

Original Research Article

Evaluation of the significance of mucin stain in a Spectrum of Colorectal Carcinoma

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Abstract: This study was conducted on 50 diagnosed cases of Colorectal Carcinoma by Histopathology irrespective of age and sex in Assam Medical College and Hospital during July 2012 to June 2013, by taking proper history, doing relevant investigations, Histopathological examination and Histochemistry using Periodic Acid Schiff's (PAS) and Alcian Blue. Our study showed the peak incidence of colorectal carcinoma in the age group of 40-49 yrs and the patients were predominantly males. Of these 50 cases mucin content was seen in 35 cases and acidic mucin was the prevalent type. Out of these 35 cases the cases which showed presence of mucin content 50% or above were categorized as mucinous Adenocarcinoma which amounted to 10% of the cases studied. This study revealed that mucinous adenocarcinoma occurred in an early age group, mostly in the Rectum and had an aggressive behaviour. Hence, categorization of the mucinous adenocarcinoma is of utmost importance for proper management of these cases. In spite of the advancement in diagnostic modalities, Mucin histochemistry is a valuable and cost effective tool for assessing the mucin content of colorectal Carcinomas. Previous studies have shown that mucin can be regarded as a prognostic indicator in these adenocarcinomas.

Keywords: colorectal carcinoma, histochemistry, mucin, PAS, Alcian Blue

INTRODUCTION

Colorectal Carcinoma has been one of the most common cancers since 1975 with one million new cases and approximately 500,000 deaths each year [1]. The incidence is high in the western world, while in India the incidence rate is 4.2 and 3.2 per 100,000 in males and females respectively [2]. Colorectal Carcinoma is the third most common cancer in men and the second in women World Wide [3]. A higher risk of colorectal carcinoma was found in persons consuming a diet poor in fibers and rich in meat [4, 5]. Over the past two decades an intense research effort has focused on elucidating the genetic defects and molecular abnormalities associated with the development and progression of colorectal adenomas and carcinomas [6, 7]. In India the incidence rate is 4.2 and 3.2 per 100,000 in males and females respectively [3].

Adenocarcinomas account for the vast majority of Colorectal Carcinomas, about 90% [8]. It is graded as well, moderately and poorly differentiated mainly on the regularity of the glandular architecture as per the

WHO classification. Mucin production varies from minimal to abundant (that is 50% or more of the Tumour area having mucin content) these cases are categorized as mucinous adenocarcinoma. About 10% of colorectal carcinomas are mucinous type. Mucin production by the Tumor cells has an impact on the Prognosis and Therapeutic management. This study was undertaken with the following aims and objectives

- To evaluate the mucin content in the Tumours Histochemically
- To correlate the mucin content with the histological gradings of the Tumours.

MATERIALS AND METHODS

A total of fifty cases of formalin fixed paraffin –embedded tissue sections diagnosed as Colorectal Carcinoma irrespective of age and sex were included in this study. The study included proper history, clinical examination, relevant investigations, histopathological examination and histochemical stains.

Ethical clearance: was taken from the Institutional ethical committee before starting the study.

Grossing Protocol:

Specimens were received after colectomy in our histopathology laboratory. For every specimen the nature of the surgical procedure was noted. The length of the entire specimen was measured. The tumour was palpated from the outer aspect and photographed from both aspects. The macroscopy of the tumour was noted (ulceroproliferative/infiltrative). Then the distance of the tumour from the proximal and the distal resected margins were measured. The involvement of the tumour circumferentially, anteriorly, posteriorly and invasion of the bowel wall was recorded. Absence or presence of perforation was noted. All lymphnodes were dissected off the specimen and were submitted according to the level of the tumour. Four or five sections of the tumour, inclusive of serosa and / or circumferential margins were taken. The terminal ileum and appendix were examined properly and any abnormality noted.

Representative sections were taken and fixed in 10% buffered formalin for histopathological examination. Sections were stained with the routine haematoxylin and eosin stain using standard protocol. The histological diagnosis was ascertained regarding the type, grade and the amount of mucin content [9]. The tissue with the mucin content was selected for special staining with PAS to assess the presence of mucin, which was again stained by Alcian blue/PAS to differentiate between neutral and Acidic mucin.

The interpretation of PAS stain was as follows:

PAS positive substances stain pink to red with blue nuclei. The PAS positive cases were further stained with AB/PAS stains to differentiate between types of mucin namely – Neutral and Acidic mucins [10]. AB/PAS Kit is a combined method utilizing the properties of both PAS and Alcian Blue to demonstrate acid mucin and neutral mucin.

The interpretation of AB/PAS staining was as follows:

Neutral mucins were stained Magenta colour while Acidic mucins were stained blue [10]. The colorectal carcinoma cases were divided into the following groups on the basis of mucin content in the whole section. The cases with less than 20% mucin were categorized as mild, those cases with 20-50% mucin as moderate and those with more than 50% mucin as marked. Sections from normal colon and rectum were taken as controls. Some studies have seen that colorectal carcinoma with marked mucin content

require wide excision, tend to recur locally and carry a poor prognosis due to imbibition of water by the mucin, which swells and cleaves the tissue planes, dispersing malignant cells [11]. Some authors consider mucin content to be an independent adverse prognostic factor, but however other researchers still debate the fact that it cannot be regarded as an independent prognostic factor [12]. Studies by Naila, Kazi and Bushra in 2009 observed that mucin content together with histological grade can be regarded as important prognostic indicators [13].

STATISTICAL ANALYSIS:

All analysis were done using SPSS version 11.

RESULTS AND OBSERVATION

A total of 50 cases of histologically confirmed colorectal carcinomas irrespective of age and sex were studied. The peak incidence was seen between 40- 49 yrs with maximum cases in between 30-70yrs of age (Table 1) and predominantly the cases were males.

Table 1 : Age Distribution

Age In Years	Male	Female	Total	Percentage
20-29	3	0	3	6
30-39	5	1	6	12
40-49	10	5	15	30
50-59	5	7	12	24
60-69	5	3	8	16
70-79	5	1	6	12
Total	33	17	50	100

Of the 50 cases of adenocarcinoma only 5 cases showed presence of mucin content more than 50 % so these cases were categorized as Mucinous adenocarcinoma. More than half of the cases (56%) were from Rectum, followed by transverse colon, sigmoid colon, ascending colon and descending colon (Table 2).

Table 2 : Site Distribution

Site Of Primary Tumour	Number	Percentage (%)
Ascending Colon	6	12
Transverse Colon	7	14
Descending Colon	2	4
Sigmoid Colon	7	14
Rectum	28	56

The mode of differentiation of these tumours according to WHO guidelines were mostly moderately differentiated (60%), well differentiated (22%) and poorly differentiated (18%) (Fig 1).

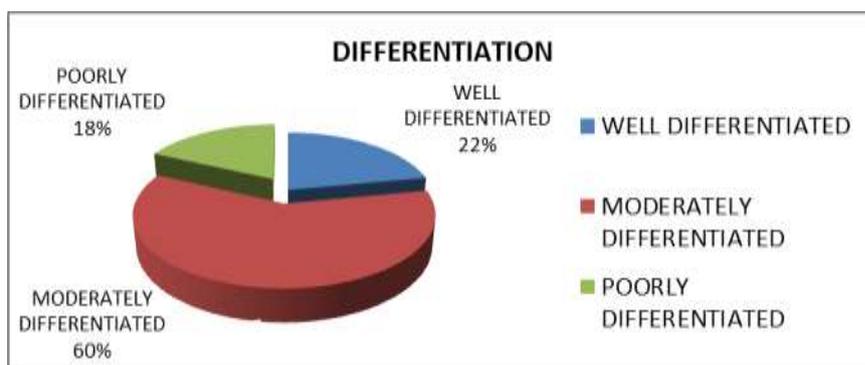


Fig. 1: Shows Differentiation of tumours

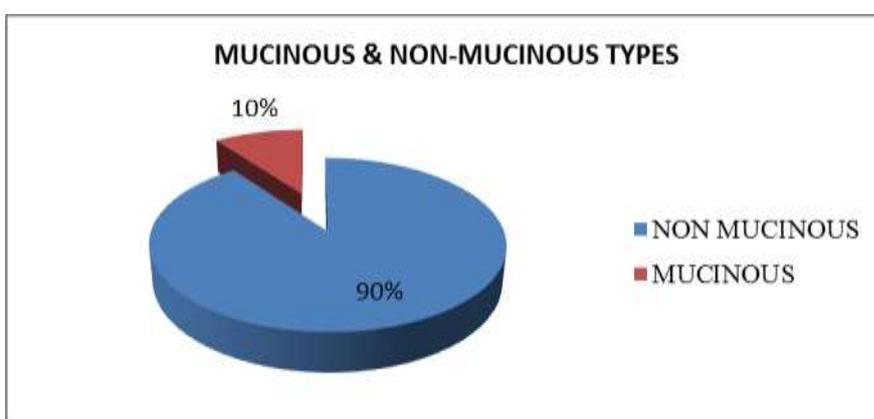


Fig 2: Shows Mucinous and Non-Mucinous Types

Regarding the mucin content, out of the 50 cases studied mucin was seen in 35 cases and was absent in 15 cases. Tumours with mucin content >50% were included in mucinous adenocarcinoma, and those with mucin content <50% were included in the non-mucinous group [14].

To detect the nature of mucin Alcian blue/PAS staining was done. Out of the 35 cases 34 showed presence of Acidic mucin, while only 1 case showed presence of Neutral mucin. Hence the predominant

mucin in this study was Acidic mucin. Mild to moderate Acidic mucins were predominantly observed in well and moderately differentiated adenocarcinoma, 5 cases of poorly differentiated adenocarcinoma showed marked Acidic mucin [15]. Out of the 50 cases studied, 34 cases showed presence of acidic mucins and only one case showed presence of neutral mucin. Of these 34 cases, 26 showed mild acidic mucin, 3 showed moderate content and only 5 cases demonstrated marked acidic mucin (Table 3).

Table-3: Staining Results

MUCIN	MILD	MODERATE	MARKED
ACIDIC MUCIN	26	3	5
NEUTRAL MUCIN	1	0	0
NO MUCIN	15		

It was observed that 3 cases of mucinous adenocarcinoma presented with ovarian metastasis, omental seeding and urethral fistula respectively. In our study it was observed that the adenocarcinomas present in the rectum were mostly associated with lymph node metastasis and had a bad prognosis. Out of the 5 cases

of mucinous adenocarcinoma in our study 4 cases were present in the Rectum and 1 case was in the colon. Lymph node metastasis was seen in 3 cases of mucinous adenocarcinoma of the Rectum while the other two cases did not have lymph node metastasis.

Gross findings:

Grossly the growths were mostly polypoidal, ulcerative and annular as shown in {Fig. 3 (a,b)}



Fig. 3: Post-operative specimen of a polypoidal growth in the rectum (a) and annular growth in the transverse colon (b)

Histological findings:

Microscopically the tumours were mostly adenocarcinomas. Of the 50 cases 30 cases were moderately differentiated {Fig.4 (b)}, (WHO) 11 cases were well differentiated {Fig. 4 (a)} (WHO) and only 9

cases were poorly differentiated {Fig. 4 (c)} (WHO). On the basis of the mucin content the tumours showing mucin content of > 50% was seen only in 5 cases which were categorized as mucinous adenocarcinoma {Fig. 4 (d)}.

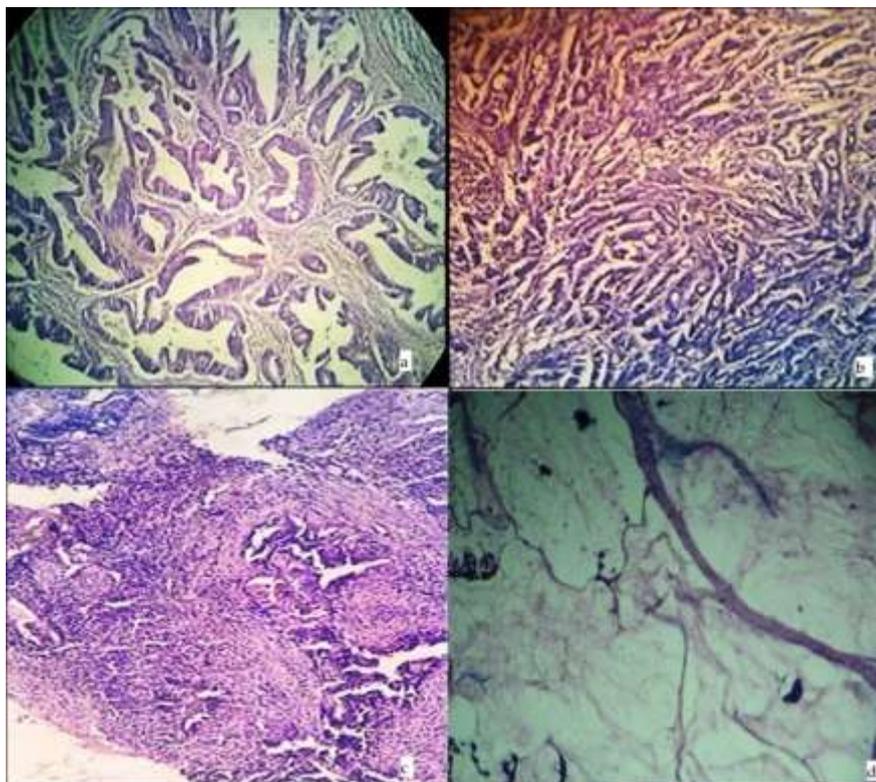


Fig. 4 Photo-micrograph showing well differentiated adenocarcinoma (a), moderately differentiated adenocarcinoma (b), poorly differentiated adenocarcinoma (c) and mucinous adenocarcinoma (d) (H&E)

Findings on PAS stain:

All the 50 cases were subjected to PAS stain of which mucin was detected in 35 cases {Fig.5 (a)}. These 35 cases were further stained with Alcian/PAS staining to detect the nature of mucin which is shown in {Fig. 5 (b) and Fig.5 (c)}.

DISCUSSION:

Amongst the gastrointestinal malignancy, Colorectal Carcinomas are the commonest variety. Many factors are responsible for its increased incidence; recent trends have shown that its incidence is even increasing in the developing countries. In our study colorectal carcinoma accounted for 0.6158% of the total patients admitted in the surgical department of our institute. However the number will be higher as some patients with advanced disease are not included as they are been referred to higher centres for treatment. In the present study the commonest age group for Colorectal Carcinomas was 40-49 years which was consistent with the findings of Shah and Wani [16], in their study the age group was 40-60 years. Out of the 50 patients 33 were males and 17 were females, hence the ratio between male: female was 1.94:1. Colorectal carcinomas were predominantly seen in males in the studies done by Qing *et al.*; in 2003 [17] and Leishram *et al.*; in 2010 [18]. Most of the cases were detected in the Rectum in our study. This finding was consistent with the findings of Qing *et al.*; in 2003 [17] and Neil & Jones [19]. In our study the commonest histological type was Adenocarcinoma amongst which mucinous adenocarcinoma accounted for 10% and was similar to the studies done by Umple *et al.*; in 1985 [20] and Oconnel *et al.*; in 2004 [21]. Previous studies have shown that mucinous adenocarcinoma comprises about 15% of colorectal carcinomas and occurs most commonly in the rectum [18, 22, 23].

Certain pathologic features have been considered important prognostic determinants in adenocarcinoma of colon and rectum. A distinctive pathologic feature is mucin production, usually extruded into the stroma with the formation of a gelatinous matrix described as mucinous carcinoma. About 10% of large bowel carcinomas secrete abundant mucus [24].

Mucins are complex carbohydrates secreted by epithelial and connective tissue cells. Mucin glycoproteins are thought to play an important role in protecting the intestine from chemical or physical injury but the mechanisms of protection and the possible relationship between mucin structure and function are incompletely understood. Structurally, purified

intestinal mucins are a heterogenous and polydisperse group of large molecular weight glycoproteins which have regional and developmental difference in composition. Mucin glycoprotein has been implicated in the pathogenesis of epithelial cell malignancies [25]. Alteration of expression pattern of mucins has been described in carcinomas as well as in their precursor's lesions. The predominance of acidic mucins have been demonstrated in the goblet cells of mucosa both around carcinomas and in more distant patches, resembles the mucus secretion pattern of human foetal gut. Such changes could therefore be further evidence of the reversion to an embryonic state, which may characterize the early stages of carcinogenesis. A positive result will lead to a more thorough examination of the patient and increase the chance of an early diagnosis of cancer [26].

On the basis of the mucin content that is > 50% mucin in the entire tumour were divided into mucinous adenocarcinoma comprising of 5 cases which accounted for 10% and non-mucinous adenocarcinoma comprising of 45 cases which accounted for 90%. The predominant mucin that was observed in our study was acidic mucin which was consistent with the findings of Hadi *et al.*; in 2009 [27], Ionila M *et al.*; in 2011 [28] and Nikumbh RD *et al.*; in 2012 [29]. Mucinous adenocarcinoma was found to be common in young patients and were associated with ulcerative colitis, anorectal fistula or malignant transformation of villous adenoma which was consistent with our findings [30]. The prognosis of mucinous adenocarcinoma was more aggressive in case of those present in the rectum [31]. In contrast mucinous adenocarcinoma of the colon frequently showed microsatellite instability and the prognosis is relatively good.

CONCLUSION:

This study was an attempt to evaluate mucin Histochemically in Colorectal carcinomas and determine its importance. In our study adenocarcinomas were found to form the main group of Colorectal Carcinomas. Mucinous adenocarcinomas occurred in an early age group and Acidic mucins were found to be the predominant type. Determining the type of Mucin (i.e. neutral or acidic) may be helpful in evaluating neoplastic changes within a tissue. Due to recent advances in diagnostic modalities such as Immunohistochemistry, the mucin histochemical study seems to lag behind. In spite of these advancements, Mucin histochemical study provides a valuable and cost-effective tool for assessing the prognosis of these mucinous adenocarcinomas and can be taken as an ancillary diagnostic tool in histopathology. To regard

mucin as a prognostic indicator, larger studies should be undertaken in the future to establish it.

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