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Mismatch Repair Proteins (MLH1, MSH2, MSH6, and PMS2) Immunohistochemical Expression and Microsatellite Instability in Endometrial Carcinoma

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Abstract

BACKGROUND: Endometrial cancer (EC) is the fourth most common female cancer worldwide constituting 7% of cancer in women. It is a disease of older, postmenopausal women. The most of these patients have an identifiable source of excess estrogen, while in a small subset the pathogenesis is related to mismatch repair abnormality and lynch syndrome (LC). Mismatch repair behave as tumor suppressors and the most clinically relevant include MLH1, MSH2, MSH6, and PMS2. mutations in mismatch repair (MMR) results in a strong mutator phenotype known as microsatellite instability, which is a hallmark of LC-associated cancers.

AIM: The aim of the study was to study microsatellite instability in endometrial cancer using the immunohistochemical expression of mismatch repair proteins (MLH1, MSH2, MSH6 and PMS2).

MATERIAL AND METHODS: Sixty EC cases were studied using MLH-1, MSH-2, MSH-6, and PMS-2 immunohistochemistry and their expression was correlated with different clinicopathologic parameters.

RESULTS: A statistically significant relationship exists between MMR immunohistochemistry (IHC) proteins and tumor grade. Intact MMR proteins profile was associated with the lower tumor grade (31.3% were Grade 1 and 46.9% were Grade 2). Combined loss of MLH1/PMS2, combined loss of MSH2/MSH6, and isolated loss of PMS2 were also associated with the lower tumor grade while isolated loss of MSH6 was associated with the high tumor grade. However, no statistically significant correlation was found between MMR IHC proteins expression and the age of patients; tumor histopathological types, or FIGO stage.

CONCLUSION: A statistically significant correlation between the tumor grade of EC cases and the MMR IHC proteins was found. Further studies are recommended to assess correlation between MMR proteins defect and different clinicopathological parameters of endometrial carcinoma.

Introduction

Endometrial cancer (EC) is the fourth most common cancer in women (7% of cancers in women). In 2019, there were estimated 61,880 new cases of and 12,160 deaths from EC [1]. EC is a disease of older, postmenopausal women and is uncommon in young women; 2% to 14% of endometrial carcinomas occur in women 40 years of age and younger. The most of these patients have an identifiable source of excess estrogen, while in a small subset the pathogenesis is related to mismatch repair abnormality and lynch syndrome (LC) [2].

In Egypt, primary malignant uterine neoplasms constituted 1.28% of total primary malignant neoplasms at National Cancer Institute and 22.83% of malignant neoplasms of female genital system. The primary malignant uterine neoplasms constituted 34.46% of all uterine lesions. The most common types were endometrioid adenocarcinoma (59.58%), followed by carcinosarcoma (10.71%); serous adenocarcinoma (7.9%); leiomyosarcoma (6.2%); endometrial stromal sarcoma (4.69 %); and choriocarcinoma (3.94%) [3].

Endometrial carcinomas are pathogenetically divisible into type 1 and type 2 tumors [4]. Type 1 tumors (Grade 1 and 2 endometrioid carcinoma) are the most common ECs. They may arise from complex atypical hyperplasia and are linked to excess of estrogen stimulation. As they are usually diagnosed at early stages, they present a relatively good prognosis. Type 2 tumors are the least common endometrial tumors. They include Grade 3 endometrioid tumors as well as tumors of non-endometrioid histology, and develop from atrophic endometrium. Type 2 tumors are less hormone sensitive. Since they are diagnosed in later stages, they are generally more aggressive [5].

Postmenopausal women with higher total concentrations of estrogens are at increased endometrial carcinoma risk as are women with polycystic ovary

syndrome or estrogen producing ovarian tumors, earlier age at menarche, later age at menopause, nulliparity, or obesity. A positive family history of endometrial carcinoma, LC or Cowden syndrome elevates the risk of endometrial carcinoma [4].

LS or hereditary non-polyposis colon cancer is an autosomal dominant inherited disease caused by germline mutations in mismatch repair (MMR) genes. MLH1, MSH2, MSH6, and PMS2 mutation in this syndrome account for approximately 37, 41, 13, and 9%, respectively. It is important to establish a diagnosis for this syndrome because of the associated elevated lifetime risk of developing cancers such as colorectal and ECs [6].

Among ECs, 2–5% are likely to be associated with LC, in women either endometrial or colorectal carcinomas could be the presenting or sentinel cancer [7].

Since, LC confers a 14–54% risk of developing EC [7]. Thus, it is clinically relevant to identify LS women among EC patients to predict and prevents the development of other LS-associated cancers. It would also provide blood relatives an opportunity for genetic analysis and surveillance for LS-associated cancers. Each of the 4 MMR germline mutations leads to distinct molecular pathologies [8], and thus individuals carrying different mutations should not be regarded as suffering from the same disease. PMS2 germline mutation is associated with later onset, weaker family history, and a lower risk for cancer compared with other MMR germline mutations [9].

Clinical criteria to predict the likelihood of LC including Amsterdam, Bethesda, and Society of Gynecologic Oncology are not accurate and molecular testing of tumors is required to confirm or exclude LC [7].

Molecular screening of the tumors for the presence of MMR proteins in the nuclei using immunohistochemistry (IHC) is an alternative method of screening with sensitivity ranging between 86 and 100% [10].

There is a growing drive for universal screening of colorectal cancer (CRC) patients for LS [11], [12], [13]. Indeed, the National Institute of Health and Care Excellence in the United Kingdom has recently introduced a LS screening pathway for all CRC patients, alongside numerous institutions in the United States [14]. LS screening pathways utilize tumor-based testing IHC for MMR protein loss, microsatellite instability (MSI) testing or MLH1 (promoter methylation testing) to triage cases to undergo germline testing to identify a pathogenic variant in one of the MMR genes. Universal screening of EC patients for LS has been recommended by numerous experts and specialist societies [15]. Such practice has already been adopted in several cancer centers across the world [16], [17], [18].

Methods

Sixty cases of endometrial carcinoma covering different age groups were retrieved from the pathology department, Ahmed Maher Teaching Hospital, Cairo, Egypt, during the period from January 2013 to December 2016. Demographic and clinical data of the patients were collected from the hospital files.

Five um thick sections were cut from formalin-fixed paraffin embedded tissue blocks and stained with hematoxylin and eosin for routine histopathological examination and determination of tumor type, grade, and stage.

Immunohistochemical staining was performed using immunostainer (Shandon Sequenza) using the labeled streptavidin biotin method with the following reagents: Diva Decloaker, pre-treatment antigen – retrieval, (Biocare Medical Catalog number: DV2004 LX, MX), hydrogen peroxide block (Lab vision, USA, Catalog number: TA-060-HP), and Ultravision large volume detection system (Lab vision, USA, Catalog number: TP-060- HL) including Ultra V block, biotinylated goat anti-polyvalent plus (link) and streptavidin peroxidase plus (label), and DAB plus substrate system (Lab vision, USA, Catalog number: TA-060-HDX) including DAB plus chromogen and DAB plus substrate. The primary antibodies were PMS-2: A mouse polyclonal antibody (Biocare Medical Catalog number: PM 344 AA), MLH-1: A mouse monoclonal antibody (Biocare Medical Catalog number: PM 220 AA), MSH-6: A mouse monoclonal antibody (Biocare Medical Catalog number: PM 265 AA), and MSH-2: A mouse monoclonal antibody (Biocare Medical Catalog number: PM 219 AA).

Lymphocytes and/or stroma were used as internal positive controls [9], [19]. Sections of the same tissue were used following the same procedure with PBS used instead of the primary antibody as internal negative controls.

Complete absence of nuclear staining in the tumor cells is interpreted as loss of MMR protein expression [9], [20].

The presence of nuclear staining in tumor cells is good evidence of retained MMR protein, even if it is focal and weak staining. This has led to neglect staining pattern interpretation, with the exception of cases that show complete absence of nuclear staining [9].

Statistical analyses were performed using Statistical Package for the Social Science (SPSS 17.0 for windows; SPSS Inc, Chicago, IL, 2010). Chi-Square test was used to examine the relationship between two qualitative variables and between one quantitative and one qualitative variable. p is significant when ≤ 0.05.

Results

Patient's ages ranged between 37 and 75 years with a mean age of 60.03 ± 9.244 years and median age is 60.8 years.

The most of the cases were endometrioid adenocarcinomas (80%) including conventional endometrioid carcinoma (66.7%), endometrioid with squamoid differentiation (8.3%), and endometrioid villoglandular subtype (5%). Twenty percent were non-endometrioid including serous carcinoma (5%), carcinosarcoma (5%), and mixed carcinoma (10%). The majority of tumors were Grade II (40 %). About 95.8% of tumors were FIGO Stage I.

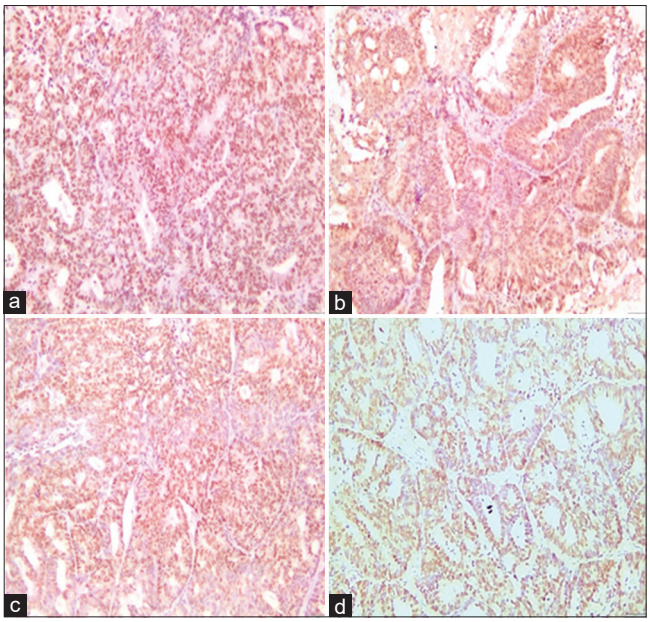

Figure 1: Endometrioid adenocarcinoma, Grade II, intact mutations in mismatch repair immunohistochemistry proteins, (a): MLH1 positive (×200), (b): PMS2 positive (×200), (c): MSH2 positive (×200), (d): MSH6 positive (×200)

Both MLH1/PMS2 were lost in 10% of cases and both MSH2/MSH6 were lost in 3.3% of cases, while all MMR proteins were lost in 15% of cases. Isolated PMS2 loss was found in 15.0% of cases and isolated MSH6 loss was found in 3.3% of cases. MMR proteins were intact in 53.3% of cases.

There was statistically significant correlation between MMR IHC expression and tumor grade (p = 0.028). Intact MMR proteins profile was associated with the lower tumor grade (31.3% were Grade 1 and 46.9% were Grade 2) (Figure 1). Combined loss of MLH1/PMS2, combined loss of MSH2/MSH6 (Figure 2) and isolated loss of PMS2 were also associated with the lower tumor grade while isolated loss of MSH6 was associated with high tumor grade (Figure 3) (Table 1).

No statistically significant correlation could be found between MMR proteins expression and age of the patients, tumor types, or FIGO stage.

Discussion

EC is the most common gynecological malignancy in high-income countries [5].

Mismatch repair proteins behave as tumor suppressors [17]. MMR loss results in a strong mutator phenotype known as MSI, which is a hallmark of LC-associated cancers [21].

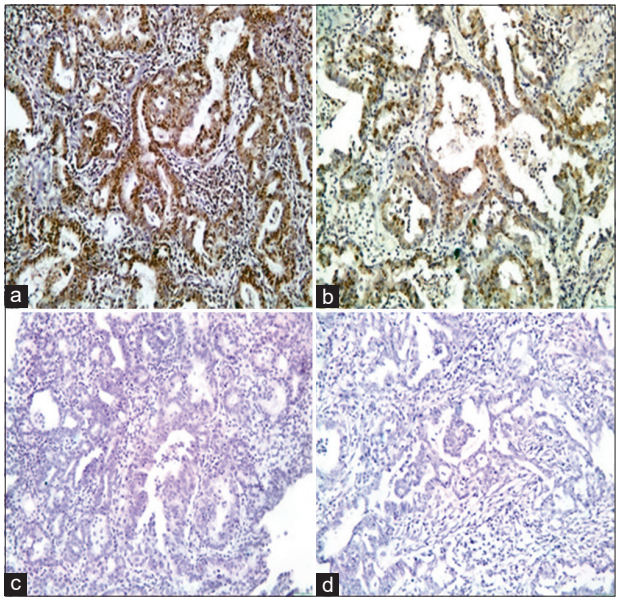

Figure 2: Endometrioid adenocarcinoma, Grade II, mutations in mismatch repair immunohistochemistry proteins, loss of MSH2 and MSH6, (a): MLH1 positive (×200), (b): PMS2 positive (×200), (c): MSH2 negative (×200), (d): MSH6 negative (×200)

Concerning the immunohistochemical expression of MMR proteins in endometrial carcinoma cases, all MMR proteins were intact in (53.3%) of cases, MLH1/PMS2 loss was in (10%) of cases, isolated PMS2 loss was in (15%) of cases, MSH2/MSH6 loss was in

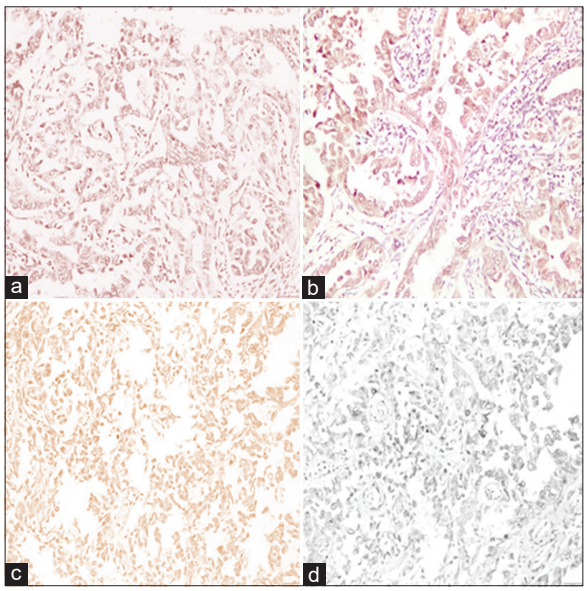

Figure 3: Serous endometrial adenocarcinoma, Grade III, FIGO Stage IB, isolated MSH6 loss, (a): MLH1 positive (×200), (b): PMS2 positive (×200), (c): MSH2 positive (×200), (d): MSH6 negative (×200).

(3.3%) of cases, isolated loss of MSH6 was in 3.3% of cases, and all MMR proteins were lost in (15%) of cases. These results are near to those obtained by Egoavil *et al*. [22], Buchanan *et al*. [23], Ferguson *et al*. [24], Joehlin-Price *et al*. [25], and Dudley *et al*. [20].

Table 1: Relationship between MMR IHC expression and the tumor grades of EC cases

IHC panel	Grade I	Grade II	Grade III
	Count (% within IHC panel)	Count (% within IHC panel)	Count (% within IHC panel)
No loss	10 (31.3)	15 (46.9)	7 (21.9)
MLH1/PMS2 loss	1 (16.7)	5 (83.3)	0 (0)
PMS2 loss	6 (66.7)	1 (11.1)	2 (22.2)
MSH2/MSH6 loss	2 (100)	0 (0)	0 (0)
MSH6 loss	0 (0)	0 (0)	2 (100)
All loss	3 (33.3)	3 (33.3)	3 (33.3)
Total	22 (100)	24 (100)	14 (100)
Sig. (p)	0.028		

MMR: Mutations in mismatch repair, EC: Endometrial cancer, IHC: Immunohistochemistry.

There was statistically significant correlation between MMR IHC expression and tumor grade. This agreed with Clarke and Cooper [26] who found significant correlation between tumor grades and MMR IHC expression (p = 0.0001) and (p = 0.009). respectively. Moreover. Hirasawa *et al*. [27] found that MSI high (MSI-H) was significantly correlated with high grade tumors (Grade 3 vs. Grades 1 and 2).

There was no statistically significant correlation between the patient's age of EC cases and MMR proteins expression. This was in agreement with the previous study of Mas-Moya *et al*. [28] but in contrast to Egoavil *et al*. [22] who found significant correlation between patient's age and MMR IHC expression and/or MSI testing that the suspected hereditary condition was more frequently found in women younger than 50 years.

There was no statistically significant correlation between the histopathological types of endometrial carcinoma and MMR IHC expression which was in agreement with the study of Egoavil *et al*. [22], Joehlin-Price *et al*. [25], and Mas-Moya *et al*. [28].

There was no statistically significant correlation between the FIGO stage of endometrial carcinoma (EC) cases and MMR IHC expression the same as Joehlin-Price *et al*. [25] study.

MMR-IHC can be performed as part of a routine surgical pathology workflow, and validation is achievable for virtually any laboratory that processes IHC [19]. It can be used to evaluate MMR proteins expression and select patients for genetic testing. Loss or abnormal protein expression may be suggestive of LS [29].

In addition to influencing health-care decisions for individual cancer patients, a diagnosis of LS affects screening strategies for related family members. Involvement of genetic counselors is critical for advising both individual cancer patients and family members about the implications of testing [30].

Conclusion

There is a statistically significant correlation between the tumor grade of EC cases and the MMR IHC proteins expression and no correlation with the other analyzed clinicopathological parameters in this study. Hence, further studies on MMR proteins expression are recommended.

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