Original Article

Correlation between immunohistochemical staining and clinicopathological findings in endometrial carcinoma

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ABSTRACT

الأهداف: تحليل حالات سرطان بطانة الرحم وذلك عن طريق دراسة نمط تلوين الصبغات الكيميائية المناعية لكل من بروتينات p53/MMR والنظر إلى أهميتها ودورها في التسبب والسلوك السريري لهذا الورم الخبيث.

المنهجية: في هذه الدراسة نقوم بالتحقيق بين نقص بروتين p53 والتشوهات الجينية ل MMR لحالات سرطان بطانة الرحم والتي يبلغ مجملها 96 حالة، منها 72 إندومتريويد و14 حليمية مصلية و 5 ذوي الخلايا الصافية و أخيرا 5 أورام ملاريان المختلطة.

النتائج: أظهرت النتائج أن 36 حالة كانت مصابة بنقص MMR، وأغلبها من بطانة الرحم النوع الانرومتريوويد. كما أظهرت أيضا الطفرة الجينية في الجين p53 في الحالات الأكثر شراسه، ومع ذلك فشلت الصبغات المذكورة أعلاه بالتنبؤ بالسرطانات المتزامنة أو المتغيرة في 5 من المرضى.

الخلاصة: هذه النتائج تسلط الضوء على أهمية التلوين المناعي لكل من MMR و p53 في التصنيف و التنبؤ بتطورات سرطان بطانة الرحم.

Objectives: To analyze the immunohistochemical staining pattern of mismatch repair (MMR) proteins and p53 in endometrial carcinoma cases, including different subtypes and stages, to gain insights into their role in the pathogenesis and clinical behaviour of this malignancy.

Methods: In this study, we investigate the association between MMR deficiency, p53 mutational status, and clinical outcomes in various subtypes of endometrial carcinoma. The immunohistochemical staining pattern of MMR proteins in 96 cases of endometrial carcinoma have been analyzed, including 72 endometrioid, 14 papillary serous, 5 clear cell, and 5 mixed Müllerian tumor.

Results: The results showed that 36 cases were MMR deficient, with the majority being of endometrioid subtype. The p53 immunostain showed a mutational pattern in a subset of cases, with a documented dismal prognosis. However, aforementioned stains failed to predict synchronous or metachronous cancers in 5 patients.

Conclusion: These findings highlight the importance of MMR and p53 immunohistochemical staining in the classification, and prognosis of endometrial carcinoma.

Keywords: endometrial carcinoma, mismatch repair proteins, immunohistochemistry, p53 mutation, clinical outcomes

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Endometrial carcinoma is the most common gynecologic malignancy in developed countries, with an estimated 67,880 new cases in the United States in 2024.[1] With 494 cases, or 6.3% of all cancer cases diagnosed among Saudi females in 2020, corpus uteri cancer was the fourth most common cancer overall among Saudi women.[2] The majority of endometrial carcinomas are of endometrioid subtype, which tend to have a favorable prognosis if diagnosed early and treated appropriately. However, other subtypes, such as papillary serous, clear cell, and mixed Müllerian tumor (MMT), have a more aggressive behavior and poor prognosis.[3] The molecular pathogenesis of endometrial carcinoma is complex and involves genetic alterations in key pathways, including DNA mismatch repair (MMR), p53, and PI3K/AKT/mTOR.[4]

Mismatch repair proteins play a critical role in maintaining genomic stability by correcting errors that occur during DNA replication. Mismatch repair deficiency leads to the accumulation of mutations and microsatellite instability (MSI), which have been

linked to carcinogenesis and intermediate prognosis in endometrial carcinoma.[5] Mismatch repair deficiency can be detected by immunohistochemical staining of MMR proteins, including MLH1, MSH2, MSH6, and PMS2.[6]

The p53 is a tumor suppressor gene that regulates cell cycle arrest and apoptosis in response to DNA damage. Mutations in p53 have been linked to malignant transformation and poor outcome.[7] Immunohistochemical staining of p53 protein can detect its overexpression or mutation, providing valuable information for diagnosis, classification, and prognosis.[8]

In this study, we aimed to analyze the immunohistochemical staining pattern of MMR proteins and p53 in endometrial carcinoma cases, including different subtypes and stages, to gain insights into their role in the pathogenesis and clinical behaviour of this malignancy.

Methods. A retrospective analysis was carried out using archival specimens obtained from the Pathology Department at King Abdulaziz University Hospital, Jeddah, Saudi Arabia, between 2007-2022. The study adheres to the ethical principles outlined in the Helsinki Declaration. We reviewed detailed clinical and pathological data, including tumor stage, grade, and subtype. The cases were classified according to the International Federation of Gynecology and Obstetrics criteria.

Patients eligible for inclusion in this study are those diagnosed with endometrial carcinoma confirmed through histopathological examination. Additionally, patients must have available clinicopathological data, including age, histological subtype, tumor grade, cancer stage, and treatment history. Furthermore, inclusion requires patients to have undergone immunohistochemical staining for relevant markers such as estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2/neu). The study encompasses patients various ethnic backgrounds, with complete medical records for analysis.

On the other hand, patients will be excluded if they have incomplete medical records or missing clinicopathological data. Additionally, patients with a history of other malignancies that could confound interpretation will be excluded. Patients who have received neoadjuvant chemotherapy or radiation therapy before immunohistochemical staining, as well as those with endometrial hyperplasia without carcinoma evidence, will also be excluded.

Archived tissue blocks were obtained with approval from Unit of Biomedical Ethics, Research Ethics Committee, Faculty of Medicine at King Abdulaziz University, Jeddah, Saudi Arabia. All samples were analyzed in a tissue microarray format utilizing tissue cylinders with a diameter of 6 mm that were punched from representative tumor regions of each donor-tissue block and transferred into recipient paraffin blocks.

Immunohistochemical staining was carried out on formalin-fixed, paraffin-embedded tissue sections using standard protocols. The MMR proteins (MLH1, MSH2, MSH6, and PMS2) were stained using primary antibody clones from Ventana/Roche, and the staining pattern was evaluated by 2 experienced pathologists. Cases showing loss of staining in one or more MMR proteins were considered MMR deficient, while cases showing intact staining for all MMR proteins were considered MMR proficient.

The p53 immunostaining was also carried out, and the staining pattern was evaluated. Cases showing overexpression or mutation of p53 were considered positive, while cases showing focal positivity or absent p53 staining were considered negative.

Statistical analysis. The Statistical Package for the Social Sciences for Windows, version 20.0 (IBM Corp., Armon, NY, USA) was used.

Results. A total of 96 cases of endometrial carcinoma were included, comprising 72 endometrioid, 14 papillary serous, 5 clear cell, 5 MMT, and one undifferentiated carcinoma. The age of patients at the time of diagnosis ranged from 33-82 years, with a mean age of 60.

Immunohistochemical staining review revealed that 60 (63%) cases of endometrial carcinoma were proficient in MMR proteins, while 36 (36.8%) cases were deficient. Among the MMR deficient cases, 29 belonged to the endometrioid subtype, 3 to MMT, 2 to papillary serous, and one to clear cell carcinoma. The rate of MMR deficiency was significantly higher in low-grade (grade 1 and 2) tumors compared to high-grade (grade 3) tumors (p=0.002). There was no significant association between MMR deficiency and tumor stage or subtype.

The p53 immunostain showed a mutational pattern in 24 (25.3%) cases, including 5/5 MMT, 11/14

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papillary serous, 1/5 clear cell, and 7/70 endometrioid endometrial carcinoma cases. Among the 24 cases associated with p53 mutation, 15 (62.5%) cases showed documented dismal prognosis, including distant metastasis stage 4 disease, recurrence, and death.

On the other hand, patients with synchronous or metachronous cancers have not shown any abnormal staining pattern with the above antibodies.

Discussion. This study provides valuable insights into the immunohistochemical staining pattern of MMR proteins and p53 in endometrial carcinoma, including different subtypes and stages. The results showed that MMR deficiency was present in a subset of cases, with the majority being of endometrioid subtype. This finding is in line with previous studies that showed MMR deficiency in up to 30% of endometrial carcinomas, particularly those with endometrioid histology.[9,10] In addition, prior studies have extensively investigated the correlation between immunohistochemical findings and endometrial carcinoma. Bounous et al[11] proposed a prognostic model based on immunohistochemistry markers, highlighting the significance of MMR-deficient tumors, p53 mutation status, and hormone receptor expression in determining prognosis. Paudice et al[12] emphasized the prognostic value of histopathological factors and immunohistochemical markers like ER, progesterone receptor, and p53 in predicting disease-free and overall survival in endometrial carcinoma patients. Kaur et al[13] focused on the association of endometrial carcinoma with age, parity, and the expression of ER, PR, and HER-2/neu, showcasing the importance of immunohistochemistry in understanding clinicopathological features. Serin et al[13] compared prognostic parameters in endometrial carcinoma patients with and without loss of nuclear expression in MMR proteins, highlighting the impact on histological grade and prognosis.

Mismatch repair deficiency has been linked to MSI, which is a hallmark of Lynch syndrome, a hereditary cancer syndrome that predisposes individuals to various malignancies, including endometrial carcinoma.[14] However, in sporadic endometrial carcinoma, MMR deficiency can occur through somatic mutations or epigenetic silencing of MMR genes.[15] The detection of MMR deficiency by immunohistochemical staining has important implications for the diagnosis, classification, and management of endometrial carcinoma.

In this study, we also analyzed the immunohistochemical staining pattern of p53, a tumor suppressor gene that is frequently mutated in various malignancies, including endometrial carcinoma. The results showed that p53 mutation was present in a subset of cases, with a higher frequency in papillary serous and MMT subtypes. This finding is consistent with previous studies that showed p53 mutations in up to 50% of papillary serous carcinomas and 80% of MMT.[16,17]

The association between p53 mutation and poor prognosis in endometrial carcinoma has been reported in several studies.[18,19] In this study, we found that 62.5% of cases with p53 mutation showed poor performance. This highlights the importance of p53 immunohistochemical staining in the prognostication and management of endometrial carcinoma.

However, it is important to note that stains failed to predict synchronous or metachronous cancers in all 5 patients. This underscores the complexity of the molecular pathogenesis of endometrial carcinoma and the need for further research to identify additional biomarkers that can improve diagnosis, classification, and prognosis.

The observed MMR deficiency in a subset of endometrial carcinomas suggests potential underlying defects in DNA repair mechanisms in these tumors. The association between MMR deficiency and specific histological subtypes, such as endometrioid and papillary serous carcinoma, highlights the distinct molecular pathways involved in their pathogenesis. Additionally, the co-occurrence of p53 mutations in MMR deficient cases suggests an overlap between these genetic alterations and further supports their role in tumor progression.

Furthermore, our findings indicate a higher prevalence of advanced stage disease in cases presenting both p53 mutations and MMR deficiency. This suggests that the combined assessment of MMR status and p53 mutational patterns can aid in risk stratification and prognostication for patients with endometrial carcinoma.

The study discussed in the provided document contributes to the existing knowledge on endometrial carcinoma in several ways. Firstly, it investigates the association between MMR deficiency and different subtypes of endometrial carcinoma. By analyzing the immunohistochemical staining pattern of MMR proteins, the study provides insights into the prevalence of MMR deficiency, particularly in the endometrioid subtype. This adds to our understanding of the molecular characteristics of endometrial carcinoma.

Secondly, the study explores the correlation between MMR deficiency, p53 mutational status, and clinical outcomes. By assessing the staining patterns of MMR proteins and p53, the study provides valuable

information on the potential prognostic implications of these molecular markers. This knowledge can contribute to refining the risk stratification and management of patients with endometrial carcinoma.

Lastly, the study highlights the importance of immunohistochemical staining in the classification of endometrial carcinoma. By demonstrating the utility of these staining techniques, the study emphasizes their role in distinguishing different subtypes of endometrial carcinoma and providing valuable prognostic information.

Overall, the study adds to the existing knowledge by providing insights into the association between MMR deficiency, p53 mutational status, and clinical outcomes in endometrial carcinoma. It underscores the significance of immunohistochemical staining in the prognosis of this malignancy, potentially informing personalized treatment approaches and patient management.

Study limitations. One potential limitation of this study is the retrospective nature of data collection, which may introduce selection bias or incomplete data. Retrospective studies rely on existing medical records, which might not always be comprehensive or standardized across different healthcare institutions. Additionally, variations in immunohistochemical staining techniques or interpretation between pathologists could impact the consistency and accuracy of results. Moreover, the study's findings may be influenced by confounding variables such as patients' comorbidities, previous treatments, or differences in tumor sampling techniques. Finally, while this study aims to explore the correlation between immunohistochemical staining and clinicopathological findings in endometrial carcinoma, establishing causality or determining the clinical significance of any observed correlations may require further prospective studies or clinical trials.

The findings of this study may provide valuable insights into the utility of immunohistochemical staining in correlating with clinicopathological features of endometrial carcinoma. However, further prospective studies are warranted to validate and expand upon these findings. Future research could focus on standardizing immunohistochemical staining protocols to enhance reproducibility and comparability of results across different laboratories. Additionally, longitudinal studies could investigate the prognostic significance of specific immunohistochemical markers in predicting patient outcomes and guiding personalized treatment strategies. Furthermore, exploring novel biomarkers or molecular signatures through advanced techniques such as next-generation sequencing may offer deeper insights into the molecular mechanisms underlying endometrial carcinoma progression and therapeutic response. Ultimately, integrating multi-omics approaches with clinicopathological data could facilitate the development of more precise diagnostic and therapeutic strategies for endometrial carcinoma patient.

In conclusion, our study provides valuable insights into the immunohistochemical staining pattern of MMR proteins and P53 in endometrial carcinoma. The observed MMR deficiency in a subset of cases, particularly in specific histological subtypes, highlights the potential predictive and prognostic implications of these markers in endometrial carcinoma. Additionally, abnormal p53 expression is a strong indicator for poor outcome. Therefore, universal immunohistochemical stains screening for women with endometrial carcinoma is recommended. Future studies should focus on elucidating the underlying mechanisms driving MMR deficiency along with TP53 mutations and its association with clinical outcomes, further optimizing risk assessment and therapeutic strategies in this population.

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