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SCREENING OF COLORECTAL CARCINOMA BY MISMATCH REPAIR DEFECT THROUGH IMMUNOHISTOCHEMISTRY A SINGLE CENTER STUDY

Original Research

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ABSTRACT

Background: Colorectal carcinoma (CRC) is the third most commonly diagnosed malignancy worldwide and develops through complex genetic and epigenetic mechanisms, including chromosomal instability, microsatellite instability, and CpG island methylator pathways. Defects in mismatch repair (MMR) genes lead to microsatellite instability, which is central to Lynch syndrome and influences prognosis and therapeutic response. While molecular assays remain the gold standard for MSI detection, immunohistochemistry (IHC) offers a practical, cost-effective, and accessible alternative, particularly in resource-limited settings. This study aimed to evaluate the utility of MMR IHC in screening for deficient MMR status in relation to clinico-pathological features in a local population.

Objective: To assess the role of immunohistochemistry in detecting MMR-deficient colorectal carcinoma and to correlate MMR status with clinico-pathological characteristics.

Methods: A descriptive cross-sectional study was conducted at the Chughtai Institute of Pathology from March 2023 to February 2024. Seventy-four colorectal carcinoma resection specimens were analyzed. Immunohistochemistry for MLH1, PMS2, MSH2, and MSH6 was performed using an automated staining platform. Demographic and pathological parameters—including age, gender, tumor site, morphology, grade, and tumor-infiltrating lymphocytes (TILs)—were recorded and correlated with MMR expression status.

Results: Loss of MMR protein expression was identified in 32 of 74 cases (43.2%). Combined loss of MLH1 and PMS2 accounted for 18 cases (24.3%), dual loss of MSH2 and MSH6 in 3 cases (4.1%), isolated loss of MSH2 in 3 cases (4.1%), isolated loss of PMS2 in 2 cases (2.7%), and complete loss of all four proteins in 6 cases (8.1%). Retained MMR expression was observed in 42 cases (56.8%). A statistically significant association was found between MMR deficiency and TILs ($p=0.02$), whereas no significant relationship was observed for age, gender, tumor site, morphology, or tumor grade.

Conclusion: Although molecular techniques remain the definitive approach for MSI detection, MMR IHC represents a reliable, economical, and accessible screening tool. The findings support universal IHC-based MMR screening for all newly diagnosed colorectal carcinomas, irrespective of clinico-pathological parameters.

Keywords: Adenocarcinoma, Colorectal Neoplasms, Immunohistochemistry, Microsatellite Instability, Mismatch Repair, Neoplasm Grading, Tumor-Infiltrating Lymphocytes.

INTRODUCTION

Colorectal carcinoma is the third most frequently diagnosed malignancy worldwide, with an estimated 1.9 million new cases reported in 2020, reflecting both its global health burden and rising incidence among individuals younger than 50 years (1). In Pakistan, it ranks as the fourth most common cancer, with an incidence rate of 5.4% and mortality rate of 3.1%, affecting men slightly more often than women (2,3). Its etiology is multifactorial, shaped by a combination of modifiable and non-modifiable risk factors. Lifestyle-related exposures—such as alcohol consumption, tobacco use, obesity, physical inactivity, high intake of red or processed meats, and chronic psychological stress—contribute significantly to disease development, whereas age, sex, family history, genetic predisposition, prior abdominopelvic radiation, inflammatory bowel disease, and gut microbiome composition represent established non-modifiable determinants (3). The molecular landscape of colorectal cancer is complex and characterized by genetic and epigenetic alterations that drive tumorigenesis through three major pathways: chromosomal instability (CIN), microsatellite instability (MSI), and the CpG island methylator phenotype (CIMP) (4). Approximately 70–80% of colorectal cancers arise through the CIN pathway, typically associated with mutations in APC, KRAS, and TP53 genes, whereas 10–15% progress through the MSI pathway, linked to defects in mismatch repair (MMR) genes (5,6). Growing evidence has highlighted distinct differences between right- and left-sided tumors, with CIN-associated cancers more frequently arising in the left colon and progressing more slowly, in contrast to right-sided MSI-high tumors, which often carry a poorer prognosis (6,7).

Microsatellite instability results from failure of the DNA mismatch repair system—a highly conserved pathway responsible for correcting base-pairing errors during replication (7). Core MMR proteins, including MLH1, PMS2, MSH2, and MSH6, function as heterodimers, with PMS2 and MSH6 relying heavily on their partners for stability. Defects may arise sporadically due to epigenetic silencing—most commonly MLH1 promoter hypermethylation—or through germline mutations, as seen in Lynch syndrome (8,9). Lynch syndrome, previously termed hereditary non-polyposis colorectal cancer, accounts for 1–3% of CRC cases and is characterized by germline mutations in MMR genes, with nearly 90% of affected individuals exhibiting MSI, in contrast to only 15–20% in sporadic tumors (10). Tumors arising in this context tend to present at younger ages, involve the proximal colon, and display distinctive histological features, including mucinous or signet-ring morphology, serrated patterns, prominent tumor-infiltrating lymphocytes, and absence of dirty necrosis (11). Assessment of MMR deficiency is essential for identifying patients with Lynch syndrome, guiding therapeutic decision-making, and predicting prognosis. While polymerase chain reaction-based MSI testing remains the gold standard, immunohistochemistry (IHC) for MMR proteins is widely employed as a rapid, cost-effective initial screening tool, reliably detecting loss of protein expression indicative of MMR dysfunction (12). Despite its clinical relevance, local data regarding the prevalence of MMR-deficient colorectal cancer and its association with clinico-pathological features remain scarce. Given the limited evidence available from Pakistan, the present study was designed to evaluate the frequency of MMR protein deficiency using immunohistochemistry in colorectal carcinoma and to analyze its relationship with the clinico-pathological characteristics of affected patients. The objective was to generate context-specific evidence that may enhance early detection strategies and improve CRC screening practices within the local population.

METHODS

This descriptive cross-sectional study was conducted at the Chughtai Institute of Pathology, Lahore, Pakistan, after obtaining approval from the Institutional Review Board (IRB). All primary colorectal carcinoma resection specimens diagnosed between March 2023 and February 2024 were included. Patients with metastatic colorectal carcinoma or those from whom only mucosal biopsies were available were excluded to ensure histological adequacy and accurate assessment of mismatch repair (MMR) protein status. A convenient sampling technique was employed, and all eligible cases were retrieved through the institutional electronic data management system (Nexus). For each case, reports and histological slides were reviewed, and relevant demographic and pathological parameters were recorded on a structured proforma. Representative hematoxylin and eosin (H&E) sections and corresponding paraffin blocks demonstrating optimal tumor morphology and sufficient tumor burden were selected for immunohistochemical evaluation. The study adhered to ethical principles of confidentiality, and informed consent had been ensured at the time of initial specimen submission as per institutional protocols. Immunohistochemistry for all four MMR proteins—MLH1, PMS2, MSH2, and MSH6—was performed using an automated

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staining platform (Dako Autostainer Link 48) with the EnVision Flex detection system. The specific antibody clones applied included MSH2 (Monoclonal Mouse Primary Antibody, Dako, Clone FE11), MSH6 (Monoclonal Mouse Primary Antibody, Dako, Clone EP49), MLH1 (Monoclonal Mouse Primary Antibody, Dako, Clone ES05), and PMS2 (Monoclonal Mouse Primary Antibody, Dako, Clone EP51). Internal positive controls were assessed in each slide, with intact nuclear staining in lymphocytes, fibroblasts, or adjacent normal epithelium taken as evidence of adequate staining quality (11). Following staining, the prepared slides were independently examined by two consultant pathologists who were blinded to each other's assessments. Nuclear expression of each MMR protein was evaluated according to the College of American Pathologists (CAP) guidelines, which classify any unequivocal nuclear staining in tumor cells—regardless of percentage—as "retained expression," whereas complete absence of tumor nuclear staining in the presence of an intact internal control is interpreted as "loss of expression." Discrepancies between observers were resolved by joint review to ensure diagnostic accuracy.

RESULTS

A total of 74 colorectal carcinoma resection specimens were analyzed. Of these, 35 patients (47.3%) were female and 39 (52.7%) were male. The mean patient age was 51.17 years (range: 11–90 years), with 35 individuals younger than 50 years and 39 older than 50 years. Tumor sites included 2 cases from the ileocecal junction, 9 from the cecum, 13 from the ascending colon, 9 from the transverse colon, 11 from the descending colon, 20 from the sigmoid colon, and 10 from the rectum. Among all clinicopathological parameters examined, only tumor-infiltrating lymphocytes demonstrated a statistically significant association with mismatch repair (MMR) deficiency (p=0.02). No significant relationship was observed between MMR status and age (p=0.59), gender (p=0.30), tumor site (p=0.80), tumor morphology (p=0.80), or tumor grade (p=0.20). Loss of MMR protein expression on immunohistochemistry was identified in 32 cases (43.2%), whereas 42 cases (56.8%) showed retained expression. Combined loss of all four MMR proteins was detected in 6 cases (8.1%). Dual loss of MLH1 and PMS2 was the most frequent pattern, present in 18 cases (24.3%). Loss of both MSH2 and MSH6 occurred in 3 cases (4.1%), while isolated loss of MSH2 was seen in 3 cases (4.1%) and isolated loss of PMS2 in 2 cases (2.7%). Regarding histologic subtype, adenocarcinoma not otherwise specified (NOS) was the most common, reported in 60 patients (81.1%). Mucinous adenocarcinoma was seen in 12 cases (16.2%), and signet ring cell carcinoma in 2 cases (2.7%). Tumor differentiation showed that 3 tumors (4.1%) were well differentiated (Grade 1), 59 (79.7%) were moderately differentiated (Grade 2), and 12 (16.2%) were poorly differentiated (Grade 3). Tumor-infiltrating lymphocytes (TILs) were present in 40 cases (54.1%) and absent in 34 cases (45.9%). Table

1: Clinico-pathological features of MSI colorectal carcinoma

Clinicopathological features	MMR-Deficient	MMR-proficient	p-value
Age(years)			0.5
>50	18	21	
<50	14	21	
Gender			0.3
Male	19	20	
Female	13	22	
Tumor site			0.8
ICJ	0	2	
Cecum	4	5	
Ascending colon	7	6	
Transverse colon	4	5	
Descending colon	5	6	

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Clinicopathological features	**MMR-Deficient**	**MMR-proficient**	**p-value**
Sigmoid colon	9	11	
Rectum	3	7	
Morphology			0.8
Adenocarcinoma Nos	25	35	
Mucinous Adenocarcinoma	6	6	
Signet ring adenocarcinoma	1	1	
Grade			0.2
1	0	3	
2	27	32	
3	5	7	
TILS			
Present	22	18	0.02*
Absent	10	24	

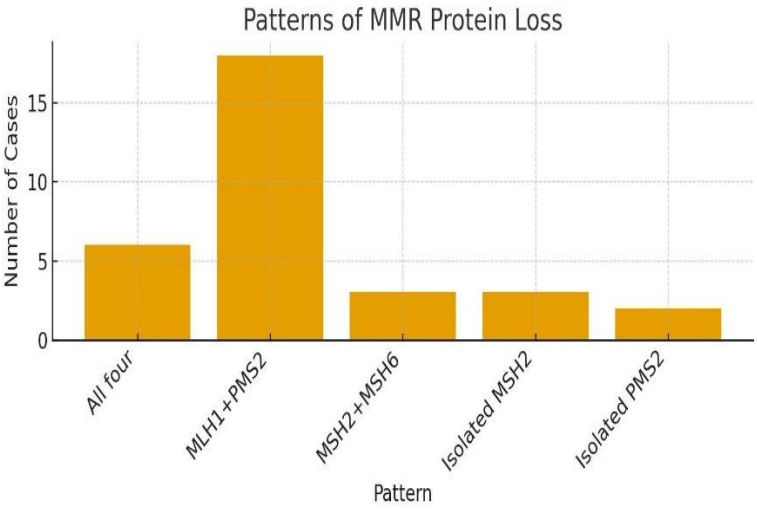

Figure 1 Patterns of MMR Protein Loss

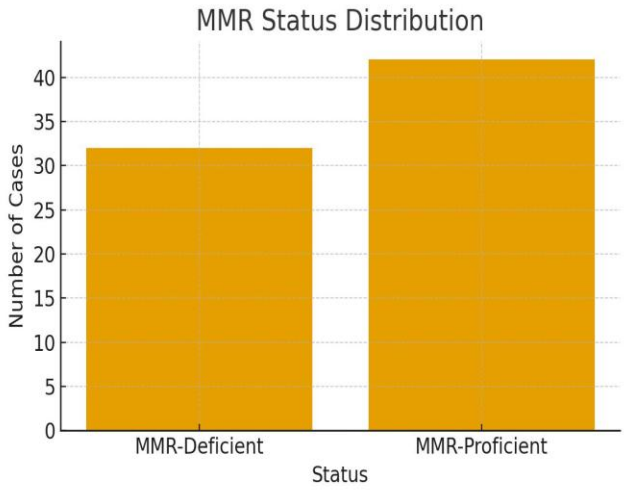

Figure 1 MMR Status Distribution

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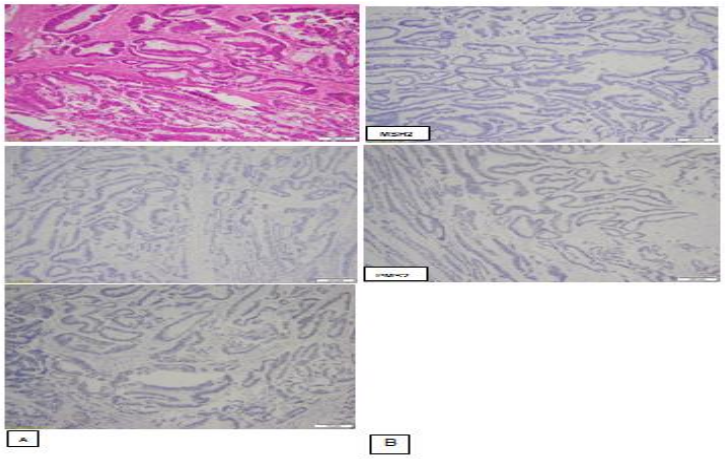
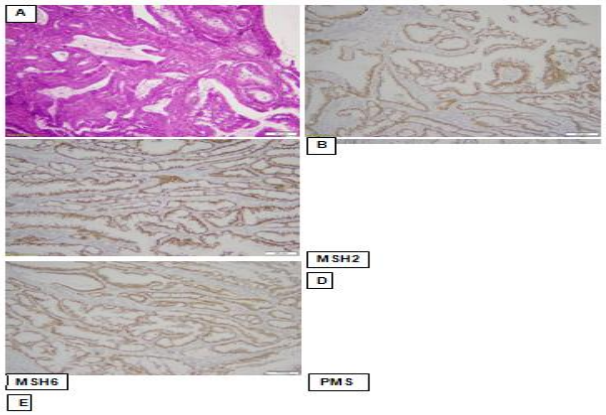


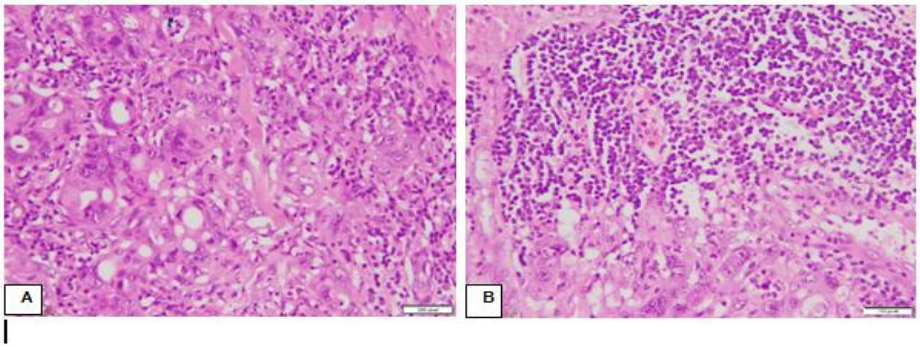
MMR-Deficient Immunohistochemical Markers

Figure 3 MMR-Deficient Immunohistochemical Markers



MMR IHC with retained Expression A) MSH2 (10X), B) MSH6 (10X), C) PMS2 (10X), D) MLH1 (10X)

Figure 4 MMR IHC with retained Expression A) MSH2(10X), B) MSH6 (10X), C) PMS2 (10X), D) MLH1 (10X)



Tumor with Tumor-infiltrating lymphocytes A, B (40X)

Figure 2 Tumor with Tumor-Infiltrating Lymphocytes A, B (40X)

DISCUSSION

MMR deficiency due to loss of functional proteins led to the accumulation of DNA replication errors, higher mutation rates, and microsatellite instability, which remained a defining feature of Lynch syndrome and required confirmation by germline DNA testing (11). Molecular techniques continued to represent the gold standard for microsatellite instability detection; however, this study reinforced that immunohistochemistry provided a practical, cost-effective, and accessible alternative in resource-limited settings (12). By demonstrating a substantial burden of MMR-deficient colorectal carcinomas in a Pakistani cohort, the study underscored the value of routine IHC screening as an entry point for Lynch syndrome identification and as a guide for therapeutic planning, including the selection of patients for immunotherapy. The absence of routine MMR evaluation in colorectal carcinoma patients at a national level indicated a critical gap in current clinical practice that this work sought to address. The pattern of MMR protein loss observed in this cohort showed

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that most deficiencies occurred in combinations rather than as isolated losses. Combined loss of MLH1 and PMS2 in 24.3% of cases, dual loss of MSH2 and MSH6 in 4.1%, isolated loss of PMS2 in 2.7%, isolated loss of MSH2 in 4.1%, and complete loss of all four proteins in 8.1% reflected disruption of the fundamental MMR heterodimer biology, with partner proteins becoming unstable when their primary counterpart was lost. The predominance of combined MLH1 and PMS2 loss aligned with patterns described by other investigators, supporting the concept that MLH1 inactivation, either through mutation or promoter hypermethylation, drove many dMMR phenotypes (13). The absence of isolated MLH1 loss in this series contrasted with some published work, suggesting possible population-specific biology or differences in case selection and technical approaches. The overall frequency of MMR-deficient colorectal carcinoma in this study, 43.2%, appeared higher than the 18.8% and 15% reported in several other series, yet remained comparable to one report that documented dMMR in 46.15% of tumors. This divergence from lower-frequency studies implied that the local population might harbor a higher proportion of tumors with MSI biology, or that referral and selection patterns enriched the cohort for cases with features prompting IHC testing (14).

Age-related patterns in this cohort showed that MMR loss occurred in both younger and older patients. Loss of MMR expression was seen in 40% of patients younger than 50 years and 46.1% of those older than 50 years. These findings mirrored one study in which dMMR tumors predominantly affected patients older than 50 years (15), but contrasted with other series where MMR-deficient colorectal carcinoma more commonly affected younger individuals, particularly those under 50 years (14,16). This contrast highlighted an ongoing debate regarding the age profile of dMMR tumors and suggested that universal or broad-based testing strategies might be preferable to age-restricted algorithms, particularly in regions where young-onset colorectal cancer incidence had risen. Given that colorectal carcinoma incidence typically increased sharply after 45 years of age and that the majority of global cases occurred beyond 50 years, the observation that dMMR was frequent in both age strata in this cohort carried practical implications for screening thresholds. The gender distribution in this study showed a slight male predominance, with 52.7% males and 47.3% females. Among these, 48.7% of males and 37.1% of females demonstrated MMR loss, indicating a higher proportion of dMMR tumors in men. This pattern was consistent with previous observations that reported a greater burden of MMR-deficient colorectal carcinoma in male patients (17). Such a pattern supported the notion that biological sex, superimposed on environmental and lifestyle factors, might modulate genetic or epigenetic vulnerability within the MMR pathway.

Topographic analysis of tumor location showed a wide distribution across the colon and rectum. Among the 32 MMR-deficient tumors, 12.5% arose in the cecum, 21.8% in the ascending colon, 12.5% in the transverse colon, 15.6% in the descending colon, 28.1% in the sigmoid colon, and 9.3% in the rectum. Overall, 55.4% of all tumors were left-sided and 44.6% were right-sided. Within these subsets, 41.4% of left-sided and 45.4% of right-sided tumors were dMMR. Although the numerical proportion of dMMR tumors was slightly higher on the right side, the study did not demonstrate a statistically significant association between tumor location and MMR deficiency, in agreement with some published work that also reported a lack of significant correlation (16). Nevertheless, the higher proportion of dMMR among right-sided tumors concurred with the broader literature, where MSI-high and Lynch-associated cancers often showed a proximal predilection (14–17). This dual observation indicated that while right-sided predilection persisted at a descriptive level, location alone did not provide sufficient discriminatory power for selecting cases for MMR testing. Histologically, adenocarcinoma not otherwise specified remained the predominant subtype, followed by mucinous and signet ring cell carcinoma. In this cohort, MMR loss occurred in 41.6% of adenocarcinoma NOS, 50% of mucinous tumors, and 50% of signet ring carcinomas. This pattern indicated that dMMR was particularly enriched in tumors with mucinous and signet ring morphology, paralleling previous studies that linked mucinous and signet ring features with MSI and MMR deficiency (15,17). In contrast, another report described a majority of dMMR tumors as non-mucinous (76.2%), underscoring the heterogeneity between study populations and suggesting that morphology-based enrichment strategies might perform differently across settings (18). In terms of grade, most tumors were moderately differentiated, and MMR loss was more frequent in this group (45.7%) compared with poorly differentiated tumors (41.6%). This pattern matched the findings of a previous study that also observed a predominance of dMMR in moderately differentiated carcinomas (18). The lack of a statistically significant association between tumor grade and MMR status in this series, consistent with several other reports, indicated that differentiation alone did not reliably predict MMR status (14–18).

A key finding of this study was the strong association between tumor-infiltrating lymphocytes and MMR deficiency. TILs were present in 40 of 74 cases, and among these, 55% demonstrated loss of MMR proteins, while 45% retained expression. This statistically significant association supported the widely accepted concept that dMMR and MSI-high tumors often elicited a robust host immune response, characterized by increased TIL density and peritumoral lymphoid reaction (14). The biological interpretation of this pattern aligned with the understanding that high neoantigen loads in MMR-deficient tumors enhanced immunogenicity and promoted

lymphocytic infiltration, which in turn related to better prognosis and heightened sensitivity to immune checkpoint inhibitors (19,20). Studies that identified TIL count as one of the strongest histologic predictors of MSI supported the present observations (21). In contrast, other authors reported no significant association between TILs and MMR deficiency, highlighting ongoing variability in histological assessment methods and cut-offs used to define lymphocytic prominence (14,15). The implications of these findings for clinical practice in Pakistan were substantial. The relatively high proportion of MMR-deficient tumors in this cohort, the clear association with TILs, and the feasibility of performing a four-antibody IHC panel supported the introduction of routine MMR screening for all colorectal carcinoma resections. Such a strategy would enhance the detection of patients at risk for Lynch syndrome, allow cascade testing of at-risk relatives, and inform therapeutic decision-making, particularly with respect to immunotherapy. Furthermore, the data contributed to a growing body of regional evidence that argued against reliance on age or single histologic features alone when deciding on MMR testing. This study possessed several strengths. It used a standardized four-antibody panel, evaluated by two consultant pathologists, and applied clear criteria for defining retained versus lost expression based on established guidelines. The inclusion of a broad range of tumor sites and histologic subtypes allowed a comprehensive exploration of clinico-pathological correlates of MMR status. The work also addressed a significant local gap, providing baseline data from a setting where systematic MMR testing had not been routinely integrated into clinical workflows.

However, important limitations were present. The non-availability of germline genetic testing represented a major constraint, as molecular confirmation of MSI and germline mutation status remained the gold standard for diagnosing Lynch syndrome. The study did not include ancillary testing for MLH1 promoter hypermethylation or BRAF mutation, which would have assisted in distinguishing sporadic MSI-high tumors from hereditary cases in those with MLH1/PMS2 loss. The single-center design and modest sample size limited the generalizability of the findings to the wider population, and the use of convenient sampling introduced the possibility of selection bias. Furthermore, the absence of long-term follow-up data precluded analysis of survival, recurrence, and treatment response in relation to MMR status. Some key prognostic variables, such as lymphovascular invasion, perineural invasion, nodal status, and pathological stage, were not fully explored, which restricted a more nuanced assessment of the prognostic impact of dMMR within this cohort. Future research in this area would benefit from multicenter collaboration, larger sample sizes, and integration of comprehensive molecular testing, including germline sequencing and methylation analysis, to distinguish sporadic from hereditary cases more accurately. Prospective studies incorporating outcome measures such as overall survival, disease-free survival, and response to chemotherapy or immunotherapy would clarify the prognostic and predictive relevance of MMR status in the local population. Expansion of this work to include family history assessment, genetic counseling, and cascade testing would deepen the impact of MMR screening on hereditary cancer prevention. Overall, this study contributed important evidence that MMR-deficient colorectal carcinoma constituted a substantial proportion of cases in a Pakistani cohort and that immunohistochemical assessment of MMR proteins provided a practical and informative tool for routine diagnostic practice. By linking MMR deficiency with specific histologic patterns and TIL prominence, the findings reinforced the role of pathology-based biomarkers in refining risk stratification and expanding access to precision oncology in resource-limited settings.

CONCLUSION

This study demonstrated that mismatch repair deficiency showed no meaningful association with most clinico-pathological parameters, apart from a clear link with the tumor-infiltrating lymphocytic response. The identification of widespread loss of MMR protein expression underscored the need for further genetic evaluation to detect Lynch syndrome and related hereditary cancers. These findings reinforce the importance of performing MMR immunohistochemistry as a reflex test for every newly diagnosed colorectal adenocarcinoma, irrespective of patient age or tumor morphology. Incorporating this approach into routine diagnostic practice would enhance early detection, guide appropriate therapeutic decisions, and support timely screening of at-risk family members.

AUTHOR CONTRIBUTION

Author	Contribution
Maryam Akhtar*	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Asma Zafar	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Saira Rathore	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Anila Chughtai	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Zubaria Rafique	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Akhtar Sohail Chughtai	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published

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