

Histopathological Evaluation of Right and Left Sided Colorectal Cancer: A Cross-sectional Study from a Tertiary Care Hospital, Assam, India

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ABSTRACT

Introduction: Colorectal cancer is a heterogeneous disease that can develop in any part of the colon including rectum, with significant differences in clinical features and survival of patient in right sided and left sided colorectal carcinoma. Hence, it is necessary to evaluate the pathological characteristics of these tumour.

Aim: To analyse the pathological features of patients with right and left sided colorectal cancer.

Materials and Methods: This hospital-based, cross-sectional study conducted in Department of Pathology at Assam Medical College, Dibrugarh, Assam, India, from July 2019 to June 2020. The study included 35 cases of all endoscopic biopsy specimens and surgically excised large specimens of carcinomatous growths of colon and rectum, whereas carcinoma of appendix was excluded from this study. Special stain like Periodic Acid Schiff (PAS) and Alcian Blue, and immunohistochemistry (CK7 and CK20) was done in selected cases. The data is shown in tables and results are expressed in terms of frequency and percentages.

Results: Left colon (74.28%) was more commonly involved than right colon (25.72%) and rectum was the most common site (31.43%). The most common gross morphology in right colon was ulcerative growth (16%) and in left colon it was polypoidal growth (28%). On histopathological examination, 27 cases were adenocarcinoma, Not Otherwise Specified (NOS) (six were well differentiated, 20 were moderately differentiated and one was poorly differentiated) and eight cases were mucinous adenocarcinoma. The mean age was 51.54 years with age distribution from 14 years to as old as 80 years. In immunohistochemistry, 9 (90%) cases show CK20 positive/CK7 negative and 1 (10%) case show CK20 positive/CK7 focally positive.

Conclusion: Routine microscopic examination of haematoxylin and eosin stained slide was still the commonly performed and vital modality of investigation in majority of the cases. The right and left sided colorectal carcinoma varies according to clinical and pathological features. However, it is still necessary to find other biological dissimilarities of adenocarcinoma located on different sides of colon.

Keywords: Adenocarcinoma, Alcian blue stain, Colorectal carcinoma, Immunohistochemistry

INTRODUCTION

Colorectal cancer is a heterogeneous disease that can develop in any part of the colon including rectum, with consequent differences in terms of risk factors, gender, clinical features, histological grades, tumour size and metastatic features. It is the second most common cancer in women and third most common cancer in male worldwide [1]. In India, colorectal cancer is the fourth most prevalent cancer in male and third most prevalent cancer in female [2].

In the recent times, great attention is being given to the pathological differences between right sided and left sided colon cancer and how much these differences will affect the survival and prognosis of colon cancer patients. Many epidemiological studies have demonstrated that right sided and left sided colorectal cancer have differences in clinical presentation, histopathological features, prognosis and survival. Tumours of the proximal part of colon (right side) and distal colon (left side) exhibit different molecular characteristics and histology. Therapy responses totally vary for the two entities. Left sided colorectal cancer patients benefit more from adjuvant chemotherapies and targeted therapies such as anti-Epidermal Growth Factor Receptor (EGFR) and have a better prognosis. Right sided colorectal cancer demonstrates more promising results with immunotherapies because these tumours have high antigenic load [3].

Distinct phenotypic variations exist between right sided colorectal cancer and left sided colorectal cancer. While right sided tumours show sessile serrated adenomas or mucinous adenocarcinomas,

left-sided tumours show tubular adenoma, villous adenoma and typical adenocarcinomas [4]. Since, left sided tumours have polypoid morphology, it is easier to detect them with colonoscopy in the early stages of carcinogenesis. Right sided colorectal carcinomas have flat morphology that is difficult to detect [5].

The aim of this study was to analyse the pathological features of patients with right sided and left sided colorectal cancer.

MATERIALS AND METHODS

This hospital-based, cross-sectional study conducted in Department of Pathology at Assam Medical College, Dibrugarh, Assam, India, from July 2019 to June 2020. Ethical clearance was obtained from Institutional Ethical Committee (letter No.AMC/EC/PG/1979; dated 22/5/2019).

Inclusion criteria: All endoscopic biopsy specimens and surgically excised large specimens of carcinomatous growths of colon and rectum were included in the study.

Exclusion criteria: All the cases of carcinoma of appendix were excluded from the study.

Study Procedure

All the patients in the present study were subjected to brief history taking, clinical examination and investigations including radiological and colonoscopy examination. The specimens were collected and fixed in 10% neutral buffer formalin, then thorough grossing was done and 4-5 sections were taken from tumour proper, sections

from the surgical margins, lymph nodes and any other grossly abnormal area, followed by routine tissue processing and staining with Haematoxylin and Eosin (H&E) stain.

Special stain like Periodic Acid Schiff (PAS) and Alcian Blue (AB), and immunohistochemistry (CK7 and CK20) was done in selected cases [6]. For Immunohistochemistry (IHC), 3-5 μ serial sections were taken in (3-Aminopropyltriethoxysilane) (APTES) coated slides. The slides were deparaffinised and exposed to heat induced antigen retrieval for five minutes in an autoclave at 121°C in pH 7 buffer. Primary antibodies specific for CK7 and CK20 (rabbit recombinant antibody) were applied at 37°C for 60 minutes at a dilution of 1:150. Bound antibodies were then visualised using the Envision kit according to the manufacturer's directions. Cells were considered positive for CK20 and CK7 when distinct cytoplasm and/or cell membrane yellow to brown staining was identified.

- Score 1: <5% staining of tumour cells
- Score 2: 5-20% staining in of tumour cells
- Score 3: 20-50% staining in of tumour cells
- Score 4: >50% staining of tumor cells

Scores 1 and 2 has low immunostaining

Scores 3 and 4 has high immunostaining

The PAS stain was done in mucinous adenocarcinoma, PAS positive substances stain pink to red/magenta with blue nuclei. The PAS positive cases were further stained with AB/PAS stain to differentiate between types of mucin namely:

- Neutral mucin stain magenta colour
- Acid mucin stain blue colour.

STATISTICAL ANALYSIS

The data is shown in tables and results are expressed in terms of frequency and percentages.

RESULTS

In the period of 12 months 35 samples were collected in the Department of Pathology, 10 endoscopic punch biopsy and 25 operatively resected specimens. The mean age was 51.54 years with age distribution from 14 years to as old as 80 years [Table/Fig-1].

Age group (years)	Number of cases	Percentage (%)
≤20	2	5.71
21-30	3	8.57
31-40	4	11.43
41-50	7	20
51-60	10	28.57
61-70	7	20
71-80	2	5.71
Total	35	100
Mean±SD	51.54±16.54	

[Table/Fig-1]: Age distribution.

Sex distribution was almost equal 18 cases were male and 17 cases were female with ratio of 1.05:1. Bleeding per rectum (40%) was the most common clinical presentation followed by abdominal pain (37.14%), weight loss (31.43%) and altered bowel habit (31.43%).

Left colon (74.28%) was more commonly involved than right colon (25.72%) and rectum was the most common site (31.43%) [Table/Fig-2].

In 25 surgically resected cases, the most common gross morphology in right colon was ulcerative growth (16%) and in left colon it was polypoidal growth (28%) [Table/Fig-3].

On histopathological examination, 27 cases were adenocarcinoma, Not Otherwise Specified (NOS) (six were well differentiated, 20 were

Site involved (Colon/Rectum)	Number of cases (n=35)	Percentage (%)
Caecum	2	5.71
Ascending colon	6	17.14
Transverse colon	2	5.71
Descending colon	4	11.43
Sigmoid colon	4	11.43
Recto sigmoid	6	17.14
Rectum	11	31.43

[Table/Fig-2]: Anatomical site involved.

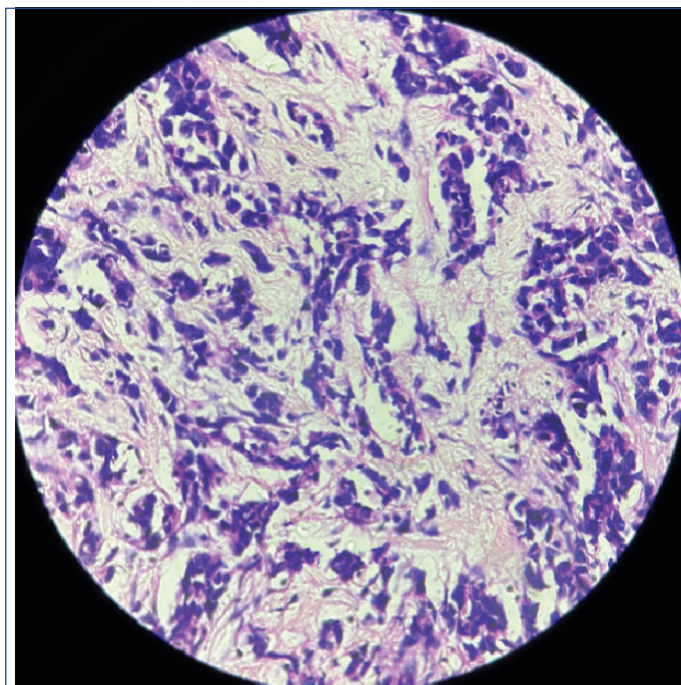
Side	Gross morphology	Number of cases	Percentage (%)
Right	Ulcerative	4	16
	Lumen obstructed by growth	2	8
	Fungating	2	8
	Polypoidal	1	4
Left	Polypoidal	7	28
	Luminal fibrosis	4	16
	Ulcerative	3	12
	Fungating	2	8

[Table/Fig-3]: Gross morphology of tumour (n=25).

moderately differentiated and one was poorly differentiated) and eight cases were mucinous adenocarcinoma [Table/Fig-4-6]. Tumour invasion upto submucosa was 8.57%, muscularis propria 62.86% and subserosa 28.57%.

Site	Adenocarcinoma NOS	Mucinous adenocarcinoma
Right side colon cancer	7	2
Left side colon cancer	20	6
Total	27 (77.17%)	8 (22.86%)

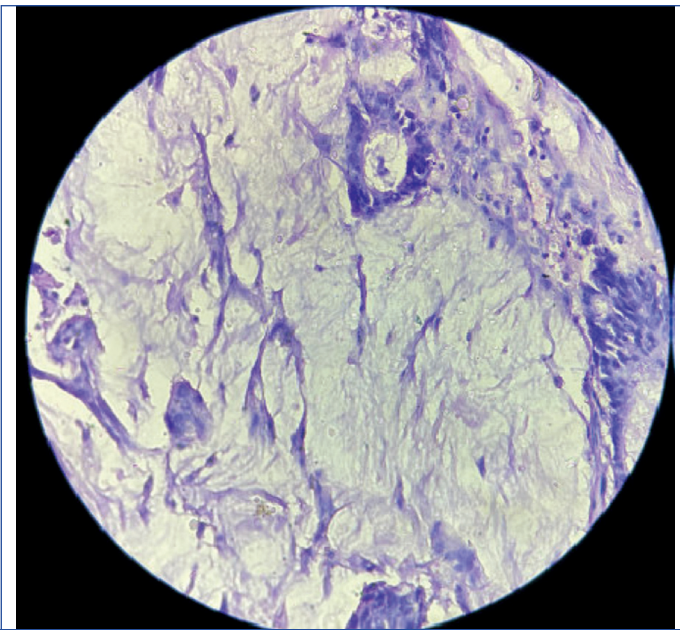
[Table/Fig-4]: Histological type of colon cancer.



[Table/Fig-5]: Microphotograph showing poorly differentiated adenocarcinoma (H&E, 40X).

In PAS and alcian blue staining, mucin was detected in 8 (22.86%) cases, among them seven show positivity to both PAS/alcian blue i.e., acidic mucin and one show positivity to PAS only i.e., neutral mucin.

Immunohistochemistry was done in four operatively resected specimens and six endoscopic tissues which pose diagnostic difficulty with histopathology, the markers used were Cytokeratin

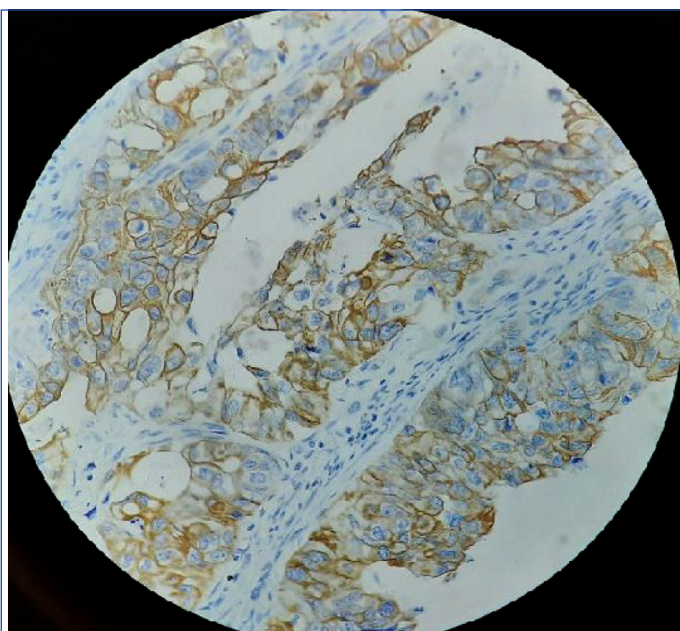


[Table/Fig-6]: Microphotograph showing mucinous adenocarcinoma (H&E, 40X).

20 (CK20) and CK7. Nine cases show CK20 positive/CK7 negative (90%) and one case show CK20 positive/CK7 focally positive [Table/Fig-7,8].

Stain	Number of cases (n=10)	Percentage
CK20 positive/CK7 negative	9	90%
CK20 positive/CK7 focally positive	1	10%
CK20 negative/CK7 negative	0	0

[Table/Fig-7]: Immunostaining in colorectal carcinoma.



[Table/Fig-8]: Microphotograph showing CK20 positivity (CK20, 40X).

DISCUSSION

The present study was aimed to analyse the pathological features of right sided and left sided colorectal cancer. Though, there were only 35 cases in a period of one year, this study is of value as it was done in a lone tertiary care referral hospital in eastern part of Assam. The most common age group affected was 6th decade of life, the youngest patient was 14 years old and the oldest was 80 years old and mean age was 51.54 years. The findings were similar with Javid G et al., and Ghanadi K et al., [7,8]. There is variation in the peak age of occurrence of colorectal cancers worldwide, and also within the country itself. This variation may be due to the geographical, ethnic and racial differences between the various populations in

which these studies were carried out. Other local and socio-cultural factors may also be involved which would need further detailed studies for proper evaluation.

Sex distribution was almost equal with male:female ratio was 1.05:1 which is in accordance with the studies done by Javid G et al., and Laishram R et al., [7,9]. The most common symptom was rectal bleeding (40%) followed by abdominal pain (37.14%) and altered bowel habits (31.43%). The study done by Poornakala S and Prema NS, has also reported similar findings in the patient of colorectal cancer [10]. Left sided colon was affected in (74.28%) cases where as involvement in right side constitute (25.71%) which was also found by Sudarshan V, et al., [11]. The most common anatomical site was rectum (31.43%) followed by recto sigmoid junction (17.14%) and ascending colon (17.14%), which is consistent with previous studies done by Patil PS et al., and Ghanadi K et al., [3,8].

The left sided tumours were mostly of polypoidal morphology (28%) followed by luminal fibrosis (16%), whereas the right sided tumour were ulcerative flat like pattern (16%) followed by growth obstructing the lumen of colon as well as fungating growth (8%). Similar findings were observed by Burcin B et al., [5]. However, Abdulkareem FB et al., in their study found that the right sided tumours were mostly fungating nodular lesions with surface ulcerations while the left sided tumours were flat and infiltrating or constricting pattern [12].

Most common histologic type noted in this study was adenocarcinoma NOS (77.14%) and 22.86% cases were mucinous adenocarcinoma. The finding is in accordance with Patil PS et al., who conducted study among 800 patients in India [3]. In the present study, 74.07% of the tumours are moderately differentiated being the most common type, followed by well differentiated 22.22% and poorly differentiated only 3.70%. In a study conducted in India by Patil PS et al., also found 2.6% well differentiated carcinoma, 47.6% moderately differentiated carcinoma, and 20.6% poorly differentiated carcinoma, while 16.6% were mucinous carcinoma [3]. Majority of the cases (62.86%) had tumour invasion upto muscularis propria, followed by serosal layer (28.57%) and limited to sub mucosa only (8.57%) which is similar with the study conducted by Poornakala S and Prema NS [10].

Special staining for mucin study was done in eight cases of mucinous colorectal carcinoma. All eight cases are PAS positive out of which seven cases were strong AB/PAS positive (87.50%) and only one case showed alcian blue weak positivity (12.50%), which indicate that acidic mucins are increased in colorectal carcinoma. Similar findings were observed by Ionila M et al., [13]. Immunohistochemistry was done in 10 cases. Out of these, 9 (90%) cases were CK20 positive and CK7 negative and only 1 (10%) cases was CK20 positive and CK7 focally positive. Chu PG and Weiss LM, reported the most common immunophenotype of colorectal adenocarcinoma was positivity for CK20 and negativity for CK7, which was a relatively specific staining pattern for colorectal origin. However, upto 20% of the tumours may exhibit a CK7 positive/CK20 negative or CK7 negative/CK20 negative staining pattern [14]. Fleming M et al., reported similar data that colorectal carcinoma are CK20 positive and CK7 negative [15]. Thus, immunohistochemical findings in this study were in consistent with other studies.

Limitation(s)

This study had a small number of cases and limited study period. Further studies with a large number of patients with colorectal carcinoma would be more helpful.

CONCLUSION(S)

In this study, the most common type of colorectal carcinoma was adenocarcinoma NOS. It was most frequent in 6th decade of life. The right sided and left sided colorectal cancer show variations in clinical and pathological features. Routine microscopic examination of H&E stained slide is still the commonly performed and vital modality of investigation in majority of the cases. However, a few

selected cases needs to be ascertained with improved diagnostic modalities like special stains and IHC.

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