Microsatellite Instability (MSI) and P16/P53 Protein Status in Different Subtypes of Endometrial Carcinoma: with Emphasis on Tumor Aggressiveness

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Abstract

Aim: We investigated microsatellite instability (MSI) in endometrial cancer (EC) and correlated results with traditional markers (p16, p53, Ki-67) to predict tumor aggressiveness.

Methods: Records of patients admitted with EC between 2010 and 2022 were reviewed, and the widest immunohistochemical (IHC) panel including (1) estrogen or progesterone receptors (ER, PR), (2) mismatch repair (MMR) proteins (MLH1, PMS2, MSH2, MSH6), (3) Ki-67, (4) p16 and (5) p53 proteins were recorded. Chi square test was used for statistical analysis.

Results: Total of 44 female patients with pathology reports containing all five IHC panel markers were included. Mean age was 64.1±12.51 years. Type I EC was the most common pathology (72%). ER or PR positivity were very prominent in type I tumors in comparison with non-endometrioid (type II) tumors (84% vs 16%, respectively; $p<0.05$). MSI was also more pronounced in type I than that of type II (46% vs 16%, respectively; $p<0.05$), but p16 and p53 expressions were more significant in patients with type II tumors ($p<0.05$). Pathological stage (pTNM) was seen to be significantly more advanced in type II and un/dedifferentiated cancers (each, 44% vs 18% in type I, $p<0.05$), and most of the tumors in these subtypes expressed Ki-67>10% ($p<0.05$).

Conclusions: A wider IHC panel including all MSI (MLH1, PMS2, MSH2, MSH6), ER, PR, p16, p53 and Ki-67 may help oncologic planning in patients with different subtypes of EC, since first three markers can be used for tumor differentiation and others indicate the necessity of aggressive treatment.

Keywords: *Endometrial cancer; microsatellite instability (MSI); mismatch repair (MMR); immunohistochemistry (IHC); p16; p53; Ki-67*

1. Introduction

Microsatellites are short segments of repetitive DNA sequences, and microsatellite instability (MSI) has emerged as one of the most important pathways in the development of endometrial carcinoma (EC)[1]. MSI results from inactivation of some intracellular proteins or cofactors that comprise the mismatch repair (MMR) system, and MLH1 hypermethylation is the most common inactivation, known as epigenetic silencing[2]. Genetic or somatic mutations of other MMR components, such as PMS2, MSH2 and MSH6, are also common in patients with MSI occurring ECs[1,2].

Endometrial endometrioid (type I) carcinomas are related to excessive estrogen exposure, and most of them are positive for estrogen or progesterone receptors (ER, PR)[3]. However, their expression can be variable in tumor tissue and this is not always explained by differences in grade of the tumor, suggesting that MMR changes may contribute to this variability. Immunohistochemistry (IHC) expression of MMR proteins can be classified in five groups: no loss of expression, isolated loss of MLH1, combined losses of MLH1/PMS2 or MSH2/MSH6, and loss of all antibodies[4].

IHC panel, other than MSI and hormone receptors, can serve as additional diagnostic markers including p16 and p53, for distinction of non-endometrioid (type II) EC (serous, papillary, mucinous, etc.) from type I carcinomas[5]. Diffuse expression of p16 and p53, and absence or focal staining of ER or PR in EC should prompt one to consider a serous carcinoma. Since the differentiation of subtypes from each other is not easy in every case and their tumoral behavior change, an advanced IHC panel including traditional proteins ER, PR, p16 and p53 enriched with MSI MMR can help clinicians in planning oncologic treatment.

Therefore, in the present study, we investigated the IHC panel in all subtypes of EC, and correlated the results with nuclear mitosis index

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(Ki-67) and pathological stage.

2. Materials and methods

After the approval of study by Ethics' Committee (University of Health Sciences, IEAH-06.05.2022/151), records of patients who were admitted with EC and underwent surgery between January 2010 and December 2022 were reviewed. Patients signed informed written consent allowing their data to be used in medical researches. All cases were biopsy proven preoperatively, and their postoperative pathology reports were investigated in detail. All data were recorded at Excel program (Microsoft 2017, Chicago, Illionis, US).

Patients with sarcomatoid lesions and with reports other than EC or not including any of the MSI/ER/PR/p16/p53/Ki-67 markers were excluded from the study. Patients with a history of neoadjuvant chemo- or radiotherapy were also excluded.

Demographics, menopausal status, types of final histopathology, IHC panel results including MSI, p16 and p53 status, and hormone receptor types (ER and/or PR), if any, were recorded. Pathologic subtypes were recorded as; 1. Endometrioid cancer (type I), 2. Non-endometrioid cancer (type II) as papillary serous, clear cell, mucinous and squamous cell, 3. Mixed type, and 4. Undifferentiated or dedifferentiated (together with low grade endometrioid carcinoma).

EC patients were also staged according to the guidelines of International Federation of Gynecology and Obstetrics (FIGO) as early disease (stages 1 and 2) and advanced disease (stages 3 and 4). Aggressiveness of tumor biology was also assessed with nuclear protein Ki-67 antigen. Ki-67>10% was regarded as aggressive biology.

2.1. Statistical Analysis

Statistical package for social sciences (Version 11, US) was used for the statistical analyses. Number (n) and median value with standard deviation (SD) were calculated for quantitative variables. Frequency and percentage (%) were evaluated for qualitative variables. Chi-square test was used to determine the probable associations. P <0.05 was taken as statistical significance value.

3. Results

EC patients with pathology reports containing all five IHC panel markers were included in the study. There were only 44 female patients, of whom 34 with early-stage EC (77.2%, mean age, 63.9±9.8), and 10 with advanced/metastatic carcinoma (22.7%, mean age, 64.7±12.7). The age difference between the groups was not statistically significant (p>0.05), and most of patients were in postmenopausal period (n=36, 81.8%).

The most common histopathology in EC was type I (endometrioid, 72% of all patients, Table 1). Hormone receptors (ER or PR) were positive in 84% and 66% of patients with type I and mixted type pathology, respectively (each, p<0.05, Table 1). Type II non-endometrioid EC showed scarce hormone receptors (only 16%). MSI was also more pronounced in type I patients than that of type II (46% vs 16%, respectively; p<0.05).

On the other hand, p16 and p53 expressions were more significant in patients with type II and un/dedifferentiated pathologies, and they were seen to be correlated with Ki-67 expressions above 10% (Table 1, each, p<0.05). The latter findings were seen to be in accordance with FIGO advanced pathological stages (44.4% in type II and undifferentiated EC vs 18.75% in type I EC, p<0.05).

4. Discussion

EC has traditionally been classified into type I and type II based on its clinical, histopathological, and molecular findings[1,6]. Type I mainly consists of endometrioid tumor that is considered to develop in an estrogen-dependent pattern[3]. It arises in atypical endometrial hyperplasia and mostly seen in perimenopausal women. This type is well-known for its more favorable prognosis, as well. Recently, in type I endometrial endometrioid carcinoma, dysfunction of DNA MMR genes have been shown to be associated with carcinogenesis of endometrium[2,4]. On the other hand, type II EC consists of serous carcinoma that is thought to be de novo carcinogenesis developing directly from the atrophic endometrium[5]. It occurs mostly in postmenopausal period, and is associated with worse prognosis[6].

Sporadic or germline mutation in at least one of the MMR enzymes (PMS2, MLH1, MSH2 or 6) and epigenetic silencing due to MLH1 gene promoter's hypermethylation can cause MSI in the DNA of tumor cells compared with normal cell DNA (4). Hashmi et al have suggested that MMR expression loss shown by IHC might be used as a possible marker for MSI[7]. MSI is usually found in endometrioid type of ECs[2]. On the other hand, non-endometrioid serous, papillary or mucinous types usually present genetic instability at chromosomal level due to primary defects in p16 and p53 genes, rather than microsatellite variations[5,8,9].

P53 plays a pivotal role in the regulation of cell proliferation, DNA repairment, apoptosis process and genomic stability, and it acts mainly as a transcriptional factor. Genetically, p53 mutation is more frequent in type II than type I EC[8]. Schultheis et al showed that p53 mutations were detected in their 64 patients (28%) of ECs[10]. In total of endometrioid and serous ECs, p53 mutation was seen in 15% and 88% of the patients, respectively[10]. In endometrioid ECs, the pattern of mutations was: frameshift, missense, and nonsense. Moreover, Netzer et al. have found p16 overexpression in 78% of patients with

Table 1

MSI, hormone receptors, p16, p53 and Ki-67 status in EC subtypes

Pathology	Total	ER/PR	MSI	p16	p53	Ki-67>10%
	n(%)					
Type I (endometrioid adenoca)	32(72.7)*	27(84.3)*	15(46.8)*	16(50)	15(46.8)	6(18.7)
Type II (non-endometrioid)	6(13.6)	1(16.6)	1(16.6)	5(83.3)*	6(100)*	5(83.3)*
Mixed type	3(6.8)	2(66.6)*	1(33.3)	2(66.6)*	1(33.3)	2(66.6)*
Un/dedifferentiated	3(6.8)	1(33.3)	2(66.6)	3(100)*	3(100)*	3(100)*

ER=estrogen receptor, PR=progesterone receptor MSI=microsatellite instability (MLH1/PMS2/MSH2/MSH6), Type II=Serous, papillary, mucinous, clear cell, non-endometrioid adenocarcinoma (adenoca), *p<0.05

serous papillary carcinomas versus that of only 36% of patients with endometrioid subtype[11]. In our study, we found similar ratios supporting the current literature. Overexpressions of p16 and p53 were evident in nearly half of the patients with type I EC, while these ratios reached up to 100% in type II.

High Ki-67 indices have long been known to be related to increased tumor proliferation, poor prognosis and shortened survival time[12-14]. Ki-67 protein, a nuclear monoclonal antibody, can be detected during all active phases of cell cycle (G1, G2, S and M), but is absent from the resting cells (G0). First, it has been shown as useful clinical marker for subtype classifications of breast cancer, its overall prognosis, and in the prediction of therapeutic response[15,16]. In a healthy mammary tissue, very low levels of Ki-67 (<3%) have been reported, and it is expressed exclusively in ER-negative cells[15]. Positive expressions of p16 and p53 are also associated with unfavorable outcomes in most kind of tumors[17-20], and this was confirmed in the present research, as well. Therefore, since the nuclear protein Ki-67 is a well-established prognostic and predictive indicator for the aggressiveness of tumor, higher ratios in our patients with type II pathology along with p16/p53 expression seems logical, as well.

MMR gene mutations have also been shown to cause a genetic predisposition to hereditary nonpolyposis colorectal cancer (HNPCC) syndrome known as Lynch' s disease[21]. These patients have an up to 80% life time risk of developing EC with MLH1 and MSH2 mutations[22]. MSI occurring after sporadic mutation or epigenetic silencing has been shown to occur in up to 20% of ECs; whereas, germline mutations account for lesser rates[7]. Our MSI results were similar, since it was found to be significantly higher in endometrioid EC than that of non-endometrioid EC.

Undifferentiated carcinoma, when associated with low-grade endometrioid carcinoma is termed as dedifferentiated carcinoma, is usually negative for hormone receptors, and commonly demonstrates loss of the expression of DNA mismatch repair proteins, as seen in our patients. Therefore, tumor biology in this subtype resembles type II EC.

The present study has some limitations like lack of the availability of recent molecular studies, such as PAX2, CK7, CK20, CD10, etc. in all histopathology reports. Furthermore, the number of patients with histopathology reports containing MSI MMR results was also very limited. Since only the available molecular markers mentioned in the pathology reports are taken into consideration, the data compared are limited. However, high ratios of MSI in endometrioid and p16 or p53 in non-endometrioid types are outstanding. The latter finding also reflects the wild type of tumor biology as proved with a higher rate of Ki67 expression and advanced pathologic stage.

5. Conclusions

In conclusion, wide IHC panel including all of the MSI MMRs (MLH1/PMS2/MSH2/MSH6), ER, PR, p16, p53 and Ki-67 may help decision-making in oncologic planning of patients with different subtypes of EC.

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Statement of ethics

The study was approved by the University of Health Sciences, Istanbul Education and Research Hospital Ethics Committee (IEAH-06.05.2022/151).

Conflict of interest statement

The authors declare that they have no financial conflict of interest with regard to the content of this report.

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Author contributions

Concept: A.F.; Design: A.F.,; Data Collection or Processing: A.F.; Literature Search: A.F.; Writing: A.F.; Critical review: A.F. All authors read and approved the final manuscript.

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