

## Histochemical Study of Mucin in the Adenomatous Transformation of Barrett's Esophagus in Human Esophageal Epithelium



### Zoology

**KEYWORDS :** Barrett's esophagus, Alcian blue, Periodic Acid Schiff.

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### ABSTRACT

*Barrett's esophagus (BE) is an early stage and predictive marker in the progression of esophageal adenocarcinoma (EAC). The prevalence and risk factors of BE and its complications are thought to be infrequent in India. There is a need for more endoscopic and histological studies on the diagnosis of BE. Mucin secretions in BE excogitate the functional state of the mucosa in human esophageal epithelium. Hence, the present study was performed to understand the mucin histochemistry using Alcian blue (AB) and Periodic Acid Schiff (PAS) staining in the progressive stages of Barrett's adenocarcinoma.*

### Introduction

Chronic exposure of esophagus to the gastro esophageal reflux leads to the variable reactive and inflammatory changes in the lower esophagus and predisposes the development of Barrett's esophagus (BE). BE is the replacement of normal stratified squamous epithelium to specialized intestinal metaplasia at distal esophagus. It is also associated with an increased risk for esophageal adenocarcinoma (EAC) which develops through dysplasia.[1-2]

BE has increased over the past four decades in western countries. When BE and EAC is still unconventional and rare in Asian populations, several Asian studies have demonstrated increase in the prevalence of EAC [2]. With the increasing incidence of EAC, much interest has been focused on better diagnosis of Barrett's esophagus (BE). Early detection of BE has become an essential pre-requisite to circumvent progression of EAC. Proper diagnosis of BE requires both endoscopic and histologic confirmation of metaplastic columnar lined epithelium.[3-5]

Novel endoscopic methods are employed in the diagnosis of BE [2] and Hematoxylin and Eosin (H&E) staining are used to assess the histological grading of biopsies, collected from the patients having symptoms of BE[4]. In particular, Alcian blue (AB) and Periodic Acid Schiff (PAS) staining are used to increase the sensitivity of the endoscopy and biopsy procedure in detecting early stage of EAC.[6] Columnar and goblet cells present in the intestinal metaplasia of BE produce mucins that can be characterised using mucin histochemistry. A change in the mucin histochemistry is the hallmark of the Barrett's adenocarcinoma.[7]

Previous reports have suggested that the presence of acidic mucins in the goblet cells of intestinal metaplasia is a characteristic feature of Barrett's esophagus. The presence of sulfomucins (stain brown-black) in columnar cells is a characteristic feature of type III intestinal metaplasia of the stomach. In BE, it is very common to have sialomucin (stain blue) containing columnar cells.[7-8]

AB and PAS staining can be exploited in the detection of acidic and neutral mucins in the esophageal tissue.[8] Hence, in the present investigation, AB and PAS staining was used to analyze the role of acidic and neutral mucins in the progression of Barrett's adenocarcinoma in all biopsied/resected samples.

### Materials and Methods

#### Sample collection

The resected/ biopsy specimens were obtained from the distal part of the esophageal epithelium of 140 specimens who underwent diagnostic gastrointestinal tract endoscopy with the symptoms of GERD (heartburn, acid regurgitation and dysphagia) at Stanley Medical College and Hospital, Chennai. Patients with all forms of liver disease, esophageal varicose and history of non steroid anti-inflammatory drug intake were excluded for

this study. All samples were obtained from each of these patients prior to the initiation of any therapy or treatment with proper consent from the patients attending the Department of Gastroenterology. Among these 97 patients were male and 43 patients were female. Collected samples were fixed in 10% formalin, processed and embedded according to standard protocol. All the cut sections (4µm thickness) were stained by Hematoxylin and Eosin (H&E) and histologically classified into Normal [N] (34), Barrett's esophagus [BE] (27), Low grade dysplasia [LGD] (29), High grade dysplasia [HGD] (24) and esophageal adenocarcinoma [EAC] (26) based on modified Savery Miller classification system.[9] The ethical committee of Stanley Medical College and Hospital, Chennai, had approved the study protocol.

#### Alcian blue staining

Tissues sections were deparaffinized in xylene and rehydrated through graded alcohols and finally washed well in running tap water for 20 min. The sections were flood in 3% acetic acid for 3 min at room temperature and washed with running tap water for 5 min, and again the sections were flood with Alcian blue solution for 30 min. The sections were washed with running tap water for 10 min and counterstained with Nuclear Fast Red for 5 min and finally washed with running tap water for 1 min then the sections were dehydrated and mounted with DPX.

#### PAS staining

Tissues sections were deparaffinized in xylene and rehydrated through graded alcohols and finally washed well in running tap water for 20 min. The sections were flood in 1% Periodic acid for 5 min at room temperature and washed with running tap water for 5 min, and again the sections were flood with Schiff's reagent for 2-5 min until pink colour develops. The sections were washed with running tap water for 3 min and counterstained with Harris Hematoxylin for 1-2 min and finally washed with running tap water for 10 min then the sections were dehydrated and mounted with DPX.

#### Statistical analysis

Chi square test was used for the percentage of samples with positive staining among histological grades using SPSS version 17.

### Results

#### AB staining

AB stain was gradually increased from the normal to adenocarcinoma stages of the esophageal tissue (Fig 1). Of the 34 normal samples, 14.7% (5/34 cases) was positively stained and 85% (29/34 cases) was negatively stained. In 27 Barrett's esophagus 29.6% (8/27 cases) was positively stained and 70.4% (19/27 cases) was negatively stained. In 29 Low grade dysplasia 34.5% (10/29 cases) was positively stained and 65.5% (19/29 cases) was negatively stained. In 24 High grade dysplasia 41.7% (10/24 cases) was positively stained and 58.3% (14/24 cases) was negatively stained. In 26 adenocarcinoma tissues 80.8%

(21/26 cases) was positively stained and 19.2% (5/27 cases) was negatively stained (Table 1). There was a significant association between normal and high grade dysplasia at 5% level and highly significant association between normal and adenocarcinoma at 1% level with respect to AB expression.

**PAS staining**

The PAS staining was high in normal esophageal tissue and it was gradually decreased in the progressive stages of esophageal adenocarcinoma (Fig 2). Of the 34 normal samples, 76.5% (26/34 cases) was positively stained and 23.5% (8/34 cases) was negatively stained. In 27 Barrett's esophagus 63.0% (17/27cases) was positively stained and 37.0% (10/27 cases) was negatively stained. In 29 Low grade dysplasia 55.2% (16/29 cases) was positively stained and 44.8% (13/29 cases) was negatively stained. In 24 High grade dysplasia 54.2% (13/24 cases) was positively stained and 45.8% (11/24 cases) was negatively stained. In 26 adenocarcinoma tissues 23.1% (6/26 cases) was positively stained and 76.9% (20/27 cases) was negatively stained (Table 2). There was a highly significant association found between normal and adenocarcinoma at 1% level with respect to PAS expression.

**Discussion**

The present study revealed, BE was now clearly discerned as a pre-neoplastic condition in EAC, which was diagnosed endoscopically and confirmed histologically. However, this was credibly not justified with an adequate number of biopsies with H&E staining. Biopsies, which were initially found to be negative in H&E staining, were found to be positive for intestinal metaplasia after AB and PAS staining and the results obtained were reproducible.

Nandurkar has also reported that AB and PAS assessment could increase the sensitivity in detecting intestinal metaplasia of BE in the pre-neoplastic condition of EAC.[6] Similarly, Cooper et al identified five additional cases of specialized intestinal epithelium in 11 children with standard BE using AB staining that were missed with H&E staining.[10] Furthermore, Gottfried et al, has also illustrated the importance of AB staining over H&E staining, in the diagnosis of intestinal metaplasia of BE.[11]

Jass depicted that intestinal metaplasia was characterized by goblet cells secreting acidic mucins in columnar epithelium of the well differentiated EAC and acidic mucin staining might permit the identification of patients at risk for developing esophageal adenocarcinoma complicating BE.[12] Peuchmaur studies on acidic mucins in patients with BE revealed its maneuver as a useful marker and considered as a form of dysplasia in the screening for adenocarcinoma.[13] Womack and Harvey also resolved that a predominance of acidic mucins was a form of low-grade dysplasia. [14] The presence of acidic mucin would therefore, help to identify individuals with BE who are at increased risk for developing adenocarcinoma.

The data obtained from the present research showed a stepwise increased level of acidic mucins stained in AB staining and decreased level of neutral mucins stained in PAS staining, which may assist in the detection of progression of Barrett's adenocarcinoma.

**Conclusion**

Combination of acidic and neutral mucins was prevailed in inflammatory lesions of the esophagus. In neoplastic conditions of EAC, most of the mucins were found to be acidic with decreased level of neutral mucins detected by AB / PAS staining at 2.5 pH. H&E staining may fail to show intestinal metaplasia in BE, hence, AB/PAS could be used as a routine stain to diagnose BE and histological confirmation in the progressive stages of EAC.

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**Table 1: Alcian blue stain of Barrett's adenocarcinoma**

Clinicopathological stages	Alcian blue stain		N	χ <sup>2</sup> value	P Value
	+ve	-ve			
Normal	5 (14.7%)	29 (85.3%)	34	-	-
Barrett's esophagus	8 (29.6%)	19 (70.4%)	27	1.999	0.157
Low grade dysplasia	10 (34.5%)	19 (65.5%)	29	3.374	0.066
High grade dysplasia	10 (41.7%)	14 (58.3%)	24	5.334	0.021*
Adenocarcinoma	21 (80.8%)	5 (19.2%)	26	26.186	<0.001**

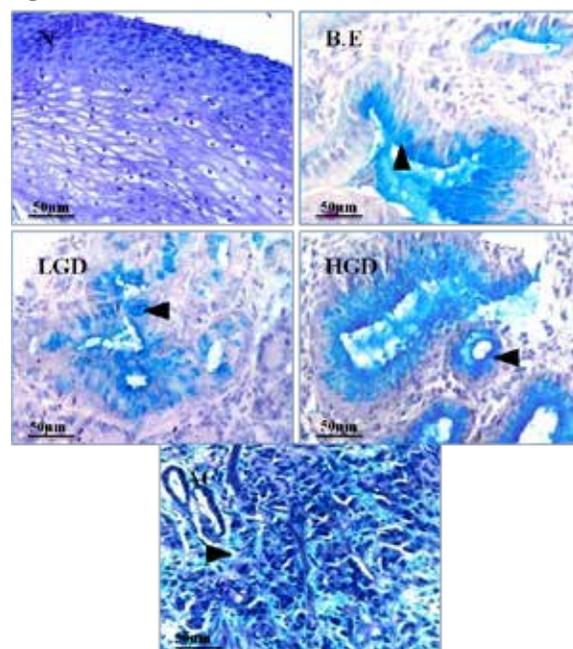
-ve, no staining or <5% cells; +ve, stained cells; \*P value<0.050- Significant; \*\* P value<0.001- highly significant; the value within bracket refers to row percentage.

**Table 2: PAS stain of Barrett's adenocarcinoma**

Clinicopathological stages	PAS stain		N	χ <sup>2</sup> value	P value
	+ve	-ve			
Normal	26 (76.5%)	8 (23.5%)	34	-	-
Barrett's esophagus	17 (63.0%)	10 (37.0%)	27	1.320	0.251
Low grade dysplasia	16 (55.2%)	13 (44.8%)	29	3.195	0.074
High grade dysplasia	13 (54.2%)	11 (45.8%)	24	3.177	0.075
Adenocarcinoma	6 (23.1%)	20 (76.9%)	26	16.876	<0.001**

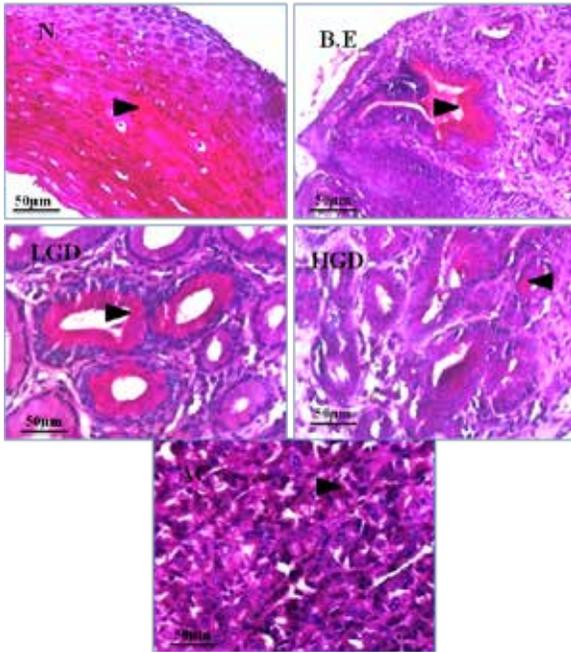
-ve, no staining or <5% cells; +ve, stained cells; \*\* P value<0.001- highly significant; the value within bracket refers to row percentage.

**Fig 1. Alcian blue stain of Barrett's adenocarcinoma**



**Abbreviations: N: normal; B.E: Barrett's esophagus; LGD: low grade dysplasia; HGD: high grade dysplasia; AC: adenocarcinoma.**

Fig 2. PAS stain of Barrett's adenocarcinoma



Abbreviations: N: normal; B.E: Barrett's esophagus; LGD: low grade dysplasia; HGD: high grade dysplasia; AC: adenocarcinoma.

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