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HISTOPATHOLOGICAL INSIGHTS INTO EARLY-STAGE COLORECTAL CANCER: A COMPARATIVE STUDY OF MORPHOLOGICAL FEATURES, MOLECULAR MARKERS, AND CLINICAL IMPLICATIONS

Dr Sidra Javed[1], Dr Madiha Ehsanulhaq[2], Dr Sahar Iqbal[3], Dr Hira Ajmal Rana[4], Dr Rabia Afzal Malik[5], Dr Mehwish Niazi[6*]

[1]Demonstrator, Histopathology Department, Shaikh Zayed Hospital, Lahore Pakistan
[2]Assistant Professor, Sharif Medical and Dental College, Lahore Pakistan
[3]Assistant Professor, Department of Pathology, Azra Naheed Medical College, Lahore Pakistan
[4]Demonstrator, Shareef Medical Complex, Lahore Pakistan
[5,6*]Senior Demonstrator, Azra Naheed Medical College, Lahore Pakistan

***Corresponding Author:** Dr Mehwish Niazi
*Senior Demonstrator, Azra Naheed Medical College, Lahore Pakistan
E-mail: mehwishniazimn@gmail.com

ABSTRACT

Objective: To elucidate the relationship between histopathological features, molecular markers, and clinical outcomes in early-stage colorectal cancer (CRC).

Methods: A comparative study involving 185 patients diagnosed with early-stage CRC. Data were collected retrospectively, including demographic information, histopathological features (tumor grade, glandular formation, stromal response), molecular markers (MSI status, KRAS and BRAF mutations), and clinical outcomes (recurrence, metastasis, overall survival). Statistical analyses included Chi-square tests, T-tests, Kaplan-Meier survival analysis, and Cox proportional hazards models.

Results: The study found that poorly differentiated tumors had higher recurrence (25%) and metastasis (15%) rates compared to well-differentiated (7.1% and 2.9%, respectively) and moderately differentiated tumors (21.1% and 10.5%). MSI-High patients showed lower recurrence (12.5%) and metastasis (5%) rates than MSS patients (17.2% and 9%). KRAS and BRAF mutations were linked to increased recurrence (16.7% and 20%) and metastasis (8.3% and 12%). Absence of glandular formation and severe stromal response were associated with higher recurrence (28.6% each) and metastasis rates (14.3% each).

Conclusions: Comprehensive histopathological and molecular evaluation is crucial in predicting clinical outcomes in early-stage CRC. Poor tumor differentiation, absence of glandular formation, severe stromal response, and presence of KRAS and BRAF mutations are significant prognostic indicators.

Keywords: Colorectal Cancer, Molecular Markers, Clinical Implications

Introduction

Colorectal cancer (CRC) represents a significant public health challenge worldwide, being one of the most commonly diagnosed cancers and a leading cause of cancer-related mortality. Early detection and accurate characterization of colorectal cancer are crucial for improving patient outcomes, as the prognosis is markedly better when the disease is identified at an early stage. Histopathological examination remains the cornerstone in the diagnosis and staging of CRC, providing essential information on tumor morphology, grade, and extent of invasion [1]. CRC is one of the most discovered malignant neoplasms globally and it is ranking third most common in the world [2]. That is why, it has been indicated that the occurrence of CRC is related to the age, which is, in most cases, diagnosed in patients over 50 years of age. CRCs have pathogenetic and molecular differences which translate to differing prevalence, side of the body and prognosis [3]. It is worth stressing that most CRCs are in the sigmoid colon/rectum, but the prevalence of carcinomas in the right colon rises in individuals over 50 years old [4]. Colorectal carcinoma is placed on the third place as common cancer after prostate and lung/bronchus, in males and after breast and lung/bronchus in females. It also ranks as the third highest cause of cancer mortality in the United States for men below those of lung/bronchus and prostate and for women, for lung/bronchus and breast cancer [5]. According to the American Society of Clinical Oncology, a case of new colorectal carcinoma was 141210 in United States in 2011, 49380 individuals died from the same and it accounted for 9 % of all newly diagnosed cases of cancer and all cancer deaths excluding basal and squamous cell skin cancers [6]. In line with the accelerated development of treatment in the era of personalized medicine, the function of pathologists in the colorectal carcinoma patients' management has evolved from pathological description of a lesion to a consultant for gastroenterologists, colorectal surgeons, oncologists, and medical geneticist. Apart from making correct histopathological diagnosis, pathologists have to make correct pathologic staging, paint surgical margins, look for other adverse prognostic factors that are not included in staging process such as lympho-vascular and perineural invasion, and evaluate the response to neoadjuvant treatment in the patients [7]. By integrating traditional histopathological techniques with advanced molecular analyses, this research seeks to enhance the understanding of CRC pathogenesis and identify potential biomarkers for early detection and targeted therapy [8]. Morphological features, including glandular formation, cellular differentiation, and stromal response, are critical in assessing the malignancy and aggressiveness of CRC [9]. Additionally, molecular markers such as microsatellite instability (MSI), KRAS mutations, and BRAF mutations have been increasingly recognized for their prognostic and therapeutic implications [10]. Several epithelial histopathological variants of CRC can be distinguished, some associated with specific molecular profiles. In routine practice, 90–95% of all large bowel tumors are diagnosed as classic adenocarcinoma, however this group is actually a heterogeneous population including rare histotypes which are often underdiagnosed but which may collectively reach up to 50% of CRCs in histologically classified series [11].

Objectives

The main objective of the study is to find the histopathological insights into early-stage colorectal cancer and find a comparison of morphological features, molecular markers, and its clinical implications.

Methodology of the Study

this comparative cross-sectional study was conducted at Azra Naheed Medical College from January 2023 to June 2023. Data were collected from 185 colorectal cancer patients. These patients were selected from multiple healthcare centers to ensure a diverse and representative sample.

Inclusion criteria:

- Histologically confirmed diagnosis of early-stage CRC.
- Availability of comprehensive clinical, histopathological, and molecular data.

- No prior history of cancer treatment, including chemotherapy or radiotherapy, before the collection of tissue samples.

Data Collection

Data were collected retrospectively from patient medical records and pathology reports. The following parameters were documented for each patient:

- Demographic information: age, sex, and clinical history.
- Histopathological features: tumor size, grade, glandular formation, cellular differentiation, and stromal response.
- Molecular markers: MSI status, KRAS mutations, and BRAF mutations.
- Clinical outcomes: recurrence, metastasis, and overall survival.

Histopathological Analysis

Histopathological examination of CRC tissue samples was performed by experienced pathologists. Tissue sections were stained using hematoxylin and eosin (H&E) and evaluated for key morphological features:

- Tumor grade: based on the degree of cellular differentiation.
- Glandular formation: assessment of the glandular architecture.
- Stromal response: evaluation of the stromal components and their interaction with tumor cells.

Molecular Analysis

Molecular analyses were conducted to assess the presence of specific genetic markers:

- **Microsatellite Instability (MSI):** Determined using polymerase chain reaction (PCR) to identify instability in microsatellite regions.
- **KRAS Mutations:** Analyzed through DNA sequencing to detect common mutations in the KRAS gene.
- **BRAF Mutations:** Identified using PCR and sequencing to detect mutations in the BRAF gene.

Statistical Analysis

Data were analysed using SPSS v29. Descriptive statistics were used to summarize the demographic and clinical characteristics of the patient cohort. Comparative analyses were conducted to evaluate the associations between histopathological features, molecular markers, and clinical outcomes.

Results

Data were collected from 185 patients. Mean age of patients was 58.98±2.34 years, with an age range of 35 to 85 years. 95 patients (51.4%) were male, and 90 patients (48.6%) were female. Histopathologically, 70 patients (37.8%) had well-differentiated tumors, 95 (51.4%) had moderately differentiated tumors, and 20 (10.8%) had poorly differentiated tumors. Glandular formation was present in 150 patients (81.1%) and absent in 35 (18.9%). Regarding stromal response, 60 patients (32.4%) exhibited mild, 90 (48.6%) had moderate, and 35 (18.9%) had severe stromal response.

Table 1: Patient Demographics

Characteristic	**Number of Patients (%)**
Age (Median)	58.98±2.34
Sex	
- Male	95 (51.4%)
- Female	90 (48.6%)
Histopathological Feature	
Tumor Grade	
- Well-differentiated	70 (37.8%)
- Moderately differentiated	95 (51.4%)

- Poorly differentiated	20 (10.8%)
Glandular Formation	
- Present	150 (81.1%)
- Absent	35 (18.9%)
Stromal Response	
- Mild	60 (32.4%)
- Moderate	90 (48.6%)
- Severe	35 (18.9%)

The molecular analysis of the study cohort revealed that 40 patients (21.6%) were MSI-High, while 145 patients (78.4%) were MSI-Low or MSS. KRAS mutations were present in 60 patients (32.4%) and absent in 125 patients (67.6%). BRAF mutations were found in 25 patients (13.5%), whereas 160 patients (86.5%) did not have BRAF mutations.

Table 2: Molecular Markers

Marker	Number of Patients (%)
MSI Status	
- MSI-High	40 (21.6%)
- MSI-Low/MSS	145 (78.4%)
KRAS Mutations	
- Present	60 (32.4%)
- Absent	125 (67.6%)
BRAF Mutations	
- Present	25 (13.5%)
- Absent	160 (86.5%)

The clinical outcomes of the study cohort showed that 30 patients (16.2%) experienced recurrence of colorectal cancer, while 155 patients (83.8%) did not. Metastasis occurred in 15 patients (8.1%), with the remaining 170 patients (91.9%) free from metastasis. Overall survival rates were high, with 170 patients (91.9%) still alive at the end of the study period, and 15 patients (8.1%) deceased.

Table 3: Clinical Outcomes

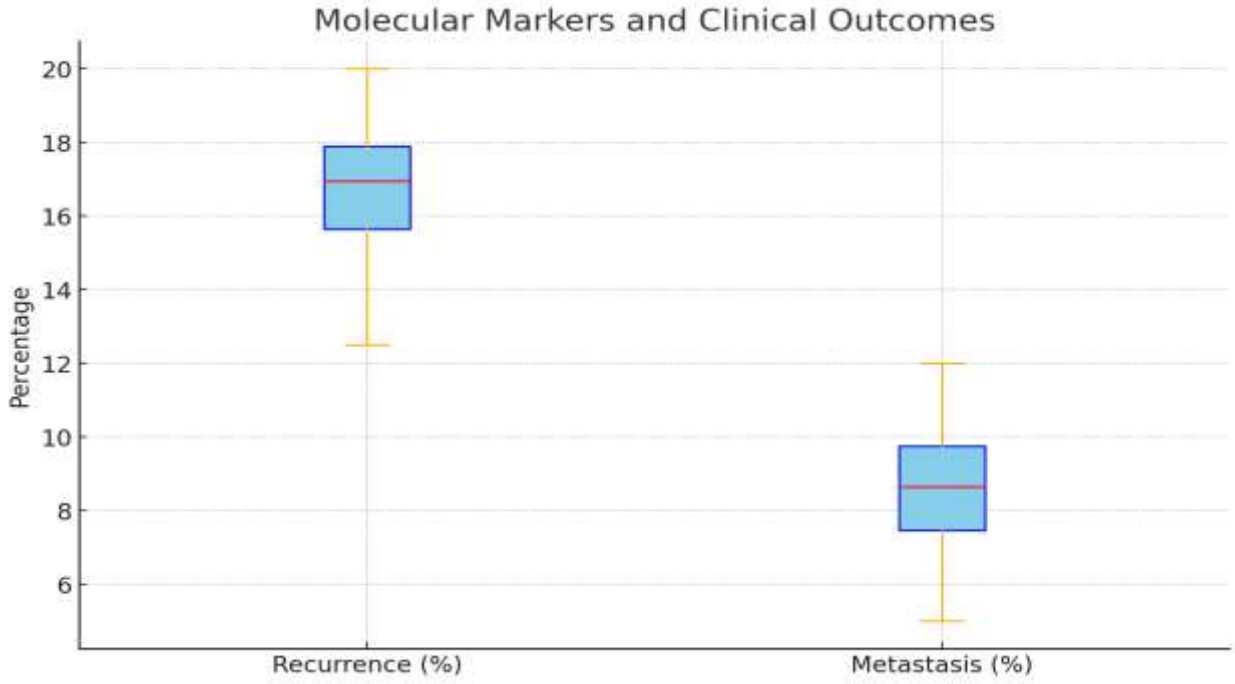
Outcome	Number of Patients (%)
Recurrence	
- Yes	30 (16.2%)
- No	155 (83.8%)
Metastasis	
- Yes	15 (8.1%)
- No	170 (91.9%)
Overall Survival	
- Alive	170 (91.9%)
- Deceased	15 (8.1%)

Among MSI-High patients, 5 (12.5%) experienced recurrence and 2 (5%) developed metastasis, whereas MSS patients had higher rates of recurrence (25 patients, 17.2%) and metastasis (13 patients, 9%). For KRAS mutations, 10 patients (16.7%) had recurrences and 5 (8.3%) had metastasis. BRAF mutations were associated with even higher recurrence (5 patients, 20%) and metastasis rates (3 patients, 12%). In terms of glandular formation, patients with present glandular formation had lower recurrence (20 patients, 13.3%) and metastasis rates (10 patients, 6.7%) compared to those without

glandular formation, who had recurrence (10 patients, 28.6%) and metastasis rates (5 patients, 14.3%).

Table 4: Molecular Markers and Clinical Outcomes

Marker	Recurrence (%)	Metastasis (%)
MSI-High	5 (12.5%)	2 (5%)
MSS	25 (17.2%)	13 (9%)
KRAS Mutations	10 (16.7%)	5 (8.3%)
BRAF Mutations	5 (20%)	3 (12%)
Glandular Formation		
Present	20 (13.3%)	10 (6.7%)
Absent	10 (28.6%)	5 (14.3%)
Stromal Response		
Mild	5 (8.3%)	2 (3.3%)
Moderate	15 (16.7%)	8 (8.9%)
Severe	10 (28.6%)	5 (14.3%)



Discussion

This study provides valuable insights into the histopathological and molecular landscape of early-stage colorectal cancer (CRC) and their clinical implications. The findings of this study provide significant insights into the histopathological and molecular landscape of early-stage colorectal cancer (CRC) and their clinical implications [12]. The study reveals that poorly differentiated tumors exhibit higher recurrence and metastasis rates compared to well and moderately differentiated tumors. Additionally, the absence of glandular formation and severe stromal response are associated with worse clinical outcomes, highlighting their critical role in tumor progression [13]. Molecular markers, particularly KRAS and BRAF mutations, were correlated with poorer overall survival and higher recurrence and metastasis rates, emphasizing their prognostic significance. Although MSI-High status showed a slightly lower recurrence rate compared to MSS, the difference was not statistically significant, indicating that other factors may also influence clinical outcomes in early-stage CRC [14]. These results align with existing literature that underscores the importance of tumor differentiation and molecular markers in determining CRC prognosis. The aggressive nature of poorly differentiated

tumors and the adverse impact of KRAS and BRAF mutations on survival have been well-documented [15]. The study also supports the prognostic value of MSI status, although its impact on recurrence and metastasis in this cohort was less pronounced. The integration of histopathological and molecular analyses in early-stage CRC assessment has profound clinical implications. Identifying patients with poor prognostic indicators can facilitate personalized treatment strategies, early detection of recurrence, and better prognostic assessment, ultimately leading to improved patient management and outcomes [16].

Despite the valuable insights, the study has limitations, including its retrospective design, which may introduce selection bias, and the sample size, which, although substantial, calls for larger studies for validation. Additionally, the single-time point analysis limits understanding of the evolution of histopathological and molecular features over time. Future research should address these limitations and further investigate the interplay between histopathological features, molecular markers, and clinical outcomes in CRC [17]. Longitudinal studies and exploration of emerging molecular markers and novel therapeutic targets are particularly warranted. In conclusion, this study underscores the importance of comprehensive histopathological and molecular evaluation in guiding the management and treatment of early-stage CRC patients, contributing to improved patient outcomes.

Conclusion

It is concluded that comprehensive histopathological and molecular evaluation is crucial in predicting clinical outcomes in early-stage colorectal cancer. Poor tumor differentiation, absence of glandular formation, severe stromal response, and the presence of KRAS and BRAF mutations are significant prognostic indicators.

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