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Tafaul-Al-Ahsa Cancer Foundation Wins EXCELLENCE AWARD for Cancer Awareness Initiatives at Gulf Cancer Awareness Week 2024





The Gulf Oncology Society meeting resulted in fruitful outcomes, with young doctors showing keen enthusiasm for assuming leadership roles and implementing impactful initiatives.

The Official Journal of the Gulf Federation For Cancer Control

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Original Article

Assessing Mismatch Repair Expression by Immunohistochemistry in Colorectal Adenocarcinoma –Insight from a Tertiary Care Centre

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ABSRACT

Background: Microsatellite instability (MSI) is a pattern of hyper mutation that occurs at microsatellite level in the genome and result due to error in the mismatch repair system. MSI is caused by defective mismatch repair (MMR) genes associated with either hyper methylation of MMR genes or BRAF mutations. Anti–MLH–1, anti–MSH–2, anti–MSH–6 and anti–PMS2 monoclonal antibodies are used for Immunohistochemical analysis.

Methods: The immunohistochemical expression of MSI proteins were assessed in 72 cases of colorectal carcinoma. These were classified based on the expression of MLH1, MSH2, MSH6 and PMS2 proteins.

Results: There were 57 percent of cases showing loss of at least one antibodies, and 43 percent cases showing intact expression of all antibodies (MLH1, MSH2, MSH6 and PMS2).

Conclusion: In conclusion, our study provides valuable insights into the expression of mismatch repair in colorectal adenocarcinoma through immunohistochemistry analysis conducted at our tertiary care centre. These findings hold significant clinical implications, suggesting further testing for BRAF and MLH1 Promoter Hypermethylation to confirm possibility of Lynch syndrome.

Key words: IHC, MMR, CRC

Introduction

Colorectal cancers (CRC) account for 10% of all cancers and 9.4% of deaths due to cancer.[(1)]About 8%–20% of all CRC tumors presents microsatellite instability (MSI) being directly linked to defective function of mismatch repair (MMR).[(2)]Mismatch repair deficiency occurs either by somatic hypermutation of MMR genes, most commonly, MLH1, an inherited germline MMR pathway mutation as in Lynch syndrome or double somatic mutations in MMR genes. [(2,3,4)]

MSI can be detected in two ways: indirectly through immunohistochemical (IHC) staining of MMR protein expression, or directly through PCR–based amplification of specific microsatellite repeats.

IHC detects the expression of MMR protein which consists of hMLH1, hPMS2, hMSH2 and hMSH6. If the result shows that any of the above MMR protein expression are absent, it is interpreted as MMR deficient (dMMR) or have high levels of microsatellite instability (dMMR–MSI–H; ~15%). If all four MMR proteins are expressed, it is interpreted as Proficient Mismatch Repair (pMMR) or mismatch–repair–proficient or microsatellite instability–low tumours (pMMR–MSI–L/MSS; ~85%).[(5,6)]

The molecular and clinicopathological characteristics of MSI–H/dMMR CRCs are distinct from those of MSI–L/proficient mismatch repair (pMMR)CRCs.[(7)] The aim of present study is to evaluate expression of mismatch repair gene deficiency (MSI) in colorectal carcinoma by using immunohistochemistry.

Methodology

A cross–sectional study of 72 cases of colorectal carcinoma (excisional biopsies and major resections) were taken. Paraffin tissue sections on pre–coated slides were used for immunohistochemistry (IHC). Anti MLH1(Dako; MLH1–clone ES05, prediluted), anti MSH2 (Dako; MSH2–

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clone FE11, prediluted), anti MSH6 (Dako; MSH6–clone EP49, pre diluted) and anti PMS2 antibody (Dako, PMS2–clone EP51, prediluted) were used. Secondary antibody Envision FLEX HRP (Dako; prediluted) were used.

The sections were deparaffinised in xylene, rehydrated in alcohol, and washed in distilled water. Antigen retrieval was done using Tris ethylenediaminetetraacetic acid (EDTA) buffer in a microwave at 800 watts for 20 min in two cycles. Primary antibodies (MLH1, MSH2, MSH6, PMS2) were added which were ready to use monoclonal antibodies.

Interpretation of MSI like histology: According to revised Bethesda guidelines[8], the histologic features associated with high–level MSI (MSI histology) were tumor–infiltrating lymphocytes (TILs), Crohn's[1] like lymphocytic reaction, mucinous histology, signet ring cell histology, and medullary growth pattern. The presence of TILs is defined as an average of ≥3 lymphocytes between tumor cells per hpf, with counts from 5 consecutive hpf. Crohn–like lymphocytic reaction is defined as at least 3 lymphoid aggregates present at the tumor periphery with at least 1 lymphoid aggregate measuring >1 mm in diameter. If any of the above histologic features are identified in the tumor, MSI histology was present.

Interpretation of IHC: The interpretation was done using the College of American Pathologists (CAP) protocol for IHC. Positive staining in nuclei of stromal, inflammatory, or nonneoplastic epithelial cell were the internal control examined. The expression of four MMR proteins (MLH1, PMS2, MSH2, and MSH6) was defined as abnormal (loss), when nuclear staining of tumor cell is absent. dMMR is defined as the loss of at least one MMR protein in tumor cells. Proficient MMR (pMMR) is defined as the expression of all four MMR proteins in tumor cells. Further, dMMR is categorized into 4 types:

1. The loss of both MLH1 and PMS2 is dMMR type 1.
2. The loss of PMS2 is dMMR type 2
3. The loss of both MSH2 and MSH6 is dMMR type 3
4. The loss of MSH6 is dMMR type 4[9]

The study was performed after approval from Institutional Ethical Committee. (Ref no: BVDUMC/IEC/140, Dated: 22/12/2020) No familial genetic studies were performed.

OBSERVATIONS AND RESULTS

Among 72 cases, mean age of the patients was 59 years ± 11.80, and included 26 females (36.1%) and 46 males (63.9%) (M: F 1.76:1). All the results are tabulated in table 1.

There were 63 cases (88.9%) of moderately differentiated adenocarcinoma followed by 07 (9.7%) cases of well differentiated adenocarcinoma and 01 case (1.4%) of poorly differentiated adenocarcinoma.

MSI like morphology showed tumor infiltrating lymphocytes in 16 (22.2%) patients, followed by mucinous areas in 5 (6.9%), Crohn's like lymphocytic reaction in 04 (5.6%) and signet ring morphology in 02 (2.8%) patients. (Fig 1)

The IHC expression in 72 cases of CRC as tabulated in table No (2).

Of the 72 cases, 40 CRC (55.55%) had loss of either 01 or more of the MMR gene proteins (dMMR/MSI–H) & 32 CRC (44.44%) had retained expressions. (pMMR/MSI–L).

These includes:

1. Loss of MLH1 only (sporadic) – 24 cases (33.33%), (Fig 2)
2. Loss of MLH1 and PMS2: 05 (6.94%), (Fig 3)
3. Loss of PMS2 only– 02 (2.77%), (Fig 4)
4. Loss of MSH2 & MSH6 only: 05 (6.94%) (Fig 5)
5. Loss of MSH6: 04 (5.55%), (Fig 6)

Discussion:

The primary defect underlying MSI is abnormal DNA MMR function secondary to either germline mutation of one of 4 MMR genes, MLH*1*, *PMS2*, *MSH2* and *MSH6* or abnormal methylation of the promoter of *MLH1*. MSI positive cancers have distinct gene regulatory patterns and frequently exhibit abnormal epigenetic alterations such as hypermethylation of various genes, including the MMR gene MLH1[10].

In present study, there was no association of MMR expression pattern with age, tumor differentiation, site of tumor, or MSI histology. With regard to gender, there was a significantly greater number of male patients with loss of MSH6 expression (p=0.044) compared to females. While MLH1, MSH2 and PMS2 expression patterns did not differ according to gender. Similar results were reported by Xiao et al [11], except for gender, there were no correlations between the expression of the proteins MLH1, MSH2, MSH6, or PMS2 and age, sex, tumor location, tumor type.

In the present study, most (88.9%) of the CRC were moderately differentiated adenocarcinomas, followed by well differentiated (9.7%) and poorly differentiated adenocarcinomas (1.4%). There was no significant correlation of grade of carcinoma with MSI IHC status (Table 1). Most of the moderately differentiated carcinomas showed loss of at least one of the MSI IHC.

		MLH1		MSH2		MSH6		PMS2	
		Intact	Loss	Intact	Loss	Intact	Loss	Intact	Loss
Age group	<50	8	10	15	3	15	3	15	3
	>50	28	26	47	7	50	4	46	8
	p value	0.7855		0.999		0.4908		0.85	
Gender	Females	13	13	23	3	26	0	24	2
	Males	23	23	39	7	39	7	37	9
	p value	0.999		0.739		0.044		0.307	
Diagnosis of adenocarcinoma	Moderately differentiated	32	32	56	8	59	5	54	10
	Well differentiated	4	3	5	2	5	2	6	1
	Poorly differentiated	0	1	1	0	1	0	1	0
	p value	0.5401		0.6461		0.3507		0.9437	
Site of tumor	Ascending colon	4	7	8	3	8	3	8	3
	Descending colon	1	1	2	0	2	0	1	1
	Rectosigmoid junction	2	5	5	2	5	2	6	1
	Rectum	8	7	12	3	15	0	12	3
	Sigmoid colon	17	15	31	1	30	2	30	2
	Transverse colon	4	1	4	1	5	0	4	1
	p value	0.5357		0.2239		0.0906		0.3634	
MSI Histology	Mucinous	2	3	5	0	5	0	5	0
	Signet ring cells	1	1	1	1	2	0	1	1
	Crohn like lymphocytic reaction	3	1	4	0	4	0	4	0
	Tumor infiltrating lymphocytes	12	4	14	2	15	1	13	3
	p value	0.4798		0.2388		0.8699		0.2929	

Table (1) Association of age, gender, diagnosis of adenocarcinoma, site of tumor and histology with MMR gene expression pattern.

In the study, moderately differentiated tumors showed loss of expression of MLH1 while Xiao et al[11] study showed moderately differentiated tumors showed loss of expression of MSH6. Also, there was no significant association of MSI histology with MSI IHC status (Table 2)

Only 01 case of poorly differentiated adenocarcinoma which showed loss of MLH1. There were 36 (50%), 62 (86.1%), 65 (90.3%) and 61(84.7%) patients showing intact expression of MLH1, MSH2, MSH6, PMS2. There were 36 cases (50%), 10 (13.9%), 07 (9.7%) and 11 (15.3%) cases showing loss of expression of MLH1, MSH2, MSH6 and PMS2. Loss of MLH1 expression suggests further testing for BRAF and MLH1 Promoter Hypermethylation to confirm possibility of Lynch syndrome.

Li et al[6] reported slightly higher percentages, with 54.4%, 66.2%, 58.6%, and 42.1% of the 133 tumor specimens for positive for MLH1, MSH2, MSH6, and PMS2, respectively.

According to the MSI phenotype of CRC patients, Soliman NA [12] et al. classified the patients into 38 cases with MSS, 23 cases with MSI–L, and 54 cases with MSI–H.

In the current study, the majority 55.6% of the patients were classified as MSI–H, and 44.4% of patients had the phenotype of MSI–L. All the MSI tumors in those patients displayed instability across all 4 markers.

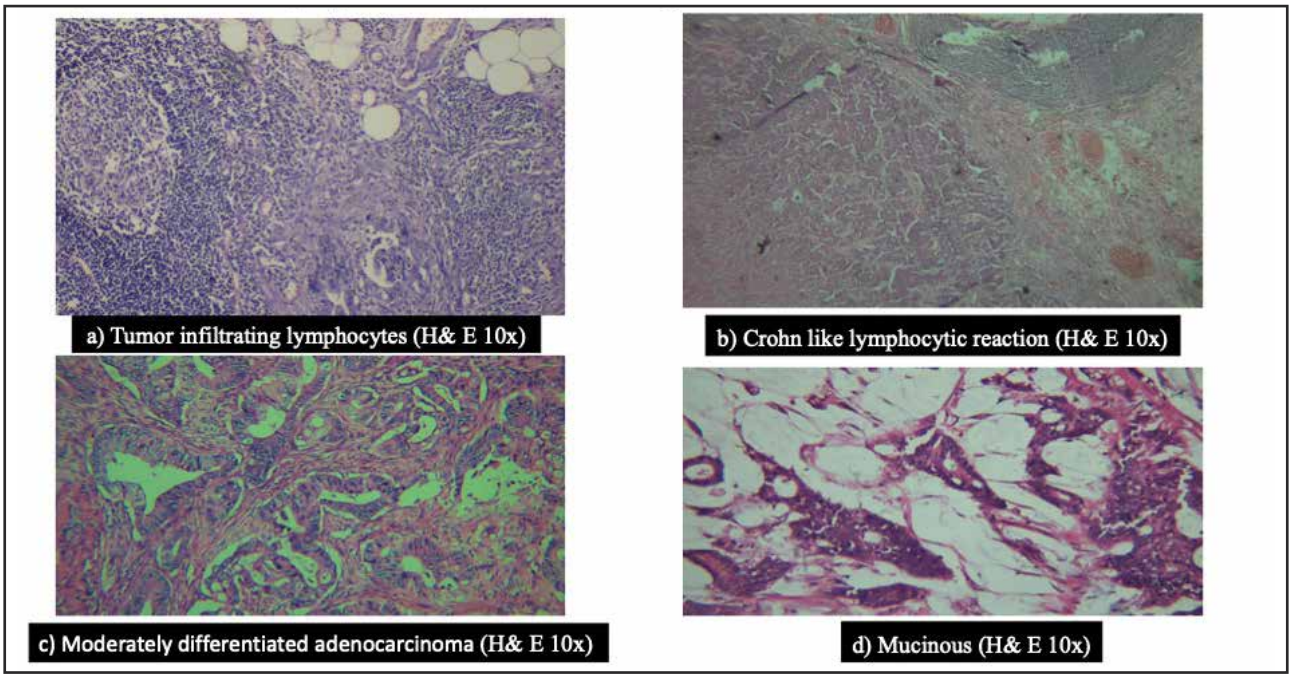


Figure 1. Microphotographs of various types of MSI Morphology in carcinoma colon

IHC Expression	Positive expression in no of cases	Negative expression in no of cases	Result
MLH1	36 (50%)	36 (50%)	55.6% MSI H with loss of one or more MMR gene. 44.4% MSI L has retained expression
MSH2	62 (86.1%)	10 (13.9%)	
MSH6	65 (90.3%)	07 (9.7%)	
PMS2	61 (84.7%)	11 (15.3%)	

Table (2). Expression of IHC in CRC.

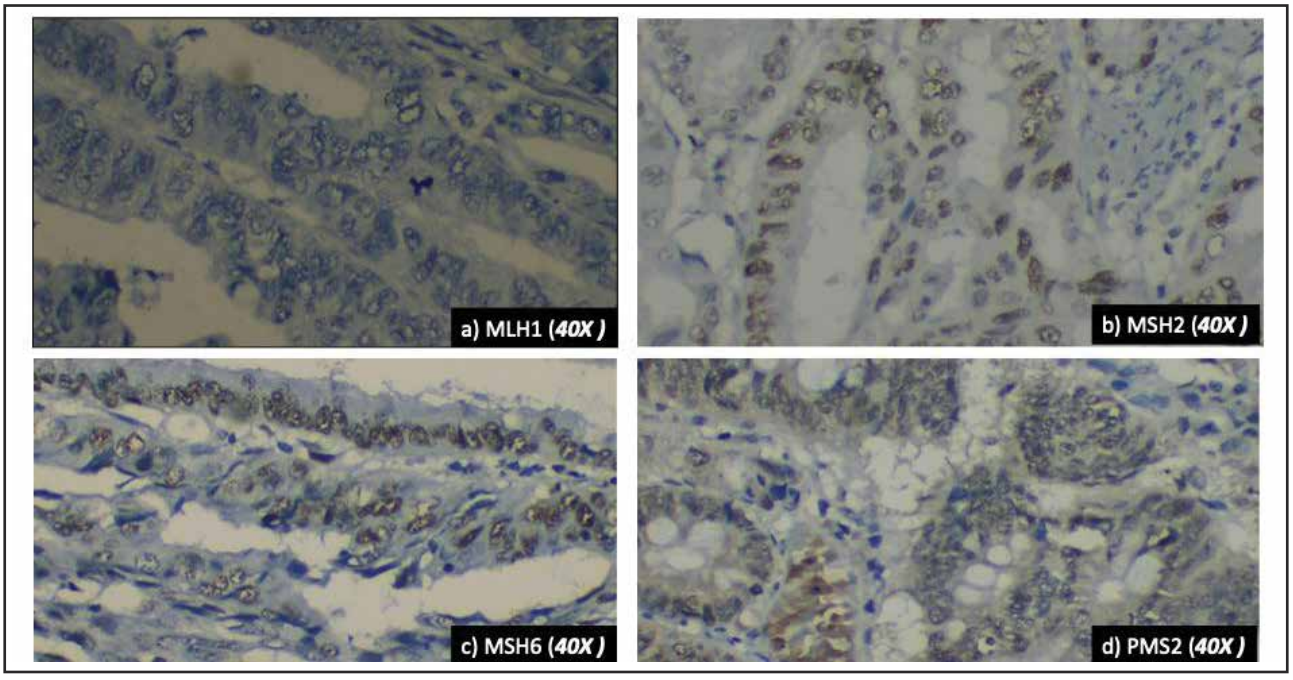


Figure 2. IHC photomicrograph showing loss of expression of MLH1 (sporadic)

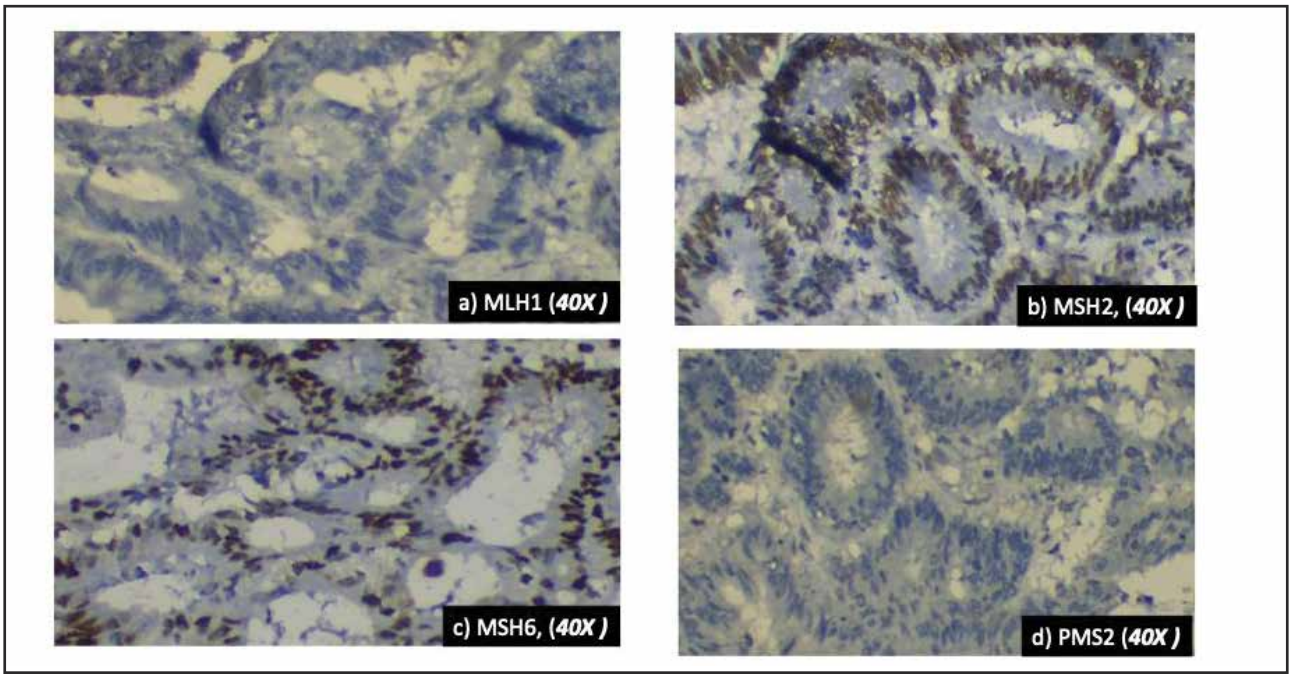


Figure 3. Photomicrograph showing dMMR – Loss of expression of MLH1 and PMS2 (type 1)

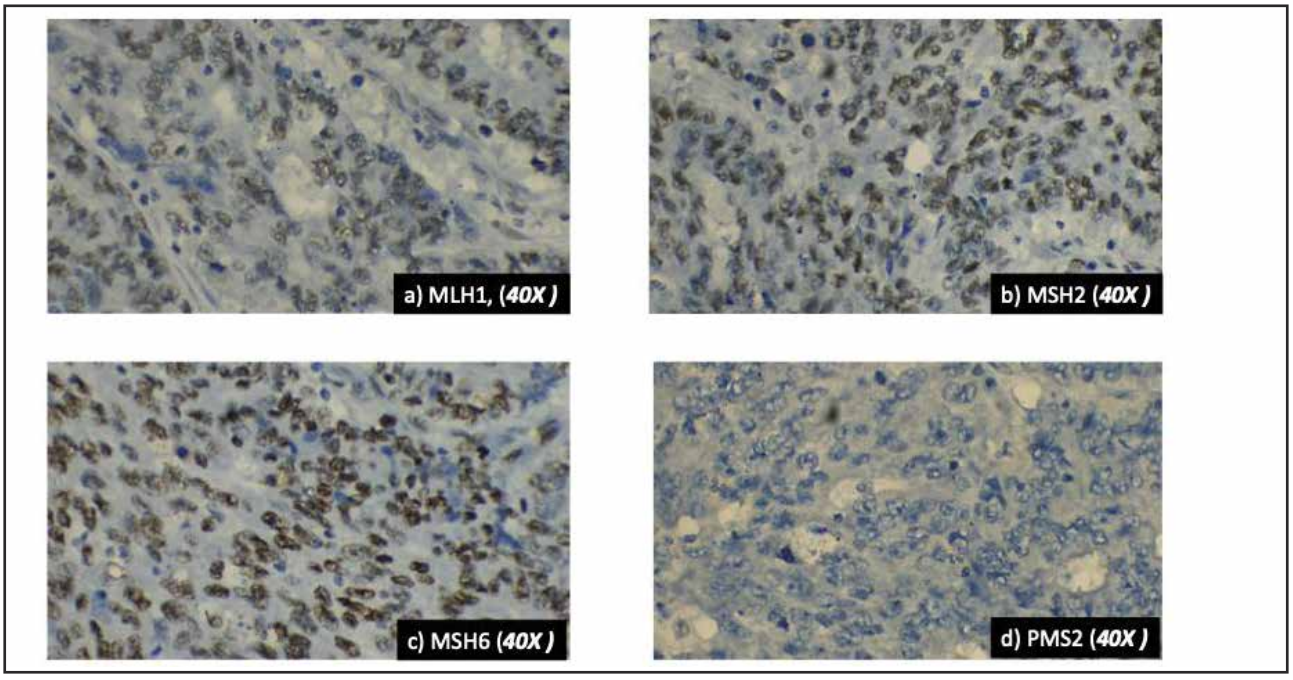


Figure 4: IHC photomicrograph showing dMMR – loss of expression in PMS2 (type 2)

Currently, the only direct methods to find mutations in genes encoding DNA repair enzymes are IHC, PCR, and DNA sequencing. Missense mutations and deletions are the most frequent changes, which helps to explain why there isn't enough protein synthesis [13]. The molecular classification of CRC may be closely associated with clinicopathological features, biological behaviors, treatment, and prognosis, as is gradually acknowledged given recent advances in molecular biology[13].

IHC minimizes the necessity of the use of a specialized laboratory, and it can aid in the detection of a potentially defective gene. It is preferred method to screen for Lynch Syndrome as IHC is equally sensitive to PCR, inexpensive, more readily available, and predicts the non–working gene, so it has a big role in limiting the number of the gene to be sequenced.

The limitation of the study is due to resource constraints for PCR based tests, comparison with IHC results could not be done.

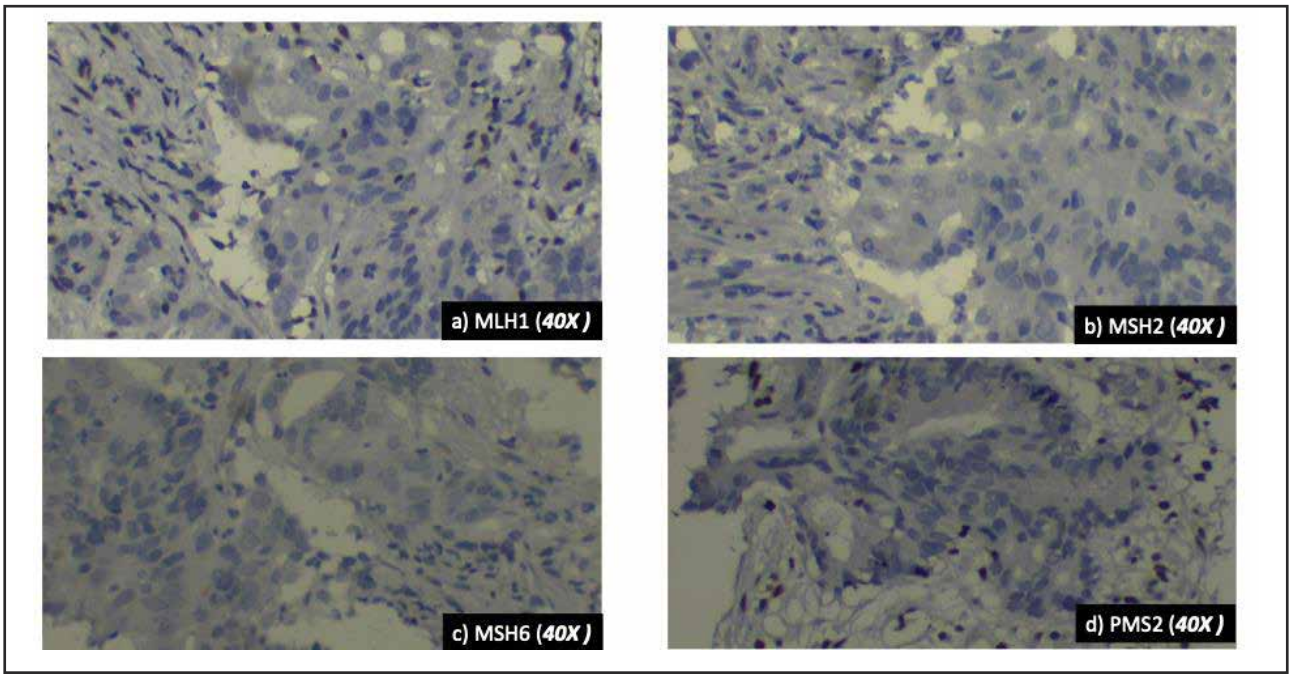


Figure 5. IHC photomicrograph showing dMMR – Loss of expression of MSH2 and MSH6 (type 3)

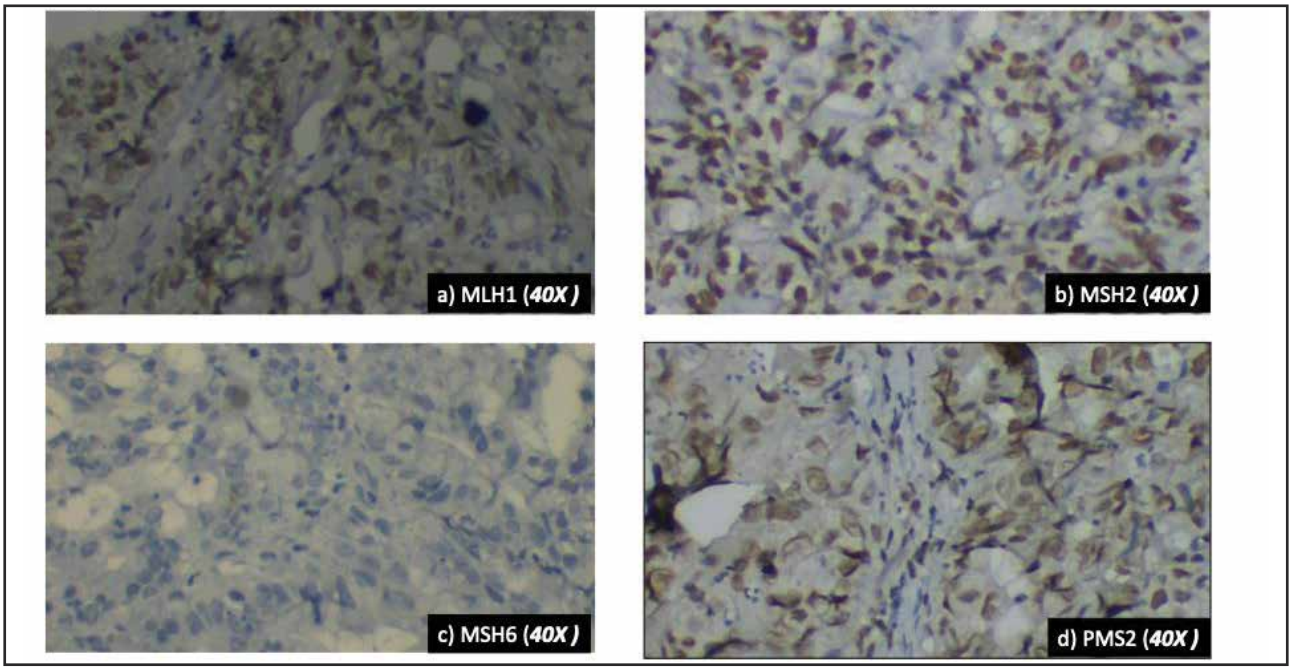


Figure 6. IHC microphotograph showing dMMR – Loss of expression of MSH6 only (type 4)

Conclusion

In conclusion, our study provides valuable insights into the expression of mismatch repair in colorectal adenocarcinoma through immunohistochemistry analysis conducted at our tertiary care centre. We found both MSI H and MSI L according to the phenotype. These findings hold significant clinical implications, suggesting further testing for BRAF and MLH1 Promoter Hypermethylation to confirm possibility of Lynch syndrome. Molecular based studies can bridge this gap and pave a way towards syndromic association and hence the prognosis. MSI is associated with distinct clinicopathological parameters and hence would be useful in the prognostic evaluation and determining therapeutic approach.

Despite the contributions of our study, it is essential to acknowledge its limitations of the study which is due to resource constraints for PCR based tests and its comparison with IHC results could not be done. In summary, our findings highlight the importance of assessing mismatch repair expression in colorectal

adenocarcinoma and pave the way for future studies aimed at improving diagnosis and treatment strategies for this disease.

Declaration

Manuscript is original and is not published or communicated for publication elsewhere either in part or full. Without the declaration the manuscripts are not considered for their publication.

Financial assistance:

Nil

Conflict of Interest:

Nil

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