Immunophenotypic Characterization of Colorectal Cancer in a Tertiary Care Hospital: A Cross sectional Study

Pralisha Maharjan[1], Gopi Aryal[1], Reena Rana[1], Anuj Poudel[1], Kricha Pande[1], Niki Thakur[1], Rajiv Mishra[2]

[1]Department of Laboratory Medicine & Pathology, Nepal Mediciti Hospital, Lalitpur, Nepal

[2]Department of Gastrointestinal Surgery, Nepal Mediciti Hospital, Lalitpur, Nepal

ABSTRACT

BACKGROUND

Colorectal carcinoma (CRC) is the third most common malignancy and the second leading cause of cancer-related deaths worldwide. It encompasses a heterogeneous group of epithelial tumors with distinct histologic, molecular, and clinical features. Immunophenotypic and molecular profiling play crucial roles in tumor classification, prognostication, and guiding targeted therapy in the era of personalized medicine. This study aimed to evaluate the clinicopathologic, immunophenotypic, and molecular characteristics of CRC in Nepal Mediciti Hospital, providing local data to enhance diagnostic and therapeutic strategies.

METHODS

A hospital-based analytical cross-sectional study was conducted on 25 histopathologically confirmed CRC cases diagnosed at Nepal Mediciti Hospital between January 2021 and December 2024. Following ethical approval, demographic, clinical, histologic, immunohistochemical and molecular data were retrieved and analyzed using statistical software SPSS version 26.

RESULTS

The mean patient age was 55.08 years (range: 24–86), with a male-to-female ratio of 1.5:1. The caecum and ascending colon were the most frequent tumor sites. Moderately differentiated adenocarcinoma was the predominant histologic type. 85.71% of resection specimen showed CK20 positivity. All of cases (100%) showed positive nuclear staining for CDX2 and SATB2. KRAS and BRAF mutations were identified in 32% and 8% of cases, respectively, with no NRAS mutations detected. Mismatch repair deficiency (dMMR) was noted in 12% of cases, all among females under 50 years.

CONCLUSIONS

The clinicopathologic, immunophenotypic, and molecular profiles of CRC in this study parallel global trends. This study did not demonstrate a significant correlation between MSI status and either tumor site or grade. It emphasizes on the relevance of molecular testing for prognosis, targeted therapy, and hereditary cancer screening.

KEY WORDS

Colorectal cancer; immunphenotype; molecular

*Corresponding Author |
Dr. Pralisha Maharjan
Department of Laboratory Medicine & Pathology
Nepal Mediciti Hospital, Sainbu, Lalitpur, Nepal
Email: Pralisha.mhzn@gmail.com

INTRODUCTION

Colorectal carcinoma (CRC) ranks as the third most common cancer and the second leading cause of cancer-related deaths worldwide as per the Global Cancer Observatory.1 The disease includes adenocarcinoma of the colon and/or rectum which are characterized by various histological features, molecular alterations, and clinical behaviors.2

The immunophenotypic (IHC) profile of CRC can reflect its biological behavior and may influence therapeutic decisions, particularly with the advent of personalized medicine strategies. Key markers such as carcinoembryonic antigen (CEA), mismatch repair proteins (MLH1, MSH2, MSH6, PMS2), KRAS, NRAS, BRAF and various cytokeratins are assessed to aid in the diagnosis and molecular subclassification of colorectal tumors.3 The role of molecular profiling in CRC has become important particularly with the development of targeted therapies and immunotherapies.4

This study helps explore potential variations in tumor biology that may arise from genetic, environmental, and dietary factors. This study aims to characterize the IHC profile of CRC in a tertiary care hospital setting, providing data that could enhance the understanding of CRC in the local population. By analyzing tumors and their IHC profiles, this study will help to provide precision oncology for effective treatment strategies.

METHODS

This study was a Quantitative Hospital Based Analytical Cross-Sectional Study conducted in 25 histopathologically confirmed CRC cases in the department of Laboratory Medicine and Pathology, Nepal Mediciti Hospital (NMC) from 1st January, 2021 to 31st December, 2024 following ethical approval from Institutional Review Board (IRB) (161/2080/81). The census sampling method was used and the sample size was all histopathologically confirmed CRC in the given timeframe based on the hospital records retrieved from the database. Total 25 cases were diagnosed as colorectal carcinoma by HPE among which 9 cases did both IHC (CK7, CK20, CDX2, SATB2, MLH1, MSH2, MSH6, PMS2), and molecular (KRAS, NRAS, BRAF) and remaining 16 cases did molecular tests only. Cases which are not diagnosed in NMC but did IHC and Molecular tests were excluded. The patients demographic factors like age and gender along with tumor location, biopsy procedure, tumor histology, pTNM classification, IHC findings and molecular findings were recorded. Tumor location were further categorised as cecum, ascending colon, hepatic flexure, transverse colon, splenic flexure, descending colon and sigmoid colon, rectosigmoid junction and rectum. Data were entered manually and stored in electronic database (MS – Excel) then entered and analysed in statistical software Statistical Package for the Social Sciences (SPSS) version 26.

RESULTS

A total of 34 cases with CRC had reports of IHC and molecular tests during the study period. Of these, 9 cases were excluded as they were diagnosed in other centers but only IHC and molecular tests were done from our center. Thus, 25 cases fulfilled the inclusion criteria and were included in our study.

Between 1st January, 2021 to 31st December, 2024, there were 25 cases of primary CRC (8 colonoscopic biopsy specimen and 17 resection specimen) (Table 1 and Table 2). The male female ratio is 1.5:1 in 3 years duration. The mean age is 55.08 years while the youngest case is 24 years and eldest case is 86 years. The most common tumor location is Caecum (n=5) and Ascending colon (n=5), followed by Sigmoid colon (n=4), Rectosigmoid junction (n=3) and Rectum (n=3), Descending colon (n=2), Hepatic flexure (n=1), Splenic flexure (n=1) and Transverse colon (n=1) (Figure 1). The procedure done were colonoscopic biopsies (n=8) and resection specimen included Right hemicolectomy (n=7) followed by Extended right hemicolectomy (n=3), Lower anterior resection (n=2) and 1 each of Left limited hemicolectomy, Near total colectomy, Sigmoidectomy, Subtotal colectomy and Total colectomy. Samples received from these procedures were processed and then the final HPE diagnosis were made. Nineteen cases were moderately differentiated adenocarcinoma, two cases were each of mucinous adenocarcinoma and well differentiated adenocarcinoma and one case each of signet ring cell carcinoma and poorly differentiated adenocarcinoma (Figure 2). In resection specimen, the largest size of the tumor was 8x6cm. Tumor budding varied from BD1-BD3: Five BD1 cases, Eight BD2 case, One BD3 cases and in three cases tumor budding was not done as two were mucinous adenocarcinoma and one was poorly differentiated carcinoma. Five cases had lymph node metastasis while two cases had distant metastasis. One case was each of tumor recurrence, multiple primary tumors and post neoadjuvent therapy respectively.

Table 1. Cases with colonoscopic biopsies:

Cases	Age (year)/ Gender	Location	Histopathological diagnosis	IHC	Molecular
1	57/M	Rectum	Signet ring cell carcinoma	Not done	KRAS: Negative NRAS: Negative BRAF: Negative MMR/MSI: Intact
2	56/M	Sigmoid colon	Moderately differentiated adenocarcinoma	Not done	KRAS: Negative NRAS: Negative BRAF: Exon 15V600R MMR/MSI: Not done
3	45/F	Rectum	Moderately differentiated adenocarcinoma	Not done	KRAS: Negative NRAS: Negative BRAF: Negative MLH1 and PMS2 Loss of expression
4	45/M	Ascending colon	Moderately differentiated adenocarcinoma	Not done	KRAS: Negative NRAS: Negative BRAF: Negative MMR/MSI: Intact
5	61/F	Sigmoid colon	Well differentiated adenocarcinoma	CDX2 and CK20 positive	KRAS: Negative NRAS: Negative BRAF: Negative MMR/MSI: Intact
6	27/M	Rectum	Moderately differentiated adenocarcinoma	Not done	KRAS: Exon 2 KRAS G12 Mutation NRAS: Negative BRAF: Negative MMR/MSI: Not done
7	30/M	Rectosigmoid junction	Moderately differentiated adenocarcinoma	CDX2 and SATB2 Positive	KRAS: Exon 2 KRAS G12 Mutation NRAS: Negative BRAF: Negative MMR/MSI: Intact
8	56/F	Sigmoid colon	Moderately differentiated adenocarcinoma	Not done	KRAS: Negative NRAS: Negative BRAF: Negative MMR/MSI: Intact

Table 2. Cases with resection specimen:

Case	Age (year) / Gender	Location	Procedure	Tumor size (cm)	HPE Diagnosis	Tumor budding	TNM Staging	IHC	Molecular
1	59/M	Descending colon	Near total colectomy	8x6	Moderately differentiated adenocarcinoma	BD1	pT2pN0	Not done	KRAS: Negative NRAS : Negative BRAF : Not done MMR : Not done
2	75/M	Ascending colon	Right hemicolectomy	3x2	Moderately differentiated adenocarcinoma	BD2	pT3pN0	Not done	KRAS: Negative NRAS : Negative BRAF : Negative MMR : Intact
3	77/M	Caecum	Right hemicolectomy	5x4	Mucinous adenocarcinoma	N/A	pT4apN1 bpM1c Mets to omental fat	CDX2 and SATB2 positive CK20 negative	KRAS: Negative NRAS : Negative BRAF : Negative MMR : Intact
4	65/F	Rectosigmoid junction	Low anterior resection	4x2.5	Moderately differentiated adenocarcinoma	BD3	pT2pN0	Not done	KRAS: Negative NRAS : Negative BRAF : Negative MMR : Intact
5	53/F	Splenic flexure	Left limited hemicolectomy	3x2	Moderately differentiated adenocarcinoma	BD2	ypT3pN0 pM1a Mets to liver	CDX2, CK20 and SATB2 positive	KRAS: Negative NRAS : Negative BRAF : Negative MMR : Intact
6	24/M	Ascending colon	Right hemicoelctomy	4.5x2	Poorly differentiated adenocarcinoma	N/A	pT3pN2b	Not done	KRAS: Negative NRAS : Negative BRAF : Negative MMR : Not done
7	57/M	Caecum	Right hemicoelctomy	2x1	Moderately differentiated adenocarcinoma	BD1	pT3pN1b	CDX2 and SATB2 positive	KRAS: Negative NRAS : Negative BRAF : Negative MMR : Intact
8	56/F	Caecum	Right hemicoelctomy	4x3	Moderately differentiated adenocarcinoma	BD2	pT4apN0	CDX2 and CK20 positive	KRAS: Exon2 KRAS G12 Mutation NRAS : Negative BRAF : Exon15 V600E Mutation MMR : Intact
9	75/M	Ascending colon	Right hemicoelctomy	5x4	Moderately differentiated adenocarcinoma	BD1	pT3pN0	Not done	KRAS: Negative NRAS : Negative BRAF : Negative MMR : Intact
10	46/F	Hepatic flexure	Extended right hemicoelctomy	3.5x2	Moderately differentiated adenocarcinoma	BD2	pT3pN0	CDX2, CK20 and SATB2 positive	KRAS: Negative NRAS : Negative BRAF : Negative MMR : MSH6 and MSH2 loss of expression
11	72/F	Rectosigmoid colon	Low anterior resection	3x2	Moderately differentiated adenocarcinoma	BD2	pT3pN0	Not done	KRAS: Exon2 KRAS G12 utation NRAS : Negative BRAF : Negative MMR : Intact
12	86/M	Transverse colon	Extended right hemicoelctomy	2x1	Moderately differentiated adenocarcinoma	BD1	pT3pN1b	CDX2,CK20 and SATB2 positive	KRAS: Exon 2 KRAS G13 Mutation NRAS : Negative BRAF : Negative MMR : Not done

13	50/M	Caecum	Extended right hemicoelctomy	One: 5.7x3 and another 4x2	Moderately differentiated adenocarcinoma	BD2	rT(m)2N0	Not done	KRAS: Not done NRAS : Not done BRAF : Negative MMR : Intact
14	28/F	Rectosigmoid junction	Total colectomy	7x3	Mucinous adenocarcinoma	N/A	pT4apN1a	CDX2,CK20 and SATB2 positive	KRAS: Negative NRAS : Negative BRAF : Negative MMR : MLH1 Loss of expression
15	47/M	Caecum	Right hemicolectomy	4x2.5	Moderately differentiated adenocarcinoma	BD2	pT4bpN0	Not done	KRAS: Exon 2 KRAS G13 Mutation NRAS : Negative BRAF : Not done MMR : Intact
16	73/M	Descending colon	Subtotal colectomy	3.5x3	Moderately differentiated adenocarcinoma	BD1	pT4apN0	Not done	KRAS: Exon 2 KRAS G12 Mutation NRAS : Not done BRAF : Negative MMR : Intact
17	57/F	Sigmoid colon	Sigmoidectomy	3x2	Well differentiated adenocarcinoma	BD2	pT3pN0	Not done	KRAS: Exon 2 KRAS G13 Mutation NRAS : Negative BRAF : Negative MMR : Intact

Figure 1. Frequency of CRC based on tumor location

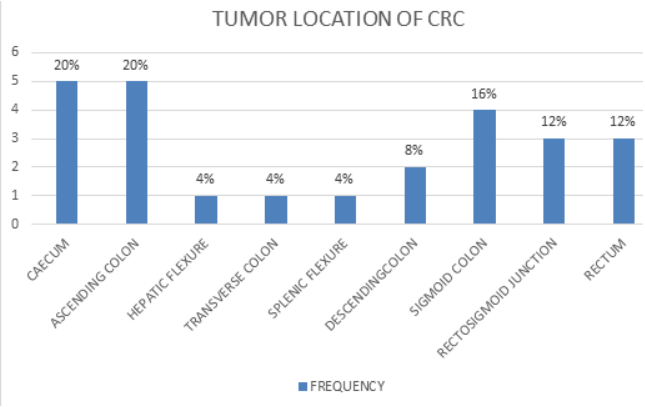
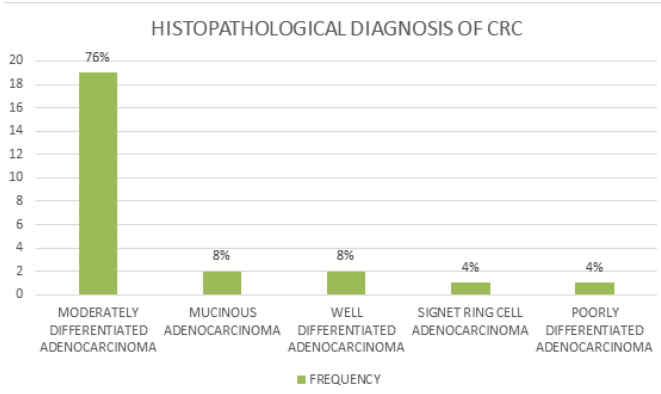


Figure 2. Frequency of CRC based of HPE diagnosis



IHC profile

Only 2 cases of colonoscopic biopsies and 7 cases of resection specimen were submitted for IHC evaluation. In colonoscopic biopsies, positive cytoplasmic and/or membranous signal for CK20 was observed in one case and was not done in another case but 85.71% of resection specimen showed CK20 positivity. All of cases (100%) showed positive nuclear staining for CDX2 and SATB2.

KRAS mutation analysis

KRAS mutation was detected in 8 (32%) cases while KRAS wild-type (Negative) in 16 (64%) cases and 1(4%) case had no KRAS Mutation data (Table 3). Of these KRAS mutated, 5 were men and 3 were women. Under 50 age group, the mutation for KRAS was observed in 3 of 8 patients, while 5 were above 50 years. The distribution of mutated vs. KRAS wild-type was in the ratio 1:2. Among KRAS mutated, Exon 2 KRAS G12V (glycine 12 to valine) was 4 cases, Exon 2 KRAS G12S (glycine 12 to serine) was 1 case, Exon 2 KRAS G13D (glycine 13 to aspartic acid) was 2 cases and Exon 2 KRAS G13X was 1 case. No correlation between microsatellite status and localization, histologic grade, and pathologic stage was observed.

Table 3. KRAS mutation in CRC

KRAS MUTATION	FREQUENCY	PERCENTAGE
EXON2 KRAS G12 MUTATION	5	20
EXON2 KRAS G13 MUTATION	3	12
NEGATIVE	16	64
NO	1	4
TOTAL	25	100

NRAS and BRAF mutation analysis: There was absence of NRAS mutation within tumor specimen of all 25 cases while BRAF mutation was detected in 2 (8%) cases and there is absence of BRAF mutation (Negative) in 21 (84%) cases (Table 4). Two of the cases had no BRAF mutation data. Male to female ratio of BRAF mutation was 1:1. Among BRAF mutated, Exon15 V600E and Exon15 V600R was one case of each.

Table 4. BRAF mutation in CRC

BRAF MUTATION	FREQUNECY	PERCENTAGE
EXON 15 V600E	1	4
EXON 15 V600R	1	4
NEGATIVE	21	84
NO	2	8
TOTAL	25	100

Mismatch repair gene status:

A mismatch repair (MMR) deficiency was found in 3 (12%) cases, 1 (4%) of which presented with loss of expression in both MLH1 and PMS2, 1 (4%) case each presented with loss of expression of MSH2 and MSH6 (Figure 3) and loss of expression of MLH1 (Table 5). Remaining 17 (68%) cases showed intact MMR/MSI status (Figure 4). All 3 cases with MMR deficiency were female. In contrast to KRAS mutation status, all patients aged less than 50 years were MMR-deficient. No correlation between localization, histologic grade, and pathologic stage was observed.

Table 5. Mismatch repair gene status in CRC

MMR Status	FREQUENCY	PERCENTAGE
INTACT	17	68
MLH1 AND PMS2 LOSS	1	4
MLH1 LOSS	1	4
MSH6 AND MSH2 LOSS	1	4
NO	5	20
TOTAL	25	100

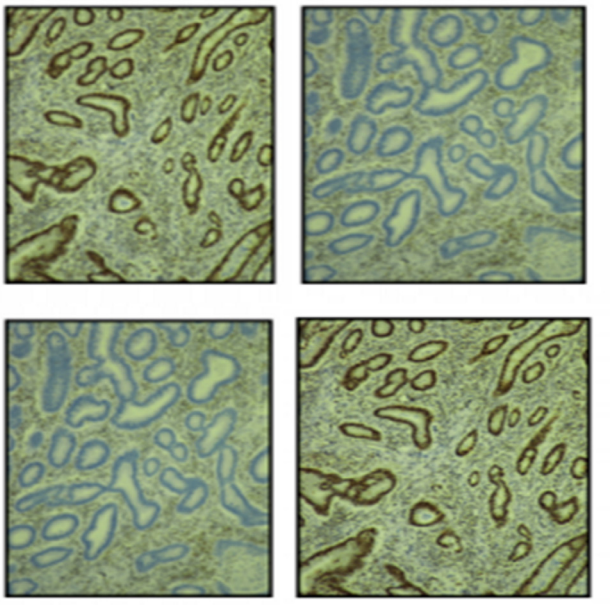


Figure 3. Image showing intact MLH-1, loss of MSH-2 and MSH-6 and intact PMS-2 status of Case 10 from Table 2.

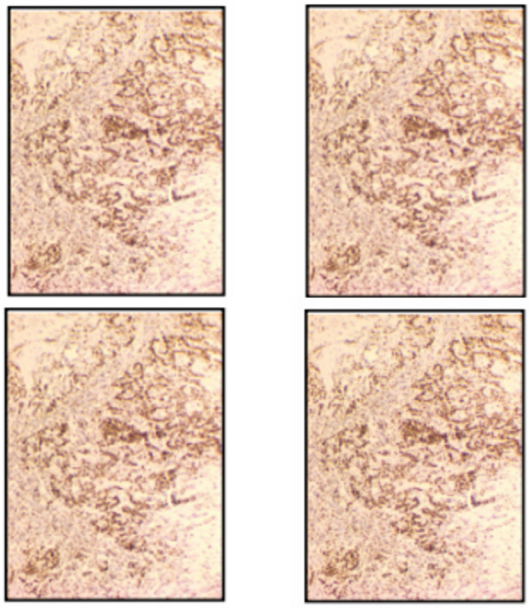


Figure 4. Image showing intact MLH-1, MSH-2, MSH-6 and PMS-2 status of Case 13 from Table 2.

DISCUSSION

CRC is one of the most prevalent malignancies globally and it also represents a significant public health concern due to its high incidence and mortality rates. The pathogenesis of colorectal cancer is multifactorial that involves both genetic predispositions and environmental factors.5 The adenoma-carcinoma sequence is the most widely recognized pathway, where benign adenomatous polyps over time progress to malignant carcinoma.6 The serrated neoplasia pathway and hereditary syndromes like Lynch syndrome are also the pathways for pathogenesis of CRC.7 Accurate diagnosis and classification of colorectal tumors are vital for effective treatment planning and its better prognosis.

Demographic and Clinicopathologic Characteristics

The mean age at diagnosis in our cohort was 55.08 years, with a male-to-female ratio of 1.5:1, consistent with global epidemiological data showing male predominance and peak incidence in the fifth to sixth decades. 8,9. Several large-scale epidemiological studies have demonstrated similar age distributions, with the majority of CRCs occurring after the age of 50.10 This age pattern reflects the accumulated impact of environmental exposures, age-related epigenetic alterations, chronic inflammation, and genetic predispositions that gradually facilitate malignant transformation.11 Early precursor lesions, including adenomatous polyps, also tend to accumulate with age, contributing to this demographic pattern.12 Similar demographic trends have been observed in South Asian populations, where CRC incidence continues to rise in both urban and rural settings.13,14 Studies from Nepal, India, Pakistan, and Bangladesh consistently report mean ages between 50 and 60 years, with a higher burden among males.15-17 Factors implicated in this regional increase include westernized diet, smoking, sedentary lifestyle, metabolic syndrome, and limited screening programs.18 However, the presence of younger patients in our study (as young as 24 years) underscores an important epidemiological shift: the rising incidence of early-onset colorectal cancer (EOCRC). Similar trends have been well documented worldwide.19,20 EOCRC patients often present with more advanced disease, aggressive tumor biology, and delayed diagnosis due to lack of screening below age 45. 21 A population-based analysis from the United States demonstrated a nearly 50% increase in CRC incidence among adults younger than 50 over the past two decades.22

The most frequent tumor sites in our study were the caecum and ascending colon (40%), demonstrating a right-sided predominance, a pattern increasingly recognized particularly among females and elderly patients.23 Right-sided CRCs are often associated with microsatellite instability (MSI) and serrated neoplasia pathways. 24

Histologically, moderately differentiated adenocarcinomas accounted for 76% of cases, consistent with previous reports. 25 Mucinous adenocarcinoma (8%) and signet ring cell carcinoma (4%) were also identified; these variants are associated with adverse outcomes and higher MSI frequency.26,27

IHC Profile: CK20, CK7, CDX2, and SATB2

The immunohistochemical profile comprising CK20, CK7, CDX2, and SATB2 remains a cornerstone in confirming colorectal origin, particularly in metastatic adenocarcinomas.28 In our study, 85.71% of resection specimen showed CK20 positivity. All of cases (100%) showed positive nuclear staining for CDX2 and SATB2. The classical CK20+/CK7− phenotype is typical of colorectal origin while CK20+/CK7+ can also be noted which have been reported, particularly in right-sided or poorly differentiated tumors.29,30 However, in this study CK7 has not been done.

CDX2 is a sensitive nuclear transcription factor for intestinal differentiation, with reduced expression correlating with poor differentiation and advanced stage.31,32 SATB2, expressed in colorectal epithelium, complements CDX2, and the combination of both markers yields the highest specificity for primary CRC.33 Our findings align with these observations, reaffirming their diagnostic utility.

Molecular Alterations: KRAS, NRAS, and BRAF

Mutations in the RAS-RAF-MEK-ERK signaling cascade have critical diagnostic and therapeutic relevance in CRC. KRAS mutations were detected in 32% of cases in our study, comparable to the global prevalence of 30–45%.34,35 The most frequent variants were KRAS G12V (50%) and G13D (25%) located in exon 2, in agreement with other reports.36 KRAS mutations are known predictors of resistance to anti-EGFR monoclonal antibody therapy (Cetuximab and Panitumumab).37

No NRAS mutations were detected, consistent with the low global prevalence (<5%).38 BRAF mutations were found in 8% of cases, involving V600E and V600R substitutions. This frequency corresponds with other studies 39 and was observed exclusively in females, a trend commonly associated with right-sided MSI-high CRCs.40

Mismatch Repair (MMR) and Microsatellite Instability (MSI) Status

Deficient mismatch repair (dMMR) was found in 12% (3/25) of cases, showing MLH1/PMS2 loss in two and MSH2/MSH6 loss in one. This frequency aligns with global estimates where 10–15% of CRCs exhibit MSI due to MMR deficiency.41,42 The presence of dMMR exclusively among younger females strengthens the suspicion for Lynch syndrome and highlights the need for integrated molecular-pathologic evaluation, genetic counseling, and personalized treatment strategies.43,44

Although MSI-H tumors have been classically associated

with right-sided location, mucinous histology, and poor differentiation,45 our cohort did not demonstrate a significant correlation between MSI status and either tumor site or grade. This discrepancy can be attributed to the modest sample size, limiting statistical associations. Larger studies consistently show that MSI-H CRCs arise predominantly in the proximal colon and often exhibit brisk tumor-infiltrating lymphocytes, Crohn-like reaction, and medullary features. 46 These immune-related characteristics reflect a high neoantigen load resulting from defective DNA repair, providing the biological basis for their strong responsiveness to immune checkpoint inhibitors.

Beyond hereditary implications, MSI testing has substantial therapeutic significance. MSI-H/dMMR tumors are now recognized as distinct molecular entities characterized by favorable stage II prognosis, limited benefit from 5-FU monotherapy, and marked sensitivity to PD-1 blockade.47 Pembrolizumab and Nivolumab have demonstrated durable responses in metastatic MSI-H CRC, leading to their incorporation into first-line treatment for advanced disease.48 This underscores the importance of routine MSI/MMR evaluation not only for genetic counseling but also for therapeutic decision-making across disease stages.

Recent large-scale profiling studies have also shed light on the heterogeneity within microsatellite-stable (MSS) CRCs. While the majority of MSS tumors follow the chromosomal instability pathway, a subset may show epigenetic alterations or POLE exonuclease domain mutations, yielding ultra-hypermutated phenotypes with immunologic profiles approaching MSI-H cancers.49,50 Although not observed in our cohort, recognition of such subcategories is increasingly relevant, as they may also respond to immune-based therapies.

CLINICAL IMPLICATIONS

Our findings reaffirm the molecular and immunophenotypic heterogeneity of CRC. The consistent positivity for CK20, CDX2, and SATB2 supports their diagnostic reliability, while KRAS and BRAF mutation profiling provide critical information for therapeutic decisions. The detection of dMMR/MSI emphasizes the need for universal MSI testing in all newly diagnosed CRCs for prognostic and therapeutic guidance and to identify patients at risk for Lynch syndrome.

LIMITATIONS

The study is limited by its retrospective design, single-institution setting, and small sample size. Additionally, germline testing and MLH1 promoter methylation analysis could not be performed due to logistic constraints. Nevertheless, this study provides valuable insight into the regional molecular and IHC patterns of CRC.

CONCLUSIONS

This study demonstrates that the clinicopathologic, immunohistochemical, and molecular features of CRC in our study closely parallel global data and it did not demonstrate a significant correlation between MSI status and either tumor site or grade. The observed frequencies of KRAS (32%), BRAF (8%), and MMR deficiency (12%) highlight the need for routine molecular testing for accurate prognostication, personalized therapy, and familial cancer risk assessment.

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