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International Journal of Molecular and Immuno Oncology

Article in Press

Original Article

Incorporation of immunohistochemistry in the assessment of survival and prognosis of endometrial cancers: Are we ready for the change?

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Received: 21 January 2022
Accepted: 23 February 2022
EPub Ahead of Print: 12 April 2022
Published:

DOI
10.25259/IJMIO_2_2022

Quick Response Code:

ABSTRACT

Objectives: The aim of the study was to determine the mismatch repair (MMR) proteins and p53 expression by immunohistochemistry in operable endometrial carcinoma (EC) patients. The study aimed to analyze and correlate clinicopathological factors and survival with MMR and p53 immunohistochemistry markers.

Material and Methods: A retrospective cohort study of 115 cases of carcinoma endometrium who underwent primary surgery in our hospital from July 1, 2013, to December 31, 2020, with a minimum follow-up of 1 year. Available tissue blocks were stained for IHC expression of MMR and p53 proteins. Patients were stratified into Type I and Type II on basis of histopathology. Clinicopathological factors, overall survival (OS), and disease-free survival (DFS) were then compared on the basis of MMR deficiency and p53 status.

Results: The mean age of study population was 58.9 years with a mean body mass index of 31.61 kg/m^2. The mean follow-up was 41.29 months. Ninety-seven patients underwent IHC staining for MMR and p53 proteins. Among these 97 patients, 79 patients belonged to Type I histopathology and 18 patients belonged to Type II histopathology. The 79 patients of Type I histopathology were further divided into MSS or microsatellite stable group and MSI or microsatellite instable group. MMR deficit status was seen in 17 (21.5%) patients and 62 (78.5%) patients were MSS. For the 18 cases of Type II ECs, 5 (27.8%) patients were p53 positive whereas 13 (72.2%) patients were p53 negative. For patients with Type I histopathology; the clinicopathological factors such as stage, age, grade of the tumor, lymph-vascular space involvement, lymph node status, and myometrial invasion were compared between the MSI and MSS groups. Patients with microsatellite instability were more likely to present with a higher grade, a positive lymph node status, and with lymph-vascular space invasion. The OS and DFS are not significantly affected in patients with loss of MMR proteins. Due to a smaller number of cases in p53 group, clinicopathological features and survival could not be compared.

Conclusion: Analyzing of immunohistochemistry status for evaluating the microsatellite instability in patients with Type I endometrioid adenocarcinomas is an alternative and efficient tool in predicting the prognosis for these patients. Further studies with more sample size can help us in studying the impact of MSI and p53 on OS and DFS and for guiding in the management of the same.

Keywords: IHC, Microsatellite instability, Endometrial carcinoma, p53 marker, Overall survival, Disease free survival

INTRODUCTION

Endometrial carcinoma (EC) has been studied extensively in the literature. There are still many unanswered questions related to the management of EC and the oncologists have to face

new challenges every day. The molecular classification of carcinoma endometrium[1] has set in a paradigm shift in the management strategies. Being more reproducible than histopathology and impacting clinical outcomes in a more predictable way, this classification looks quite promising. However, the cost and complexity of genetic sequencing needed for molecular staging are a major limiting factor for its widespread clinical use. Immunohistochemistry markers appear to be an attractive, promising, and cost-effective replacement for these genetic assays with wider clinical applicability aiding in classification, planning surgery, and guiding adjuvant therapies.

In this study, our aim was to determine the mismatch repair (MMR) proteins and p53 expression by immunohistochemistry in operable EC patients. Clinicopathological factors and survival were then analyzed and correlated with these immunohistochemistry markers.

MATERIAL AND METHODS

A retrospective cohort study of 115 cases of carcinoma endometrium who underwent primary surgery in our hospital from July 1, 2013, to December 31, 2020, and had a minimum follow-up period of 1 year was included in the study. Patients with a pre-cancer diagnosis of endometrial intraepithelial neoplasia or complex atypical hyperplasia or advanced cases which did not undergo definitive surgery were excluded from the study. History of any other cancer in the past or concurrent cancer at the time of diagnosis of endometrial cancer was also taken as exclusion criteria. Ethics Committee approval was taken from Institutional Review Board. Informed consent was taken from all patients for use of their tissue blocks for research purpose. Available tissue blocks were stained for IHC expression of MMR and p53 proteins using VENTANA BENCHMARK XT system. MMR staining was done for MLH1, MSH2, MSH6, and PMS2 proteins. Microsatellite instability (MSI) was reported when there was loss of any one or more of these proteins whereas microsatellite stable (MSS) was reported when there was no loss of the above proteins. p53 staining was also done using IHC for all patients and p53 positivity for the mutant type was reported when there was diffuse staining (>80% cells positively stained) for the marker whereas all other staining patterns were reported as negative.

The clinicopathological characteristics of the entire study population were studied. Patients were stratified into Type I and Type II histopathology. Clinicopathological factors, overall survival (OS), and disease-free survival (DFS) were then compared on the basis of MMR deficiency and p53 status after stratifying the population into two groups of MSI and MSS and p53 positive and p53 negative divisions.

Statistical analysis

Kaplan–Meier curves were used to estimate OS and DFS. Fischer exact test and Pearson Chi-square test were used to compare clinicopathological variables after applying yates' correction. Survival was compared using cox regression model and log rank test. $P < 0.05$ indicated a significant statistical difference. All statistical analysis was performed using the IBM SPSS software; version 24.

RESULTS AND OBSERVATIONS

One hundred and fifteen patients of carcinoma endometrium were studied retrospectively. The mean age of our study population was 58.9 years (30–88 years) with a mean body mass index of 31.61 kg/m^2 (20.30–52.44). The mean follow-up of the study population was 41.29 months (12–102 months).

The histopathological characteristics of our study population were then studied [Table 1]. It was seen that 72.2% patients belonged to Stage I of FIGO 2009 staging for carcinoma endometrium, followed by 16.5% belonging to Stage III, 7% patients were of Stage IV, and only 4.3% cases belonged to Stage II. About 53.9% of the study population had more than 50% myometrial involvement with only 22.6% patients having a positive lymph-vascular space invasion. Furthermore, 16.5% of our study population had a positive lymph node status, that is, either pelvic or para-aortic node positivity.

Endometrial cancer is divided into two types of patterns based on the histopathology, that is, Type I and Type II.[2] About 81.7% patients of our study population belonged to Type I histopathology group and 18.3% patients belonged to Type II histology group.

Table 1: Histopathological characteristics of the study population.

Histopathological parameters	Number of Patients (115)	Percentage
Stage		
I	83	72.2
II	5	4.3
III	19	16.5
IV	8	7.0
Type of histopathology		
Type I	94	81.7
Type II	21	18.3
Myometrial invasion		
<1/2	53	46.1
>1/2	62	53.9
Lymph-vascular space invasion		
Negative	89	77.4
Positive	26	22.6
Lymph node status (pelvic and or para-aortic nodes)		
Negative	96	83.5
Positive	19	16.5

Out of the 115 patients of our study population, 97 underwent IHC staining for MMR and p53 proteins; as for 18 patients enough tumor tissue could not be obtained for IHC purposes.

Among these 97 patients, 79 patients belonged to Type I histopathology and 18 patients belonged to Type II histopathology.

The 79 patients who belonged to Type I histopathology were further divided into MSS or MSS group and MSI or microsatellite instable group [Table 2]. MMR deficit status was seen in 17 (21.5%) patients and 62 (78.5%) patients in this group were MSS. All the 79 cases showed a negative or normal expression for the p53 protein.

For the 18 cases of Type II ECs, 5 (27.8%) patients showed diffuse staining for p53 protein whereas 13 (72.2%) patients were p53 negative. Furthermore, no patient in the Type II tumors showed loss of any MMR protein and thus all were MSS or microsatellite stable. Above results are summarized in [Figure 1].

For patients with Type I histopathology; the clinicopathological factors such as stage, age, grade of the tumor, lymph-vascular space involvement, lymph node status, and myometrial invasion were compared between the MSI and MSS groups [Table 3] and it was seen that patients with MSI were more likely to present with a higher grade (Grade III tumors), a positive lymph node status, and with lymph-vascular space invasion as compared to the MSS group. The results for these three factors were significant as $P < 0.05$.

Kaplan–Meier curves were then plotted using the SPSS software and OS and recurrence free survival for 79 cases belonging to Type I histopathology calculated [Figure 2]. The OS was 92.7% (95% CI: 85.31–100.22) and the recurrence free survival was 94.3% (95% CI: 87.83–100.78) after a median follow-up of 33 months.

The OS and recurrence free survival between MSS versus MSI groups were compared and p value calculated using the Chi-square test. It was seen that OS and DFS are not significantly affected in patients with loss of MMR proteins [Figure 3].

OS for Type II histopathology group in our study was 73.23% after a median follow-up of 30 months [Figure 4]. We compared the

Table 2: MMR status for the Type I histopathology group.

MSS	62 (78.5%)
MSI	17 (21.5%)
Loss of MLH1/MSH2/MSH6/PMS2	3
Loss of MLH1/PMS2	5
Loss of MSH2/MSH6	2
Loss of PMS2	5
Loss of MLH1	1
Loss of MLH1/MSH2	1

MMR: Mismatch repair, MSS: Microsatellite stable, MSI: Microsatellite instable

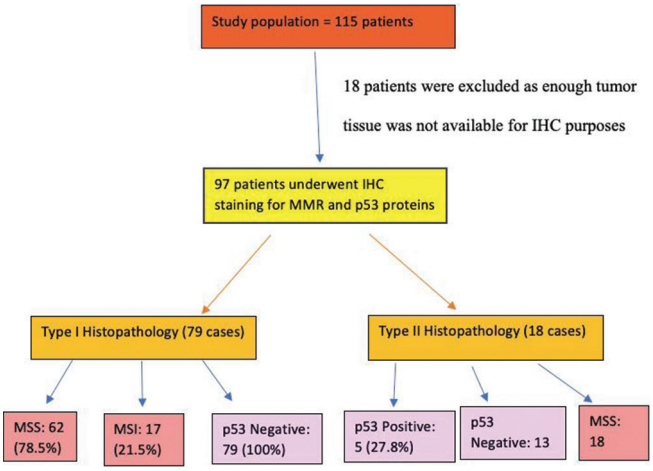

Figure 1: Immunohistochemistry staining of study population.

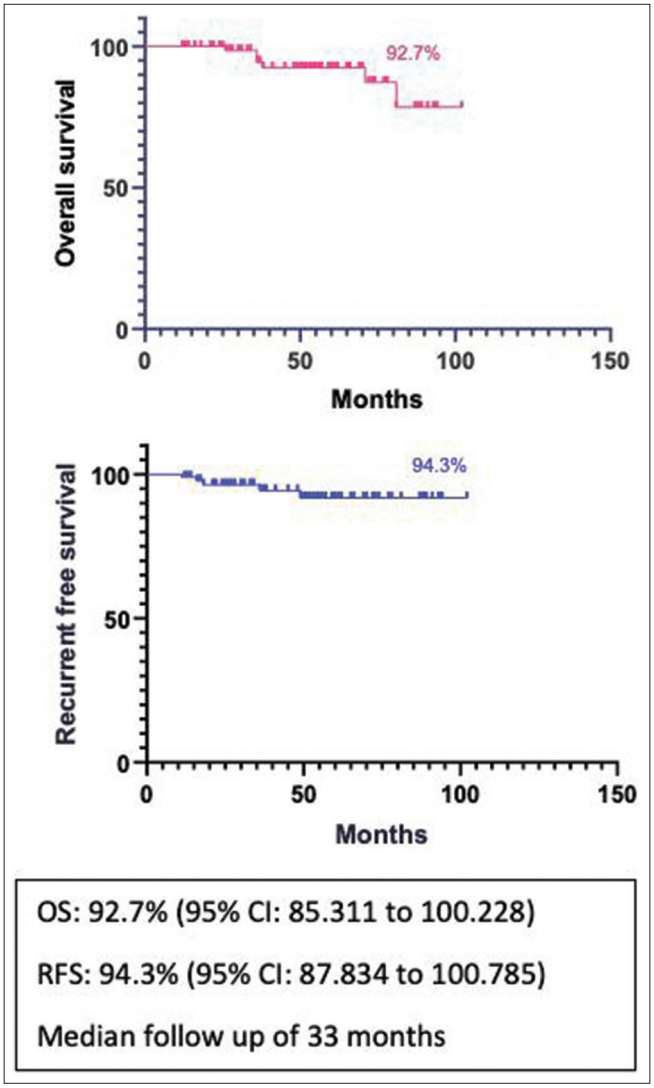

Figure 2: Overall survival and recurrence free survival of Type I histopathology group.

Table 3: Clinicopathological factors for Type I histology in MSI versus MSS status and their level of significance.

Feature	MSI (17)	MSS (62)	*P* value
Stage			
I	11 (64.7%)	52 (83.8%)	0.0745
II	0 (0%)	2 (3.2%)	
III	4 (23.5%)	7 (11.3%)	
IV	2 (11.8%)	1 (1.7%)	
Age (years)			
≤60	10 (58.8%)	33 (53.2%)	0.891
>60	07 (41.2%)	29 (46.8%)	
Grade			
I	5 (29.4%)	37 (59.6%)	0.0062
II	5 (29.4%)	19 (30.7%)	
III	7 (41.2%)	6 (9.7%)	
Lymph-vascular space invasion			
No	10 (58.8%)	54 (87.1%)	0.0223
Yes	7 (41.2%)	8 (12.9%)	
Myometrial invasion			
<1/2	6 (35.3%)	36 (58.1%)	0.163
>1/2	11 (64.7%)	26 (41.9%)	
Lymph node status			
Negative	11 (64.7%)	55 (88.7%)	0.045
Positive	6 (35.3%)	07 (11.3%)	

MSS: Microsatellite stable, MSI: Microsatellite instable

Table 4: Histopathological factors for Type II histopathology group.

Feature	p53 Positive (5)	p53 Negative (13)	*P* value
Stage			
I	3 (60%)	6 (46.1%)	0.631
II	0 (0%)	2 (15.4%)	
III	1 (20%)	4 (30.8%)	
IV	1 (20%)	1 (7.7%)	
Lymph-vascular space invasion			
No	3 (60%)	5 (38.5%)	0.768
Yes	2 (40%)	8 (61.5%)	
Myometrial invasion			
<1/2	1 (20%)	4 (30.8%)	0.896
>1/2	4 (80%)	9 (69.2%)	
Lymph node status			
Negative	3 (60%)	9 (69.2%)	0.852
Positive	2 (40%)	4 (30.8%)	

histopathological features between p53 positive and p53 negative groups [Table 4]. No significant statistical difference could be obtained as only five cases were p53 positive. Furthermore, we were not able to correlate the OS and DFS between the two groups due to the smaller number of cases in this category.

DISCUSSION

EC has been classified into four subgroups according to the molecular characteristics that include the POLE ultra-mutated type, MSI, copy number high, and copy number low.[1] Genetic testing is required for evaluation of the molecular status for patients with endometrial cancer. Due to the unaffordability of genetic testing, immunohistochemistry is commonly used for predicting prognosis for these patients. After reviewing the literature, at present, the only reliable test for detection of POLE mutation is through sequencing of the POLE gene exonuclease domain and IHC has not been tried for the same. For detection of MSI and p53; IHC has been used in the past and its effectiveness proven in various studies. P53 IHC can be interpreted as normal/wild type and abnormal/aberrant/mutant type. The specificity of p53 immunohistochemistry is 100% that has been validated in various studies using ovarian carcinoma and thus depicting the presence of an underlying mutation in the gene when positive for the mutant type staining pattern.[3,4]

MSI has been extensively studied with respect to endometrial cancers and they have proven to be of prognostic significance in the past. The methods that are used for detecting MSI are next generation sequencing, polymerase chain reaction (PCR), capillary electrophoresis (capillary electrophoresis), IHC, and single molecule molecular inversion probes. The gold standard for detection of MSI is PCR and CE through fluorescent multiplex techniques with an accuracy of 100%.[5,6] However, genetic testing is cost-prohibitive and unrealistic in many middle- and low-income group countries. Hence, to detect MMR status for patients with endometrial cancer, many utilize immunohistochemistry tools. The accuracy of IHC has been reported to be in range of 89–95% in various studies.[7] The impact of MSI on prognostic factors and survival of patients has also shown comparable results with respect to genetic testing.[8] Evaluation of patients for MSI status and p53 positivity not only helps in predicting prognosis and survival but can further guide us in the management and planning of adjuvant therapy in these patients. On scrutinizing the literature sources, we found similar outcomes from various studies conducted on MSI and p53 marker proteins. The methods that they used for analysis (IHC or genetic testing) did not seem to influence the final results.

One hundred and nine patients of carcinoma endometrium were studied by Kanopiene *et al.*[9] from April 2010 to June 2011. They studied the MSI status for their study population using genetic testing and observed that 100 patients who belonged to endometrioid adenocarcinoma group had 17% MSI-high status. Furthermore, the clinicopathological parameters which were significantly associated with MSI-high status were grade and myometrial invasion. The nine patients in their study who belonged to Type II histopathology of ECs were all MSS. Their study results are comparable to the present study even though the method used for analysis was different.

The role of MSI in patients of endometrioid adenocarcinomas who received post-operative radiation therapy was studied by Sahinturk *et al.*[10] from January 2002 to December 2012.

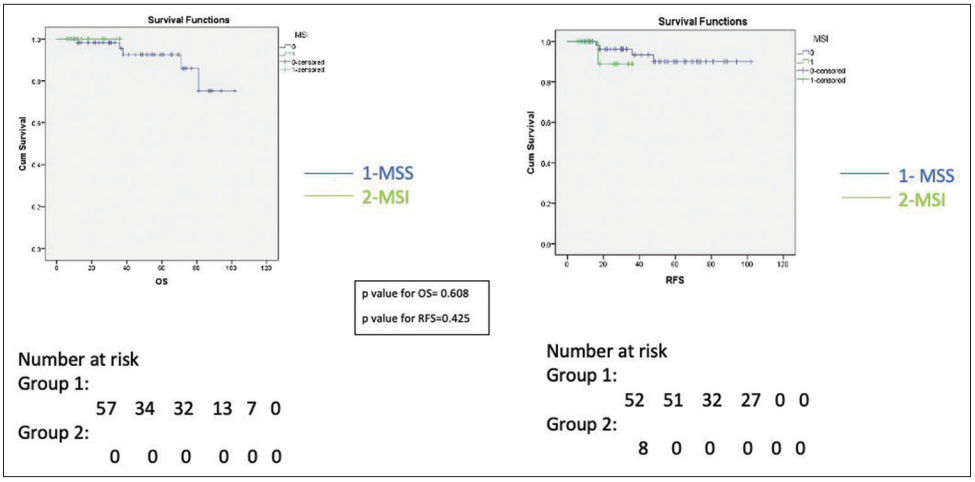

Figure 3: Comparison of overall survival and recurrence free survival between MSS and MSI status of Type I histology (P>0.05; Not significant). MSS: Microsatellite stable, MSI: Microsatellite instable.

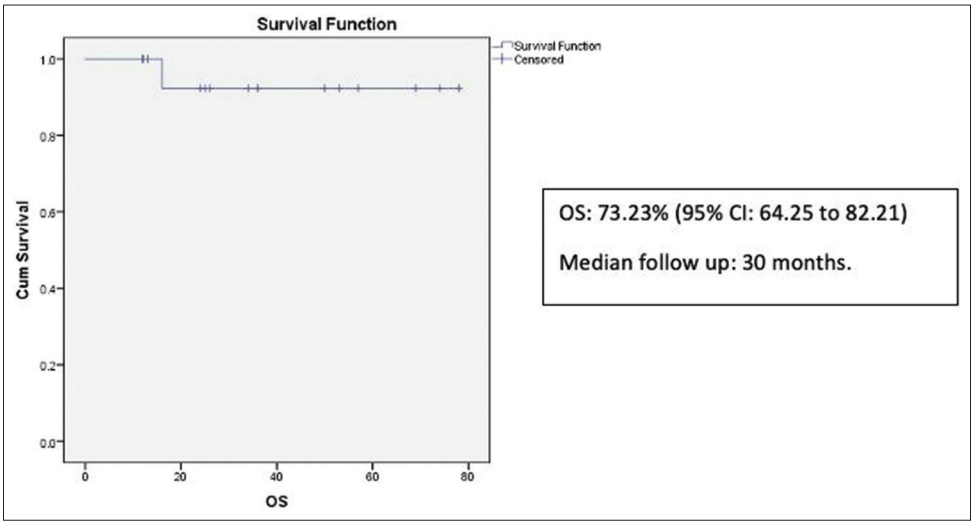

Figure 4: Overall survival for Type II histopathology group.

A total of 124 cases were included and MSI analysis was done using DNA extraction techniques by genetic testing. They also analyzed the OS and DFS between the MSI and MSS groups which were not significant. The clinicopathological factors that were significantly found to influence the prognosis were age, lymph node involvement, and advanced stage.

Use of immunohistochemistry for assessment of MMR protein expression was studied by Sharma *et al.*[11] for 102 cases of ECs. The prevalence of MMR protein deficit in their study population was 21.6% and for endometrioid adenocarcinomas was 25.3%. The most common MMR loss in their study was for MLH1 and PMS2. The factors that were significantly affected due to the MMR loss in their patients were stage and grade of the tumor. In our study, also the most common MMR loss was of MLH1 and PMS2 combined and PMS2 alone that was seen in five patients each.

The impact of MSI on survival in high-grade EC was studied by Arabi *et al.*[12] in 119 patients. Out of the 119 patients; 57 belonged to the Type I category and 62 belonged to the Type II category of Bokhman classification[2] of carcinoma endometrium. In their study, they immune stained using antibodies against MLH 1, MSH2 and MSH6 MMR protein and correlated the overall survival between MSI and MSS groups, but could not find a statistically significant result. The MSI patients in their study had 13.2 times more risk of death when all the three proteins (MLH1, MSH2, and MSH6) were lost as compared to MSS status patients.

p53 is also a common immunohistochemistry marker for predicting survival in patients with EC. It is most commonly seen to be involved in high-grade types of endometrioid adenocarcinomas or Type II histopathology. Brett *et al.*[13] conducted a study to evaluate the survival in p53 mutated

endometrial cancers. Grade 3 endometrioid carcinomas and endometrial serous carcinomas were analyzed. A total of 326 patients underwent IHC staining for p53 and the OS compared between the two groups. p53 positive status for mutant type was seen in all 100% patients of the serous carcinoma group whereas only 23.5% of endometrioid Grade 3 carcinomas showed a similar status. No significant difference in the OS was observed in the two groups. In our study, 27.8% patients belonging to Type II histopathology showed p53 diffuse staining for the mutant type and no case in the Type I histopathology group showed p53 mutant positive status. This difference of results especially for the Type II histopathology group can be due to differences in demographic profile and stage of the patients as in our study, all the cases presented at an earlier stage and underwent primary surgery.

Our study helps in predicting prognosis of patients with Type I ECs and opens avenues in modifying the post-surgical management of these patients. Although the present study has its own limitations still, we were able to show a significant influence of microsatellite deficient status in predicting a higher grade, lymph node positivity, and lymph-vascular space invasion for Type I ECs.

The drawbacks of our study were that it was a retrospective study and only a small number of patients underwent IHC staining in the Type II histopathology group. Furthermore, for 18 slides as adequate tumor tissue was not available for IHC purposes, they were excluded from the final evaluation. Thus, we could not obtain significant results for the same.

CONCLUSION

Analyzing of immunohistochemistry status for evaluating the MSI in patients with Type I endometrioid adenocarcinomas is an alternative and efficient tool in predicting the prognosis for these patients. In our study, MSI significantly affected the grade, lymph node status, and lymph-vascular space invasion for our study population, though there was no significant difference in the OS and recurrence free survival in the MSI versus MSS arms. Further studies with more sample size can help us in studying the impact of MSI and p53 on OS and DFS and for guiding in the management of the same.

Declaration of patient consent

Institutional Review Board (IRB) permission obtained for the study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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How to cite this article: Kaur S, Nandwani M, Garg H, Aiyer HM, Aggarwal P. Incorporation of immunohistochemistry in assessment of survival and prognosis of endometrial cancers: Are we ready for the change? Int J Mol Immuno Oncol, doi 10.25259/IJMIO_2_2022