Original Article

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Microsatellite instability in Endometrial carcinoma.

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ABSTRACT

Background: Endometrial carcinoma is the commonest gynaecological malignancy in the western countries with a standardised incidence of 8 per 100000 women. In India and Southeast Asia, the incidence of endometrial carcinoma is low but it is increasing due to increasing prevalence of obesity, diabetes, early menarche, late menopause, late marriage and declining birth rate. The study aimed to identify the role of microsatellite instability in endometrial carcinoma. Although MSI has been studied extensively in colorectal carcinoma there have been very few studies in southeast asian region regarding association of MSI and prognosis in endometrial carcinoma. To the best of our knowledge this is first study in India.

Method: A Descriptive study in which 40 patients of endometrial carcinoma were studied. MSI was detected using immunohistochemistry (MSH-1, PMS-2, MSH-2, MSH-6). Statistical Analysis was done using SPSS software and fisher exact test was used to calculate p value. p value less than.05% was considered significant.

Results: Overall prevalence of microsatellite instability was 40%. Microsatellite instability was associated with higher tumor grade, myometrial invasion>50% and presented in initial stages compared to microsatellite stable tumors.

Conclusion: Our study showed statistically significant association between microsatellite instability and Figo staging, tumor grade and myometrial invasion.

Keywords: Endometrial Carcinoma, Microsatellite Instability, Lynch Syndrome, Colorectal Carcinoma, Carcinogenesis.

Introduction

Microsatellites are repeat sequences of several DNA bases. They are generally used for paternity testing and other forensic investigations as they are found both in exon and intron regions. As they have repeat structure they are more prone to slippage and thus any error in these regions that take place during replication are repaired by DNA mismatch repair genes (MMR) which are mainly MLH1, MSH2, MSH6, PMS2.[1] In tumors, microsatellite repeat number is different from that in normal tissues which is known as microsatellite instability. MSI has been related to carcinogenicity in various tumors including Lynch syndrome. The role of MSI in colorectal carcinoma has been very well studied with a prevalence of 12-24% and it is documented that colorectal tumors with MSI behave in a different way clinically when compared to tumors without MSI. [2,3]MSI positivity in colorectal carcinoma is associated with favourable prognosis. Studies of MSI in endometrial carcinoma have been very few. In a study by Caduff et al it was found that endometrial tumors positive for MSI have a high grade and a poor prognosis.[4] On the basis of tumor histology, biology and clinical features endometrial cancers are divided into two major types. Type 1 endometrial cancer is more common (70-80%), is hormone-sensitive and occurs commonly in women exposed to estrogen. It is generally associated with a higher level of tumor differentiation and has a good prognosis. Type 2 endometrial cancer is less common (20-30%) and includes serous and clear cell histology. It is characterized by poor level of differentiation and has a higher probability of myometrial invasion with poor prognosis and behaves more aggressively. In addition to these histological subtypes endometrial carcinoma is also classified on basis of molecular alterations one of which is MSI. In 1998 the National Cancer Institute (USA) recommended panel of 05 MSI markers for the determination of MSI. The tumor is called MSI-high if it shows instability in at least 02 markers out of 05; MSI-low if 1 out of 05 and MSI-stable if none. MSI association with prognosis in endometrial carcinoma is not clearly understood. The purpose of our study is to add upon existing knowledge and to compare clinical characteristics and prognosis in endometrial tumors with and without MSI.

Materials and Methods

Data Collection: 40 patients who were treated for endometrial carcinoma at tertiary care hospital in western Maharashtra between January 2017 and March 2020 were included in the study. The institute ethical clearance was sought before initiation of study. Review blocks were used for study of MSI after permission from head of the institution. The histological type was classified using World Health Organisation criteria and surgical staging

was determined using the International Federation of Obstetrics and Gynaecology (FIGO).[5]

Immunohisochemistry: Immunohistochemical staining was performed on formalin –fixed paraffin embedded specimens using standard avidin-biotin method. Positive controls were sections known to express the investigated antigens, whereas negative controls were obtained by omitting the primary antibodies. Normal staining pattern for MLH1, MSH2, MSH6 and PMS2 is nuclear (Fig1-5). Evaluable staining was available for all 40 cases. Some cases showed a weak cytoplasmic staining but they were considered negative. To investigate the observer reproducibility all cases were seen by same observer twice and by two different observers.

Statistics: Comparisons of groups was done using fisher exact test. Only cases with conclusive results available for all four IHCs were included. Data was analysed using SPSS software package.

Results

Of the total 40 endometrial tumors, 34were endometrioid while others 6 included serous papillary (4), high grade serous (1) and clear cell (1). Out of 40 patients 24 (60%) were MS stable whereas 16(40%) were MS instable (Table 1). Amongst MS instable tumors, 10 out of 16 showed loss of only 2 markers, 1 out of 16 showed loss of single marker, 2 out of 16 showed loss 3 markers and 3 out of 16 showed loss of all 4 markers. Age in our study ranged from 35 to 80 years with mean age of 57 years. 18 (45%) of 40 patients were less than 57 years of age. 37.50% of MS stable patients were less than 57 years of age compared to 56.25% of MS instable patients which shows that MS instable tumors tend to occur in younger age group. 03 of 24 (12.50%) MS stable patients were premenopausal compared to 06 of 16(37.50%) of MS instable patients who were premenopausal.

We also found that 02 of 16 (12.50%) of MS instable tumor presented in advanced stage III-IV disease, whereas 11 out of 24(45.84%) MS stable patients presented with advanced stage disease. Conversely 87% of MS instable patients presented in Stage I-II versus 54% of MSI stable tumors. There was also a trend for higher grade (G2 and G3) in MS instable tumors (68.75%) compared to MS stable tumors (25%). Myometrial invasion greater than 50 percent was seen in 50% of MS instable tumors compared to 12.50% of MS stable tumors. Lymphovascular invasion was present in 25% of MS instable tumors compared to 8.34% of MS stable tumors. We found out that 04 of 40 patients (10%) had family history of carcinoma.

One of our patients who presented with endometrial carcinoma at age of 45 also gave history of carcinoma colon and had all four markers negative. Out of total 06 non endometrioid tumors (Table 2) five were MS stable whereas one was MS instable (Small cell).

Discussion

Modern medicine has found out molecular basis for carcinogenesis in every tumor and this has led to the discovery of biomarkers for each tumor.[6] Tumorigenesis of colorectal carcinoma has been known to proceed through a series of genetic alterations involving protooncogenes and tumor suppressor genes.[7] One such biomarker involved in colorectal carcinoma is MSI, germline mutations of which have been found in patients with hereditary non-polyposis colorectal cancer(HNPCC). In the present study, we evaluated the MSI status of endometrial cancers in Indian patients. To the best of our knowledge this is the first study of this type in India. About 20% endometrial carcinoma have microsatellite instability though only 2-5% of these are associated with Lynch syndrome. [8,9] Most of MS instable endometrial cancers are endometrioid with occasional other types being MS instable. [10,11] Abnormal expression of MSI in our population was 40% which is quite high as compared to studies in other parts of the world. A Study by Atif Ali Hashmi et al in Pakistan found MSI instability in 44% of patients.[12]

In colorectal carcinoma, MSI is associated with older age, female sex, and other clinicopathological parameters such as prevalence in the proximal colon, mucinous differentiation, lymphocytic infiltration and low pathological stage.[13,14] In endometrial carcinoma most of research has concluded that MS instable phenotype has been associated with Type 1 or endometrioid type, high tumor grade and, greater myometrial invasion.[15,16] Our study too showed a greater percentage of MS instable patients with higher tumor grade as compared to MS stable patients. Our study also showed that majority of MS instable tumors present in lower FIGO stage compared to MS stable which present at a late stage. As per our study MS instable tumors have a higher prevalence in premenopausal compared to MS stable tumors in pre-menopausal patients.

The MMR protein occurs in two complexes MLH1/PMS2 and MSH2/MSH6. MLH1 and MSH2 are stable without their counterparts but PMS2 and MSH6 require their counterparts for stability.[17] Thus PMS2 and MSH6 are not expressed if their counterparts are not expressed, hence causing loss of MLH1/PMS2 in case of MLH1 defect and loss of MSH2/MSH6 in case of MSH2 defect. With the loss of MSH6 only or PMS2 only germline testing for each is carried.[18,19] In case MSH2 is negative further testing for germline mutations in EPCAM is indicated.[20] In case of loss of MLH1 on IHC, it can be

Table 1: Association of various clinicopathological factors in endometrial tumors with and without microsatellite instability.

Category	No of Patients	% of Total	MS Stable	%	MSI Instable	%	p value
	40	100	24	60.00	16	40.00	
Age							
<57	18	45.00	09	37.50	09	56.25	0.334
>57	22	55.00	15	62.50	07	43.75	
Menopause							
Pre	09	22.50	03	12.50	06	37.50	0.119
Post	31	77.50	21	87.50	10	62.50	
Figo Stage							
I+II	27	70.00	13	54.16	14	87.50	0.040
III+IV	13	30.00	11	45.84	02	12.50	
Tumor grade							
G1	23	57.50	18	75.00	05	31.25	0.009
G2+G3	17	42.50	06	25.00	11	68.75	
Myometrial Invasion							
<50%	29	72.50	21	87.50	08	50.00	0.013
>50%	11	27.50	03	12.50	08	50.00	
Lymphovascular Invasion							
Absent	34	85.00	22	91.66	12	75.00	0.195
Present	06	15.00	02	08.34	04	25.00	
Lymph Node Metastasis							
Absent	37	95.00	23	95.83	14	87.50	0.553
Present	03	05.00	01	04.17	02	12.50	
Family History							
Absent	36	90.00	21	87.50	15	93.75	0.637
Present	04	10.00	03	12.50	01	06.25	
Tumor Histology							
Endometrioid	34	87.50	19	79.16	15	93.75	0.372
Others	06	12.50	05	20.84	01	06.25	

Fisher Exact Test used to calculate p value

Table 2: Pattern of expression of MSI Markers in endometroid and non-endometroid endometrial tumors.

Total No of cases	Endometrioid (n=34)	Others (n=06)
All markers retained	19	05
Isolated loss of PMS2	01	00
Loss of MLH1, PMS2	10	00
Loss of 3 Markers (MLH1, PMS2, MSH6)	02	00
Loss of all 4 markers	02	01
Loss of MSH2, MSH6	00	00

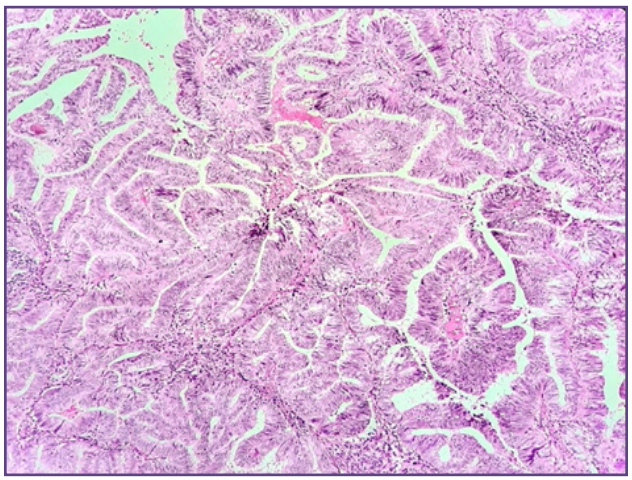

Fig. 1: (a) Well differentiated endometrioid adenocarcinoma. (H&E 200X).

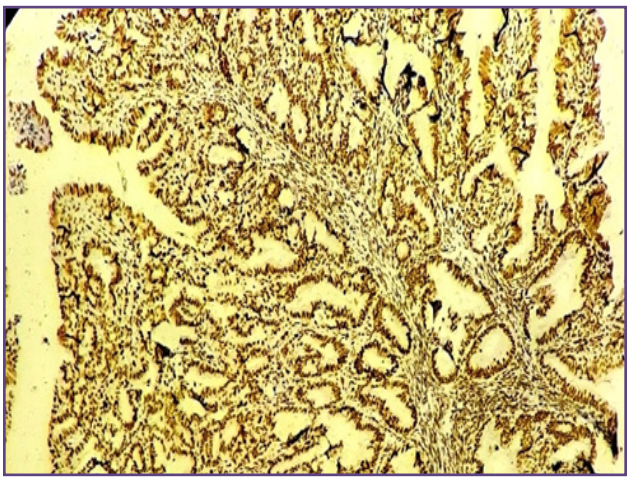

Fig. 2: Tumor cells with retained positivity for MLH1. (200x, DAB used as chromogen).

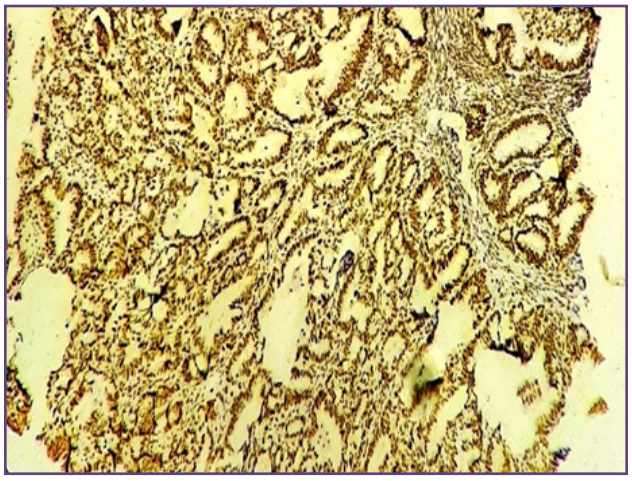

Fig. 3: Tumor cells with retained positivity for PMS2. (200x, DAB used as chromogen).

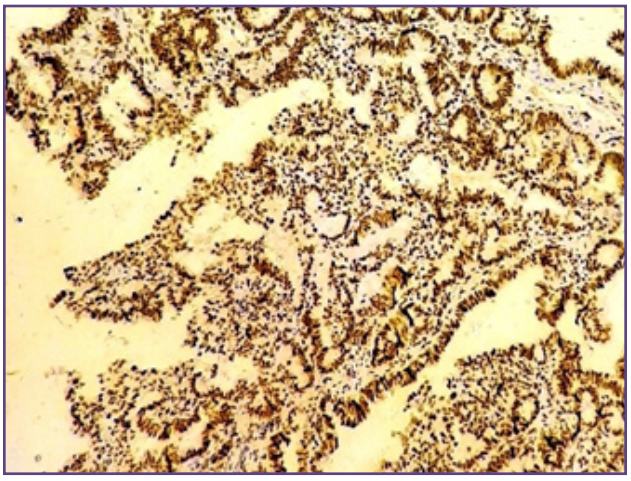

Fig. 4: Tumor cells with retained positivity for MSH2. (200x, DAB used as chromogen).

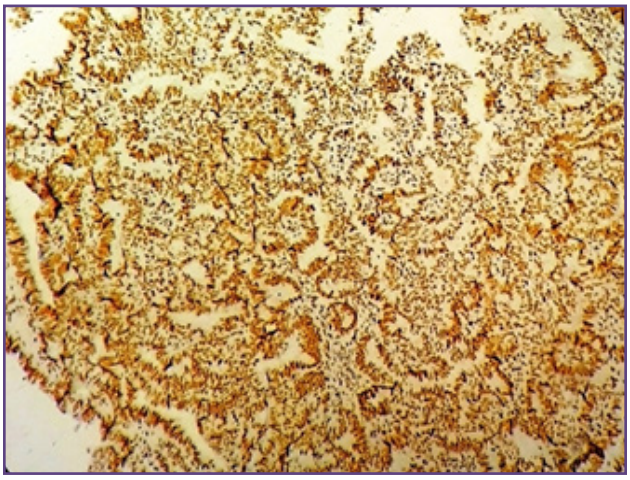

Fig. 5: Tumor cells with retained positivity for MSH6. (200x, DAB used as chromogen).

due to MLH1 hypermethylation likely sporadic and does not require further testing for Lynch syndrome.[18,19] In such cases, MLH1 hypermethylation is checked and if present it indicates a sporadic cause for MMR defect. MLH1 hypermethylation in endometrial carcinoma is not associated with BRAF and hence BRAF cannot be used as an indicator for sporadic MMR defect to rule out Lynch syndrome.[19] If MLH1 hypermethylation is absent further germline testing for MLH1 is indicated. In cases with strong suspicion for Lynch syndrome with MLH1 hypermethylation germline methylation or germline epimutation is likely.[20] Our study had loss of MLH1/PMS2 pattern highest among MS instable tumors.

Another significance of MSI testing is the benefit of PDL-1 antagonists. MSI-high tumors generally harbour high neoantigen loads, increased immune checkpoint expression such as programmed cell death protein (PD-1), and programmed cell death ligand-1(PDL-1) and an increased number of tumor-infiltrating lymphocytes. Thus, these tumors are good candidates for immunotherapy. It has been seen that MS instable tumors have higher response rates to anti PDL1 therapy compared to MS stable tumors.[21]

However, the main limitation of our study was the small sample size. Thus, it is necessary to continue the investigation including a greater number of patients with MS instable tumors.

Conclusion

Although MSI has been associated with favourable prognosis in colorectal carcinoma patients, the MSI overall impact in endometrial carcinoma is still controversial. According to our study MS instable phenotype is more common in tumors with higher tumor grade, tumors with deep myometrial invasion and those which present in early stage of disease; thus, it may be associated with worse prognosis.

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Competing Interests

Nil.

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