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# Histochemical Detection of O-acetyl Sialomucin in Adenocarcinoma in Gastric Biopsies: An Old Mucin with New Perspective

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#### Abstract

**Objective:** To determine the O-acetyl sialomucin content in different type of adenocarcinomas in gastric biopsies.

Study design: Descriptive, Cross sectional study.

Place and duration of study: Histopathology department, Sheikh Zaid hospital, Lahore, Pakistan from January 2012 to July 2014.

**Materials and methods:** Total sixty cases of gastric adenocarcinoma diagnosed on H&E were included in this study. Patient's gender and age were noted. Periodate borohydrate PAS saponification technique to detect sialomucin was applied and the results were recorded. The data was analyzed by using computer software program SPSS version 20.

**Results:** Out of 60 patients of gastric adenocarcinoma, the male to female ratio was 2:1. The age of the patients ranged between 30 to 80 years with an average age of  $51.4 \pm 11.4$ . Out of the 60 cases, 31 were of intestinal type adenocarcinomas, rest 29 cases were diagnosed as diffuse type adenocarcinomas according to Laurén classification. On histochemical examination, 13 out of 31 cases were O acetyl sialomucin positive in intestinal adenocarcinoma group where as only 3 cases were positive in diffuse type adenocarcinoma by combined Periodate borohydride PAS saponification technique.

**Conclusion:** The clinicopathological and histochemical results in our study not only ascertain prognosis at the time of initial diagnosis but also incorporate sialomucin as therapeutic target in advanced cases of gastric adenocarcinoma.

**Keywords**: Sialomucin; Gastric adenocarcinoma; Periodate borohydride; PAS (Periodic shciff stain); Saponification technique; H&E (Hematoxylin and eosin)

## Introduction

Gastric carcinoma is a paramount health problem, owing to reduced overall survival despite advanced diagnostic and operative techniques. Global cancer statistics made in 2012 refers an incidence of 6.8% of gastric cancer, which places it in fifth rank, following cancers of the lung, breast, colorectal and prostate, with broad international variation on geological distribution [1] Regardless of therapeutic intervention; it still holds third place in cancer related death in both sexes worldwide [2].

A local study by Bhurgri et al. in his study on gastric carcinoma in Pakistan, revealed that the frequency of gastric cancer in males were 2.9% and 1.7% in females respectively during the years 1995-2002. In the course of years from 1995 to 2002, an 18% boost was observed in gastric carcinoma in males and 14% in females respectively [3] In our country, gastric carcinoma holds a dismal prognosis with only 13.3% survival rate over 5 years in one case series due to advanced stage of gastric cancer at time of presentation [4].

Gastric adenocarcinoma is the predominant malignant neoplasm of stomach making up to 90% of total cases. Laurén classified adenocarcinoma into two histological subtypes, intestinal and diffuse, depending on the degree of gland formation by the tumour cells [5] Intestinal type tumours are preceded by a precancerous stage, which is represented by the consecutive steps of superficial gastritis, atrophic gastritis, intestinal metaplasia, dysplasia and intramucosal carcinoma. Diffuse type gastric adenocarcinomas are also interrelated with gastritis but without atrophy [6] Goseki, in 1985 proposed another classification which was based on tumor histology and further subdivided into four subtypes based on tubular differentiation and intracellular mucin content [7]

Several modifications in mucin associated carbohydrates are observed in neoplastic epithelial tissues in patients with gastric adenocarcinomas [8]. In the multistep pathway of intestinal type adenocarcinoma, acid mucins replace the neutral gastric mucins. These acid mucins, in turn, can be sialic (sialomucin) or sulphated (sulfomucin) [9] Sialomucins are large, heavily O-glycosylated molecules containing terminal sialic acid molecule. Sialomucins are categorized histochemically into two types based upon the presence or absence of the O acetyl group. Sialidase resistant (O acetyl form) are not detectable by routine PAS technique, for which various histochemical techniques are used [10].

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In tumor cells two types of sialomucins have been observed. One type of sialomucin is released from the tumor cells whereas other is on the cell surface as a heterodimeric complex [11]. Sialomucins protect tumor cells from the immune system due to their large size, extended structure and negative charge preventing immune and tumor cells interactions [12] Presence of sialomucin leads to decreased adhesion of tumor cells to the connective tissue, increasing tumor chances to metastatize [13,14] Various studies done on sialomucins showed its presence in metaplastic and neoplastic epithelium, but not in normal gastric mucosa. A study by Mull revealed presence of O acetyl sialomucin in 42.9% cases of gastric adenocarcinoma [11]. In a study conducted at China, the incidence of O acetyl sialomucin was higher in intestinal adenocarcinoma [15]. Mandal in 2013 illustrated the presence of sialomucins in intestinal type of gastric adenocarcinomas [16]. In the light of the above-mentioned facts this study was designed to see the morphological types of gastric adenocarcinoma and amount of sialomucin in adenocarcinoma in gastric biopsies [17].

### Material and Methods

A total of sixty cases, diagnosed in Histopathology department, Sheikh Zaid hospital, Lahore, Pakistan from January 2012 to July 2014 were included in this study. Clinical data such as age, gender, tumor size, tumor location in stomach on endoscopic examination were noted. Histopathological features such as degree of neoplastic glandular formation was recorded following CAP guidelines. Cases were divided into intestinal and diffuse type adenocarcinoma. Specimen received without formalin and patients who received prior chemotherapy were excluded from the study.

O-acetyl Sialomucin was detected by applying Periodate borohydride PAS saponification technique on formalin fixed and paraffin embedded sections. The section was treated with 0.5 percent aqueous periodic acid, for 30 minutes. After Washing well in distilled water, freshly prepared borohydride solution was applied for 30 minutes. The section was washed in running tap water and than in 70% alcohol, followed by the alcoholic potassium hydroxide solution for 30 minutes. Finally the section was washed in 70% alcohol, distilled water and then treated with 0.5% periodic acid for 5 minutes, followed by washing in distilled water. The section was treated with Schiff's reagent for 15minutes [10]. Statistical analysis was done by using SPSS calculator version 20. Mean and standard deviation was calculated for age of the patient and size of the tumor. Percentage of Sialomucin was calculated using 4 tier scheme of Absent, Mild, moderate and marked [18].

## Results

Out of 60 patients of gastric adenocarcinoma, the male to female ratio was 2:1. The age of the patients ranged between 30 to 80 years with an average age of  $51.4 \pm 11.4$ . Most patients were found to be in the 5th and 6th decade (Figure 1). The size of the tumour ranged from 1.8 cm to 14 cm with an average diameter of  $5.2 \pm 7.2$  cm. Out of the 60 cases, 31 were of intestinal type adenocarcinomas, rest 19 cases were diagnosed as diffuse type adenocarcinomas according to Laurén classification. On histochemical examination, 13 out of 31 cases were O acetyl sialomucin positive in intestinal adenocarcinoma group where as only 3 cases were positive in diffuse type adenocarcinoma by combined Periodate borohydride PAS saponification technique (Table 1)

# Discussion

In Pakistan, 3840 per million new cases of gastric carcinoma are diagnosed each year with an incidence rate of 3.8%. Jamal et al. in his study, over a period of fourteen years, found an increased trend of gastric carcinoma in male patients, in whom the incidence rate has raised from 2.8% to 4.1% [19]. In our study, more than 50% of patients were above 51 years of age. The results are compatible with local study and international studies in which same frequency was noted for patients of gastric adenocarcinoma [4,20] Other studies by Jamal, Burghari et al. and Peng et al. on gastric carcinoma, revealed double ratio of male patients as compared to female patients supporting our results which showed similar trend [3,19,21,22] (Figures 2 and 3).

Body and antrum was the commonest site to be involved by the

		Frequency	Percent	Valid Percent	Cumulative Percent	
Valid	Male	41	68.3	68.5	68	
n=60	Female	19	31.6	31.5	32	
	Total	60	100	100	100	

 Table 1: Gender distribution in gastric adenocarcinoma (n=60).



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gastric adenocarcinoma. Our study is similar to an international study by Shi in which distal stomach was the commonest site to be involved [23]. Most cases were of intestinal adenocarcinoma 31 (51.6%), whereas 29 (48.3%) cases were of diffuse type of gastric adenocarcinoma. Our result was supported by recent conference in 2014 which showed nearly same observation [24]. In contrast to our results Afridi SP observed 66.6% of diffuse type adenocarcinoma and 20% of intestinal adenocarcinoma in gastric carcinoma which may be due to small sample size (n=15) [25] (Table 2).

In our study O acetyl sialomucin was detected in 13 out of 31 (41.9%) cases of intestinal adenocarcinoma patients comparable with study by PJ Mull in which the expression of O acetyl sialomucin was 42.9%.15 Saez C, in a study on 17 cases of gastric adenocarcinoma patients, demonstrated O acetyl sialomucin as the predominant type of acidic mucin in carcinomatous gastric mucosa [26]. Only 3 (10.3%) cases of diffuse type adenocarcinoma showed O acetyl sialomucin expression. Cheah demonstrated sialomucin in 13% of cases of diffuse gastric adenocarcinoma. Furthermore his study concluded that loss of neutral mucin and presence of sialomucin in metaplastic



Figure 2: Intestinal adenocarcinoma (Well differentiated). Positive result (Combined Periodate borohydride saponification PAS stain 40x).



Figure 3: Photomicrograph of section of diffuse gastric adenocarcinoma. Positive result (Combined period ate borohydride saponification PAS stain, 40x).

epithelium should raise an alarm for underlying carcinomatous focus [27] (Table 3).

Presence of O-acetyl sialomucin in cases of gastric adenocarcionoma has both prognostic and therapeutic implications. Its presence in well differentiated adenocarcinoma will lead to aggressive behavior despite favourable histology as its presence not only masks tumour cells but also serve as growth factor for them. Presence of O acetyl sialomucin in our study was an independent adverse prognostic factor as Rivera-Hueto et al. found in their study on gastric adenocarcinoma [28]. Carcinomas producing O acetyl sialomucin require more aggressive surgical excision as chances of their recurrence increases with decrease overall survival [29] (Table 4).

### Conclusion

O-acetyl sialomucin presence provides tumor cells several attributes, which contributes to tumor progression and metastasis. In an era where standard prognostic, predictive and diagnostic parameters are ever changing, simultaneous assessment of clinicopathological and mucin characteristics at the time of initial diagnosis, can provide a beneficial role in individual therapeutic strategies in cases of gastric carcinoma in Pakistan.

Clinicopathological	Number of cases (n=60)			
Characteristics	%			
Age (years)				
Mean ± SD	51 ± 11.4			
Range	30-80			
Gender	41			
Male	19			
Female				
Tumor size (cm)				
Mean ± SD	5.2 ± 7.2			
Range	1.8-14			
Stomach location				
Proximal	64%			
Distal	36%			

Table 2: Clinicopathological characteristics of gastric adenocarcinoma (n=60).

	Gastric adenocarcinoma	Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Intestinal type	31	51.66	52	52
	Diffuse type	29	48.33	48	48
	Total	60	100	100	100

Table 3: Histologic types and percentage of gastric adenocarcinomas (n=60).

	O acetyl sialomucin			
Gastric Adenocarcinoma	Absent	Mild	Moderate	Marked
(n=60)	0	+	++	+++
Well differentiated adenocarcinoma (n=12)	8	2	1	2
Moderately differentiated adenocarcinoma (n=9)	3	3	2	1
Poorly differentiated adenocarcinoma (n=10)	8	1	1	0
Diffuse type adenocarcinoma (n=29)	26	1	2	0

Table 4: Presence and Amount of O acetyl sialomucin in various types of Gastric adenocarcinoma (n=40).

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