Sulphomucins and neutral mucins concentration is more compared to sialomucins. In group 2 - Increase in sailo mucins concentration compared to sulpho mucins and neutral mucin. In group 3 - increase in sailomucins. In adults -more acidic mucin especially sulpho mucin compared to sialo and neutral mucin and in sinonasal carcinoma- increase in sialo mucins compared to neutral and sulpho mucins.

Conclusions: Change in functional collaboration of the cell before change in structure and hence affect the formation of mucins. Hence early detection of mucinal changes may be the first step before structural changes occurring in many diseases.

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INTRODUCTION

Mucins form major component of mucus which is secreted by the goblet cells of respiratory, uro-genital and gastrointestinal systems. These serve as first line of defence and acts as protective barrier against pathogens and toxins which contributes to the innate defence system in mucosal immunology (Corfield *et al.,* 2004). Apart from its role in protection, mucins also play a role in detection of metaplasia and helps in the classification of metaplasia which is a precancerous condition. Mucins are complex carbohydrate secreted by different types of epithelial cells and glandular tissue of respiratory and alimentary tract. There has been growing recognition in recent years that demonstration of these substances is difficult and complex and is affected by types of mucins present. The term mucin is a traditional one. It is commonly used as it implies precise meaning and easily recognized. Mucin is a designation for variety of compounds from the viscid, clear, mucoid secretions of goblet and other secretory epithelial cells such as those of intestinal mucosa glands to the cement / ground substances between cells and fibres of connective tissue. Mucins are classified into neutral mucins and acidic mucins.

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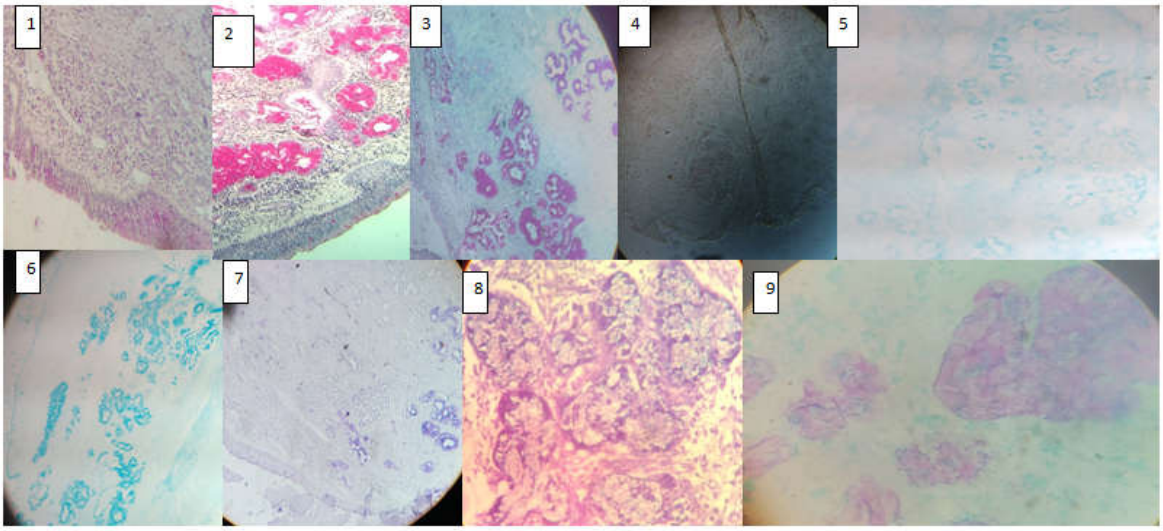
Acidic mucins again divided into sialomucins (sailic acid group) and sulpho mucins (sulphate group) (Filipe and Branfoot, 1976). Mucins and glycogen are the two main entities to be considered in the tissue carbohydrate demonstration, of which mucin is the largest group consisting of mucopolysaccharides (MPS), mucosubstances and Glycoconjugates. There are studies on mucin histochemistry of respiratory tract done on adult normal and malignant conditions. But hardly there are any studies on histochemistry of developing human nasal cavity and its comparison with adults and malignant conditions. So that histochemistry plays an important role in early detection of mucinal changes (Ganga, 2003). So histochemistry may provide a valuable and cost-effective tool for the diagnostic histopathology and for the researchers in histology. Diagnostic problems which are difficult to solve by routine histological methods, but which may be resolved by histochemical techniques. With this review we propose to detect mucins which are present in nasal cavity of human fetus, adult and in carcinoma related to human nasal cavity.

MATERIALS AND METHODS

After obtaining IEC letter 50 normal nasal samples (lateral wall) from foetus, 30 samples (lateral wall) from adults and 10 sinonasal carcinoma samples were collected from S N Medical College and HSK &RC Bagalkot.



1 .Haematoxylin and Eosin (H&E) 10X .2. Periodic acid Schiff (PAS) 10X. 3. PAS-Diastase(PAS-D) 10X .
4. PAS-Phenyl hydrazine (PAS-PH) 10X .5 .Alcian Blue (ph-2.5)- (AB) 10X. 6. Alcian Blue (ph-1.0) 10X
7 . Aldehyde Fuschin Stain (AF) 10X .8. Aldehyde Fuschin with Alcian Blue (AF-AB 2.5) 40X .9. Alcian Blue 2.5 -PAS- (PAS-AB 2.5) 40X

Table 1. Comparison Table between Foetus, Adults and Carcinoma - Nose shown in figure no 1

S.No.	Stains	Colours	Intensity of colour				
			Foetus Group1	Group 2	Group3	Adult	Ca
1	PAS	Magenta	+++	+++	+ + +	+ + +	+ + +
2	PAS- D	Magenta	++	+++	++		++
3	PAS –PH	Absence of Magenta	-	+/-	+/-	+/-	+/-
4	AB (ph2.5)	Blue	+++	+++	+++	++	+++
5	AB –(ph 1)	Blue	++	++	++	+	++
6	AF	Purple	++	+	++	++	++
7	AB-PAS (Ph -2.5)	BP	++	+ ++	++	++	++
		Blue	+	++	+++	+++	+++
		Magenta	++	++	++	++	++
8	AF-AB (2.5)	Purple	++	++	++	+++	++
		Blue	+	+++	+++	++	+++

- In this sampling method, we have included only normal aborted foetus.
- Normal adult respiratory tract
- Excluded Smokers and persons having respiratory diseases
- Excluded foetus with congenital anomalies.

In foetus we have divided into 3 groups according to gestational age

- Group 1- GA between 16weeks-26weeks
- Group 2 -GA between 27 to 32 weeks
- Group3- GA between 33weeks to full term

The tissues obtained were fixed in 2% calcium acetate in 10% formalin and pinch of phosphotungstic acid to preserve mucin kept it for 3 to 4 days in room temperature. After that tissues were processed and embedded in paraffin wax and prepared sectioned at 6 microns. The sectioned were stained with the following standard techniques to demonstrate the different epithelial mucins.

- Haematoxylin and Eosin- know the normal architect of the tissue,
- Periodic acid Schiff (PAS) - PAS positive substance,
- PAS-Diastase- Glycogen and confirmation of neutral mucins,
- PAS-Phenyl hydrazine- Confirmation of neutral mucin,
- Alcian Blue (ph-2.5)- Acidic mucins contains both sulphated and sailo mucins,
- Alcian Blue (ph-1.0)- Demonstration of sailo mucins,
- Aldehyde Fuschin Stain- Confirmation of sulphated mucin,
- Alcian Blue-PAS-To distinguish between acidic mucins and neutral mucins
- Aldehyde Fuschin with Alcian Blue--Distinguish between sulphated mucin and carboxylated (Sailo mucin) mucin.

Scoring system was devised based on the percentage positivity of cells in each field under low power examination (10X) + Mild, ++ Moderate, +++ Marked All quality control measures were followed carefully.

RESULTS

In all 3 groups of foetal nasal glands contain glycogen, neutral mucins, sulphomucins and carboxylated mucins (sailomucins) in different concentration. As shown in table no 1.

Foetus

- **Group 1**: Prescence of more amounts of neutral and acidic mucins. In acidic mucins more sulpho mucins compared to sialo mucins.

- **Group 2:** Large amount of both acidic and neutral mucins are present with increased sialo mucins in acidic mucins.
- **Group 3:** Neutral and acidic both the mucins are in large amount with increased sialo mucins compared to sulpho mucins

Adults: Prescence of acidic and neutral mucins with more sulpho mucins and less sialo mucins

Sinonasal carcinoma: Large quantity of acidic mucins with large amount of sialo mucins compared to sulpho mucins and along with moderate amount of neutral mucins

DISCUSSION

In 1865 E. Eichwald, a Russian physician reported for the first time, that mucins are proteins bound to carbohydrates and these create a medium in which most macromolecules and large particles are insoluble whereas small molecules are soluble and free to diffuse. (Brockhausen *et al.,* 2009) Mucins does antibacterial action (Forstner *et al.,* 1981). And it has role in lubrication, waterproofing action (Forstner, 1970). Sialomucins have viscoelastic property, cell recognition whereas sulpho mucins acts as antiulcerogenic property (Forstner *et al.,* 1981). The predominance of epithelial glycogen in first half of foetal life prior to the appearance of hepatic glycogen. Glycogen synthesizing enzymes are more prominent in foetal than in adult specimens including man. Synthesis of mucin is initiated in rough endoplasmic reticulum of the producing cells and in the Golgi apparatus. Role of glycogen or its breakdown products helps in different species synthesis of glycoprotein (Robert Lev and Herbert Weisberg, 1969). Neutral mucins are present in nasal mucosa of different species. In general sulphated; sailo mucins are more predominant in all parts of the respiratory tract .The ciliated epithelium of the nasal respiratory mucosa has also been found to elaborate acidic mucosubstances (Taylor, 1958). Neutral mucins, sialomucins and sulpho mucin in varying proportion were present in different stages of cellular differentiation and foetal maturation (Gad, 1982). Recently Sialic acids are of particular interest because of reported variations in diseases. Sialomucins have role in cell protection, regulation of cell growth and cellular recognition. Molecular mechanism of mucin synthesis may help in understanding pathophysiology of diseases. The mucins reflect activity or location of enzymes such as sialyl transferase. Depending upon the presence of two sialyl transferase sialation of individual mucins would very with different glycosylation may marked influence on ultimate physical properties of secreted product (Forstner, 1970). The aberrant expression of mucins, as found in many cancers, is likely associated with cancer biology as alterations in the expression and/or glycosylation patterns of various mucins influence cellular growth, differentiation, transformation, adhesion, invasion, and immune surveillance (Hollingsworth, 2004).

Conclusion

- In all 3 groups of fetus, adults and carcinoma contain neutral mucins, sulpho mucins and carboxylated mucins (sailomucins) in different concentration.
- Increase in sailomucins compared to neutral and sulfomucins in 2nd trimester and increase in sialo mucins in 3rd trimester of foetal life. And in adults more sulpho mucins and neutral mucins and in carcinoma there is increase in large quantity of sialo mucins and neutral mucins compared to sulpho mucins.
- Malignant transformation may alter existing regular mechanism of glycoprotein synthesis and release inactive genes so that various in complete or abnormal glycoproteins are formed .variation in mucin composition in foetal life, adult life and its relationship with early changes in carcinogenesis is subject of interest still today.
- Change in functional collaboration of the cell before change in structure and hence affect the formation of mucins. Hence early detection of mucinal changes may be the first step before structural changes occurring in many diseases like carcinoma.

Abbreviations

AB- Alcian blue
PAS- Periodic acid Schiff reaction
PAS- D-periodic acid Schiff reaction –diastase
PAS- PH – periodic acid Schiff reaction -phenyl hydrazine
AF- Aldehyde fuschion
H&E- Haematoxyline and Eosin
Ca- Carcinoma

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