Original Article

SCREENING OF ENDOMETRIOID ADENOCARCINOMA AND ATYPICAL HYPERPLASIA FOR MISMATCH REPAIR GENES BY IMMUNOHISTO-CHEMISTRY: A SINGLE INSTITUTE EXPERIENCE

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ABSTRACT

Objective: Mutation of mismatch repair gene (MMR) is one of the proven molecular etiology of endometrioid adenocarcinoma. The aim of our study is to find frequency of MMR deficient cases by immunohistochemistry (IHC) and statistically significant clinico-pathological parameters of these cases.

Material and Methods: A cross-sectional study was carried out in Chughtai Institute of Pathology from January 2018 to August 2020. A panel of four antibodies against MLH1, PMS 2, MSH2 and MSH6 MMR proteins was applied on 62 cases.

Results: Loss of MMR protein by IHC was seen in 22 out of 62 cases (35.5%). 15 cases (24.5%) showed combined loss of MLH1/PMS2, 6 cases (9.7%) showed combined loss of MLH2 /PMS in 9.7 % and isolated loss of MSH2 was seen in 1 case (1.6%). Statistically significant relationship was found between MMR protein loss and lymphocytic response around tumor. No statistically significant relationship was found for age, FIGO grade, tumor stage and location in uterine corpus.

Conclusion: Our study showed combined loss of MMR proteins in most of the cases which supports the use of a cost effective two antibody panel instead of four antibody panel approach for screening purpose. Lymphocytic response is more commonly seen in MMR deficient cases. Young age, tumor grade, stage and lower uterine segment involvement has no relation with MMR deficient expression. Our study concludes that regardless of age, grade, stage and tumor location, all newly diagnosed cases of endometrial carcinoma should be screened for MMR status by IHC.

Key Words: Endometrioid adenocarcinoma, Mismatch repair, Screening, Immunohistochemistry.

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INTRODUCTION

Endometrial cancer (EC) is the most common genital tract malignancy both in developed and developing countries. The USA cancer journal reports it as the fourth most common tumor in females after breast, lung and colorectal tumor [1]. According to a report by Punjab cancer registry of Pakistan published in 2018 uterine malignancy is the second most common tumor after breast cancer in Pakistani females [2].

The well-known risk factors for endometrial carcinoma are obesity, diabetes, hypertension, nulliparity, prolonged estrogen exposure as in cases with early menarche and tamoxifen use for treatment of breast cancer. A number of these cases are also related to hereditary cancer syndromes such as Lynch syndrome. For a long period of time alteration in various genes was thought to be involved in the development of endometrial cancer. Recent advances in the field of genetics and molecular biology has led to a newer classification of endometrial cancer based on genetic alterations. The Cancer Genome Atlas (TCGA) working group has divided these tumors into four molecular classes as POLE and MSI mutated along with two classes of low mutation rate cancers further divided into low-frequency DNA copy number and high- frequency DNA copy number [3]. Rationale for this molecular classification is the independent association of these subtypes with clinical outcome. This has opened a new discussion for prognostication and prediction in MSI-related tumors in particular [4]. MSI (microsatellite instability) related cancers is caused by somatic or germline alteration in DNA mismatch repair (MMR) genes. Only the cancers with germline mutation in these genes are associated with Lynch syndrome. MMR gene status can be assessed by MSI testing which is, although gold standard, an expensive modality or by IHC analysis using a panel of antibodies against MMR proteins which on the other hand is cost effective and helpful for stratification of cases for further genetic studies [4, 5]. E. Stelloo *et al* study proves a high concordance

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between IHC and MSI (94 %) [5]. The MMR protein assessment of colorectal tumor using IHC is a routine practice in western countries since 2009, with subsequent genetic testing to identify sporadic or germline mutations [6].

Patients with Lynch syndrome have high probability of developing endometrial cancer at a younger age and up to 50 percent cases of this syndrome develop uterine tumor at some period of their life [7]. The overall percentage of Lynch associated endometrial cancer ranges from 2 to 5 % [4, 8]. The identification of these cases would help the patient and family for screening and surveillance for other Lynch associated cancers. Moreover, knowledge of MSI related tumor whether sporadic or germline has an impact on treatment modality. Newly diagnosed MSI related endometrial cancer as well as recurrent tumors show good response to immunotherapeutic agents.

The society of gynecologic oncology (SGO) states that the endometrioid tumor which show loss of MMR protein expression on IHC should be further subjected to MSI testing but there is no recommendation for universal screening of all the cases [9]. Even In the developed countries this is still a matter of discussion. Several researches have also highlighted importance of MMR IHC use for precancerous cases as atypical hyperplasia [10,11]. This study was performed to assess the MMR protein expression in all the diagnosed cases of endometrial using IHC to determine the frequency of MMR deficient cases in our study population and to study clinic pathological features of these tumors.

MATERIAL AND METHODS

The study was conducted at Chughtai Institute of Pathology on the cases received between January 2018 to August 2020. Using lab electronic data system (nexus) all the cases were retrieved. History sheets, reports and slides of all these cases were reviewed. Relevant details such as the patient´s age, clinical presentation, gross location of tumor, FIGO grade, peritumoral lymphocytic response and pathologic stage (pT) in resection samples were recorded. The suitable representative section and corresponding block was selected for IHC application. A panel of four antibodies against MLH1, PMS2, MSH2 and MSH6 MMR proteins (provided by DAKO) was applied by manual technique on 3 to 4 μm thick sections. The positivity in endometrial stromal cells / lymphocytes was assured in every case and was considered as positive internal control. Nuclear expression of all MMR proteins (either intact or loss) as well as its percentage was noted. The cases showing complete loss of nuclear expression (100% tumor cells) of one or more proteins were categorized as MMR deficient. If a single tumor cell expressed either dim or moderate nuclear staining, it was classified as tumor with intact protein. All the cases were re-evaluated independently by a second histopathologist. Results were then studied in relation with clinical and morphologic features to establish any significant relation between various features and MMR deficiency.

RESULTS

A total of 62 cases (60 of EC and 2 of atypical hyperplasia) were included in this study. Majority of the specimens were resection type (hysterectomy and bilateral salpingoopherectomy (36 cases), hysterectomy (9 cases), hysterectomy, BSO and omentum (3 cases) and hysterectomy with unilateral adnexa (1 case) with thirteen endometrial curettage samples. The age range was 32-65years with mean age 52.90 years. For EC, the most common grade was FIGO grade II. Postmenopausal bleeding was the most common complaint in 40 cases (64.5%) followed by menorrhagia/irregular bleeding in 22 cases (35.5%). Out of 62 cases, complete loss of nuclear expression of MMR proteins was seen in 22 (35.5%). 15 cases (24.2%) showed combined loss of MLH1& PMS2, 6 cases (9.7%) showed combined loss of MSH 2 & MSH6 and isolated loss of MSH2 was seen in 1 case (1.6%). Amongst clinico-pathologic features, only peritumoral lymphocytic response showed statistically significant relationship with MMR deficiency (p value: 0.026). Other parameters such as young age (<50 years), tumor grade, pathologic stage and lower uterine segment location were statistically non-significant. (Table-I).

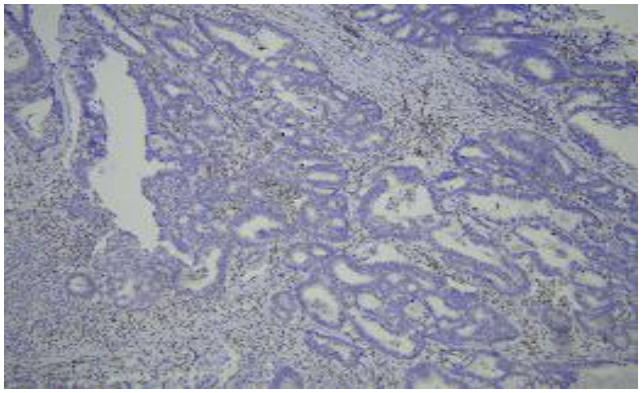
Table-I: Relationship of age, lymphocytic response, FIGO grade and lower uterine segment with MMR expression on immunohistochemistry.

Age			
MMR deficient by IHC		**MMR intact by IHC**	**p-value (exact sig 2-sided)**
≤50 years	06	17	0.281
>50 years	16	23	
Total	**22**	**40**	

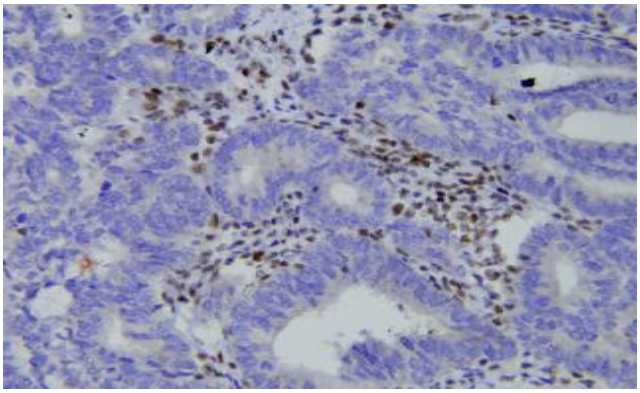
Lymphocytic Response					
MMR deficient by IHC			**MMR intact by IHC**		
Present	07		03		
Absent	15		37		0.026
Total	22		40		
Figo Grade					
MMR deficient by IHC			**MMR intact by IHC**		
Low grade	18		35		
High grade	03		04		0.687
Total	21		39		
Lower Uterine Segment					
MMR deficient by IHC			**MMR intact by IHC**		**P value**
Cases	Valid	missing	Valid	missing	
Involved	10	06	19	08	0.790
Uninvolved	06		13		
Total	**16**	**06**	**32**	**08**	
Pathologic Stage					
	MMR deficient by IHC		**MMR intact by IHC**		**p-value**
Early stage (Pt1)	13		23		
Late stage (Pt2, pt3, pt4)	04		09		1.00
Total	**17**		**32**		

Table-II: Pathologic stages representation.

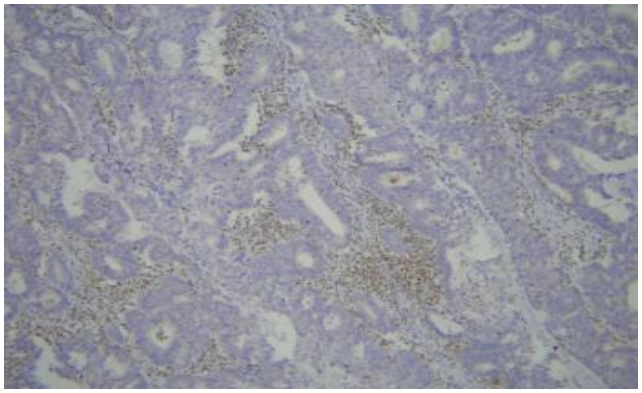
	Pt1a	**Pt1b**	**Pt2**	**Pt3a**	**Pt3b**	**Total**
MMR Deficient	10	04	02	01	0	17
MMR Intact	15	08	02	05	02	32
Total	**25**	**12**	**04**	**06**	**02**	**49**



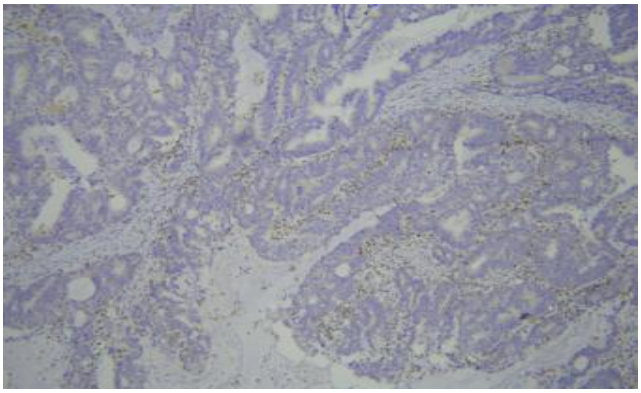
MSH6 LOSS



MSH2 LOSS

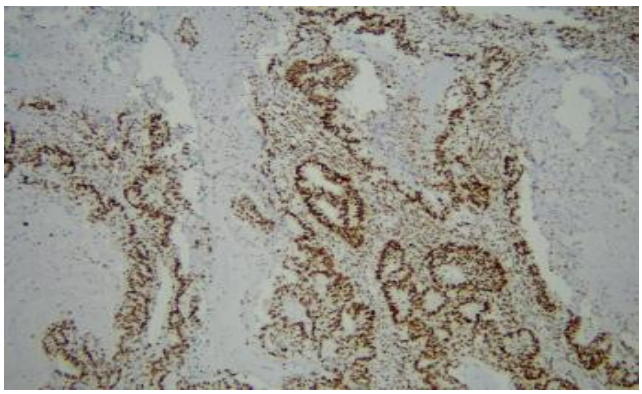


MLH1 LOSS

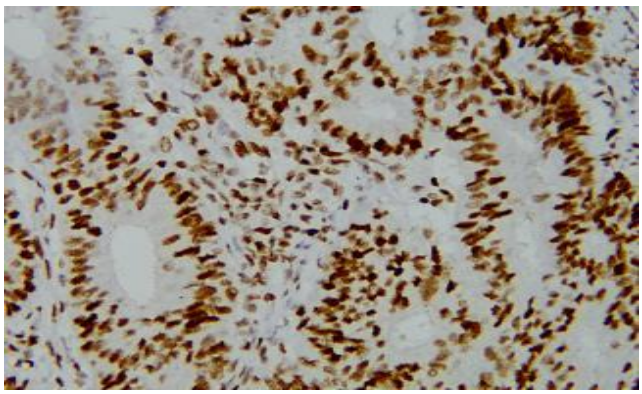


PMS2 LOSS

Figure-I: MMR deficient immunohistochemical expression.

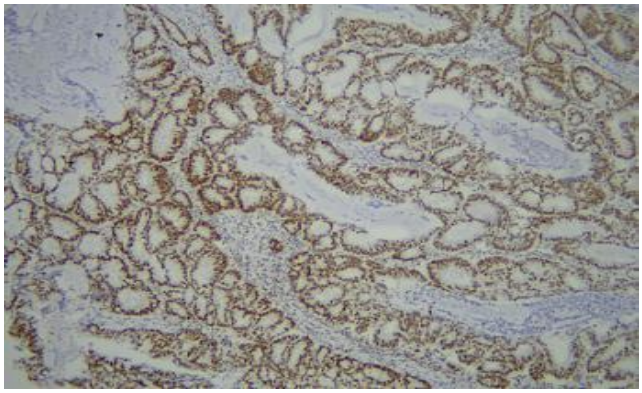


STRONG INTENSITY (10 X)



STRONG INTENSITY (40X)

WEAK INTENSITY



MODERATE INTENSITY

Figure-II: MSH6 intact immunohistochemical expression with intensity pattern.

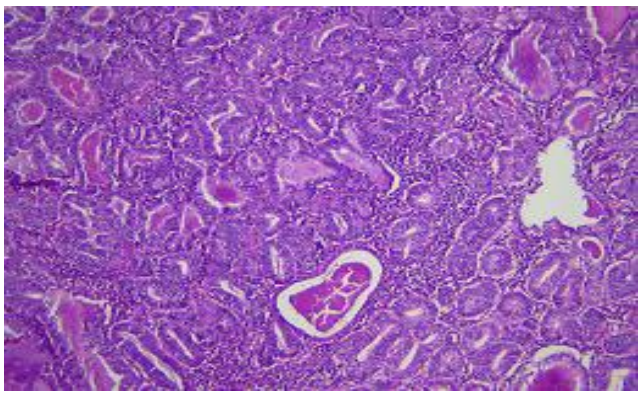


Figure-III: Tumor with lymphocytic response 10x.

DISCUSSION

The practice of mismatch repair gene evaluation by IHC on endometrioid adenocarcinoma as a screening tool is variable among different institutes all over the world. The benefit of this screening is to subcategorize tumors for further mutational analysis (sporadic or germline). The germline cases (Lynch syndrome) are further subjected to genetic counselling of the family and follow up of the patient. The predictive benefit is the use of anti-PD1 drugs especially on recurrence of MSI related endometrioid tumors which has shown good response.

In Pakistan, MMR evaluation is not a routine practice on endometrioid adenocarcinoma. One study by Hashmi *et al* analyzed MMR status of endometrioid adenocarcinoma in Pakistani population by IHC [12]. Our study showed the pattern of protein loss in the combination of MLH1/PMS2 in 15 cases (24.2%), MSH2 /MSH6 in 6 cases (9.7%) and isolated loss of MSH2 in 1(1.6%) cases which highlight the fact that majority of the MMR deficiency existed in the combination pattern in our study population. A total of 22 out of 62 cases (35.5%) showed complete loss of expression. The frequency of MMR deficient cases in our study was quite lower than Hashmi *et al* [12] and Kumar *et al* [13] study while higher than E.stello study [5]. E.stello *et al* study showed 26 % cases with MMR deficiency, overall concordance of 94% between IHC and MSI & a p value of <0.001. Similar to our study majority of these cases show combined loss of proteins as compared to isolated protein loss. Our results support E.stello proposal that using a two-antibody panel against PMS2 and MSH6 protein would be as effective as the four-antibody panel in detecting MMR protein abnormalities. The study by Hashmi *et al* on endometrioid adenocarcinoma in Pakistani females showed higher percentage (44.4%) of MMR loss as compared to our study (35.5%). The combination

pattern loss was similarly highlighted by Hashmi *et al* study with majority of cases exhibiting MLH1/PMS2 loss. As compared to Hashmi study, our study did not show loss of all four proteins in any case while Hashmi's study showed 16 out of 56(28%) cases with loss of all four proteins. The whole panel loss of proteins was not seen in our study and in E.stello study. Kumar *et al* study stated a higher frequency of MMR deficiency which was 62.7% with most frequent PMS2 loss followed by MLH1, however combination loss pattern was not stated in his study.

Our study showed a mean age of 52.90±7.58 and the most common presenting complaint was postmenopausal bleeding (64.5%). Only 37.1% cases presented below 50 years which shows endometrioid adenocarcinoma is more prevalent in Pakistani females of older age. No statistically significant relationship was found between young age (< 50) and MMR deficiency with a p value of 0.28 which was similar to results of Hashmi and Kumar study. The previous studies by Mills *et al* [14] proved 75% women with germline mutation of MMR gene were older than 50 years. Goodfellow *et al* [15] study concluded that 24% women were older than 60 years with germline MMR mutation. Results of our study emphasize that MMR abnormalities either due to sporadic or germline mutation have no relation with age at presentation and propose that all the cases should be studied for MMR deficiency irrespective of age. The high occurrence of MSH2/MSH6 protein abnormalities in young age was depicted in the previous studies, which was not supported by our results which showed that the combination of MSH2/MSH6 was lost only in 1 patient below 50 years of age and p value (0.247) was not significant.

Tumor infiltrating lymphocytes (TILs) was a constant finding in all MMR deficient cases with a statistically significant p value (0.026) (Figure-III). These findings were similar to the study conducted by Shia *et al* [16] which stated TILs count to be the most significant predictor of MSI. In contrast, studies by Kumar and Honore *et al* [17] found a no significant relationship between TILs and MMR deficiency.

Majority of the cases in our study are FIGO grade II with low pathologic stage (pT1). Hashmi *et al* [12] study showed a statistically significant relationship of MMR deficient cases with high pathologic and FIGO stage, however no such relationship was found in our study. There was no relationship between lower uterine segment involvement and MMR deficiency in our study. This was similar to the findings of Kumar *et al* [13], Masuda *et al* [17] and several other studies which rejected the association of Lower uterine segment with MMR related tumors. The MSI-positive frequency in Masuda study [19] was 22.2% in the LUS and 25.9% in the non-LUS groups, with no statistically significant difference between the two groups. Westin *et al* demonstrated high incidence of MMR mutations in lower uterine segment tumors [18].

CONCLUSION

In our study a number of cases showed loss of MMR expression on IHC which warrants further workup for Lynch syndrome as proactive approach towards identification of lynch associated cancers can significantly reduce morbidity and mortality in such patients. All the cases of endometrioid carcinoma irrespective of clinical and morphological findings must be screened for MMR expression and only cases with loss of expression should be subjected to mutational analysis. Furthermore, use of IHC panel of two antibodies PMS2 and MSH6 can prove be as beneficial as four panel approach. The non-availability of further genetic testing is limitation of our study which could have assessed Lynch syndrome /sporadic mutation in these patients.

AUTHOR CONTRIBUTION

Aafia Qasim: Original concept, study design, data analysis and paper write up.

Asma Zafar: Data collection, analysis and interpretation, edited the manuscript.

Safana Sadaf and Saima Batool: Discussion and result interpretation.

Zunaira Rathore and Saira Rathore: Proof reading.

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