Original Article

Utility of Assessing Mucin Characteristics in Colorectal Adenocarcinoma by Applying Cost Effective Combined Periodic Acid Schiff and Alcian Blue

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Abstract

Background: Colorectal carcinoma is the most common malignancy of the gastrointestinal tract. Adenocarcinomas account for 90% of the colorectal carcinomas. Mucinous adenocarcinoma is a subtype of colorectal adenocarcinoma. **Objective:** This is a study to see mucin characteristics in colorectal adenocarcinoma to help in diagnosis and predict prognosis. **Materials and methods:** The study comprises of 50 paraffin blocks of all patients who were histopathologically diagnosed as having colorectal adenocarcinoma in the Department of Pathology, Rajshahi Medical College. **Results:** Out of total 50 cases, most 13 (26%) cases belonged to the age group of 51-60 years and the patients were predominantly males. Mucin content was seen in 43 cases and acidic mucin was the prevalent type. Out of these 43 cases, the cases which showed presence of mucin content above 50% are categorized as mucinous. Rectum being the most common site of involvement (36%) followed by ascending colon, sigmoid colon and descending colon. In this study, most 20 (40%) were grade 3 tumors, followed by 18 (36%) grade 2 tumors and 12 (24%) grade 1 tumors. Marked mucin positivity was found mostly in grade 3 tumor. **Conclusion:** This study has showed that predominance of acid mucin increases along with the increase in tumor grade. So, mucin may be a helpful marker for early recognition of cancerous transformation and prediction of future prognosis.

Keywords: Adenocarcinoma, Tumor grade, mucin, PAS, Alcian Blue.

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Introduction

Colorectal cancer is the most common malignancy of the gastrointestinal tract and ranks third in terms of incidence and second in mortality, overall. More than 1.9 million new colorectal cancer cases and 9,35,000 deaths are estimated to occur in 2020, representing about 1 in 10 cancer cases and deaths [1].

Adenocarcinomas account for 90% of the colorectal carcinomas [2]. Mucinous adenocarcinoma is a subtype of colorectal adenocarcinoma with more than 50% of the lesion composed of mucin [3]. The colorectal carcinoma cases with less than 20% mucin are categorized as mild, those cases with 20-50% mucin as moderate and those with more than 50% mucin as marked [2].

Colorectal carcinoma with marked mucin carries a poor prognosis due to imbibition of water by the mucin, which swells and cleaves the tissue planes, dispersing malignant cells [4].

Though immunohistochemistry is an advanced diagnostic modality alongside the routine histopathology, but mucin study is a cost effec-

tive tool for diagnostic histopathology of colorectal carcinoma [5].

This study was undertaken to assess the mucin characteristics in colorectal adenocarcinoma and to compare our experience with findings in this literature.

Materials and methods

The present study was carried out in the Department of Pathology, Rajshahi Medical College, Rajshahi, Bangladesh from March 2021 to February 2023. It comprises 50 paraffin blocks of all patients, who were histopathologically diagnosed as having colorectal adenocarcinomas in this department. All histopathologically confirmed colorectal adenocarcinoma cases were included in this study. Poorly fixed sample were excluded.

Both H&E stain and combined PAS/AB stain were done according to the protocol followed in the Department of Pathology, Rajshahi Medical college. Slides of all cases were examined and then they were graded as well (grade 1), moderate (grade 2) and poorly (grade 3) differentiated carcinomas. On the basis of mucin content, colorectal adenocarcinomas were categorized as mild (<20% mucin), moderate (20- 50% mucin) and marked (>50% mucin). For the comparison of mucin content, a control section of combined PAS/AB stained normal colonic wall was used.

Results

The present study includes 50 cases of colorectal adenocarcinomas reported in the Department of Pathology, Rajshahi Medical College. The age group ranged from 20-75 years with a mean age 51.2±13.74. Most 13 (26%) cases belonged to the age group of 51-60 years. 3 (6%) cases were less than 30 years, 10 (20%) cases were within 31-40 years, 12 (24%) cases were within 41-50 years and 12 (24%) were in the age group of more than 60 years. Table-1 shows the age wise distribution of colorectal adenocarcinomas in our study.

Table I. Distribution of the study subjects by their age (n = 50).

Age (year)	Frequency n (%)
<30	3 (6)
31-40	10 (20)
41-50	12 (24)
51-60	13 (26)
>60	12 (24)
Mean ± SD	51.2 ± 13.74
Range (Min-Max)	(20-75)

There were 40 (80%) males and 10 (20%) females with a male to female ratio 4:1. Figure 1 shows gender distribution.

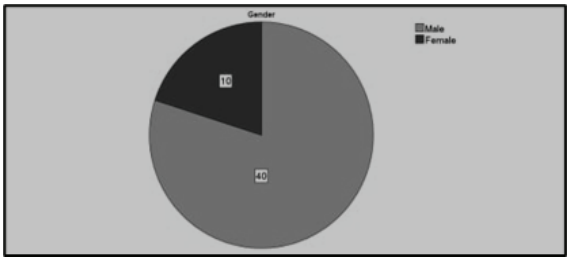


Fig. 1: Pie chart showing gender distribution (n = 50).

Out of 50 cases, most were located in the rectum 18 (36%), followed by ascending Colon 16 (32%), sigmoid colon 13 (26%) and descending colon 3(6%). Figure 2 shows distribution of cases according to the site of the tumor.

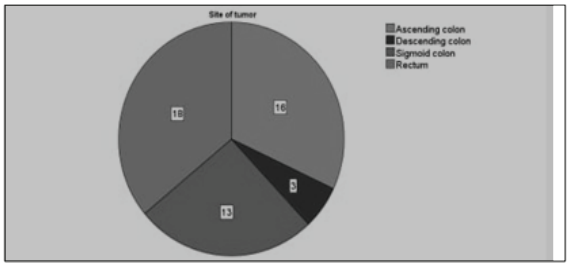


Fig. 2: Pie chart showing distribution of cases according to the site of the tumor (n = 50).

Most 20 (40%) were grade-3 tumors, followed by 18 (36%) grade 2 tumors and 12 (24%) grade 1 tumors. Figure 3 shows distribution of study subjects according to tumor grade.

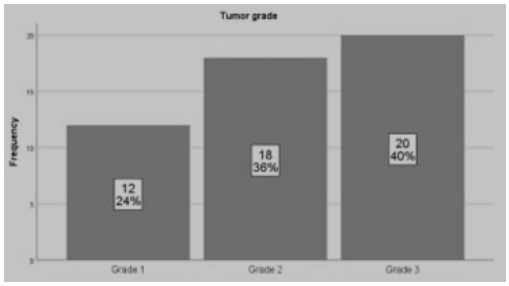


Fig. 3: Bar diagram showing distribution of study subjects according to tumor grade (n=50).

Out of 50 colorectal adenocarcinoma cases mucin positivity was found in 43 (86%) cases and 07 (14%) were mucin negative. Among the mucin positive cases mild mucin expression was found in 12 (24%) cases, moderate expression was found in 12 (24%) cases and strong expression was found in 19 (38%) cases. Figure 4 shows distribution of cases according to mucin content.

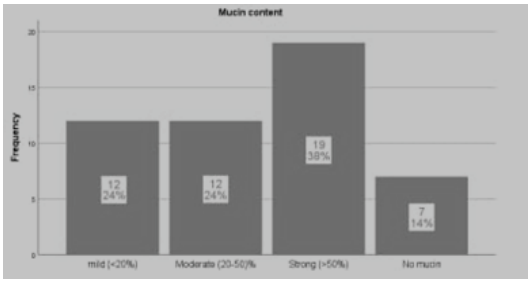


Fig. 4: Bar diagram showing mucin content (n = 50).

Out of 43 colorectal adenocarcinoma cases, marked mucin positivity was found mostly in grade 3 tumor that is 15 (83.3%) cases. 03 (16.7%) cases of grade 3 tumors showed mild mucin positivity. Moderate mucin positivity was found mostly in grade 2 in 07 (50.0%) cases and mild mucin positivity was found in grade 1 in 05 (45.5%) cases. Association of mucin content with tumor grade was statistically significant (p-value = 0.001) that is mucin content increases with the increase of tumor grade. Table-II shows association of mucin content with tumor grade.

Table II. Association of mucin with tumor type (n = 43).

Mucin content	Tumor Grade			*p value*
	Grade I	Grade II	Grade III	
Mild (<20%)	5 45.5%	4 28.5%	3 16.7%	0.001[s]
Moderate (20-50) %	5 45.5%	7 50.0%	0 0%	
Marked (>50%)	1 9.0%	3 21.5%	15 83.3%	

Both Mucinous and non-mucinous adenocarcinoma showed predominantly acidic mucin in 18 (94.7%) cases and 18 (75%) cases respectively. This association was not statistically significant. Table-III shows association of mucin type with tumor type.

Table III. Association of mucin content with tumor grade (n = 43).

Mucin Type	Tumor Type		*p value*
	Non mucinous	Mucinous	
Mixed with predominance of neutral mucin	6 25.0%	1 5.3%	0.082[ns]
Mixed with predominance of acidic mucin	18 75.0%	18 94.7%	

Discussion

Colorectal cancer is the most common malignancy of the gastrointestinal tract and ranks third in terms of incidence and second in terms of mortality [1]. Adenocarcinomas account for most colorectal carcinomas, about 90%.2 Colorectal adenocarcinoma with marked mucin content requires wide excision, tend to recur locally, and carry a poor prognosis. Mucinous adenocarcinomas are associated with poorer response to chemotherapy and chemoradiotherapy [4]. Though immunohistochemis-

try is a recent advanced diagnostic modality alongside the gold standard routine histopathology, but mucin study by combined PAS and AB stain may provide a valuable and cost effective tool for diagnostic histopathology of colorectal adenocarcinoma [5]. This study was planned to evaluate the mucin characteristics in colorectal adenocarcinoma by applying combined PAS/AB stain in the Pathology Department of Rajshahi Medical College, to help in diagnosis, predict prognosis and plan management.

This present study included 50 colorectal adenocarcinoma cases. After PAS/AB stain they were classified into mild, moderate and marked. Afterwards mucinous and non-mucinous subtyping was done.

The age group ranged from 20-75 years with a mean age 51.2 ± 13.74. Out of 50 total study cases, most 40 (80%) were males and 10 (20%) were females. In India, Borgohain et al. [2] conducted a similar study on colorectal adenocarcinoma in 2017. A total of 50 cases of histologically confirmed colorectal carcinoma irrespective of age and sex were studied. The peak incidence was seen between 40-49 years with maximum cases in between 30-70 years of age and predominantly the cases were males. Another study conducted by Parul et al. (2014) [3] showed that the age interval of patients was from 23 to 78 year, the median age being 53 years. Most (82-22%) of the cases occurred in patients above 40 years of age. The male: female ratio was 1.4:1. There were 52 (57.77%) male patients and 38 (42.22%) female patients. In current study most tumors were in the rectum 18 (36%), followed by ascending colon 16 (32%), sigmoid colon 13 (26%) and descending colon 3 (6%). Another study, was conducted by Lucky et al. (2016) [6] on colorectal adenocarcinoma and it was seen that the commonest site of growth was rectum (59%) followed by ascending colon (16%), transverse colon (12%), sigmoid colon (8%) and descending colon (6%). Parul et al. (2014) [3] found that most (40%) of the carcinomas had a right colonic location followed by rectum with 24.44% of cases. Left colon accounted for 20% of the cases, whereas rectosigmoid region accounted for 15.55% cases.

Out of 50 colorectal adenocarcinoma cases mucin positivity was found in 43 (86%) cases and 7 (14%) were mucin negative. Among the mucin positive cases mild mucin expression was found in 12 (24%) cases, moderate expression was found in 12 (24%) cases and strong expression was found in 19 (38%) cases. 31 (62%) cases were non-mucinous tumor and 19 (38%) were mucinous. 36 (83.72%) mucin positive cases showed predominantly acidic mucin and 7% (16.28%) showed predominantly neutral type mucin. Ali et al. (2013) [7] studied 16 cases of colorectal adenocarcinoma by combined PAS/AB and found 67.6% cases with mild acidic mucin, 20.6% cases with moderate acidic mucin, 5.9% cases with marked acidic mucin, 2.9% cases with mild neutral mucin and 2.9% cases with moderate neutral mucin.

In another study conducted by Borghain et al. (2017) [2] out of the 50 cases mucin was seen in 35 cases and was absent in 15 cases. 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. Of these 34 cases, 26 showed mild acidic mucin, 3 showed moderate content and only 5 cases demonstrated marked acidic mucin. In a study conducted by Parul et al. (2014) [3] on 90 colorectal carcinoma patients, combined PAS/AB staining was positive for both stains in 68.88% cases, positive for only one stain in 28.88% cases, and negative for both stains in 2.22% cases indicating that both neutral and acidic mucins are increased in CRC.

In this current study out of 50 colorectal adenocarcinomas, most 20 (40%) were grade 3 tumors, followed by 18 (36%) grade 2 tumors and 12 (24%) grade 1 tumors. Marked mucin positivity was found in most of the grade 3 tumor, in 15 (83.3%) cases; moderate mucin positivity was found in grade 2 in 07 (50.0%) cases and mild mucin positivity was found in grade 1 in 05 (45.5%) cases. In a study conducted by lucky et al. (2016) [6] it was found that out of the 50 cases there were 41 (82%) low grade tumors and 9 (18%) high grade tumors. Mucinous carcinoma showed a higher proportion of high-grade tumors 5 cases (55%) as compared to non-mucinous which was 4 cases. In a study conducted by Duara et al. (2016) [5] it was seen that 5 (55%) grade 3 tumors are mucinous and 4 (44.4%) grade 3 tumor are non-mucinous. Seventeen (94.4%) grade 3 tumors are predominantly acidic mucin followed by grade 2 (78.5%) and grade 1 (72.8%).

Conclusion

This study concludes that predominance of acid mucin increases along with the increase in tumor grades. So, mucin may be a helpful marker for the early recognition of cancerous transformation, and prediction of future prognosis. It can be used as an adjunct to H&E stain in detection of mucin producing colorectal adenocarcinomas, which sometimes may be difficult to diagnose on H&E stain alone.

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