THE FREQUENCY OF MISMATCH REPAIR DEFICIENCY IN COLORECTAL CARCINOMA DETERMINED BY IMMUNOHISTOCHEMISTRY

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ABSTRACT

Objective: To determine the frequency of mismatch repair deficiency in colorectal carcinoma determined by immunohistochemistry.

Study Design: Cross-sectional study.

Place and Duration of Study: Department of Histopathology, Armed Forces Institute of Pathology, Rawalpindi, from Aug 2018 to Jan 2019.

Methodology: A total of 101 patients of adenocarcinoma of colorectum who underwent surgical resections and their characteristic and clinical data were recorded. Immunohistochemical stains were performed using antibodies MLH1, MSH2, PMS2 and MSH6. Results were interpreted and mismatch repair deficiency status of all patients was determined. Patients with loss of expression for MLH1, MSH2, PMS2 and MSH6 antibodies were observed and noted.

Results: In this study, out of 101 patients with CRC, 71 (70.3%) were male and 30 (29.7%) female. The mean age was (54 years ± 15.9). Amongst the 101 cases loss of immunohistochemical staining for MMR proteins was noted in 19 patients (18.8%). The combined loss of all four antibodies was seen in one case, loss of MLH1 and PMS2 in 7, MSH2 and MSH6 in 5 and MLH1 only in 6 patients. However, no mismatch repair deficiency was detected in remaining 82 cases. According to statistical analysis no significant association between mismatch repair deficiency and variables was found.

Conclusion: The frequency of mismatch repair deficiency in colorectal carcinoma patients was found to be 18.8% in our population.

Keywords: CpG island methylation phenotype, Colorectum, Immunohistochemistry, Mismatch repair deficiency, Microsatellite stable, Microsatellite instable.

INTRODUCTION

Colorectal carcinoma (CRC) is one of the most common malignancies in the developed countries including; North America, Russia and Australia. Each year around 500,000 new cases of CRC are diagnosed worldwide[1].

Several factors have been considered responsible for the significantly increased incidence such as; smoking, poor nutrition and obesity. However, the burden of colorectal cancer is preventable to a larger extent by early detection, spreading awareness related to healthy dietary patterns, tobacco control, reducing the utilization of red and processed meat encouraging physical activity and maintaining a healthy body weight[2].

Colorectal cancer being insidious in onset follow a sequence of genetic alterations that includes; activation of proto-oncogene such as loss of APC gene on 5q21 region of chromosome, and inactivation of tumor suppressor genes such as loss of *p*53 gene on 17*p*13 and loss of heterozygosity on chromosome 18 at long arm i.e. 18q LOH[3]. According to the molecular analyses, there are two principles that support that genetic alterations are responsible for pathogenesis in the CRC i.e. Familial adenomatous polyposis is the result in the Adenomatous polyposis coli (APC) gene mutation and the factors that are associated with CRC have proved to have carcinogenic effects[4]. In general, the alterations in length observed within the simple repeated sequences are known as microsatellite. However, the stability of microsatellite is dependent on DNA Mismatch repair (MMR) system. MMR enables recognition and repairing of the damages occurring during DNA replication and recombination such as deletions, inaccurate insertions and mis-assimilation of the DNA bases. Microsatellite instability (MSI) is a genetic hypermutable state as a result from defect in the DNA mismatch repair (MMR). The cells with defective MMR fail to remove the errors occurring during DNA replication and hence errors accumulate leading to mutation. However, MSI operates as second major pathway after chromosomal instability or microsatellite stable pathway in the carcinogenesis of CRC[5]. Three groups of tumors are defined on the basis of mutation of MSI markers; MLH1, MSH2, PMS2 and MSH[6]. The tumors showing no instability are called microsatellite stable (MSS); tumors possessing <30% of the mutated microsatellite marker

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panel are called MSI-Low, and tumors consisting >30% of the mutated microsatellite marker panel are called MSI-High. Another type of MSI called "Elevated microsatellite alterations at selected tetranucleotide repeat" (EMAST) have been reported, these repeats have been found to be associated with p53 mutations[6].

Colorectal cancers with mismatch repair deficiency is characterized with distinguished features; involvement of proximal colon, tumor infiltrating lymphocytes (activated and cytotoxic), mucinous and signet ring cell component and poor differentiation. These tumors have a favorable prognosis compared to CRC without mismatch repair deficiency and show different response to chemotherapy[7]. In general, evaluation of mismatch repair deficiency status in CRC is considered for Lynch syndrome (hereditary nonpolyposis colorectal cancer, or HNPCC), that is a hereditary disorder caused by inactivation of the germline mutations in genes coding mismatch repair factors. Patients with Lynch syndrome have unique and inherited features including tumor development at early ages (usually between 20-30 years of age), occurrence of multiple tumors in the body (including colon, rectum, stomach, small intestine, urinary tract, ovary, endometrium and other sites), low-stage disease at the time of diagnosis and less metastatic potential. The analyses of mismatch repair deficiency and its related molecular alterations in the clinical settings are currently increasing, and mismatch repair deficiency status is considered a useful marker for screening the patients with Lynch syndrome and as a prognostic factor in the field of chemotherapeutic interventions[8]. There are four genes that are mutated in the Lynch syndrome including MLH1, MSH2, PMS2, and MSH6. On the basis of mismatch repair (MMR) capacity, the CRC are classified into MMR-proficient (with intact MMR proteins) and MMR-deficient tumors (with loss of function of MMR pathway)[9].

The molecular MSI testing is considered as "gold standard" for assessing the tumor DNA mismatch repair system competency. However, this test is labor-intensive and time consuming involving the DNA extraction both from tumor and normal tissue, polymerase chain reaction (PCR) amplification of DNA, running the amplified products of tumor and normal tissue followed by comparing and scoring the difference which makes this test clinically troublesome as surgeon would preoperatively like to know the likelihood of HNPCC to decide the extent of surgery. In contrast, monoclonal antibodies are commercially available to the protein products of hMLH1, hMSH2, hPMS2 and hMSH6. In comparison to molecular MSI testing, IHC detection of MMR proteins is less labor intensive and less time consuming and provides an alternative method for detection of MMR deficiency[10].

There are different therapeutic responses in MSI-H CRCs depending on type of adjuvant chemotherapy. Although, use of mismatch repair deficiency status have been controversial for its response towards adjuvant chemotherapy, but tumor with MSI-high, is diagnosed with either Lynch syndrome or Methylated. In case of IHC done and the unexpressed protein is MSH2, PMS2 or MSH6, germline testing is required for deleterious mutation in mismatch repair to diagnose it to be Lynch syndrome. If the unexpressed protein is MLH1, possibility could be of CIMP tumor with hypermethylation of MLH1 promoter, or Lynch syndrome.

In this study, we determined the frequency of mismatch repair deficiency status by immunohistochemistry in 101 patients who went through surgical resection for CRC. As a part of this study, we evaluated the possible association with age, gender, location of tumor and smoking as variables. However, the patient's family history of CRC, young age at the time of diagnosis proximal tumor location was considered the independent predictors of mismatch repair deficiency status.

METHODOLOGY

This was a cross-sectional study, conducted at department of Histopathology, Armed Forces Institute of Pathology (AFIP) Rawalpindi, Pakistan from August 2018 to January 2019. The sample size was determined using WHO calculator and based on the given prevalence of 34% inmismatch repair deficiency positive patients in a study by Hashmi *et al*[11]. The estimated sample size was calculated and found to be 101 cases. All the parameters; including age at diagnosis, gender, family history and frequency of smoking (smokers/non-smokers) were recorded from institutional tumor registry section. All those cases that were diagnosed as poorly differentiated adenocarcinoma or either showed tumor infiltrating lymphocytes and/or mucinous component were selected. Patients with inadequate record of diagnosis of CRC and those who did not undergo surgical resections were excluded. The formalin-fixed paraffin-embedded tissue sections of tumors with their matched normal tissues were prepared for 101 cases. Ethical approval for this study was obtained from the Institutional review board of the Armed Forces Institute of Pathology (AFIP) Rawalpindi (Ref:

FC-HSP 17-2/READ-IRB/18/595). Immunohistochemical staining was performed on representative tissue blocks by using Dako en Vision method using antibodies MLH1, MSH2, PMS2 and MSH6. Results were interpreted and mismatch repair deficiency status of all the patients was determined. The patients were divided into 4 groups; those lacking expression of at least one MMR gene (mismatch repair deficiency status positive), with combined loss of expression, with loss of expression of all four antibodies and those with no loss of expression (mismatch repair deficiency status negative). The data was analyzed using SPSS-20. The quantitative variables including age were presented by calculating mean and standard deviation while qualitative variable including gender and expression of MSI markers were presented by calculating frequency and percentages. Post stratification, we applied chi-square test and *p*-value ≤0.05 was taken as significant.

RESULTS

In this study, out of 101 patients with CRC, 71 (70.3%) were male and 30 (29.7%) female. Themean age was calculated and found to be 54 ± 15.9 years (fig-1).

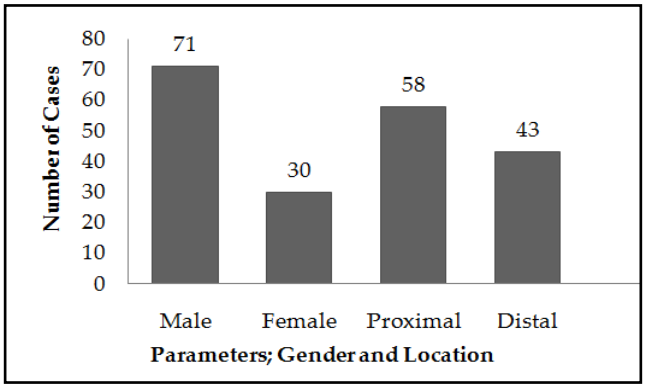

Figure-1: Represent the parameter of gender and location.

Amongst the 101 patients, the loss of IHC staining for MMR proteins was noted in 19 patients (18.8%). The combined loss of all four antibodies was seen in one patient out of 101 cases (fig-2), loss of MLH1 and PMS2 in 7 patients, MSH2 and MSH6 in 5 cases and MLH1 only in 6 patients. However,no loss of IHC staining for MMR proteins wasdetected in 82 cases. These results were tabulated and presented as shown in the table-I. According to the statistical analysis the *p*-value of each variable; smoking (*p*-value 1.39), gender (*p*-value 0.284) and age (*p*-value 1.105) was found to be >0.05 hence no significant evidence of association between mismatch repair deficiency status and variables was found (table-II).

Fig-1 representation of parameters; gender and location of tumor for corresponding number of cases under investigation with (mean age 54 years ± 15.9). Out of 101 cases, 65 were found out to be smokers and 36 nonsmokers.

Fig-2(A-J) represents Immunohistochemical staining performed on representative tissue blocks by using Dakoen Vision method using antibodies MLH1 (C and D), PMS2 (E and F), MSH2 (G and H) and MSH6 (I and J) (A-I=100X magnification, B-J=400X magnification) and its expression in the colorectal tumor (A&B). The combined loss of all four antibodies seen in one patient out of 101 cases.

DISCUSSION

The frequency of mismatch repair deficiency in 101 cases of Colorectal carcinoma (CRC) was observed in our study. The four essential parameters (variables) including; age, gender, location of tumor and smoking were taken under consideration as part of study. However, using the online software, SPSS the mean age of the 101 patients was calculated as 54 years ± 15.9. In

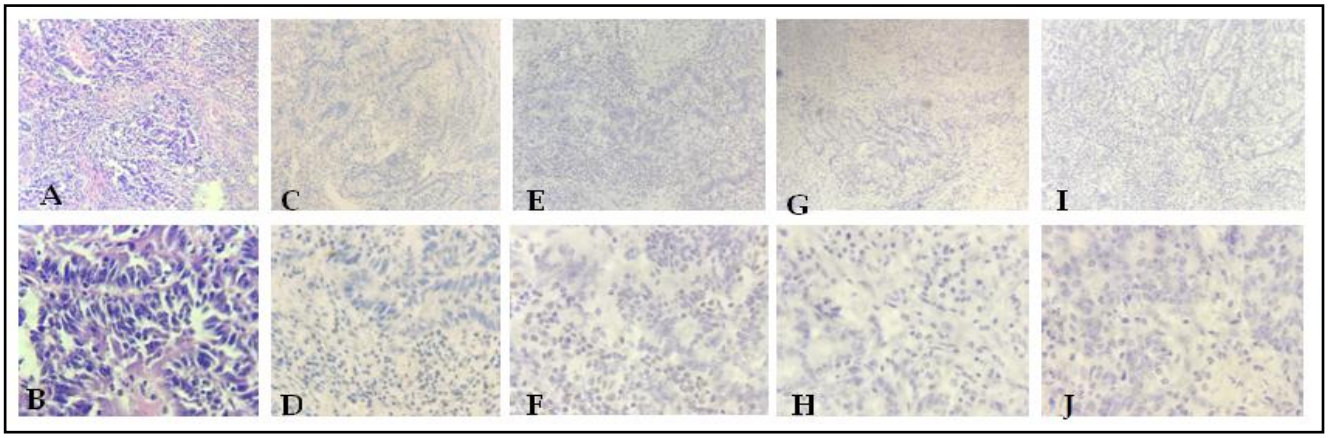

Figure-II: Immunohistochemical expression of MSI markers in colorectal carcinoma.

this study, the frequency of mismatch repair deficiency was determined among patients of Colorectal carcinoma which was found to be 18.8% (19 cases out of 101) as shown in table-I. The loss of expression of all four antibodies was found in 1% of the cases (n=1out of 101 cases), which is lower than the frequency found in a studyconducted by Hashmi *et al*, that reported thefrequency of loss of expression of all four antibodies as 7% (n=7 out of 100 cases)[11]. On the other hand, combined loss of expression of MLH1/PMS2 was found in 7% (n=7 out of 101 cases) which is lower than the frequency found by Hashmi *et al*, as loss of expression in 16% (n=16 out of 100 cases)[11]. The loss of MSH2/ MSH6 and isolated loss of MLH1 is observed in only 5% (n=5 out of 101 cases) and 6% (n=6 out of 101 cases) respectively. This result was comparable with the prior study conducted by Hashmi *et al*, reporting the loss of MSH2/MSH6 in 6% (n=6 out of 100 cases) and isolated loss of MLH1 in 5 cases (n=5 out of 100 cases)[11]. In two separate studies conducted by Gafa *et al*, and Vilar *et al*, the frequency of mismatch repair deficiency was found to be as nearly 15% being slightly lower thanthe result of our study as mentioned above[12,13]. As shown in table-II, the above study comparison is summarized indicating; frequency of mismatch repair deficiency, loss of expression of antibodies in varied patterns including; all four antibodies, combined and combined as well as isolated.

Table-I: Frequency of loss of IHC staining of MLH1, PMS2, MSH2 and MSH6 observed in 19 cases (n=19) out of 101.

Patterns	Number of Cases
MLH1+PMS2+MSH2+MSH6	1
MLH1+PMS2	7
MSH2 and MSH6	5
MLH1only	6
No loss of IHC staining	82

However, a comparison was drawn in the study conducted by Shen *et al*, stating that right-sided colon cancer had increased frequency of mismatched repair as compared to left-sided[14]. In general, large number of tumor-infiltrating cytotoxic lymphocytes was found to be an associating factor between tumors with mismatch repair deficiency possessing longer survival as observed in one study[15]. In general, evidence suggested, the incidence rates of CRC are maximum in Europe, northern America, Australia and New Zealand as compared to South-central Asia and Africa[17]. However, the economically transitioning countries also have increased incidence and mortality rates[16]. In Pakistan, the estimated incidence of CRC is 3.6%[17].

While few prior studies found association between mismatch repair deficiency and variables, this results did not lead to any such conclusion. One of such few studies was conducted by Slattery *et al*, where strong association of smoking and mismatch repair deficiency positive colon cancer was observed whereas no significant association of smoking with mismatch repair deficiency status was found in this results[19]. Another group of study conducted by Slattery *et al*, Yang *et al*, and Samowitz *et al*, reported the association of smoking with mismatch repair deficiency in sporadic colon cancer. This cancer showed the V600E BRAF mutations and widespread methylation of CIMP (CpG island methylator phenotype)[19-21]. The results were then investigated by the study conducted by Samowitz *et al* in 1315 cases of colon cancer and concluded that irrespective of the mismatch repair deficiency status, the cigarette smoking is associated with the colon cancer due to V600E BRAF mutations and/or CIMP methylation[19]. In this study they also reported no association with gender whereas, study conducted by Zisman *et al*, reported that CRC is associated with younger age group and male predominance[19,22].

Table-II: Summary of prior study comparisons with current findings in contrast with; frequency of mismatch repair deficiency, loss of expression of all four antibodies, combined as well as combined and isolated antibody expression.

Author	Year	Frequency of MSI	Loss of Antibody Expression	Combined Loss of Antibody Expression	Combined and Isolated loss of Antibody Expression
Vilar *et al*	2010	15%	-	-	
Gafa *et al*	2010	15%	-	-	
Hashmi *et al*	2017	-	MLH1, PMS2, MSH2 & MSH6 7% (7out of 100 cases)	MLH1/PMS2 16% (16 out of 100cases)	MSH2/ MSH6 6% (6 cases) MLH1 5% (5 out of 100 cases)
AFIP	2019	18% (19 out of 101 cases)	MLH1, PMS2, MSH2 & MSH6 5% (1 out of 101 cases)	MLH1/PMS2 7% (7 out of 101 cases)	MSH2/ MSH6 5% (5 out of 101 cases) MLH1 6% (6 out of 101cases)

An enhanced responsiveness to adjuvant chemotherapy by CRC with mismatch repair deficiency was

observed in a study conducted by Elsaleh *et al*[23]. This responsiveness was considered with an overall significantly better survival than tumors without mismatch repair deficiency and similar results by Lawes *et al*, in another study[24].

CONCLUSION

In our population the frequency of mismatch repair deficiency in CRC patients was found to be 18.8% that can be used as a platform to perform genetic testing for monitoring the therapeutic response and to predict the overall survival rate in patients with CRC.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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