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Mismatch repair status in Endometrioid type of Endometrial Carcinoma: association with clinicopathological parameters

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**ABSTRACT**

**Introduction:** Endometrial carcinoma [EC], particularly the most predominant endometrioid type [EEC] is a major contributor to cancer burden globally, and its molecular classification has gained much importance recently. Aim: This study aimed to determine the immunohistochemical expression of mismatch repair proteins 'MLH1 and MSH2' in relation to clinicopathologic parameters in EEC and to characterize clinicopathologic features of mismatch repair protein (MMRP) deficient EEC. Material and Methods: The current work was carried out on 80 cases of EEC retrieved [with clinical data] from the Department of Pathology, Faculty of Medicine, Tanta University in the period from June 2018 to December 2019. H&E staining and immunohistochemical staining with MLH1 and MSH2 were done for each case. Results: 29 (36.3%) carcinomas showed abnormal MMRP expression (11 cases showed isolated MLH1 deficiency (37.93%), 10 cases showed isolated MSH2 deficiency (34.48%), and 8 cases (27.59) showed a combined loss of both proteins), whereas the remaining 51 (63.7%) of cases demonstrated normal MLH1/MSH2 immunoreactivity (MMRP intact). MLH1, MSH2 expression, and MMRP status were closely related to some clinicopathologic features (patient’s age, histopathological tumor grade, and tumor stage) with a statistically significant relation. Conclusions: A subset of endometrioid type EC demonstrates MMRP defect; the MMRP deficient EEC often displays adverse clinicopathological parameters as poorly differentiated or undifferentiated histology, an advanced stage with young age at presentation.

Keywords: Endometrioid type endometrial cancer; mismatch repair proteins; MLH1 and MSH2

**INTRODUCTION**

Endometrial carcinoma is the most common gynecologic malignancy in the world in which incidence is rapidly increasing (Ferlay et al, 2019). Endometrial cancer arises from the inner layer (endometrium) of the uterus that represents approximately 90% of uterine malignancies (Ritterhouse and Howitt, 2016). Endometrial carcinoma is classically classified into two types. Type I tumors, estrogen–related, characterized by endometrioid histology and favorable prognosis. On the contrary, type II tumors, estrogen–unrelated, are characterized by non-endometrioid histology and poor prognosis (Alvarez et al, 2012). Recently, the genetic and molecular basis of EC classification has gained much importance; with microsatellite instability (MSI) being a major cornerstone.

The Mismatch repair (MMR) system is a strand–specific DNA repair mechanism that functions to maintain genomic integrity by correcting base substitution as well as small insertion–deletion mismatches. Such mismatches are generated by errors during DNA replication in tandem repeats known as microsatellites. Several MMR genes are identified, but four only are of most clinical interest [MLH1, MSH2, MSH6, and PMS2] (McAlpine et al, 2018).

Tumor DNA can be classified as microsatellite–stable (MSS), low-level MSI (MSI–L) and high-level MSI (MSI–H). Mismatch repair deficiencies can result from i) an inherited cancer syndrome (e.g., Lynch syndrome), ii) acquired/somatic mutations or iii) epigenetic events e.g. methylation of one of the genes involved in mismatch DNA repair (Hegde et al, 2014). MMR
function defect has been detected in approximately 20% to 30% of endometrial cancers (McAlpine et al, 2018). Histologically, endometrial carcinoma with MSI or MMRP defect is mostly of endometrioid type (Köbel et al, 2017). Although genetic testing is important for detection of MSI, loss of expression of mismatch repair proteins MLH1, MSH2, MSH6 and PMS2 by immunohistochemistry (IHC) may be used as a surrogate marker for MSI (Hashmi et al, 2018). Detection of patients with MSI–high endometrial cancers due to germline mutations is important; as these patients are predisposed to develop a variety of other malignancies, especially colorectal carcinoma (Okoye et al, 2016). Suggested universal screening targeting the four MMR proteins (MLH1, MSH2, MSH6 and PMS2) is recommended (Goodfellow et al, 2015).

The association between MMR status and outcome in endometrial carcinoma remains unclear. The aim of the current study was to evaluate the MMRP status in relation to clinicopathological parameters in endometrioid type of endometrial carcinoma.

MATERIAL AND METHODS

This retrospective study included 80 specimens of endometrial carcinomas of endometrioid subtype. Formalin–fixed paraffin embedded blocks were collected from Pathology Department, Faculty of Medicine, Tanta University in the period from June 2018 to December 2019. For each specimen, the block best representing the tumor was selected. Detailed clinicopathological data of these patients were obtained from their medical reports. Endometrioid adenocarcinomas were subclassified into three grades (G1, G2 and G3) according to the International Federation of Gynecology and Obstetrics (FIGO) criteria (Kurman, 2014). The staging was performed based on FIGO 2009 criteria (Creasman, 2009).

Immunohistochemical staining

Sections were deparaffinized in xylene then rehydrated in a series of ethanol for 5 min each. For antigen retrieval, slides were subsequently incubated in modified citrate buffer for 40 minutes. After blocking of endogenous peroxidase activity with 3% hydrogen peroxidase for 10 min, slides were incubated with the primary antibody for 2h at room temperature. *Rabbit polyclonal antibody against MLH1* (Kit no. E17810. Spring bioscience, USA), and *a rabbit polyclonal antibody against MSH2* (Kit no, E17790. Spring bioscience, USA) were used. Then, slides were washed in phosphate-buffered solution (PBS), incubated with the biotinylated secondary antibody, followed by avidin–biotinylated peroxidase complex, and finally, DAB (diaminobenzidine tetrachloride) was developed as the chromogen. The sections were counterstained with hematoxylin, dehydrated with graded ethanols and xylene and then mounted with a coverslip.

Evaluation of immunohistochemical staining

The corresponding normal tissue was considered as an internal positive control [stromal cells and infiltrating tumor lymphocytes]. Loss of MMR proteins expression was defined as the absence of nuclear staining in tumor cells (less than 10% of cells), with preservation of nuclear staining in adjacent non neoplastic cells (Shikama et al, 2016). Cases with positive IHC staining of MLH1 and MSH2 proteins were regarded as intact MMR protein (MMRP intact). Cases with negative staining of one or both MMR proteins were interpreted as MMRP deficient (Tangjitgamol et al, 2017).

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Science (SPSS version 23, IBM corp., Armonk, New York, USA). Categorical variables were expressed as frequencies and percentages, whereas mean ± SD was used to express continuous variables. Chi-square (X2) test was performed for comparing categorical variables. Fisher’s exact test was applied when one of the expected frequencies was ≤ 5. P values of < 0.05* were considered statistically significant.

RESULTS

1. Clinicopathological Characteristics

The clinical and pathologic features of the included cases are represented in Table 1. Forty-five (56.3%) of the patients aged ≥ 60 years. 45 cases were moderately differentiated carcinomas [grade 2]; representing 56.3% of
cases, stage I was the predominant stage among cases (51.2%); 46 cases (57.5%) showed lymphovascular invasion, whereas myometrial invasion [more than 1/2 myometrial thickness] was reported in 41 (51.3%) of cases.

Table 1. Clinicopathological features of the studied cases

<table>
<thead>
<tr>
<th>Age</th>
<th>&lt;60</th>
<th>35 (43.7%)</th>
<th>≥60</th>
<th>45 (56.3%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade</td>
<td>Grade I</td>
<td>14 (17.5 %)</td>
<td>Grade II</td>
<td>45(56.3 %)</td>
</tr>
<tr>
<td></td>
<td>Grade III</td>
<td>21(26.2 %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage</td>
<td>Stage I</td>
<td>41 (51.2%)</td>
<td>Stage II</td>
<td>25 (31.2%)</td>
</tr>
<tr>
<td></td>
<td>Stage III</td>
<td>9 (11.2 %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stage IV</td>
<td>5 (6.2 %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myometrial invasion</td>
<td>&lt;1/2</td>
<td>39 (48.7%)</td>
<td>&gt;1/2</td>
<td>41 (51.3%)</td>
</tr>
<tr>
<td>Lymphovascular invasion</td>
<td>Positive</td>
<td>46 (57.5%)</td>
<td>Negative</td>
<td>34(42.5%)</td>
</tr>
</tbody>
</table>

2. Immunohistochemical results

The immunohistochemical expression of MMRP in relation to clinicopathological features of the patients in this study was illustrated in Table 2 and Figure 1.

Among 80 cases, 29 (36.3%) were considered MMRP deficient, isolated loss of MLH1 expression was detected in 11 cases (37.93%) and isolated loss of MSH2 expression was detected in 10 cases (34.48%); combined loss of MLH1/MSH2 was detected in 8 cases (27.59%). MMRP deficient was found to be significantly associated with patients aged <60 years than the older age group (p=0.001*). MMRP deficient was also found to be significantly related with advanced FIGO stage and high tumor grade (p=0.004*, p<0.001*), respectively. No significant association was found regarding myometrial invasion and lymphovascular invasion (p=0.054 and 0.431), respectively.

DISCUSSION

Type 1 endometrial carcinoma or the so-called endometrioid type endometrial carcinoma [EEC] typically arises through the progression of a precursor lesion. DNA mismatch repair system helps to prevent tumor progression by correcting errors that occur continuously during DNA replication; and accounts for a considerable proportion of endometrial cancers (Wong et al, 2016). Microsatellite instability (MSI) is a hyper–mutable phenotype caused by defects in DNA mismatch repair due to the inactivation of one of mismatch repair genes: most importantly MLH1, MSH2, MSH6 and PMS2 (Coppedè et al, 2014).

Table 2. Expression of MMRP in relation to Clinical and pathological features of EEC cases

<table>
<thead>
<tr>
<th>MMRP</th>
<th>Total</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative 29 (36.25%)</td>
<td>51 (63.75%)</td>
<td>80 (100%)</td>
</tr>
<tr>
<td>Age</td>
<td>&lt;60</td>
<td>20 (57.1%)</td>
</tr>
<tr>
<td></td>
<td>≥60</td>
<td>9 (20.0%)</td>
</tr>
<tr>
<td>Grade</td>
<td>Grade I</td>
<td>1 (7.1%)</td>
</tr>
<tr>
<td></td>
<td>Grade II</td>
<td>11 (24.4%)</td>
</tr>
<tr>
<td></td>
<td>Grade III</td>
<td>17 (81.3%)</td>
</tr>
<tr>
<td>Stage</td>
<td>Stage I</td>
<td>8 (19.5%)</td>
</tr>
<tr>
<td></td>
<td>Stage II</td>
<td>11 (44%)</td>
</tr>
<tr>
<td></td>
<td>Stage III</td>
<td>7 (77.8%)</td>
</tr>
<tr>
<td></td>
<td>Stage IV</td>
<td>3 (60%)</td>
</tr>
<tr>
<td>Myometrial invasion</td>
<td>&lt;1/2:</td>
<td>10 (25.6%)</td>
</tr>
<tr>
<td></td>
<td>&gt;1/2:</td>
<td>19 (46.3%)</td>
</tr>
<tr>
<td>Lymphovascular invasion</td>
<td>Positive</td>
<td>15 (32.6%)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>14 (41.2%)</td>
</tr>
</tbody>
</table>

*p<0.001 *
Figure 1. Immunohistochemical expression of mismatch repair proteins (MMRP) in endometroid type of endometrial carcinoma showing intact nuclear expression of (A) MLH 1 in grade I EEC (x200), (B) MSH2 in grade II EEC (x200), and (C) MLH1 in grade II EEC (x400). Loss of expression of (D) MSH2 in grade II EEC (x400), (E) MLH1 in grade III (x200) and (F) MLH1 in grade III (x400).

A possible potential genetic background is the argued phenomena of genetic instability. Genetic instability is thought to be a stamp of some human malignancies (Rajagopalan et al, 2003). Lack of genetic stability occurs frequently in many types of cancers, including endometrial carcinoma and colorectal carcinoma (Black et al, 2006).

Tumor microenvironment suppresses DNA repair pathways to help genomic instability and encourage tumor proliferation and survival (Tian et al, 2015). In colorectal carcinoma, MSI may be associated with lower stages despite poor differentiation (Perea et al, 2014).
MSI is corresponding to the loss of immunohistochemistry (IHC) staining of one of the mismatch repair genes; most frequently MSH2 and MLH1 (De la Chapelle and Hampel, 2010). Genetic analysis of MSI status is expensive and requires long turnaround time and specialized laboratories. Therefore, recently, it cannot be performed routinely on various carcinomas. Immunohistochemical analysis of MLH1 and MSH2 protein expression representing a rapid, easier, less costly, as well as sensitive and specific alternative method for the detection of tumors of the mutator phenotype, and it could be performed by histopathology laboratories as a routine diagnostic test (Tangjitgamol et al., 2017).

The present study investigated the immunohistochemical expression of mismatch repair proteins (MLH1 and MSH2) in EEC; abnormal MMR proteins (MMRP) expression was detected in 29 (36.3%) of the included cases. Previous reports investigating the expression of MMR proteins using IHC have shown that approximately 16% to 45% of endometrial cancer had MMRP deficient status (Grzankowski et al., 2012 and Kato et al., 2015). In a study by Long et al., 2014, approximately 23.7% of EC patients showed abnormal expression of MMRP by IHC, also, Buchanan et al., 2014 reported a rate of IHC abnormality in 29% of their cases. The different proportions encountered between various reports may be due to contribution of other features: for example, age, history of other cancers in individuals and cancers in the family which are associated with higher genetic risk, histopathological tumor types and grades of differentiation, and even screening techniques used. There was a similarity in the frequency of MMRP deficient status in the present study and the previous reports, suggesting no ethnic difference of frequency in MMRP among the studied endometrial cancers.

In the current work, the percentage of MMRP deficient tumors was associated significantly with young aged patients (<60) as p-value =0.001. Similar results were observed by Shikama et al., 2016 and Tangjitgamol et al., 2017; who reported increased rates of MMRP defects among young EC patients. In contrast to their data, Kim et al., 2018 demonstrated that MMRP deficiency was significantly associated with postmenopausal status. Ring et al., 2013 and Ruiz et al., 2014 found no significant association between age and MMRP defect. The great differences encountered between various reports and the current study may be explained by including other histopathological tumor variants.

Concerning the relationship of MMRP and other clinicopathological features; the current study observed significant or highly significant results with inverse relationship; regarding both tumor grade and stage. Abnormal MMRP is characterized by a high proportion of poorly differentiated tumors and advanced stage at diagnosis; regarding grading of EEC, MMRP defect increase in frequencies with increasing grades as follows 7.1% vs. 24.4% vs. 81.0% in grade I, II and III, respectively, P<0.001*.

Regarding myometrial invasion and lymphovascular invasion, there was no significant relation between MMR status and them. Previous studies reported that the MMRP defect EC had more aggressive features as grade III cancer, deep myometrial invasion, more frequent LVI, and more advanced stage (Ring et al., 2013 and Shia et al., 2013). A close study to the current work done by An et al., 2007, which included only the endometrioid subtype, showed an increase in the frequencies of MSI–high phenotype in higher histologic grade (13% vs. 21% vs. 50% in histologic grade I, II, and III, respectively. The MSI–high phenotype was related to the presence of lymphovascular invasion, deep myometrial invasion, and the higher clinical stages. Hashmi et al., 2019, reported that MMRP defect was related to high FIGO stage, but no relation with tumor grade. Another study by Tangjitgamol et al., 2017 found that early stage, more endometrioid histology, and lower grade tumor were associated with MMRP deficiency. Kim et al., 2018 pointed to a contradictory data as they reported that MMRP defect was associated with a higher histologic grade (G2–3), yet a lower FIGO stage (I–II). On the other hand, Ruiz et al., 2014 did not find a correlation between MMRP and histopathological grade, myometrial invasion, lymphovascular invasion or clinical stage.

A significance of MSI testing is the therapeutic benefit of various targeted therapies (including
In conclusion, MMR protein defect was associated with the younger age group (<60) years, high grade and advanced-stage tumors of EEC, however more large-scale studies with molecular tests is required to validate these findings and to determine their clinical value.

**Conflict of interest**

The authors declare that they have no competing interests.

**References**


immunohistochemistry and MLH1 promotor methylation testing for practical molecular classification and the prediction of prognosis in endometrial cancer. Cancers, 10(9):279-293.


EACR is an NGO society that was declared by the Ministry of Social Solidarity (Egypt) No. 1938 in 19/11/2014 based on the initiative of Prof. Mohamed Labib Salem, the current Chairman of EACR. EACR aims primarily to assist researchers, in particular young researchers in the field of cancer research through workshops, seminars and conferences. Its first international annual conference entitled "Anti-Cancer Drug Discovery" was successfully organized in April 2019 (http://acdd.tanta.edu.eg). Additionally, EACR aims to raise the awareness of the society about the importance of scientific research in the field of cancer research in prediction, early diagnosis and treatment of cancer. EACR is also keen to outreach the scientific community with periodicals and news on cancer research including peer-reviewed scientific journals for the publication of cutting-edge research. The official scientific journal of EACR is "International Journal of Cancer and biomedical Research (IJCBR: https://jcbrr.journals.ekb.eg) was successfully issued in 2017 and has been sponsored by the Egyptian Knowledge Bank (EKB: www.ekb.eg).

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