IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 21, Issue 4 Ser.8 (April. 2022), PP 14-25
www.iosrjournals.org

Immunohistochemical Analysis of Microsatellite Instability and Its Associated Clinicopathological Factors In Colorectal cancers

Anitha Wilson[1], Lekha T[2], Sherin E K[3], Sathi P P[4]
[1]*Department of Pathology, Government Medical college Kozhikode, India*
[2] *Department of Pathology, Government Medical college Kozhikode, India*
[3] *Department of Surgery, Government Medical college Kozhikode, India*
[4] *Department of Pathology, Government Medical college Kozhikode, India*

Abstract
Background*: Microsatellite instability status (MSI) accounts for 12-15% of colorectal cancers. Detection of MSI has significant therapeutic and prognostic implications. Immunohistochemistry can be used as a first line screening test in the detection of tumours showing MSI.*
Materials and Methods: *Ours was a descriptive study conducted in the Department of Pathology, Government Medical College, Kozhikode, for a period of 18 months, including all resected specimens of CRC received in our department. A total of 104 consecutive cases were included in the study. Clinicopathological correlation and IHC for MLH1, MSH2, MSH6 and PMS2 was done for all the cases and those satisfying Revised Bethesda Criteria were identified. Data were analysed using IBM SPSS statistics software.*
Results: *Out of the 104 cases studied, 14 showed a loss of two markers and 90 showed no loss of MMR genes. 10 out of the 14 MMR deficient cases showed a combined loss of MSH2 and MSH6, while the rest 4 showed a loss of MLH1 AND MSH2. There was no statistically significant association between age and microsatellite status (p value - 0.706), as well as gender and microsatellite status (p value – 0.132). The most common presenting symptom was melena (43%), followed by constipation (15%) and intestinal obstruction (12%). 86% of the dMMR and 92% of the MSS tumours were ulceroproliferative. There was a statistically significant association between side of the tumour and microsatellite status (p value: 0.0001) with 61.1% of microsatellite stable tumours being left sided and 57.1% of dMMR tumours being right sided. dMMR tumours were commonly located in ascending colon and majority of the MSS tumours were located in the sigmoid. 71.4% of dMMR tumours and 76.7% of MSS tumours were adenocarcinoma, (p value - 0.663). There was a statistically significant association between Crohns like lymphoid aggregate and microsatellite status with a p value of 0.001. None of the dMMR tumours showed lymph node metastasis. There was no statistically significant association between stage of the tumour and microsatellite status (p value: 0.066). 78.6% of the microsatellite unstable tumours and 18.9% of the stable tumours satisfied atleast one of the 5 Modified Bethesda criteria. This association between Modified Bethesda criteria and microsatellite status was statistically significant with a p value of 0.0001.*
Conclusion*: Microsatellite instability accounts for 14% of colorectal carcinoma in our population. From this study we incurred that, the tumours showing dMMR presented at higher ages (>50 years), with no sex predilection. These tumours were more often right sided and showed a significant Crohns like lymphoid aggregate with lower grade at presentation. Majority of our cases with dMMR also satisfied the Modified Bethesda criteria. There was no significant association between microsatellite status and gender as well as histological subtype of the tumour.*
Key word*: Microsatellite instability, IHC, revised Bethesda criteria, dMMR*

Date of Submission: 04-04-2022 Date of Acceptance: 19-04-2022

I. Introduction

Colorectal cancer is the third most deadly and fourth most commonly diagnosed cancers worldwide according to the GLOBOCAN 2018 data. The recent advances in early detection screening and treatment options have significantly reduced the mortality in developed nations. Families with hereditary predisposition for gastrointestinal malignancies can be identified by family history and genetic testing so that they can take necessary preventive measures. (1)

The molecular classification of colorectal cancer is based on the cumulative study of precursor lesions (like adenomas and sessile serrated polyps), inherited colon cancer syndromes (such as Familial Adenomatous

Polyposis Syndrome and Lynch Syndrome) and molecular profiling of colorectal cancers. Colorectal cancers can be broadly divided into two general groups based on their genomic differences – Chromosomal instability pathway which accounts for 75 – 80% of all colorectal cancers and Microsatellite Instability pathway (MSI) accounting for 15 -20% of colorectal cancers.(2)

Microsatellites are tandem repeats of one to six dinucleotides found throughout the genome. Their instability is characterized by contractions or expansions of these sequences within the DNA.(3) Microsatellite instability(MSI) ,is detected in about 15 % of all colorectal cancers(CRC); 3% of which is associated with Lynch Syndrome and the other 12% are sporadic.(4) Detection of MSI is important, as clinical prognosis and management of patients are closely related to molecular mechanism underlying cancer development.(5)

The Mismatch Repair proteins include MLH 1, MSH 2, MSH 6, PMS1 and PMS 2.Any inherited or somatic mutations or epigenetic silencing of any of the aforementioned genes lead to MSI.(3) CRC with dMMR have distinct clinical and pathological features that commonly include early age at onset, proximal colon predominance, Crohn's like lymphocytic reaction and mucinous or signet ring cell differentiation.(5)MMR status is used increasingly to guide clinical management and studies have shown that CRC with dMMR have better stage adjusted survival than others.(6)

Although genetic testing remains the gold standard for detection of MSI, College of American Pathologists recommends an initial workup using a four antibody panel including MLH 1, MSH 2, MSH 6 and PMS 2.(7) IHC for Mismatch Repair (MMR) proteins is simple and economical with ideal specificity and sensitivity compared to MSI analysis using PCR and should be carried out universally for MMR deficiency screening and genetic counseling for Lynch Syndrome.(8)

Lynch Syndrome (previously known as Hereditary Non Polyposis Colon Cancer Syndrome), is a dominantly inherited cancer syndrome in which patients have an increased life time risk for CRC(70-80%), as well as carcinomas of endometrium, stomach, ovaries, small intestine, the biliary tract, brain , ureters and renal pelvis. The revised Bethesda guidelines, is used to identify families that are very likely to represent Lynch Syndrome. Identification of these patients is important because of implications for genetic counseling, increased risk of a second malignancy of colon or other organs.

II. Materials and Methods

Study Design: Descriptive Study
Study Setting: Department of Pathology, Government Medical College, Kozhikode
Duration: From Jan 1st 2019 to June 30 2020 (18 months)
Sample size: 104 cases
Sample size calculation: Considering the proportion of CRC showing MSI as 15%(4),
$N= 4pq/d^2$
Prevalence(p)=15%
q =100-15 =85%
Absolute precision (d) =7
Sample size =104
Inclusion criteria: All resected specimens diagnosed as Colorectal carcinoma, received in the Department of Pathology, Government Medical College, Kozhikode
Exclusion criteria: Patients with history of preoperative chemoradiotherapy
Sampling procedure: Consecutive sampling

Procedure methodology:

The resected specimens of CRC received in the Department of Pathology, Government Medical College Kozhikode, were considered for the study. Relevant history from the case records and histopathology requisition forms were noted which included the age, location of tumour, history of synchronous or metachronous tumours and family history. Specimen received was fixed in 10% formalin for 24 hours and grossed as per standard guidelines. Tissue blocks were made and sections of 4-5 micrometer will be cut and stained with Hematoxylin and Eosin. Histological findings recorded were morphological subtype of carcinoma, grade of the tumour, presence of crohns like lymphoid aggregate and lymph node status. The stage of tumour was assessed. Microsatellite status was assessed using IHC markers MLH1, MSH2 and PMS6. Microsatellite status and histopathological findings were correlated. Patients identified to satisfy the Revised Bethesda criteria were identified and correlated with Microsatellite status.

IHC was performed after standardization with a positive and negative control using antibodies from Pathn Situ. The vials contained 6ml of mouse monoclonal antibody. The antibody was ready to use and pretitrated. Any staining of >1% in tumour cells was considered positive provided the internal control showed strong nuclear positivity (normal colonic mucosa and lymphoid aggregates). A complete loss of staining with

normal internal control was taken as loss of MMR expression and if the internal control didn't show positivity, the procedure was repeated.

Statistical analysis:
Data was entered in Microsoft Excel and analyzed using SPSS version 18. The association between MSI and various clinicopathological factors were assessed by chi square test and p value <0.05 was considered statistically significant.

III. Result

MSI Status of Cases
Out of the 104 cases studied, 14 showed a loss of atleast one marker and the rest 90 showed no loss of MMR genes.

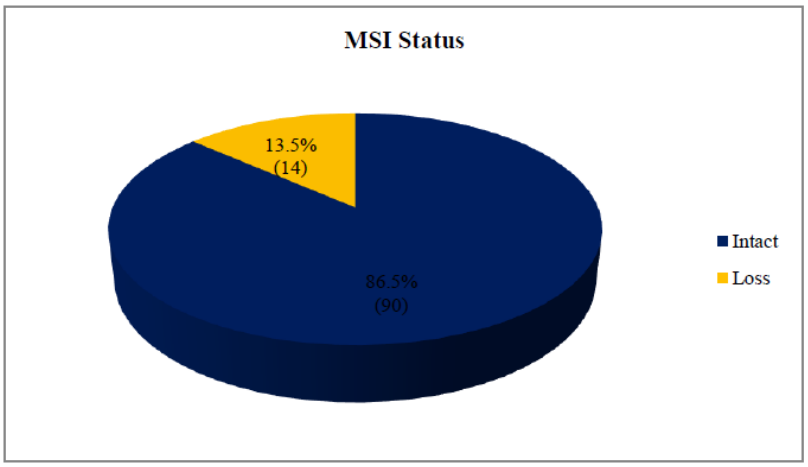
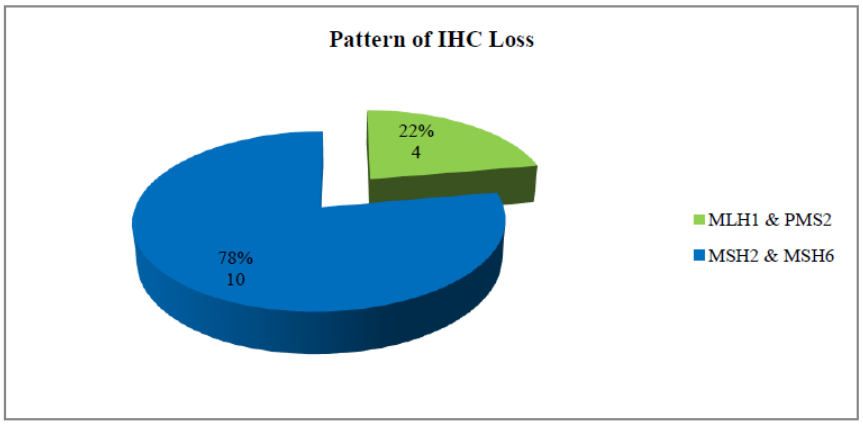


Figure 1: MSI Status of cases under study

Distribution of cases based on the pattern of IHC loss
10 out of the 14 cases which showed loss of MMR proteins showed a combined loss of MSH 2 and MSH 6, while the rest 4 showed a loss of MLH 1 AND MSH 2.

Figure2: Pattern of IHC loss



Age wise distribution of dMMR cases
The age of patients who showed a deficient MMR ranged from 39 to 70 years with a standard deviation of 8.6.

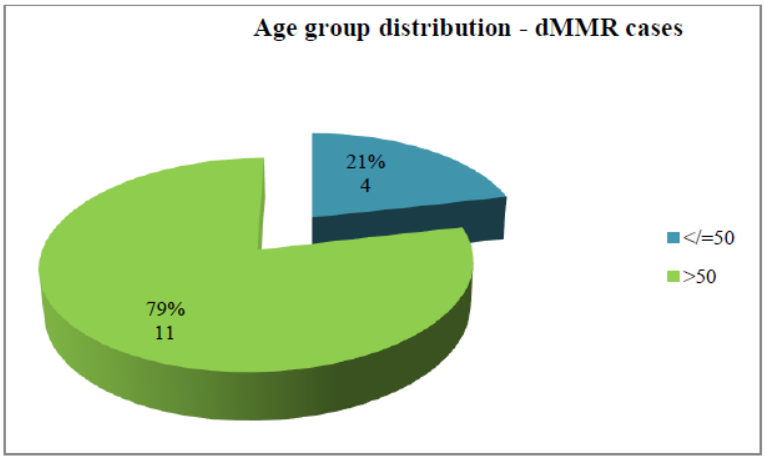
Table 1: Age wise distribution of dMMR cases

	Maximum	Minimum	Mean	Standard Deviation
Age	70	39	56.93	8.606

Age group distribution of cases

The patients involved in the study were divided into </= 50 years and >50 years. Among the 104 cases, 18 were </= 50years, of which 4 were MMR deficient and 86 were more than 50 years, of which 11 were MMR deficient.

Figure 3: Age group distribution of dMMR cases



Microsatellite status and age

In our study, 3(21.4%) out of 14 patients with microsatellite instability were </= 50 years and rest 11(78.6%) were >50 years. 15(16.7%) of the 90 patients who were microsatellite stable were </=50years while rest 75(83.3%) were>50 years

Table 2: Microsatellite status and age

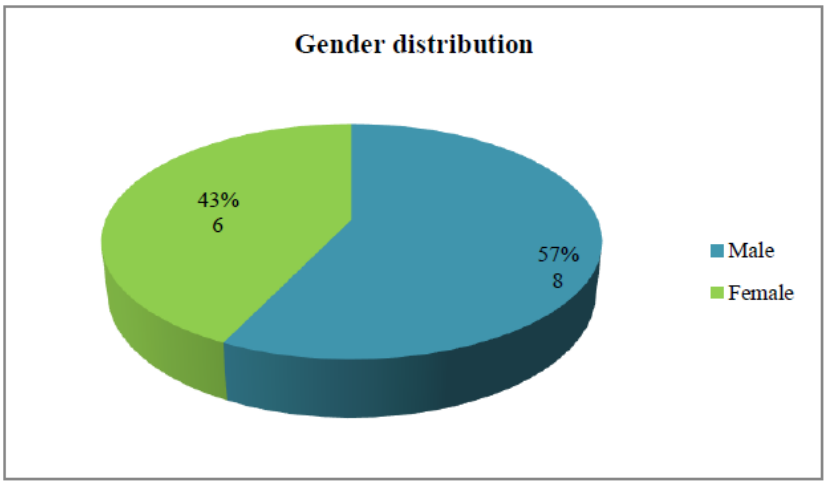
Age	MSI	MSS
</= 50 years	3(21.4%)	15(16.7%)
>50 years	11(78.6)	75(83.3%)
Total	14	90

P value by fisher's exact test :0.706

Gender wise distribution of cases

Of the 14 patients showing microsatellite instability, 8 were male and 6 were female

Figure 4: Gender wise distribution of cases



Microsatellite status and gender

In our study, 8(57.1%) out of the 14 patients showing microsatellite instability were male and rest 6(42.9%) were female. 56(62.2%) of the microsatellite stable patients were male and 34(37.8%) of them were female.

Table 3 : Microsatellite status and gender

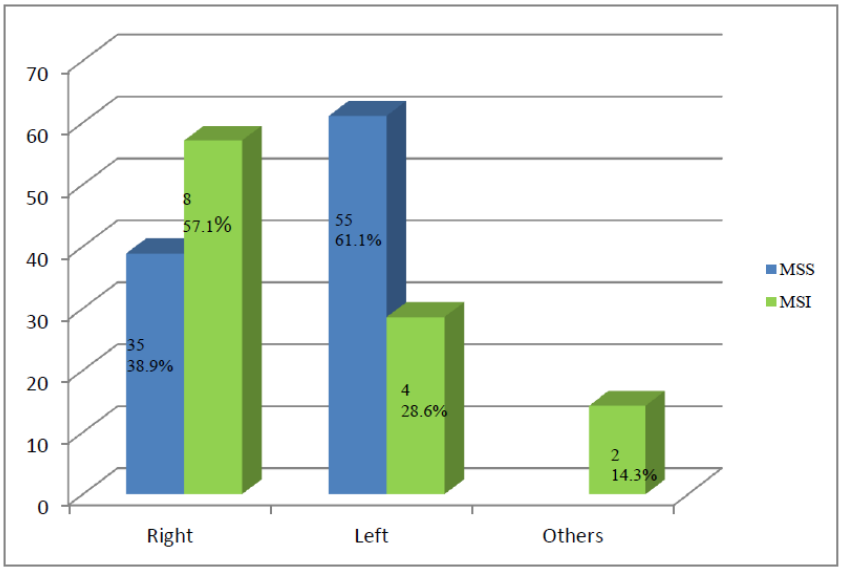
Gender	MSI	MSS
Male	8(57.1%)	56(62.2%)
Female	6(42.9%)	34(37.8%)

P value by chi square test : 0.132

Microsatellite status and side of tumour

Out of the 14 cases with deficient mismatch repair proteins, 8(57.1%) were right sided, 4(28.6%) were left sided and 2(14.3%) patients had synchronous tumours in the colon. While 55(61.1%) of the microsatellite stable tumours were left sided and 35(38.9%) were right sided.

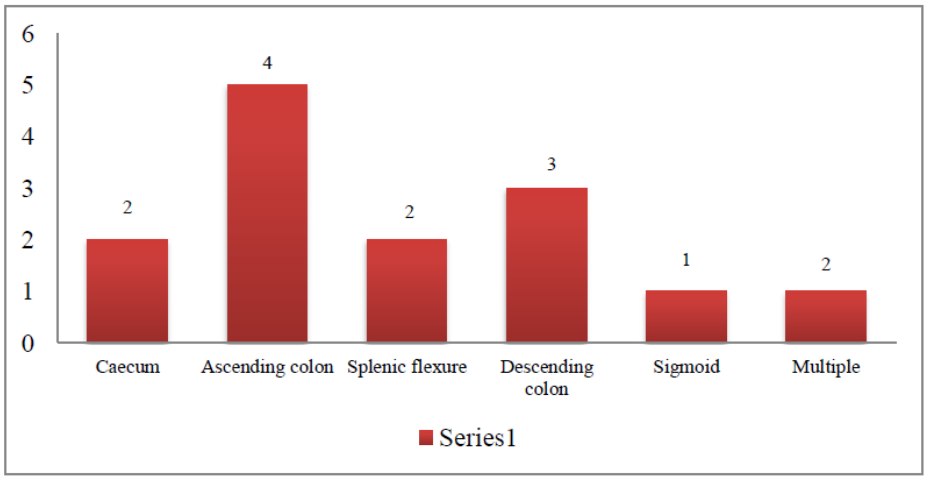
Figure 5: Microsatellite status and side of tumour



Site of tumour

Out of the 14 cases which showed microsatellite instability, 4 were in ascending colon, 3 were in descending colon, 2 each in caecum and splenic flexure, 1 in sigmoid colon, 1 had tumour in ascending colon and rectum and 1 patient had 2 lesions in rectum

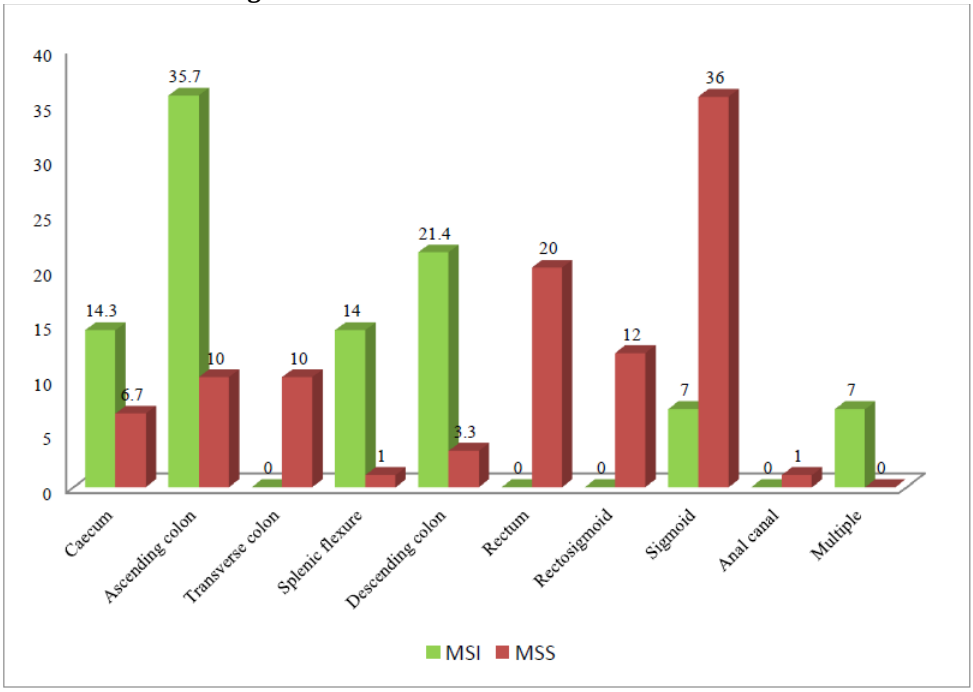
Figure 6: Site of dMMR tumours



Microsatellite status and site of tumour

4(35.7%) out of the 14 Microsatellite unstable tumours were located in the ascending colon and 3(21.4%) were located in descending colon, 2(14.3%) each were located in the splenic flexure and caecum, 1(7.1%) was located in sigmoid colon and 2 patients had synchronous tumours in ascending colon and rectum. Among the microsatellite stable tumours, 32(35.6%) cases were located in the sigmoid, 18(20%) were located in the rectum, 11(12.2%) were located in the rectosigmoid, 9(10%) cases each were located in ascending colon and transvere colon respectively, 6(6.6%) cases were located in the caecum, 3(3.3%) cases were located in the descending colon, 1(1.1%) case each were located in splenic flexure and anal canal respectively.

Figure 7: Microsatellite status and site of tumour

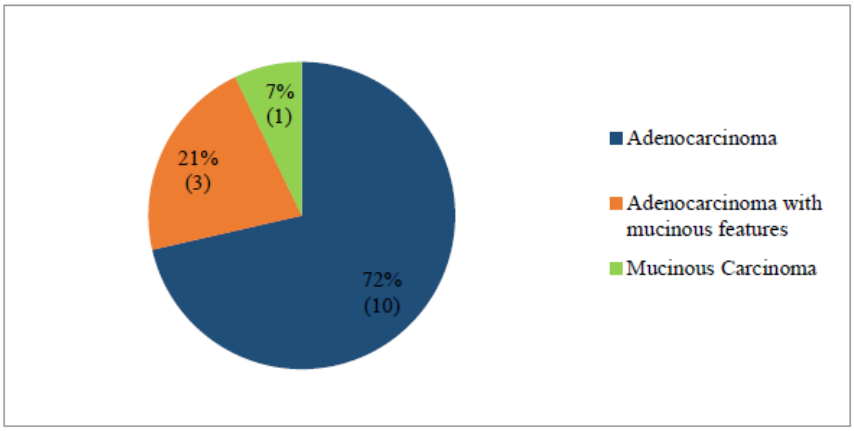


P value : 0.0001

Histological subtype of carcinoma

10/14 cases with microsatellite instability were adenocarcinoma, 3 were adenocarcinoma with mucinous features and 1 was mucinous carcinoma

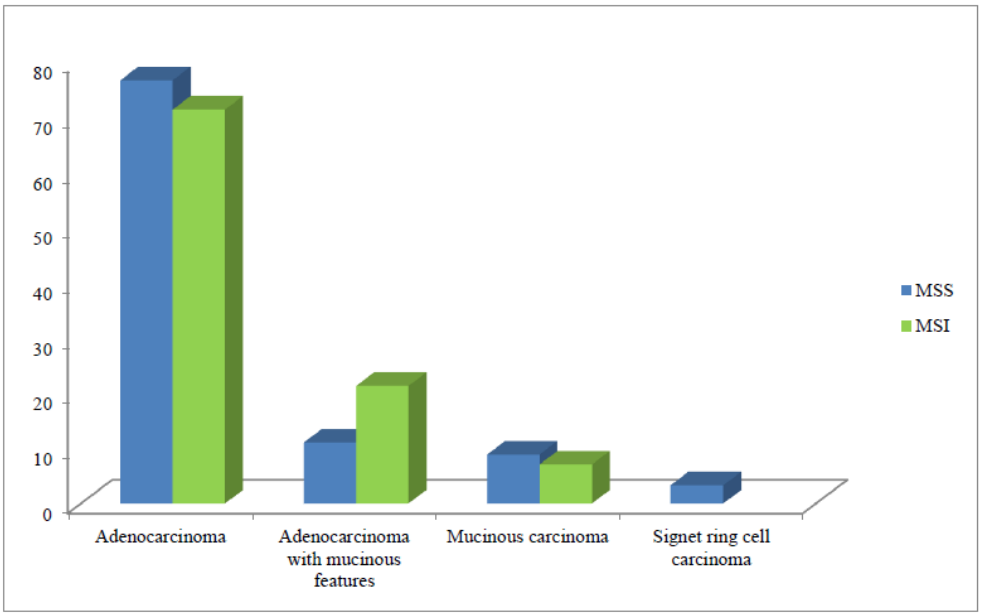
Figure 8: Histological subtype of dMMR tumours



Microsatellite status and histological subtype

Out of the 14 cases which showed loss of mismatch repair proteins, 10(71.4%) were adenocarcinoma, 3(21.4%) were adenocarcinoma with mucinous features and 1(7.1%) was mucinous carcinoma. 69(76.7%) of the microsatellite stable cases were adenocarcinoma, 10(11.1%) were adenocarcinoma with mucinous differentiation, 8(8.9%) were mucinous carcinoma and 3(3.3%) were signet ring cell carcinoma.

Figure 9: Microsatellite and status and histological subtype

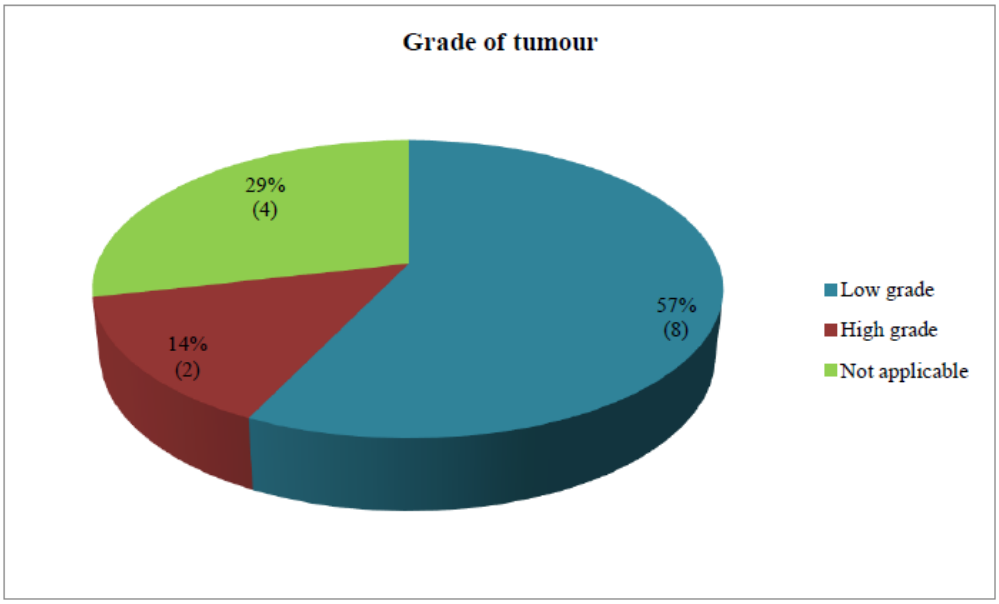


P value: 0.663

Grading of tumour :

Out of the 10 adenocarcinomas showing microsatellite instability, 8 were low grade and 2 were high grade

Figure 10: Grade of dMMR tumours



MSI status and grade of tumour

69(76.6%) of Microsatellite stable cancers and 8(57.2%) of microsatellite unstable were low grade and only 2 tumours were high grade. All others were of other histomorphology.

Table 4: MSI status and grade of tumour

Grade	MSI	MSS
Low grade	8(57.2%)	69(76.6%)
High grade	2(14.3%)	0
Not applicable	4(28.6%)	21(23.4%)

P value 0.001

MSI status and Crohns like lymphoid aggregate

67(67.8%) of the microsatellite stable tumours had a score of 0, 22(24.4%) score 1 and 7(7.8%) Score 2. Out of the 14 tumours with MSI, 6 each had a score of 0 and 2 and 2 (14.3%)had a score of 1.

Table 5: MSI status and Crohns like lymphoid infiltrate

Crohns like aggregate	MSI Loss	MSS
Score – 0	6(42.9%)	61(67.8%)
Score – 1	2(14.3%)	22(24.4%)
Score - 2	6(42.9%)	7(7.8%)

P value – 0.001, statistically significant

MSI Status and Lymph node status

None of Microsatellite unstable cancers showed lymph node metastasis. 33(36.7%) out of 90 microstaellite stable tumours showed lymph node metastasis and 56(62.2%) showed no metastasis.

Table 6: Microsatellite status and Lymph node status

Lymph node metastasis	MSI	MSS
Present	0	33(36.7%)
Absent	14(100%)	56(62.2%)
No lymph nodes sampled	0	1(1.1%)

P value: 0.020

MSI status and stage of tumour

9(64.3%) of the microsatellite unstable tumours were stage IIA and 5(35.7%) were stage I. 51(56.7%) of the microsatellite stable tumours were stage IIA, 23(25.6%) were stage IIIB, 11(12.2%) were stage I, 3(3.3%) were stage IIIC and 2(2.2%) were stage IIIA.

Table 7: Microsatellite status and stage of tumour

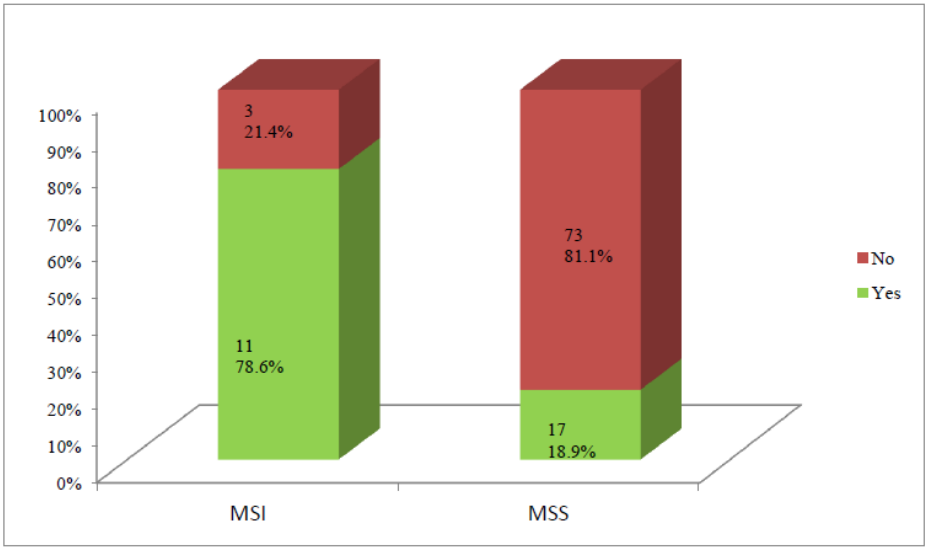
Stage	MSI	MSS
I	5(35.7%)	11(12.2%)
II A	9(64.3%)	51(56.7%)
III A	0	2(2.2%)
III B	0	23(25.6%)
III C	0	3(3.3%)

P value – 0.066

MSI status and Bethesda criteria

78.6%(11) of the microsatellite unstable tumours and 18.9%(17) of the microsatellite stable tumours satisfied atleast one of the 5 Bethesda criteria**.** Of the MSI cases, 4 cases met 4/5 Bethesda criteria, 2 cases met 3/5 criteria, 2 cases met 2 criteria and 2 cases met 1 criteria. 5 patients had CRC diagnosed at <50 years, 6 had history of synchronous or metachronous tumours, 7 were less than 60 years with MSI –H pathological features, 6 had first degree relatives with Lynch associated tumours and 5 had Lynch associated tumours in two first degree or second degree relatives.15 MSS tumours were identified in patients less than 50 years. 2 cases had a family history of Lynch associated tumour in their first degree relatives. 2 patients satisfied 2/5 criteria(age </= 50 years and presence of a synchronous or metachronous CRC).

Figure 11: MSI status and Bethesda Criteria



P value 0.0001

IV. Discussion

The DNA MMR system corrects base – base mispairs and insertions/deletions in the short repetitive DNA sequences known as microsatellites(65). Studies show that tumours showing dMMR have specific clinicopathologic characteristics(66). Identification of dMMR cancers is important as they have therapeutic and prognostic implications.MSI can be detected by IHC or by polymerase chain reaction based amplification of the repeat sequences. While both PCR and IHC serve as sensitive and specific method for detection of MSI, IHC can be employed as a rapid and cost effective tool with respect to PCR which helps in identifying cases which require genetic testing (65).

In our study, 14% of cases showed a loss of atleast one MMR protein. The proportion of cases showing loss of MMR proteins is slightly less in our study compared to other Indian studies. However there are various published studies conducted outside India which show a lower proportion of cases showing dMMR ranging between 9 – 10%(67,71)

78% of the tumours showed a combined loss of MSH2 and MSH6, while 22% showed a combined loss of MLH1 and PMS2. None of the tumours showed isolated loss of any marker. While some studies showed a higher proportion of tumours showing loss of MLH1 and PMS2(65), there were few reported studies in India showing a higher proportion of MSH2 and PMS2 loss(67).

11 out of the 14 patients who showed a deficient MMR and 75 of the 90 MSS patients were above 50 years. This is in contrast to Indian studies which showed MSI patients with a younger median age at presentation(28).The p value obtained was 0.706, which implies that there is no statistically significant association between MSI and age. This is in par with the study conducted by Kumar et al, where there was no statistically significant association between age and MSI status(68). However a study done on South India on dMMR status and its prognostic and predictive significance showed a significant association between age and MSI, with MSI tumours being diagnosed at an earlier age compared to MSS tumours(65).In a study conducted by Nayak et al. in North India, there was a significant association between age and microsatellite status(69).

There was striking male preponderance in both MSI and MSS tumours with 57.1% and 62.2% of the patients being male in each group, respectively. However, this association was not statistically significant(p value :0.132). While some Indian studies showed a male preponderance(67,68), there were studies in South India which showed a female preponderance for tumours showing MSI(65).

There was no statistically significant association between type of growth and Microsatellite status. Majority of the dMMR and MSS tumours in our study were ulceroproliferative lesion. p value for the same was 0.278.

While, 61.1% of the MSS tumours were left sided, only 28.6% of dMMR tumours were located on the left side. 57.1% of the dMMR tumours were right sided and 14.3%(2 cases), presented with synchronous lesions. This association between side of the tumour and microsatellite status was statistically significant with p value being 0.0001. This is in concordance with various Indian studies (65,67,68).

35.7% of the dMMR cases were located in the ascending colon, while the most common location of MSS tumours were sigmoid(36.7%). A statistically significant association was found between MSI status and location of tumour emphazising the fact that dMMR tumours are more often right sided. The p value of this

association as calculated by chi square test was 0.0001. This is in concordance with a South Indian study which showed 44.4% of dMMR tumours proximal to the splenic flexure (67).

While tumours with MSI usually show a mucinous or medullary histology with poor differentiation, our study had only one case of mucinous carcinoma and 3 cases of adenocarcinoma with mucinous features showing MSI. 8 of the 10 adenocarcinomas showing MSI were low grade and 2 were high grade. Among the MSS tumours, majority were adenocarcinomas amounting to 76.7%. There was no statistically significant association between histological type of tumour and MSI status, p value being 0.663. However in the study conducted by Paulose et al., a significant proportion of cases showing MSI were poorly differentiated and showed mucinous features also(65).

There was a statistically significant association between MSI status and grade of tumour with a p value of 0.001. Tumours showing dMMR usually tend to be of lower grade when compared to MSS tumours.

Our study used the Graham Appelman grading criteria for assessing the lymphoid aggregate and we could arrive at a statistically significant association with MSI and presence of Crohns like lymphoid aggregate with a p value of 0.001.

All 14 of our cases showing MSI had no evidence of lymph node metastasis, which ascertains the fact that MSI tumours present earlier compared to MSS tumours. Most of the dMMR tumours were stage IIA at the time of presentation but there was no statistically significant association between stage of tumour and microsatellite status (p value: 0.06). Similar to another Indian study conducted by Nayak et al., the most common stage at which CRC were diagnosed was stage II(69). This may be attributed to early diagnosis of CRC in our population.

There was statistically significant association between microsatellite status and satisfaction of Bethesda criteria with a p value of 0.0001. Family history of Lynch associated tumours in first and second degree relatives, as well as presence of synchronous or metachronous tumours were significantly higher in patients with MSI than others. This is in concordance with the study conducted by Dubey et al(28). From our study we could incur that Bethesda criteria can be used as a powerful tool to identify the group of patients who needs to be screened for Lynch syndrome.

Thus tumours showing dMMR presented in our setting at higher ages (>50 years), with no sex predilection. These tumours were more often right sided with ascending colon being the most common site. These tumours showed a significant Crohns like lymphoid aggregate with lower grade at presentation. Majority of our cases with dMMR also satisfied the Bethesda criteria.

There was no significant association between gender as well as histological subtype of the tumour. This may be due to the low sample size of our study.

V. Conclusion

Microsatellite instability is an important pathway in pathogenesis of CRC. Tumours showing MSI have distinctive clinicopathological features. Identification of microsatellite status is important because of prognosis and treatment implications. Modified Bethesda criteria can be used as a powerful tool in identifying patients who warrants MSI testing. IHC remains as a powerful tool in determining the microsatellite status.

Microsatellite instability accounts for 14% of colorectal carcinoma in our population. The tumours showing dMMR presented in our setting at higher ages (>50 years), with no sex predilection. These tumours were more often right sided with ascending colon being the most common site. These tumours showed a significant Crohns like lymphoid aggregate with lower grade at presentation. Majority of our cases with dMMR also satisfied the Modified Bethesda criteria. There was no significant association between gender as well as histological subtype of the tumour.

References

[1]. Rawla P, Sunkara T, Barsouk A. Epidemiology of colorectal cancer: incidence, mortality, survival, and risk factors. Przeglad Gastroenterol. 2019;14(2):89–103.
[2]. Joanna Gibson, Jill Lacy, Ellen Matloff, and Marie Robert. Microsatellite Instability Testing in Colorectal Carcinoma : A Practical Guide. Clin Gastroenterol 2014.
[3]. Hashmi AA, Ali R, Hussain ZF, Faridi N, Khan EY, Bakar SMA, et al. Mismatch repair deficiency screening in colorectal carcinoma by a four-antibody immunohistochemical panel in Pakistani population and its correlation with histopathological parameters. World J Surg Oncol. 2017 Jun 26;15(1):116.
[4]. Boland CR, Goel A. Microsatellite Instability in Colorectal Cancer. Gastroenterology. 2010 Jun;138(6):2073-2087.e3.
[5]. Yuan L, Chi Y, Chen W, Chen X, Wei P, Sheng W, et al. Immunohistochemistry and microsatellite instability analysis in molecular subtyping of colorectal carcinoma based on mismatch repair competency. Int J Clin Exp Med. 2015 Nov 15;8(11):20988–1000.
[6]. Li P, Xiao Z, Braciak TA, Ou Q, Chen G, Oduncu FS. Systematic immunohistochemical screening for mismatch repair and ERCC1 gene expression from colorectal cancers in China: Clinicopathological characteristics and effects on survival. PloS One. 2017;12(8):e0181615.
[7]. Hashmi AA, Ali R, Hussain ZF, Faridi N, Khan EY, Bakar SMA, et al. Mismatch repair deficiency screening in colorectal carcinoma by a four-antibody immunohistochemical panel in Pakistani population and its correlation with histopathological parameters. World J Surg Oncol. 2017 Jun 26;15(1):116.

[8]. Yuan L, Chi Y, Chen W, Chen X, Wei P, Sheng W, et al. Immunohistochemistry and microsatellite instability analysis in molecular subtyping of colorectal carcinoma based on mismatch repair competency. :13.
[9]. Favoriti P, Carbone G, Greco M, Pirozzi F, Pirozzi REM, Corcione F. Worldwide burden of colorectal cancer: a review. Updat Surg. 2016 Mar;68(1):7–11.
[10]. Meyer B, Are C. Current Status and Future Directions in Colorectal Cancer. Indian J Surg Oncol. 2017 Dec 1;8(4):455–6.
[11]. A study on the frequency and clinicopathological correlates of mismatch repair-deficient colorectal cancer. 2001 [cited 2020 Sep 3]; Available from: http://www.cancerjournal.net/preprintarticle.asp?id=269747
[12]. Kuipers EJ, Grady WM, Lieberman D, Seufferlein T, Sung JJ, Boelens PG, et al. COLORECTAL CANCER. Nat Rev Dis Primer. 2015 Nov 5;1:15065.
[13]. Colorectal Cancer - Screening [Internet]. Cancer.Net. 2015 [cited 2020 Sep 3]. Available from: https://www.cancer.net/cancer-types/colorectal-cancer/screening
[14]. Lanzi A, Pagès F, Lagorce-Pagès C, Galon J. The consensus immunoscore: toward a new classification of colorectal cancer. Oncoimmunology [Internet]. [cited 2020 Sep 3];9(1). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7466865/
[15]. Fed T Bosman, Fatima Carneiro, Ralph H Hruban, NEil D Theise. WHO classification of tumours of the digestive system. 4th, 2010th ed.
[16]. Fleming M, Ravula S, Tatishchev SF, Wang HL. Colorectal carcinoma: Pathologic aspects. J Gastrointest Oncol. 2012 Sep;3(3):153–73.
[17]. Harris E, Lewin D, Wang H, Lauwers G, Srivastava A, Shyr Y, et al. LYMPHOVASCULAR INVASION IN COLORECTAL CANCER: AN INTEROBSERVER VARIABILITY STUDY. Am J Surg Pathol. 2008 Dec;32(12):1816–21.
[18]. Betge J, Pollheimer MJ, Lindtner RA, Kornprat P, Schlemmer A, Rehak P, et al. Intramural and extramural vascular invasion in colorectal cancer. Cancer. 2012;118(3):628–38.
[19]. Liebig C, Ayala G, Wilks J, Verstovsek G, Liu H, Agarwal N, et al. Perineural Invasion Is an Independent Predictor of Outcome in Colorectal Cancer. J Clin Oncol. 2009 Nov 1;27(31):5131–7.
[20]. Zlobec I, Lugli A. Tumour budding in colorectal cancer: molecular rationale for clinical translation. Nat Rev Cancer. 2018 Apr 1;18(4):203–5.
[21]. Lugli A, Kirsch R, Ajioka Y, Bosman F, Cathomas G, Dawson H, et al. Recommendations for reporting tumor budding in colorectal cancer based on the International Tumor Budding Consensus Conference (ITBCC) 2016. Mod Pathol Off J U S Can Acad Pathol Inc. 2017;30(9):1299–311.
[22]. Kong JC, Guerra GR, Pham T, Mitchell C, Lynch AC, Warrier SK, et al. Prognostic Impact of Tumor-Infiltrating Lymphocytes in Primary and Metastatic Colorectal Cancer: A Systematic Review and Meta-analysis. Dis Colon Rectum. 2019;62(4):498–508.
[23]. Evrard C, Tachon G, Randrian V, Karayan-Tapon L, Tougeron D. Microsatellite Instability: Diagnosis, Heterogeneity, Discordance, and Clinical Impact in Colorectal Cancer. Cancers [Internet]. 2019 Oct 15 [cited 2020 Feb 20];11(10). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6826728/
[24]. Colorectal Cancer.
[25]. Kim SH, Park KH, Shin SJ, Lee KY, Kim TI, Kim NK, et al. CpG Island Methylator Phenotype and Methylation of Wnt Pathway Genes Together Predict Survival in Patients with Colorectal Cancer. Yonsei Med J. 2018 Jul 1;59(5):588–94.
[26]. Boland CR, Goel A. Microsatellite Instability in Colorectal Cancer. Gastroenterology. 2010 Jun;138(6):2073-2087.e3.
[27]. Peltomäki P, Lothe RA, Aaltonen LA, Pylkkänen L, Nyström-Lahti M, Seruca R, et al. Microsatellite instability is associated with tumors that characterize the hereditary non-polyposis colorectal carcinoma syndrome. Cancer Res. 1993 Dec 15;53(24):5853–5.
[28]. Dubey AP, Vishwanath S, Nikhil P, Rathore A, Pathak A. Microsatellite instability in stage II colorectal cancer: An Indian perspective. Indian J Cancer. 2016 Dec;53(4):513–7.
[29]. Culligan KM, Meyer-Gauen G, Lyons-Weiler J, Hays JB. Evolutionary origin, diversification and specialization of eukaryotic MutS homolog mismatch repair proteins. Nucleic Acids Res. 2000 Jan 15;28(2):463–71.
[30]. Cr B, A G. Microsatellite instability in colorectal cancer. Gastroenterology. 2010 Jun 1;138(6):2073-2087.e3.
[31]. Tamura K, Kaneda M, Futagawa M, Takeshita M, Kim S, Nakama M, et al. Genetic and genomic basis of the mismatch repair system involved in Lynch syndrome. Int J Clin Oncol. 2019 Sep;24(9):999–1011.
[32]. Vilar E, Gruber SB. Microsatellite instability in colorectal cancer—the stable evidence. Nat Rev Clin Oncol. 2010 Mar;7(3):153–62.
[33]. Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, et al. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res. 1998 Nov 15;58(22):5248–57.
[34]. Greenson JK, Huang S-C, Herron C, Moreno V, Bonner JD, Tomsho LP, et al. Pathologic predictors of microsatellite instability in colorectal cancer. Am J Surg Pathol. 2009 Jan;33(1):126–33.
[35]. Gian L de'Angelis, Lorena B, Cinzia A, Nicola de'Angelis, Gioacchino L, Francesco DM, et al. Microsatellite instability in colorectal cancer. Acta Bio Medica Atenei Parm. 2018;89(Suppl 9):97–101.
[36]. Luo C, Cen S, Ding G, Wu W. Mucinous colorectal adenocarcinoma: clinical pathology and treatment options. Cancer Commun [Internet]. 2019 Mar 29 [cited 2020 Sep 4];39. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6440160/
[37]. Wei Q, Wang X, Gao J, Li J, Li J, Qi C, et al. Clinicopathologic and Molecular Features of Colorectal Adenocarcinoma with Signet-Ring Cell Component. PloS One. 2016;11(6):e0156659.
[38]. Hendry S, Salgado R, Gevaert T, Russell PA, John T, Thapa B, et al. Assessing tumor infiltrating lymphocytes in solid tumors: a practical review for pathologists and proposal for a standardized method from the International Immuno-Oncology Biomarkers Working Group. Adv Anat Pathol. 2017 Nov;24(6):311–35.
[39]. Greenson JK, Bonner JD, Ben-Yzhak O, Cohen HI, Miselevich I, Resnick MB, et al. Phenotype of microsatellite unstable colorectal carcinomas: Well-differentiated and focally mucinous tumors and the absence of dirty necrosis correlate with microsatellite instability. Am J Surg Pathol. 2003 May;27(5):563–70.
[40]. Maoz A, Dennis M, Greenson JK. The Crohn's-Like Lymphoid Reaction to Colorectal Cancer-Tertiary Lymphoid Structures With Immunologic and Potentially Therapeutic Relevance in Colorectal Cancer. Front Immunol [Internet]. 2019 [cited 2020 Sep 4];10. Available from: https://www.frontiersin.org/articles/10.3389/fimmu.2019.01884/full#B10
[41]. Bai W, Ma J, Liu Y, Liang J, Wu Y, Yang X, et al. Screening of MSI detection loci and their heterogeneity in East Asian colorectal cancer patients. Cancer Med. 2019 Apr 3;8(5):2157–66.
[42]. Yuan L, Chi Y, Chen W, Chen X, Wei P, Sheng W, et al. Immunohistochemistry and microsatellite instability analysis in molecular subtyping of colorectal carcinoma based on mismatch repair competency. Int J Clin Exp Med. 2015 Nov 15;8(11):20988–1000.
[43]. RICHMAN S. Deficient mismatch repair: Read all about it (Review). Int J Oncol. 2015 Aug 12;47(4):1189–202.

[44]. Kang S, Na Y, Joung SY, Lee SI, Oh SC, Min BW. The significance of microsatellite instability in colorectal cancer after controlling for clinicopathological factors. Medicine (Baltimore) [Internet]. 2018 Mar 2 [cited 2020 Sep 4];97(9). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5851768/

[45]. Guang Yang1 · Ru-yi Zheng2 · Zai-shun Jin3. Correlations between microsatellite instability and the biological behaviour of tumours.

[46]. Therapy implications of DNA mismatch repair deficiency, Microsatellite Instability adn tumour mutation burden.

[47]. Huyghe N, Baldin P, Van den Eynde M. Immunotherapy with immune checkpoint inhibitors in colorectal cancer: what is the future beyond deficient mismatch-repair tumours? Gastroenterol Rep. 2019 Nov 25;8(1):11–24.

[48]. Koehler-Santos P, Izetti P, Abud J, Pitroski CE, Cossio SL, Camey SA, et al. Identification of patients at-risk for Lynch syndrome in a hospital-based colorectal surgery clinic. World J Gastroenterol WJG. 2011 Feb 14;17(6):766–73.

[49]. Jin H-Y, Liu X, Li VKM, Ding Y, Yang B, Geng J, et al. Detection of mismatch repair gene germline mutation carrier among Chinese population with colorectal cancer. BMC Cancer. 2008 Feb 7;8:44.

[50]. Lam AK-Y, Chan SS-Y, Leung M. Synchronous colorectal cancer: clinical, pathological and molecular implications. World J Gastroenterol. 2014 Jun 14;20(22):6815–20.

[51]. Aslanian HR, Burgart LJ, Harrington JJ, Mahoney DW, Zinsmeister AR, Thibodeau SN, et al. Altered DNA mismatch repair expression in synchronous and metachronous colorectal cancers. Clin Gastroenterol Hepatol Off Clin Pract J Am Gastroenterol Assoc. 2008 Dec;6(12):1385–8.

[52]. Mf K, Se K, B H, Ca B, Jm C. Defining the adenoma burden in lynch syndrome. Dis Colon Rectum. 2015 Apr 1;58(4):388–92.

[53]. Husain S, Hassell LA. Microsatellite Instability (MSI) Testing in Extra-colonic Tumors. J Clin Exp Pathol. 2015 Mar 15;5(2):1–3.

[54]. Møller P, Seppälä T, Bernstein I, Holinski-Feder E, Sala P, Evans DG, et al. Cancer incidence and survival in Lynch syndrome patients receiving colonoscopic and gynaecological surveillance: first report from the prospective Lynch syndrome database. Gut. 2017 Mar 1;66(3):464–72.

[55]. Higgins HJ, Voutsalath M, Holland JM. Muir-Torre Syndrome. J Clin Aesthetic Dermatol. 2009 Aug;2(8):30–2.

[56]. Dipro S, Al-Otaibi F, Alzahrani A, Ulhaq A, Al Shail E. Turcot Syndrome: A Synchronous Clinical Presentation of Glioblastoma Multiforme and Adenocarcinoma of the Colon. Case Rep Oncol Med [Internet]. 2012 [cited 2020 Sep 4];2012. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3479943/

[57]. Fred T. Bosman, Fatima Carneiro, Ralph H. Hruban,Neil D. Theise. WHo Classification of tumours Digestive system Tumours. 5th ed.

[58]. Pouya F, Mojtabanezhad Shariatpanahi A, Ghaffarzadegan K, Tabatabaee Yazdi SA, Golmohammadzadeh H, Soltani G, et al. A novel large germ line deletion in adenomatous polyposis coli (APC) gene associated with familial adenomatous polyposis. Mol Genet Genomic Med. 2018 Sep 26;6(6):1031–40.

[59]. Rudloff U. Gastric adenocarcinoma and proximal polyposis of the stomach: diagnosis and clinical perspectives. Clin Exp Gastroenterol. 2018 Dec 3;11:447–59.

[60]. He EY, Wyld L, Sloane MA, Canfell K, Ward RL. The molecular characteristics of colonic neoplasms in serrated polyposis: a systematic review and meta-analysis. J Pathol Clin Res. 2016 Apr 22;2(3):127–37.

[61]. Ratti M, Lampis A, Hahne JC, Passalacqua R, Valeri N. Microsatellite instability in gastric cancer: molecular bases, clinical perspectives, and new treatment approaches. Cell Mol Life Sci. 2018;75(22):4151–62.

[62]. Walker CJ, Eisfeld A-K, Genutis LK, Bainazar M, Kohlschmidt J, Mrózek K, et al. No evidence for microsatellite instability in acute myeloid leukemia. Leukemia. 2017 Jun;31(6):1474–6.

[63]. Tian T, Li J, Xue T, Yu B, Li X, Zhou X. Microsatellite instability and its associations with the clinicopathologic characteristics of diffuse large B-cell lymphoma. Cancer Med. 2020;9(7):2330–42.

[64]. Guang Yang1 · Ru-yi Zheng2 · Zai-shun Jin3. Correlations between microsatellite instability and biological behaviour of tmours. J Cancer Res Clin Oncol 2019 1452891–2899 Httpsdoiorg101007s00432-019-03053-4. Received: 24 July 2019 / Accepted: 4 October 2019 / Published online: 15 October 2019 © The Author(s) 2019.

[65]. Paulose RR, Ail DA, Biradar S, Vasudevan A, Sundaram KR. Prognostic and predictive significance of microsatellite instability in stage II colorectal carcinoma: An 8-year study from a tertiary center in South India. Indian J Cancer. 2019 Dec;56(4):302–8.

[66]. Ward R, Meagher A, Tomlinson I, O'Connor T, Norrie M, Wu R, et al. Microsatellite instability and the clinicopathological features of sporadic colorectal cancer. Gut. 2001 Jun 1;48(6):821–9.

[67]. A study on the frequency and clinicopathological correlates of mismatch repair-deficient colorectal cancer. 2001 [cited 2020 Sep 3]; Available from: http://www.cancerjournal.net/preprintarticle.asp?id=269747

[68]. Kumar A, Jain M, Yadav A, Kumari N, Krishnani N. Pattern of mismatch repair protein loss and its clinicopathological correlation in colorectal cancer in North India. South Afr J Surg Suid-Afr Tydskr Vir Chir. 2018 Mar;56(1):25–9.

[69]. Nayak SS, Roy P, Arora N, Arun I, Roy MK, Banerjee S, et al. Prevalence estimation of microsatellite instability in colorectal cancers using tissue microarray based methods – A tertiary care center experience. Indian J Pathol Microbiol. 2018 Oct 1;61(4):520.

[70]. Arpitha Shetty PRR. A study on the frequency and clinicopathological correlates of mismatch repair-deficient colorectal cancer.

Anitha Wilson, et. al. "Immunohistochemical Analysis of Microsatellite Instability and Its Associated Clinicopathological Factors In Colorectalcancers." *IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)*, 21(04), 2022, pp. 14-25