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Original Research Article.

Significance of Specified Set of Histomorphological Features in Prognosis of Sporadic Colorectal Adenocarcinomas and Their Importance as Predictor of Microsatellite Instability (MSI-H): Study of 179 Cases at Tertiary Care Centre, Jaipur

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ABSTRACT

Introduction: Incidence of colorectal carcinoma (CRC) is rising among young adults. CRC is caused by combination of genetic and environmental factors. Majority develop via chromosomal instability pathway whereas 12-15% arise from Microsatellite instability (MSI). They exhibit considerable variability in clinical outcome, so IHC, molecular and genetic studies are must to individualize the therapy. Aims of study is to 1. Highlight conventional specified set of histomorphological features favouring MSI-H and their significance as better prognostic factors. 2. Role of Genetic and molecular studies.

Methods: The study was conducted in Department of Pathology, SMS Medical College, Jaipur. The study is retrospective type carried out between period of 2014 to 2019 with Sample size 179. In our study, Histopathological features were studied in detail for prognostic stratification and those which discriminate between Microsatellite instability – High (MSI-H) and Microsatellite stable (MSS) tumors were used in combination to attempt increased sensitivity for identification of MSI-H tumors.

Results: In our study, like many centres were molecular and genetic study isn't available, we concluded that tumors with certain features (young females, right sided colon involvement, few mucinous tumors, medullary carcinomas and inflammatory responses like - chron's reaction, intra-tumoral / intraepithelial lymphocytic infiltration) have better prognosis/survival. It was also compared with the studies where MSI testing was done. Detailed study of histomorphological features may be of help in prioritizing sporadic colon cancer for further MSI studies. These features form the basis of Revised Bethesda Guidelines for selecting candidates for MSI testing.

Conclusion: Molecular testing plays an important role in era of personalized medicine. Pathologists play a central role in analyzing MSI-H phenotype which constitutes pathologically and clinically distinct subtype, selecting appropriate sections for MSI testing and mutational analysis for KRAS and BRAF, and their interpretation for prognostic and therapeutic tests.

Keywords: Colorectal Carcinoma, Microsatellite Instability, Histopathology.

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INTRODUCTION

Colorectal carcinomas are the fourth most common type of cancer in males and third common cause of cancer in females in india.[1] Worldwide, the incidence of early onset colon cancer is found to be increasing.[2,3] Globally, incidence of CRC is 9.4% in males and 10.1% in female.[4] Majority of CRC develop via chromosomal instability pathway, whereas 12-15 % arise from Micro satellite Instability.[5] Phenomenon of DNA Microsatellite Instability (MSI) resulting from extensive nucleotide insertions or deletions with failure of repair of DNA mismatches. It is caused by inactivation of one of group of genes responsible for nucleotide mismatches, including hMSH2, hMLH1, PMS1, PMS2, hHMSH6/GTBP and hMSH3.[6-8] MSI-H tumors are hallmark of hereditary nonpolyposis colorectal cancer syndrome (HNPCC) and has its significance in management and prognosis of patients.[5]

CRC is caused by combination of genetic and environmental factors. The revised Bethesda Criteria of Clinical and Pathological

characteristics are also developed to identify those patients for whom MSI analysis should be done.[9]

As per various studies, MSI-H cancers have better stage specific survival after surgical and adjuvant therapies. However, molecular analysis is currently time consuming, expensive to perform, and presently available in premiere institutes.

In a setup where molecular analysis is a constraint, we have tried to focus on Importance of conventional histomorphology as an important predictor of MSI-H (screening tool) to start with appropriate therapy. Detailed histopathological evaluation of tumor features and host responses can be used as tool for identification of MSI-H tumors and selecting appropriate sections for MSI testing and mutation analysis for KRAS AND BRAF.[10]

More than 90% of colorectal carcinomas are adenocarcinomas. In well differentiated adenocarcinoma >95% of tumor is gland forming. Moderately differentiated adenocarcinomas show 50-95% gland formation and poorly differentiated are mostly solid with <50% gland formation. Mucinous adenocarcinoma, when >50% of lesion is composed of extracellular pools of mucin. While carcinomas with mucinous areas of <50% are categorized as having mucinous component. Those which are Microsatellite stable are more aggressive. Signet ring carcinomas constitutes <1% of all colon carcinomas, are defined by presence of > 50% of tumor cells showing signet rings. They are poorly differentiated (high grade) and carries worse outcome. However, some may be MSI-H tumors and behave as low grade.

Medullary is extremely rare and is strongly associated with MSI_H. It usually has favourable prognosis despite it is poorly differentiated. It is also characteristically associated with tumor infiltrating lymphocytes.

MATERIALS AND METHODS

The study was conducted in Department of Pathology, SMS Medical college, Jaipur. The study is retrospective type carried out between period of 2014 to 2019. Sample size was taken is 179. All colorectal adenocarcinomas and their variants operated in SMS hospital, were 3 years follow up available were the cases included for study. Autolysed sample & cases for those Follow-up not available were excluded from study. Tissue received in 10% formalin, Clinical features, age, sex, site of involvement, type of growth were noted. Gross examination done; routine processing paraffin embedded blocks were prepared. All sections were stained with Hematoxylin and Eosin. Complete histomorphological assessment was done. IHC markers CK7, CK 20 and CDX2 were done on as and when required basis. Features of host immune responses like chron's like lymphoid reaction, intratumoral lymphocytic infiltrate & intraepithelial T-cells by IHC for CD 3 positivity. Special stains like mucicarmine wherever required. The cases were followed after the surgery and chemotherapy for 3 survivals. In a setup where molecular analysis is a constraint, we have tried to focus on importance of conventional histomorphology as an important predictor of MSI-H (screening tools) and correlated with various studies were MSI-H testing was done.

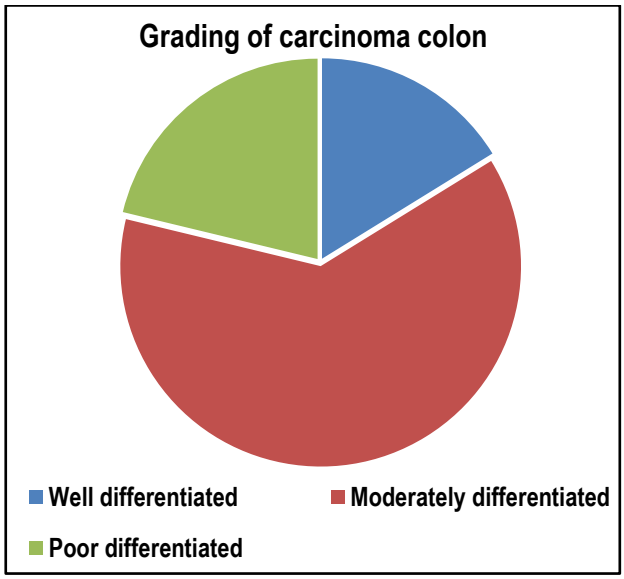

Figure 1: Grading of Colon Carcinoma

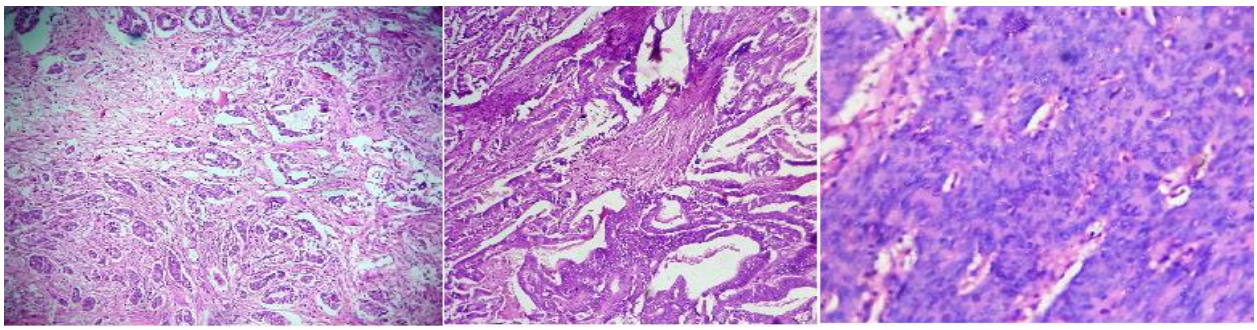

Fig 2: Histopathology images (Right to left) of Well-differentiated (>90% gland formation), Moderately differentiated (50-90% Glands) and poorly differentiated carcinoma (<50% gland formation).

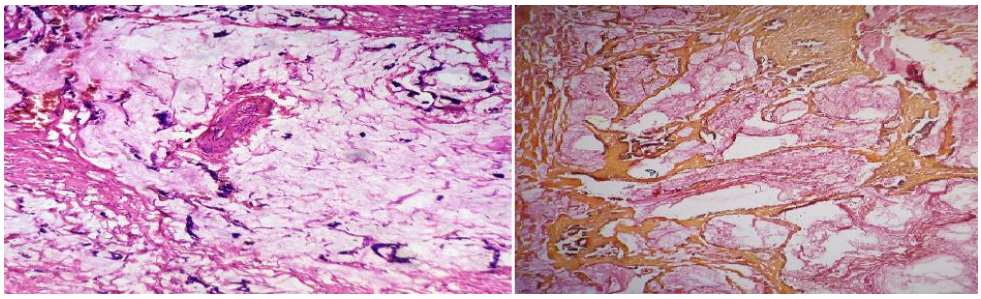

Fig.3 (Right) Mucinous adenocarcinoma showing abundant extracellular mucin. (Left) Mucicarmine staining was done to confirm mucinous morphology.

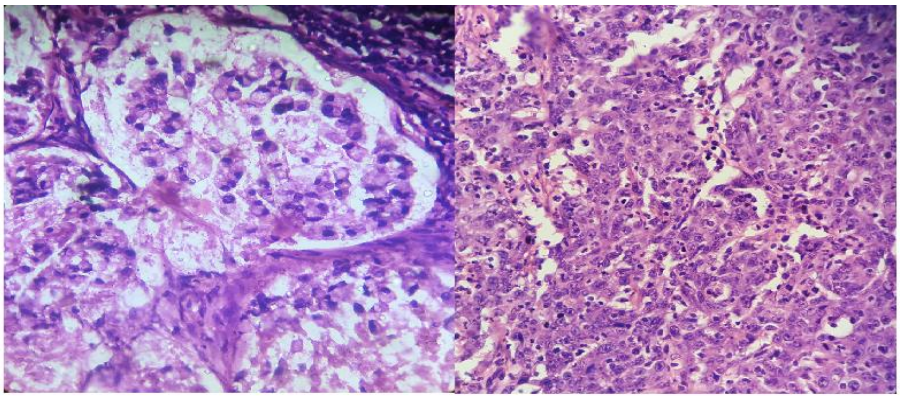

Fig. 4: (Right) Signet ring carcinoma showing intracellular mucin. (Left) Medullary carcinoma shows large malignant cells with abundant pink cytoplasm and vesicular nuclei with prominent nucleoli. Numerous lymphocytes were evident in the malignant epithelium.

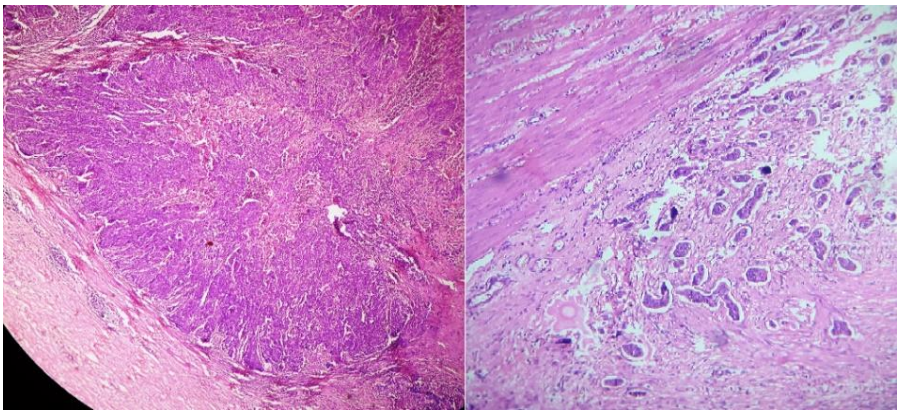

Fig. 5: (Right) Medullary carcinoma showing a pushing border (reaching upto serosa) at tumor edge. (Left) Infiltrative margins seen, tumor reaching upto serosa.

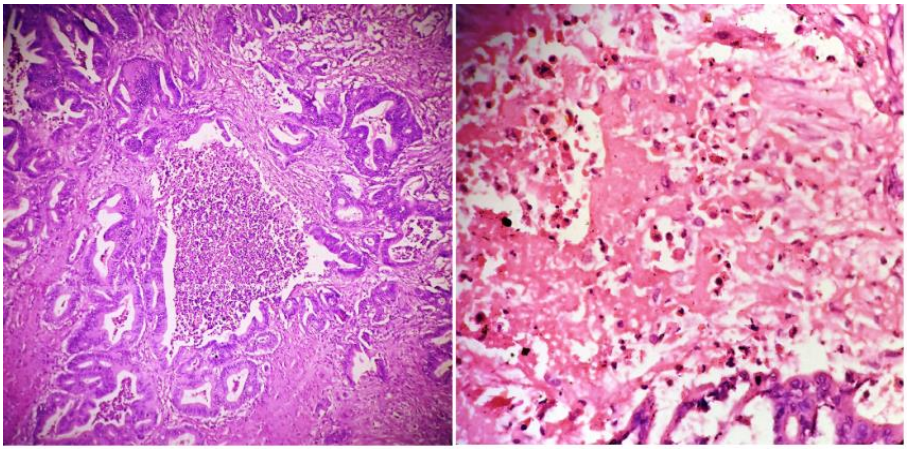

Fig. 6: (Right) Dirty Necrosis – S/o Primary. (Left) Dirty necrosis seen within the lumina of adenocarcinomatous glands.

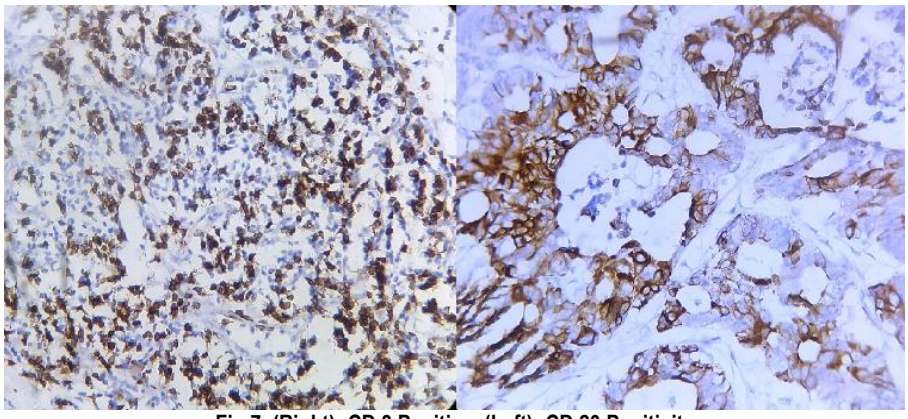

Fig 7. (Right): CD 3 Positive, (Left): CD 20 Positivity

Table 1: Histomorphological features in correlation with IHC findings and MSI

HPE Of total cases (179)	No. of cases seen	% of cases	IHC CK 20	CDX 2	CK7	Cases in study of Alexander J et al with MSI-H	Cases in study of Alexander et al with MSS
CLASSICAL TYPES:							
Well differentiated	28		+	+	-	-	-
Moderately differentiated	112		+	+	-	-	-
Poorly differentiated	38		+	+	-/+	-	-
VARIANTS:							
Mucinous carcinoma	12	6.70%	+	+	-	15%	5%
Mucinous type >10%	22	12.29%	+	+	-	21.73%	7%
Signet-ring	13	7.2%	+	+	-	13%	5%
Medullary carcinoma	4	2.2%	-	+/-	-/+	38%	13%
Medullary type >10%	10	5.58%	+/-	+	-	25%	3%
Poor differentiation	29	16.20%		+/-	-	25%	3%
OTHER PROGNOSTIC FACTORS:							
Cribriforming	40	22.3%					More frequent
Invasive margins	101	56.42%				74%	73%
Pushing margins	33	18.4%				15%	8%
Tumor necrosis/Dirty necrosis	64	35.75%				-	-
Lymphocytic infiltration (intratumoral, intraepithelial, chron's like)	41	22.91%				**21%**	3%

RESULTS

In our study out of 179 cases studied, 99 (55.31%) were males and 80 (44.69%) were females. Predominantly the age group involved was Middle age group (40-60 years) with a mean age of 56 years. No significant difference was observed between the early onset and late onset in terms of gender distribution or side of colon involved. Left side of colon i.e., descending colon, sigmoid colon and rectum was more frequently involved. Growth ranged from 2.5 to 22.5 cm. And most commonly were fungating /ulcerative type. Conventionally classifying them, M/C was Moderately differentiated adenocarcinoma – 112 cases (62.56%), followed by well differentiated –29 cases (16.20%) and poorly differentiated - 38 cases (21.22%) (Figure-1, 2). No statistically significant correlation between age group involved and grade of tumor was seen.

Histological features like mucinous carcinoma (Figure-3), mucinous-type, signet ring cell carcinoma (Figure-4), medullary cancers (Figure-5), tumors with poor differentiation, features of host immune responses like-chron's reaction, intra-tumoral / intraepithelial lymphocytic infiltration, invasive/ pushing margins, dirty necrosis (Figure-6) were all studied a detail. IHC was applied in necessary cases. (Table-1) We have found that medullary carcinoma, intraepithelial lymphocytes and poor differentiation were best discriminators between MSI-H and MSS.

IHC was applied wherever required. Most common immunophenotype of CRCs is CK 20 +ve & CK 7 –ve. However, 20% are CK20 –ve and CK7 +ve. CDX2 +ve in >90%, is suggestive of Enteric Differentiation. In many of Medullary CRCs, reduced or absent expression of CK 20 is seen. (Figure-7)

DISCUSSION

In present study, similar to other studies show male preponderance in both early onset and late onset tumors.[11-13] However, study done by chimmen et al shows female preponderance.[4]

No significant difference was observed between the early onset and late onset in terms of gender distribution or side of colon involved. In present study, left sided colon involvement was seen more commonly. Many Studies show early onset patients have predilection for left sided colon involvement[11,13], while right sided involvement in young was seen in some other studies.[14,15]

Most common was well differentiated adenocarcinomas.

Mucinous adenocarcinoma constituted 6.7 % in our study with variable prognosis. Study by chimmen et al, were mucinous constituted 4-19% and showed poor prognosis.[4] Gao et al analyzed primary tumor site and outcome.[16] Mucinous differentiation was associated with poor outcome for left sided tumors, and independent protective survival indicator for right sided tumors. It was due to fact that right sided tumors are MSI-H.[17] Signet ring carcinoma is identified as independent predictor of poor outcome. They are right sided, show poor differentiation and high rate of lymphatic invasion.[17-20] Overall, 1% are Signet ring cell carcinomas upon histology. Medullary arise frequently in proximal colon with female preponderance and has good prognosis. On Molecular level, majority are MSI-H cancers.[21]

Inflammatory response was based on four distinct features of tumor infiltrating lymphocytes, lymphocytic infiltration of intra and peri tumoral stroma and chron's like lymphoid reaction.

Klintrup et al demonstrated that high grade inflammation in node negative CRC is associated with five-year survival compared to low grade inflammation.[22]

It is associated with an MSI-H phenotype[23,24] and favorable prognosis in several studies. [25-26]

Tumor necrosis correlates with increased metastatic potential and worse prognosis.[27-29]

Tumor budding was described as independent predicter of poor survival [30-32] and high risk of recurrence.[33-35]

Leisbig et al reported fourfold greater 5-year disease free survival rates for PNI –negative cancers.[36,37]

CONCLUSION

Colorectal adenocarcinomas are heterogenous diseases that involves multiple tumorigenic pathways. Molecular testing plays an important role in era of personalized medicine. Pathologists play a central role in analyzing MSI-H phenotype which constitutes pathologically and clinically distinct subtype, selecting appropriate sections for MSI testing and mutational analysis for KRAS and BRAF, and their interpretation for prognostic and therapeutic tests.

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