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Pathology

ANALYSIS OF MLH1 AND MSH2 IMMUNOHISTOCHEMISTRY IN COLORECTAL CARCINOMAS

Dr. P. Pavethira* M.D Pathology, Assistant professor , Department of pathology, GMC and ESI hospital, Coimbatore-15. *Corresponding Author

Dr. Suma. B. Pillai Professor, Department of pathology, Velammal Medical college hospital and research institute, Madurai.

ABSTRACT The aim of this study is to determine the frequency of loss of mismatch repair protein MLH1 and MSH2 expression by immunohistochemical method in colorectal carcinomas.and to study is correlation with various clinical and histopathological characteristics so as to determine if there is any significant association between them.In our study population 40% of the cases lacked the expression of mismatch repair proteins MLH1 and MSH2 which is significantly high when compared to other studies done in Indian population. MMR protein expression statistically associated with histological type and grade of the tumor.MSH2 negative tumors were mostly poorly differentiated adenocarcinomas which included mucinous and signet ring cell carcinomas.Immunohistochemical method for mismatch repair proteins expression can be used as a screening method for detection of colorectal carcinoma with MSI.

KEYWORDS : colonic carcinomas, immunohistochemistry, MLH1 , MSH2,MSI

INTRODUCTION:

Colorectal cancer is the 3rd most common malignancy reported worldwide. Countries like North America, Australia, and Europe are facing an increased incidence of colorectal carcinoma. (1) When compared to the western world, colonic cancer is less common in India. (2) Colorectal carcinomas are mostly associated with diet, genetics and environmental factors. With westernization of life style, India is facing an increase in incidence of colorectal cancer. (1)Two different pathogenetic pathways are implicated in colorectal carcinomas. The microsatellite stable pathway (MSS) where there is inactivation of tumor suppressor genes like APC, p53, and DCC. The other pathway which plays a major role in colorectal carcinomas is inactivation of mismatch repair genes such as MLH1, MSH2, MSH6, PMS2, and MSH3. These belong to microsatellite instability pathway (MSI). Literature states that virtually all the cases of Hereditary Non-Polyposis Colorectal Cancer (HNPCC) / Lynch syndrome and 15% of sporadic cases of colorectal carcinomas have MSI. Mutation in two MMR genes, MLH1 and MSH2 accounts for majority of cases of HNPCC [1]It is postulated that testing for MSI would serve two purposes:

1. It is a powerful tool to screen for HNPCC and therefore members of the family with HNPCC can benefit from clinical survey by colonoscopy.
2. Though MSI colorectal carcinomas are relatively insensitive to treatment with 5- flurouracil based chemotherapy, they have a better prognosis.(1)

Hence the knowledge of the MSI status in colorectal carcinoma cases would help the clinician to assess the prognosis and also to guide in therapy.

Therefore we propose to study the deficiency of MMR protein expression by immunohistochemical method in a series of colorectal carcinoma cases reported from our institute and to correlate with various clinicopathological characteristics.

MATERIALS AND METHODS:

The cases diagnosed as colorectal carcinomas from colonic resection specimens in a period of three years in the department of pathology, PSGIMSR were included in the study. The clinical details like age, sex of the patients and other gross findings necessary such as site, size of the tumor were taken from the requisition slips. The representative paraffin blocks and H&E slides were retrieved from the archives of pathology. Paraffin blocks of the slides with high tumor density were chosen for the study. 4µ thick sections were made from the chosen blocks for routine hematoxylin and eosin staining. Tissue blocks of normal colon send for non – neoplastic lesions were taken as controls. The blocks were then cut to 5µ thick sections and taken on a Poly-L-lysine coated slide to serve as positive control for MLH1 and MSH2 antibody.

Immunohistochemistry for detection of expression of MLH1 and MSH2 was performed using the supersensitive HRP-polymer detection system with the appropriate control.The stained sections were screened to analyze the expression of the two antigens, MLH 1 and MSH2. Apart from the control sections taken from the specimen send for non-neoplastic lesion, the cells in the crypt epithelium and lymphocytes in the normal mucosa of the respective sections were also be used as positive control. The presence of these two antigens are expressed as brown colour nuclear staining.The slides were scored as positive or negative staining. The slides were scored positive, when there is presence of brown colour nuclear staining in the tumor cells. Negative score denotes the absence of this brown colour nuclear staining , which also includes the slides with less than 5% of cells showing weak nuclear staining.(3)

Results: Of the 40 cases, 12 (30%) patients had lack of expression of MSH2 protein, 4 (10%) patients had lack of both MLH1 and MSH2 protein while the remaining 24 cases (60%) showed both MLH1 and MSH2 positivity.(Table 1)

Table 1

Lack of MLH1	Lack of MSH2	Lack of MLH1 and MSH2	Normal MLH1 and MSH2 expression
0	12(30%)	4(10%)	24(60%)

Out 40 cases, 16 (40%) cases showed lack of expression of either MSH2 or both MLH1 and MSH2. There were no cases with only lack of MLH1 expression. Among these 16 cases there was equal distribution of males and females. (table2)

Table 2:

Sex	Total	Total no cases lacking MLH1/MSH2	Lack of MLH1	Lack of MSH2	Lack of MLH1 & MSH2
Males	21	8	0	7 (33%)	1 (5%)
Females	19	8	0	5 (26%)	3 (16%)

The sizes of the tumors were ranging from 1cm to 9 cm in greater dimension. Out of 5 cases with size less than 3 cm, 3 cases (60%) showed isolated negative staining for MSH2 protein. Lack of expression of both MSH2 and MLH1 was common among tumors measuring > 6 cm in size .In our present study 32/ 40 cases were conventional adenocarcinomas and among these only 10 cases showed alteration in expression of MLH1 / MSH2 protein. When lack of expression of MLH1 and MSH2 was correlated with tumor grade it was found to be associated with higher grade. Of the 16 cases which showed MLH1 / MSH2 alteration or both, 7 cases were moderately differentiated and remaining 7 cases were poorly differentiated. In our study mucinous adenocarcinoma and signet ring cell carcinoma were considered as poorly differentiated and 83% of mucinous adenocarcinomas (5/6) showed lack of MSH2 expression.(table 3)

Table 3:

S.no	variable	total cases	Lack of MLH1	Lack of MSH2	Lack of MLH1 and MSH2
1.size of tumor	Less than 3 cm	5	0	3(60%)	0
	3< size <6 cm	29	0	8(25%)	2(7%)
	> 6cm	6	0	1(16.7%)	2(33%)
1.Histological type	Adenocarcinoma	32	0	6 (19%)	4 (12.5%)
	Mucinous adenocarcinoma	6	0	5 (83%)	0
	Signet ring cell carcinoma	2	0	1 (50%)	0
2. Histological grade	Well	4	0	2 (50%)	0
	Moderately	27	0	4 (15%)	3(11%)
	Poorly	9	0	6 (67%)	1(11%)

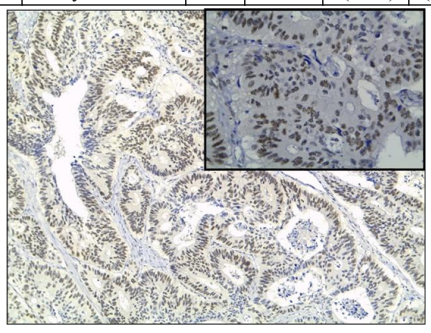

Figure 1:moderately differentiated adenocarcinoma nuclear staining for MLH1 (IHC,10X)

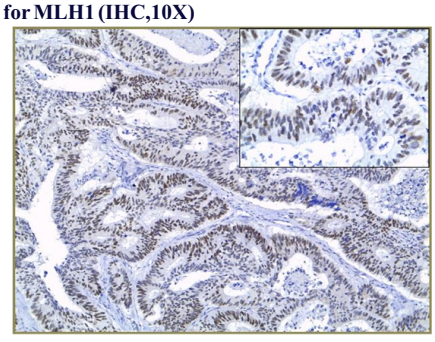

Figure 2:moderately differentiated adenocarcinoma nuclear staining for MSH2(IHC,10X)

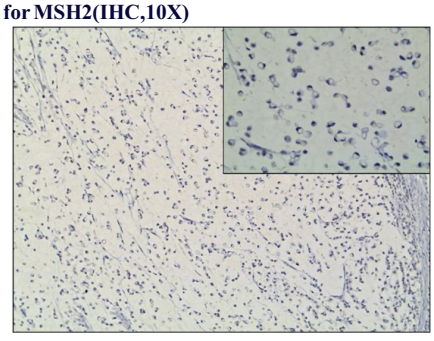

Figure 3:signet ring cell carcinoma negative nuclear staining for MLH1(IHC,10X

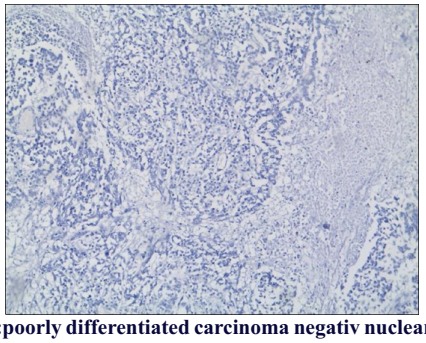

Figure 4:poorly differentiated carcinoma negativ nuclear staining for MSH2(IHC,10X)

While correlating lack of MLH1 and MSH2 expression with site of occurrence, it was found to be associated mostly with rectal origin (77%) and it showed isolated loss of MSH2 in 33% and remaining 44% showed alteration in both MLH1 and MSH2 expression. Loss of expression was more common on tumors on proximal colon compared to distal colon.Of the 16 cases with alteration in MLH1 and MSH2, 11 cases were in T3 stage showing that the loss of expression was associated with higher stage. Among the 40 cases in study group, 28 were of T3 grade among which 11 showed lack of mismatch repair proteins. One case was in stage T4, which also showed lack of MLH1 and MSH2.In our study expression of MLH1 and MSH2 did not have significant correlation with the lymph node involvement.(table 4)

Table 4:

S.no	variable	Tota cases	Lack of MLH1	Lack of MSH2	Lack of MLH1 and MSH2
1.site of tumor	caecum	4	0	3(75%)	0
	Asc, colon	4	0	1(25%)	1(2%)
	Tr.colon	7	0	2(28%)	1(14%)
	Desc.colon	3	0	1(33%)	0
	sigmoid	10	0	2(20%)	0
	rectum	12	0	3(25%)	2(16%)
2.stage of tumor	T0	0	0	0	0
	T1	1	0	0	0
	T2	10	0	3(30%)	1(10%)
	T3	28	0	9(32%)	2(7%)
	T4	1	0	0	1(100%)
3.Node involvement	N0	29	0	7(24%)	4(14%)
	N1	8	0	4(50%)	0
	N2	3	0	1(33%)	0

Statistical analyses.

The data obtained were entered in the master chart , coded and edited. The inferential and descriptive analysis of these data were computed using a software SSPS-17. The association between the variables and the lack of expression of MLH1 and MSH2 were determined by using chi- square test.

Table 5:

S.no	variable	P value	significance
1	Sex	0.496	Not significant
2	Size of tumor	0.162	Not significant
3	Histological type	<0.05	significant
4	Degree of differentiation	<0.05	significant
5	Site	0.626	Not significant
6	Stage of tumor	0.127	Not significant
7	Node status	0.541	Not significant

With reference to p value there was a strong association between the histological subtype and degree of differentiation of the tumor with the loss of expression of MMR genes.

CONCLUSION

- In our study population 40% of the cases lacked the expression of mismatch repair proteins MLH1 and MSH2 which is significantly high when compared to other studies done in Indian population.
- MMR protein expression statistically associated with histological type and grade of the tumor.
- MSH2 negative tumors were mostly poorly differentiated adenocarcinomas which included mucinous and signet ring cell carcinomas.
- Immunohistochemical method for mismatch repair proteins expression can be used as a screening method for detection of colorectal carcinoma with MSI.

A small proportion of MSI colorectal tumors are due to mutations in other MMR genes like MSH6, MSH3 and PMS 2. Hence, addition of antibodies against these proteins is also recommended in screening of colorectal carcinomas with MSI.

DISCUSSION

Colorectal cancer is the third most common type of malignancy in western countries. 15% of sporadic cases are due to MSI. In MSI, there are frame shift mutations and base pair substitutions in microsatellites. Microsatellites are repetitive genetic loci with 1 to 5 base pairs repeated 15 to 30 times. These mutations in microsatellites occur mainly during DNA replication and are normally controlled by the

DNA mismatch repair genes such as MLH1, MSH2, MSH6, PMS2 and MSH3. MLH1 recruits its binding partner PMS2 to the site of DNA injury, so if the expression of MLH1 is lost then PMS2 will also be lost. Similarly, when MSH2 is lost, MSH6 will be lost. Several studies have reported that defect in MMR genes forms the basis for MSI. MSI is seen in all cases of HNPCC and a subset of sporadic colorectal carcinoma. (4) Mutations of MLH1 and MSH2 are more common and they occur in exon 16 and exon 12 of the genes respectively. It is important to screen all colorectal carcinomas for MSI, regardless of patient's age or family history. This helps in detecting sporadic cases with MMR protein defect and also potential cases of Lynch syndrome. Sporadic tumors with microsatellite instability have a better prognosis when compared to microsatellite stable tumors (MSS). MSI tumors may respond less favorably to 5- fluorouracil- based chemotherapy. Therefore, the knowledge of MSI status may pave the way to assess the prognosis and for therapy. Molecular method is considered to be the gold standard for diagnosing mismatch repair genes but several recent studies have shown > 95% specificity of immunohistochemical analysis for mismatch repair genes. Other advantage with IHC is the availability of tissues to evaluate histopathological features of the tumors.(5)

We observed that in our study population there is 10% cases(4/40) lacked the expression of both the mismatch repair proteins. It was also observed that isolated loss of MSH2 was seen in 30% (12/40) cases. This is significantly higher when compared to the study done by Vijay pandey et al which quotes an incidence of 17% in an Indian cohort. (6). Majority of the tumors were located in the distal colon, mostly from rectum which was in agreement with the previous study. (6) The sizes of the tumors varied from 1.0 cm to 8.0 cm in greatest dimension. Out of 40 cases, 32 were conventional adenocarcinomas, 6 were mucinous adenocarcinomas and 2 cases were signet ring cell type carcinomas. With respect to differentiation, 27 cases were moderately differentiated, 4 cases were well differentiated and 9 cases were poorly differentiated (including mucinous and signet ring cell carcinomas). Lymph node metastases were noted in eleven cases.While correlating the loss of expression of MLH1 and MSH2 with various clinicopathologic characters , we found that lack of expression of MSH2 and both of MLH1 and MSH2 were more often seen in females (46%) when compared to males(38%), similar to the study by K.Ohrling. They had stated that there is a relationship between the lack of MMR gene and gender.(6) However in our statistical analysis, we found that there is no significant (p=0.496) association between these two variables as evolved by a chi- square value of 1.40 .

In our study MMR defective tumors developed in the age group between 35 years and 85 years with a more frequent incidence in the 7th decade. This was in correlation with the study of Rodrigo jover et al (4) where the mean age at diagnosis was high (70.5 yrs). Yet another study by Valerie Rigau showed that loss of MLH1 expression was frequent in elderly women. This proves that advanced age at the time of diagnosis does not rule out the possibility of hereditary non-polyposis colorectal carcinoma (HNPCC). (7) In the present study majority of the tumors (29/40) were between 3and 6 cm in size and 5 cases were less than 3cm in size. Of the 5 cases which are smaller than 3 cm in size, 3 cases (60%) showed isolated negative staining for MSH2 protein. Of the six cases larger than 6 cm in size only one case showed lack of MSH2 protein and two cases showed alteration in expression of both the proteins. This finding was different with previously reported studies which states that MSI tumors more often display larger size.(8)But in our study statistical analysis did not show any association between the tumor size and lack of MMR protein expression, the chi square value being 6.53 and p=0.162.

In our study, lack of MLH1 and MSH2 was more common on tumors on proximal colon compared to distal colon. In the present study, abnormality of MMR protein expression was observed in 53.3% of the proximal colorectal carcinomas when compared to only 32% of distal colorectal carcinomas. Few studies correlating with these findings have been recorded in literature. The various studies suggested that early onset microsatellite instable tumors were located in proximal colon and mostly showed a poorly differentiated grade. Eventhough the lack of expression of MMR protein was more among the tumors on proximal colon, statistically it was not significant.(9,10).

With regard to the histological type of the tumor, lack of MSH2 protein was more associated with mucinous adenocarcinoma (83%) and signet ring cell carcinoma (50%) compared to conventional adenocarcinoma.This was in agreement with a previous study done by Roberta Gafa et al (11), where it was found that in a series of 216 cases, MSI tumors were found to be closely related to poorly differentiated tumors and mostly in mucinous adenocarcinoma.Statiscal analysis showed that there is a strong association between the histological type and MMR protein loss. MMR protein expression was also found to be closely related to grade of the tumor. Of the 9cases of poorly differentiated adenocarcinomas, 6 cases showed loss of expression of MSH2 protein and one case showed lack of both MLH1 and MSH2 protein. The association between degree of differentiation and expression of MLH1 and MSH2 was found to be statistically significant.

A Previously reported study showed poorly differentiated tumors was associated with loss of MLH1 and the mucinous and medullary carcinomas were more frequently MLH1 negative and MSH2 positive.(7) Our present study however has contradicted these observations. In our study, poorly differentiated tumors including the mucinous adenocarcinomas were more often MSH2 negative. In contrast to Adrian Gologan, our study showed no statistically significant association between tumor stage and nodal metastases (12)

Screening for MSI helps in detecting sporadic cases with MMR protein defect and also potential cases of Lynch syndrome. In the present study 40% (16/40) cases showed lack of expression of either MSH2 or both MLH1 and MSH2 by immunohistochemical method. Among the various variables studied, only histological type and grade of the tumor showed statistically significant correlation with lack of mismatch repair protein. In colorectal carcinomas with MSI the common deficit proteins are MLH1 and MSH2. However a small proportion of MSI colorectal tumors are due to mutations in other MMR genes like MSH6, PMS1 and PMS 2. Hence, addition of antibodies against these proteins is also recommended in screening of colorectal carcinomas with MSI.

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