Investigation of the Relationship Between Immunohistochemical Mismatch Repair (MMR) Protein Expression and Prognostic Parameters in Endometrial Carcinomas

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Abstract: Objective: Molecular classification has been the most important development in endometrial carcinomas in recent years. This classification divides tumors into four groups: (i) POLE mutant group, (ii) microsatellite instable (MSI) group, (iii) high copy number group (P53 mutation), and (iv) low copy number group. Among these groups, POLE and MSI groups stand out with their better prognosis and potential to benefit from immune-control inhibitor therapy. In our study, we aimed to compare the prognostic parameters of patients with and without nuclear expression loss in MMR proteins (MLH-1, PMS-2, MSH-2, MSH-6) by immunohistochemical (IHC) method. Methods: Between 2017 and 2020, 80 patients who were diagnosed with endometrial carcinoma in hysterectomy material and whose MMR proteins were evaluated as IHC were included in the study. Patients with and without MMR loss were compared in terms of tumor size, histological grade (HD), depth of myometrial invasion, lymphovascular invasion (LVI), and cervical involvement. Results: Loss of any of the MMR proteins was present in 37 cases (46.3%), while 43 cases (53.7%) had no loss. When the cases were compared in terms of loss of MMR protein nuclear expression, 45.9% (17/37) of the cases with loss and 27.9% (12/43) of the cases without loss had histologic grade III (P = 0.03). There was no statistically significant difference between the two groups in terms of myometrium 1/2 external invasion, cervical stromal involvement, and LVI. Conclusion: Approximately half of the patients in our study lost at least one of the MMR proteins. The HD of patients with loss of nuclear expression of MMR proteins tended to be statistically significantly higher than those without loss.

Keywords: Endometrium cancer; Mismatch repair; Microsatellite instability

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1. Introduction

Endometrial cancer (EC) is the most common gynecologic malignancy in Western countries, with increasing incidence and mortality in recent years [1]. The low reproducibility of the current histopathological classification and the inadequacy of risk stratification based on this classification in some cases may cause some patients to receive excessive or inadequate treatment [2]. The Cancer Genome Atlas (TCGA) Research Network classified endometrial tumors into four new prognostic groups according to their molecular characteristics in 2013 (Table 1) [3].
Table 1. Molecular classification of endometrial tumors

<table>
<thead>
<tr>
<th>No.</th>
<th>Molecular classification</th>
<th>Alternative classification (ProMisE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ultramutant group</td>
<td>POLE mutated tumors</td>
</tr>
<tr>
<td>2</td>
<td>Hypermutant group</td>
<td>Microsatellite unstable tumors (MMR-deficit)</td>
</tr>
<tr>
<td>3</td>
<td>High copy number group</td>
<td>P53 mutated tumors (Serous-like)</td>
</tr>
<tr>
<td>4</td>
<td>Low copy number group</td>
<td>Other tumors (P53-wild-type)</td>
</tr>
</tbody>
</table>

However, immunohistochemical (IHC) classifications have also been defined as an alternative to molecular classification based on these sequencing analyses due to the fact that molecular examinations cannot be performed in every laboratory, technical difficulties, and cost \([4,5]\). According to the Proactive Molecular Risk Classifier for EC (ProMisE), endometrial tumors include: (i) POLE-mutant tumors (POLE-nt), (ii) Mismatch repair (MMR) defective tumors, (iii) P53-abnormal tumors (P53-abn), and (iv) P53-wild-type tumors (P53-wt). In this alternative classification, sequencing analysis is required only for POLE mutations, while IHC examination is sufficient for MMR-deficiency (MMR-d) and P53 mutations. The POLE-nt group includes ECs with the best prognosis and the highest mutation burden. The MMR-deficient group has an intermediate prognosis, while P53-wt tumors have a good-intermediate prognosis. The p53-abn group is the tumor group with the worst prognosis \([4-8]\).

A defect in the MMR mechanism results in the accumulation of mismatches, insertions, and deletions in repeated sequences (microsatellites). This is termed microsatellite instability (MSI). MSI can be observed in approximately 20%–40% of endometrial carcinomas. The most common reason for this is the silencing of the MLH1 gene as a result of hypermethylation \([3]\). Lynch syndrome, one of the tumor predisposition syndromes, is caused by pathogenic germline mutations in MMR genes, and the resulting MMR deficiency (MMR-d) is observed in approximately 2% of ECs \([9]\). MMR-d can be detected by molecular MSI analyses and/or IHC with antibodies to four MMR proteins (MLH1, PMS2, MSH2, and MSH6). IHC is a much more cost-effective method than MSI analysis, and studies have shown a high concordance between MSI analysis and loss of MMR protein expression \([10,11]\). Recent guidelines recommend routine evaluation of MMR expression in ECs by IHC \([12]\).

For a tumor to be declared MMR-d by IHC, loss of nuclear expression of one or more of the MLH1, PMS2, MSH2, and MSH6 proteins must be observed. Loss of nuclear expression of these proteins is usually observed in all or nearly all tumor cells in MMR-d tumors. However, in recent years, there are publications showing that subclonal losses are also valuable \([13]\). MLH1 protein is required for PMS2 to function. Therefore, PMS2 loss is also observed in tumors with MLH1 loss. Similarly, MSH2 protein is required for MSH6 to function, and MSH6 loss is also observed in tumors with MSH2 loss. Sample staining patterns that occur as IHC as a result of mutations in MMR genes are shown in Figure 1.

Determination of MMR status in endometrial carcinomas is important because it provides information about tumor prognosis and may help to guide adjuvant treatment. It may also contribute to the accurate detection of ECs arising due to Lynch syndrome (LS) \([4]\).

In our study, we aimed to document the MMR protein expression status in ECs previously analyzed in our department and to investigate its relationship with prognostic parameters.
Figure 1. Sample staining patterns that appear as IHC as a result of mutations in the mismatch repair (MMR) genes. In MLH1 gene mutations, nuclear expression of MLH1 and PMS2 proteins are lost together. In PMS2 gene mutations, only the nuclear expression of PMS2 protein is lost. In MSH2 gene mutations, nuclear expression of MSH2 and MSH6 proteins are lost together. In MSH6 gene mutations, only the nuclear expression of MSH6 protein is lost.

2. Methods
Between 2017 and 2020, 80 patients diagnosed with EC and had routine IHC examinations of MMR proteins in hysterectomy material in the Department of Pathology, Ege University Faculty of Medicine, were retrospectively evaluated. IHC monoclonal antibodies for MMR status during routine pathologic examination are shown in Table 2.

Table 2. Monoclonal antibodies to mismatch repair proteins used in our department

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clone</th>
<th>Dilution</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLH1</td>
<td>m1</td>
<td>Ready-to-use solution</td>
<td>Ventana</td>
</tr>
<tr>
<td>PMS2</td>
<td>G219-1129</td>
<td>Ready-to-use solution</td>
<td>Ventana</td>
</tr>
<tr>
<td>MSH2</td>
<td>sp93</td>
<td>Ready-to-use solution</td>
<td>Ventana</td>
</tr>
<tr>
<td>MSH6</td>
<td>A16-4</td>
<td>Ready-to-use solution</td>
<td>Ventana</td>
</tr>
</tbody>
</table>

Demographic and pathologic data were obtained from pathology reports. Cases were divided into two groups: with and without loss of nuclear expression of MMR proteins by IHC method. The two groups were compared in terms of the largest tumor size, FIGO histological grade of the tumor, 1/2 internal or external myometrial invasion, presence of lymphovascular invasion, and uterine cervix involvement.
Statistical evaluation was performed using SPSS 25.0. Cross-tabulation and chi-square test were used to compare the two groups. \( P < 0.05 \) was considered significant.

3. Findings
In our study, the number of patients with nuclear expression loss in any MMR protein by IHC method was 37 (46.3%). In the remaining 43 (53.7%) cases, no loss was detected. Out of 37 cases, the following observations were made regarding the loss of different MMR proteins: loss of MLH1 and PMS2 together was observed in 30 cases, loss of MSH2 and MSH6 together was observed in 4 cases, loss of MLH1, PMS2, and MSH6 together was observed in 1 case, loss of all MMR proteins was observed in 1 case, and isolated loss of MSH6 was observed in 1 case. The distribution of the cases according to MMR protein expression is shown in Table 3.

Table 3. Distribution of cases according to immunohistochemical MMR protein expressions

<table>
<thead>
<tr>
<th>MLH1</th>
<th>PMS2</th>
<th>MSH2</th>
<th>MSH6</th>
<th>OLGU SAYISI</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>30</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>4</td>
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<tr>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>1</td>
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<td>-</td>
<td>1</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>43</td>
<td></td>
</tr>
</tbody>
</table>

Note: (+) no loss of nuclear expression. (-) loss of nuclear expression.

Table 4 shows the relationship between prognostic parameters in cases with and without immunohistochemical loss of nuclear expression of MMR proteins. When the cases with and without loss were compared, the tumor size was 4.1 cm in both groups. FIGO HD III was in 17 (45.9%) of 37 patients with loss and in 12 (27.9%) of 43 patients without loss, and the difference is statistically significant \( P = 0.03 \). There was no statistically significant difference between the groups of cases with and without MMR protein loss in terms of ½ outer myometrial invasion, stromal tumor involvement in the uterine cervix, and the presence of lymphovascular invasion.

Table 4. Associations of immunohistochemical MMR protein loss and non-loss cases with prognostic parameters

<table>
<thead>
<tr>
<th>FIGO</th>
<th>MMR loss</th>
<th>No loss of MMR</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade (HD): 1</td>
<td>0 (0%)</td>
<td>6 (14.0%)</td>
<td>0.03</td>
</tr>
<tr>
<td>Grade (HD): 2</td>
<td>20 (54.1%)</td>
<td>25 (58.1%)</td>
<td>0.03</td>
</tr>
<tr>
<td>Grade (HD): 3</td>
<td>17 (45.9%)</td>
<td>12 (27.9%)</td>
<td>0.03</td>
</tr>
<tr>
<td>LVI available</td>
<td>20 (54.1%)</td>
<td>19 (44.2%)</td>
<td>0.05</td>
</tr>
<tr>
<td>Myometrium ½ external invasion</td>
<td>16 (43.2%)</td>
<td>18 (41.9%)</td>
<td>0.05</td>
</tr>
<tr>
<td>Presence of cervical stromal invasion</td>
<td>7 (18.9%)</td>
<td>9 (20.0%)</td>
<td>0.05</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
<td>43</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Abbreviations: HD, histologic grade; LVI, lymphovascular invasion; MMR, mismatch repair protein.

4. Discussion
With the emergence of molecular classification in endometrial carcinomas and the subsequent clinical trials
based on this molecular classification, it is evident that the evaluation of some new parameters in routine pathologic evaluation will become mandatory. Among these parameters, the easiest and currently used in routine practice are the evaluation of P53 as an IHC and the evaluation of MMR protein expressions. MMR deficiency is caused by germline mutations in MLH1, PMS2, MHS2, and MHS6 genes with a rate of 15–25%, and the majority (more than 75%) is caused by methylation in the promoter region of the MLH1 gene [14,15].

Loss of MMR proteins in endometrial carcinomas is mostly seen in endometrioid carcinomas but can also be seen in other tumor types. In a study investigating MMR-d in high-grade ECs [16], a deficiency was found in approximately one-third of ECs, while no deficiency was found in serous and clear cell tumors, but in another study [17], it was found in 4% of serous carcinomas, 6% of clear cell carcinomas and 40% of undifferentiated carcinomas. Since routine MMR evaluation has not yet been performed in our department except for ECs, all of the cases in our study were ECs.

When we examined the relationship between prognostic parameters and MSI, MMR protein loss was observed at higher rates in grade III tumors in studies comparing tumors with MMR protein loss with tumors without loss in terms of HD [18,19]. In our study, loss of MMR protein expression was found to be statistically significantly higher in histologic grade III tumors (P = 0.03). The results indicate that tumors with loss of nuclear expression in MMR proteins tend to have higher HD compared to tumors without any loss. When tumors are compared according to MMR protein expression in terms of lymphovascular invasion, lymphovascular invasion is more common in tumors with MMR-d [17-19]. In our study, LVI was found to be higher in patients with loss of MMR protein expression. However, this difference was not statistically significant. With these results, it can be said that MSI tumors show more aggressive histopathological features.

In terms of myometrial invasion, although ½ external invasion is observed at a lower rate in patients with MSI, there is usually no significant difference between the two groups [17,18]. In our study, no significant difference was found between the two groups in accordance with the literature.

Our study found cervical stromal invasion at similar rates in patients with and without MMR protein expression loss. Although it has been reported that tumors with MMR deficiency tend to be located in the lower uterine segment, there is no comparison between these two groups in terms of the presence of cervical stromal invasion in the literature.

Evaluation of microsatellite instability, either molecularly or as IHC, contributes to prognosis prediction and treatment planning. It has been shown that the presence of MMR-d in early-stage ECs, which are considered to have a good prognosis, may be a negative prognostic indicator [20]. On the other hand, the presence of MMR-d in histologic grade 3 ECs, which are considered to have a poor prognosis, is seen as a positive prognostic factor [21]. In the study by Raffone et al., approximately 30% of patients with MMR-d were classified as low-risk, and it was reported that these patients might have been inadequately treated [22]. In this study, almost half of the ECs with MMR-d were shown to have FIGO grade 3, LVI, and deep myometrial invasion. Therefore, these patients were classified as high risk according to both 2013 [23] and 2016 [23] ESMO risk assessment systems. Considering the intermediate prognosis of the MMR-d group, these patients may have been overtreated.

In a recent study on ECs including 29 cases [19], the MMR status between the tumor in the metastatic or recurrent focus and the primary tumor was evaluated. MMR-d was detected in the primary tumor or at the site of recurrence in 48.2% of cases. In only about 7% of the cases (2 cases), loss of PMS2 was observed in metastatic tumors even though MMR-d was absent in the primary tumor. In these two cases, subclonal loss was observed in the primary tumor. As a result, MMR evaluation may be necessary for recurrent tumors of patients with subclonal loss in the primary tumor since MMR-d may occur. Another study [13] showed that subclonal loss of MMR protein expression was observed in less than 3% of tumors. Although the
number of cases is limited, it has been reported that there is no relationship between subclonal losses in MMR proteins and LS. Although the frequent presence of MSI and MLH1 hypermethylation in patients with subclonal MLH1 and PMS2 protein losses is accepted as a finding of sporadic intratumoral heterogeneity, germline mutations cannot be completely excluded. This study also suggests that subclonal losses of 10% or more should be considered MMR defective. Therefore, it may be useful to include subclonal losses in pathology reports when evaluating MMR in primary tumors. In our study, subclonal loss was detected in only two cases that were not evaluated as MMR-d, and this was noted in the pathology report.

Some studies recommend MMR evaluation with IHC not only in ECs but also in precursor lesions of ECs. It has been reported that MMR staining as IHC in atypia endometrial hyperplasia may be useful in identifying patients at high risk for LS and thus may provide early follow-up and diagnostic advantage for the patient and affected family members before EC develops [24].

The success of immune control inhibitor therapies in recent years has led to an increased interest in this subject and, as a result, has encouraged the investigation of molecular properties that can be used to predict tumor response to these agents [21]. The treatment of tumors with MMR-d has been shown to benefit from immune control inhibitors [25]. As a result, the FDA recently approved programmed cell death protein 1 (PD-1) pathway blockade for ECs with MMR-d in relapse but not in first-line treatment. Defective DNA repair in these tumors significantly increases the number of somatic mutations in tumor cells. Some of these mutations result in the synthesis of new antigens that can increase sensitivity to radio/chemotherapy. The appearance of new antigens results in an intense lymphocyte response in and around the tumor. The emergence of a large number of new antigens suggests that POLE mutant tumors and tumors with MMR deficiency may be excellent candidates for anti-PDL1 target therapies [26].

5. Conclusion
In conclusion, routine evaluation of the endometrium for MMR proteins by IHC method, especially in ECs, is critical. Its importance is increasing daily because it can guide treatment planning, provide information about prognosis and contribute to LS screening.

Disclosure statement
The authors declare no conflict of interest.

References


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