Clinicopathological Characteristics of Colorectal Carcinoma in Adults and Demonstration of Microsatellite Instability

Sungjemla Longkumar¹, Seena A. R.²

¹,²Department of Pathology, Government Medical College, Thiruvananthapuram, Kerala, India.

ABSTRACT

BACKGROUND
Colorectal cancer (CRC) is one of the leading causes of mortality and morbidity from cancer. The risk group ranges from 69-79 years and the mean age being 62 years. Studies have shown that the percentage of patients under 50 years of age has increased to approximately 12%. Colorectal carcinoma in young is usually located in the distal colon and is associated with aggressive behaviour, diagnosed at an advanced stage or maybe part of one of the hereditary colorectal syndrome. 12 - 15% of carcinomas develop through microsatellite instability and 2 - 5% are hereditary. Tumours with microsatellite instability are recognised by the absence of immunohistocchemical staining for mismatch repair proteins (MLH1, MSH2, MSH6, PMS 1, and PMS 2) which is associated with Lynch syndrome. It is important to recognise tumours with MSI because of implications for genetic counselling, increased risk of secondary malignancy of colon or other organs, and in some settings, differences in prognosis and management. The study was intended to assess and compare the clinicopathological characteristics of colorectal carcinoma in adults above and below 50 years and also study the expression of MLH1 for demonstration of microsatellite instability in colorectal carcinoma.

METHODS
It was a retrospective study carried out in the Department of Radiodiagnosis, at Justice K. S. Hegde Charitable Hospital, from March 2020 to March 2021 after receiving ethical clearance from the institutional ethical committee. The study included a total of 90 patients. Patients were categorized into three different age groups, 18 - 25 years, 26 - 40 years and 41 - 55 years of age.

RESULTS
Clinicopathological features of 61 cases were studied and the mean age was 61 years. Most cases were left-sided cancer with moderately differentiated classical adenocarcinoma being the most common histopathological subtype. Individuals less than 50 years presented with adenocarcinoma with mucinous and signet ring cell morphology and with poor differentiation. 67.2% had no lymphovascular invasion. The majority of the cases were positive for MLH1. The cases which showed negativity for MLH1 were cases with mucinous differentiation or signet ring cell type and were predominantly moderate to poorly differentiated and this relation was found to be significant.

CONCLUSION
The study concludes that the majority of CRC occurred in individuals between 61 - 70 years of age, they were left-sided moderately differentiated adenocarcinomas with stage IIb disease, most were negative for lymphovascular invasion and tumour infiltrating lymphocytes. Younger patients had a higher percentage of mucinous and signet-ring cell histology. 78.7% were positive for MLH1.

KEY WORDS
Colorectal carcinoma, MLH1, Microsatellite instability
Colorectal cancers are among the leading causes of death and morbidity, being the third commonest cancer in men and second commonest in women. Though distributed worldwide, the incidence was seen to be higher in western countries. Many Asian countries like China, Japan and Singapore also show an increased incidence of carcinoma colon. Gross appearance may vary from ulceroproliferative growth to a stricture.

In India, the annual incidence for colorectal carcinoma is 4.4 in males and 3.8, in females. The Indian council of medical research (ICMR) consensus document for management of colorectal cancer found that the annual incidence rate in males was highest in Thiruvananthapuram, in the year 2013. The rate was 4.1. The city of Bangalore was second with 3.9. The risk group ranged from 69 to 79 yrs. However, recent studies show that the percentage among people below 50 yrs has increased to 12.

Age group that was affected the most was 62 - 79 years. Risk factors for the development of colon cancers include age, environmental factors like smoking, unhealthy food habits and genetic factors like FAP (familial adenomatous polyposis) and HNPPC (hereditary nonpolyposis colorectal cancer). Recently the studies have shown that young patients are usually associated with aggressive behaviour, advanced disease or hereditary colorectal syndrome. These include FAP, Lynch syndrome or HNPPC. This may be through chromosomal defects or microsatellite instability. Twelve to fifteen percent of colorectal cancers are due to microsatellite instability and two to five percent are hereditary. These tumours have different methods of treatment. The relatives of these people need to be screened for MSI.

MSI is a hallmark of Lynch syndrome. Microsatellites are short tandem repeats in DNA, accounting for 3% of the human genome and 15% of sporadic colorectal carcinomas are associated with microsatellite instability. The instability may be characterized by deletions or insertions resulting in defects of DNA mismatch repair proteins. Hereditary mutation in any mismatch repair genes (MLH1, MSH2, MSH6, PMS1 and PMS2) results in HNPPC or Lynch syndrome. Tumours with MSI show some common features like predilection to proximal colon, mucinous histology and lymphocytic infiltrate. Hence it is a valuable biomarker to detect MSI in colorectal cancer. It can be detected by IHC marker or with PCR. Other factors that may be considered as aetiological or risk factors include:

**Nutritional Habits**
A diet rich in fibre lowers the pH, provides antioxidants, and decreases the transit time thus reducing the risk for carcinoma, whereas red meat and saturated fat increase the risk and alcohol also increases the risk.

**Medical History**
Diseases like diabetes and inflammatory bowel disease and previous radiotherapy increase the risk for carcinoma. Obesity and lack of physical activity make people more susceptible to develop cancer. Drugs like tobacco, aspirin and NSAIDs are found to have a very strong association with colorectal cancer.

The study was conducted to find the difference in clinicopathological features in younger and older age group patients and demonstrate MSI in these tumours. This can be tested by immunohistochemistry or PCR. Both methods are sensitive and specific and have a high concordance value (more than 92%).

**Methods**
It was a retrospective study carried out in the Department of Radiodiagnosis, at Justice K. S. Hegde Charitable Hospital, from March 2020 to March 2021 after receiving ethical clearance from the institutional ethical committee. The study included a total of 90 patients (Males-49 (54.4 %) and Females- 41 (45.6 %)) from 18 - 55 years of age referred for CT PNS in our institute. Patients were excluded based on the history of trauma-related to MS. All scans were performed on GE Elite Bright Speed (16 Slice MDCT) and data was collected from DICOM software. Patients were categorized into three different age groups, 18 - 25 years, 26 - 40 years and 41 - 55 years of age.

The measurements were taken on 2D and 3D reconstructed images of both maxillary sinuses. Measurements were done using an inbuilt electronic caliper into DICOM viewer software. The largest linear measurements of the diameters like anteroposterior (AP), superoinferior (SI), and mediolateral (ML) and distance between right and left MS were performed on coronal & sagittal planes and volume of the sinus was calculated using formula (AP x ML x SI x 0.625).

**The Measurements Were as Follows**

**AP Diameter**
It was measured on the sagittal images from the most anterior point to the most posterior point of the right and left maxillary sinus on 2D and 3D images.

**ML Diameter**
It was measured on coronal images from the longest distance perpendicular from the medial wall of the MS to the outer most point of the lateral process of both right and left MS on 2D and 3D images.

**Results**
In this study, the age group affected ranged from 30 to 90 years, the majority being between 61 and 70 years. There was a mild male preponderance and 77 % were left-sided. A predominant variant is a classical adenocarcinoma, mostly of moderate differentiation. Among the MSI positive cases, 79.2 % were left-sided and only 20 % were right-sided. Regarding differentiation of the tumours, moderately differentiated tumours showed maximum expression of MLH1, followed by poorly differentiated tumours. No expression was detected on well-differentiated tumours.
The stage at the time of diagnosis was mostly IIA followed by IIIB.

| Stage | Number | Percent | Number | Percent |
|-------|--------|---------|--------|---------|
| I     | 4      | 8.7     | 2      | 13.3    |
| IIA   | 21     | 45.7    | 5      | 33.3    |
| IIIB  | 1      | 2.2     | 1      | 6.7     |
| IIIA  | 2      | 4.3     | 0      | 0       |
| IIIB  | 13     | 28.3    | 6      | 40.0    |
| IIIC  | 4      | 8.7     | 1      | 6.7     |
| IVA   | 1      | 2.2     | 0      | 0       |

Table 2: Stage of Tumour at the Time of Diagnosis in Relation to Age

78.7% i.e., 48 cases were showing MLH1 expression.

70% of them were left sided. 83.3% of positive cases were classical adenocarcinomas followed by mucin secreting and mucinous carcinomas. At the same time, signet ring cell carcinoma was not showing MSI.

**DISCUSSION**

The primary objective of this study was to evaluate the clinicopathological features of colorectal carcinoma in terms of age, sex, site, histopathological type, grade, differentiation, stage at the time of diagnosis, lymphovascular invasion and tumour infiltrating lymphocytes and to compare these features in patients above and below 50 years of age. Further, it was intended to detect and correlate the expression of MLH1 in these tumours with the clinicopathological parameters.

**Age**

75% of patients were of the age group above 50 years. Maximum cases were between 61 and 70 yrs with a mean age of 61 yrs. This was similar to some studies previously done.

**Relation to Sex**

Literature states that males and females are equally affected. However, in this study, there was a predilection for the male sex, especially in the younger age group.

**Site of the lesion**

Most of the colorectal carcinomas were located in the left side of the colon (rectosigmoid) and two-thirds of the cases associated with Lynch syndrome were located in the proximal colon. Seventy-seven percent of the cases were in the left side colon which was similar to observations in some previous studies.5

**Histopathological Type**

The majority of the cases in the present study (72.1%) were classical adenocarcinoma. The other histological types in the study included adenocarcinoma with mucinous differentiation, adenocarcinoma with signet ring cells, mucinous adenocarcinoma and signet ring carcinoma. Adenocarcinoma with mucinous and signet ring cells was more prevalent in the younger age group (less than 50 years).

**Stage of the disease at the time of diagnosis**

The majority of the patients (42.6%) were diagnosed to be in the stage IIA (T3 N0). Individuals less than 50 years of age however presented at a higher stage IIIB. This may be due to the advanced end-stage for younger individuals.

**Differentiation**

The majority of the cases in the present study were moderately differentiated adenocarcinoma (67.2%) unlike the study conducted by some other centres where the predominant type was well-differentiated carcinoma. However, the individuals younger than 50 years predominately presented with poorly differentiated carcinoma. However, the relation between age and differentiation was not very significant due to a small number of patients below the age of 50 years.

**Lymphovascular Invasion**

Most persons were negative for lymphovascular invasion while younger individuals showed an increased tendency for early invasion. This could also explain the higher stage at the
time of diagnosis in these patients. This relation was found to be significant. P-value was 0.05

**Tumour Infiltrating Lymphocytes**

An inflammatory reaction at the invasive borders of the tumour is considered as a good prognostic indicator. Our study showed only 24.6% cases as positive for tumour-infiltrating lymphocytes. In the younger population, tumour-infiltrating lymphocytes were absent. This can be related to poor differentiation and higher stage of the tumour.

**Immunohistochemistry - MLH1**

MLH1 is a DNA mismatch repair protein that becomes defective in Lynch syndrome. This can be detected using immunohistochemistry or PCR but IHC is comparatively cheaper. In the present study, 78.7% of cases showed positivity for MLH1 and the rest were negative. On comparing MLH1 expression to the site of lesion, right-sided lesions had a higher loss of MLH1 expression. Most of the classical adenocarcinoma cases showed positive expression whereas those with mucinous or signet ring cell histology showed loss of MLH1 expression. The relation between histopathological type and MLH1 expression was significant (P < 0.01).

The present study found out that all well-differentiated tumours were positive for MLH1 whereas moderately differentiated and poorly differentiated tumours showed higher loss of MLH1 expression, and this relation was found significant.

In our study, the absence of MLH1 expression suggested MSI but it is recommended that a panel of four MMR proteins must be used to increase the specificity and sensitivity. And should be accompanied by PCR based germline mutational analysis.

**CONCLUSIONS**

The relation between histological types, differentiation and MLH1 was found to be consistent and significant. Colorectal carcinomas are found to be commoner in males, age group affected the most was between 60 and 70 yrs. The left side of the colon was preferred by carcinoma. The carcinoma was mostly moderately differentiated classical adenocarcinoma, younger individuals showing adenocarcinomas with mucinous and signet ring cell differentiation, predominantly. The cases were mostly detected at stage II A. However, younger patients were in stage II B at the time of detection.

Right-sided colorectal carcinoma, poorly differentiated carcinoma, signet ring cell carcinoma and mucinous carcinoma showed higher loss of MLH1 expression. This relation between MLH1 and tumour grade is significant, suggesting microsatellite instability. However, evaluation of other proteins like MSH2 and MSH6 should be done along with MLH1 for the sensitivity and specificity.

Data sharing statement provided by the authors is available with the full text of this article at jemds.com.

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The support staff of labour room and cardiac ICU.

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