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Original Article

Study on the prognostic value of p53 and PTEN immunomarkers for endometrial cancer using immunohistochemistry

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Abstract

Introduction: Mutation in p53 and phosphatase and tensin homolog (PTEN) genes are reported to be prevalent in endometrial cancer. The present study aimed to evaluate the immunohistochemical expression of p53 and PTEN proteins in endometrial cancer among women with hysterectomy.

Methods: In this cross-sectional study, 40 paraffin-embedded endometrial cancer samples were collected during 2015 to 2016, from women with hysterectomy in Al Zahra Hospital, Tabriz, Iran. The histopathological observation was performed to confirm endometrial cancer and its grade. Immunohistochemistry (IHC) was done for p53 and PTEN biomarkers. Data were analyzed by SPSS.

Results: Thirty-three (82.5%), six (15.0%) and one (2.5%) out of 40 samples were endometrioid endometrial adenocarcinoma, serous carcinoma and clear cell adenocarcinoma, respectively. Furthermore, 5, 16 and 19 out of 40 studied samples belonged to grade I, II and III, respectively. The IHC observation showed that p53 expression in 9 (22.5%) was positive, while the rest 31 (77.5%) samples were p53 negative. Moreover, PTEN expression was observed in 10 (25%) samples and 30 (75.0%) samples were PTEN negative. The sensitivity of p53 and PTEN for diagnosis of endometrial cancer was calculated as 56.3% and 80%, respectively.

Conclusion: The IHC markers, p53 and PTEN, show heterogeneous results as diagnostic and prognostic markers for endometrial carcinoma and are suggested to be used along with other markers for such purposes.

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Introduction

Endometrial cancer is the fourth common malignancy in women. It is also the most common gynecological malignancy in developed and the second common in developing countries.[1,2] In the 1970s, the incidence of endometrial cancer showed an elevation after increased use of menopausal estrogen therapy.[2,3] Obesity causes higher relative risk of endometrial cancer as compared to any other obesity-associated cancer.[4,5] Another risk factor is the earlier onset of menarche and also decreasing of the other protective factors like multiparity. The incidence of endometrial cancer is expected to increase by nearly 50-100% in the next two decades.[2,6]

Two major types of endometrial cancer have been described. Type I is the endometrioid adenocarcinoma, which is mostly well-differentiated tumor and comprises of the large majority of endometrial cancers. Type I endometrial cancer is associated with the unopposed estrogen stimulation and is mostly progressed by endometrial hyperplasia. Type II includes papillary serous adenocarcinomas, clear cell adenocarcinomas, carcinosarcomas, and grade III endometrioid carcinomas. This type is commonly described as estrogen independent,

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preceded from atrophic endometrium or endometrial polyps. The type II endometrial cancers are derived from intraepithelial carcinoma that is a precancerous lesion. This type, which accounts for almost 10–20% of endometrial cancers, is less-differentiated and as a result, shows poorer prognosis and accounts for a considerable number of endometrial cancer deaths.[2,7,8]

The different genetic alterations found in type I and type II tumors suggest that these subtypes may have distinct etiologies.[2,8,9] Type I endometrial cancer shows microsatellite instability (MSI) and mutations in K-ras, phosphatase and tensin homolog (PTEN), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA), and catenin beta-1 (CTNNB1) genes. However, type II displays p53 mutations and also chromosomal instability.[10]

The histopathological diagnosis of obstetrical and gynecologic cancers was mostly based on morphologic criteria. Thus, inter-observer variations for entities such as endometrial cancers were inevitable. The advent of immunohistochemistry (IHC) and the recent discovery of new genes and their functions in cancer have led to the discovery of cellular proteins or nucleic acids that are exclusively expressed in tumors. These biomarkers have the potential of enhancing diagnostic consistency and reproducibility of cancers.[11] Some of these biomarkers are PTEN, L1 cell adhesion molecule (L1CAM), MutL homolog 1 (MLH1), estrogen receptor, progesterone receptor, p53, postmeiotic segregation increased 2 (PMS2), β-catenin and E-cadherin.[12] The present study aimed to evaluate the IHC profile of the diagnostic and prognostic value of p53 and PTEN biomarkers in patients with endometrial cancer.

Methods

In this cross-sectional study, 40 paraffin-embedded tissue blocks were collected from women underwent a hysterectomy during 2015-2016 in Al Zahra hospital, Tabriz, Iran. The samples had been previously diagnosed with endometrial cancer. The type and grade of these samples were recorded using the International Federation of Gynecology and Obstetrics (FIGO) grading system.[13]

IHC for p53 and PTEN biomarkers was performed on all 40 collected samples. The procedure of IHC has done according to the instruction of purchased antibodies and the detection system. The laboratory work out, in brief, is as follow: the 4 µm-thick tissue sections were cut on the positive charged adhesive slides (Leica Biosystems, USA) and incubated at 37 °C for 24 hours. The tissue sections were dewaxed with xylene, followed by hydration with different ethanol compositions. The antigen retrieval process was performed using target retrieval solution, pH 9 (Tris/EDTA buffer, pH = 9, DAKO®, Denmark) in 95 °C for 30 minutes, cooled to room temperature (RT) for 15 minutes, and then the endogenous peroxidase was blocked using 30% H_2O_2 for 10 minutes. Primary antibodies, p53 (Dako®, Denmark) and PTEN (Abcam, GB) were added to the slides, incubated for 20 minutes at RT, washed two times in IHC wash buffer (Tris-buffered saline tween 20) for 10 minutes each, and once in phosphate-buffered saline (PBS) for 5 minutes. The slides were incubated with the secondary antibody, anti-mouse/rabbit EnVision® Duo FLEX double stain system, horseradish peroxidase (HRP)/3,3'-diaminobenzidine (DAB) (Dako, Denmark) at RT for 30 minutes.

The washing procedure was repeated again as the previous step. DAB was diluted (35 µl in 1000 µl of its buffer), added to the slides and incubated at RT for 5-7 minutes for enzymatic reaction to be developed. Then the slides were washed in distilled water, dehydrated with ethanol and mounted with Entellan®. IHC staining for p53 was interpreted as positive when a strong nuclear staining in 75% of cells was observed. IHC staining for PTEN was interpreted as positive when a diffuse cytoplasmic staining of cells was observed.

Table 1. p53 immunohistochemistry staining among different types of studied endometrioid carcinomas cases

Cancer type	p53		P*
	Positive (n = 9) n (%)	Negative (n = 31) n (%)	
Endometrioid adenocarcinoma (n = 33)	2 (5.0)	31 (77.5)	< 0.001
Serous adenocarcinoma (n = 6)	6 (15.0)	0 (0)	
Clear cell adenocarcinoma (n = 1)	1 (2.5)	0 (0)	

*Chi-square test

Breast invasive ductal carcinoma was used as a positive control for both p53 and PTEN. The same slide without adding the primary antibodies was also used as a negative control.

The sensitivity of p53 and PTEN IHC tests for diagnosis of endometrial carcinoma was calculated using the following equation: Sensitivity = TP / (TP + FN), where TP is true positive and FN is false negative. Data were analyzed by SPSS software (version 20, IBM Corporation, Armonk, NY, USA) using the chi-square test.

Results

In the present study, 40 women suffering from endometrial carcinoma with mean ± standard deviation (SD) age of 57.67 ± 12.34 years were studied. Among the 40 studied samples, 33 (82.5%), six (15.0%) and one (2.5%) were endometrioid, serous and clear cell adenocarcinomas, respectively. Furthermore, five (12.5%), 16 (40.0%) and 19 (58.5%) out of 40 samples were categorized in grade I, II and III, respectively.

Results of p53 IHC staining showed the expression of this biomarker in 9 (22.5%) out of 40 samples, while 31 (77.5%) were p53 negative. Moreover, the PTEN IHC showed positive staining in 10 (25.0%), whereas other 30 (75.0%) samples were negative.

All of the 6 serous adenocarcinoma samples and the only clear cell adenocarcinoma were IHC positive for p53 staining. However, it was positive only in two (6.06%) out of 33 endometrioid carcinoma samples (Table 1). The difference between the histopathological findings with the p53 IHC staining was significant statistically (P < 0.001). The PTEN IHC result showed the positive and negative staining in 9 (27.27%) and 24 (72.73%) out of 33 endometrioid adenocarcinoma samples, respectively (Table 2). The difference between the histopathological findings with the PTEN IHC staining result was not statistically significant (P = 0.078).

A significant difference was observed in the frequency of p53 IHC positivity among the different grades of the studied endometrial cancer samples (P = 0.010). The highest frequency of p53 expression was seen in grade III endometrial cancer (Table 3). Furthermore, no significant difference was observed in the frequency of PTEN IHC positivity among the different grades of the studied endometrial cancer samples (P = 0.757). The highest frequency of p53 expression was seen in grade II endometrial cancer (Table 4).

The sensitivity of the p53 and PTEN IHC for diagnosis of endometrial cancers with the histopathological gold standard is as follow: Sensitivity of P53 IHC for overall endometrial cancer was 56.3% (40 samples were studied), for endometrioid carcinoma was 51.5% (33 samples were studied), for serous carcinoma was 100% (six samples were studied), for grade II endometrial cancer was 51.6% (16 samples were studied), and for

Table 2. PTEN (Phosphatase and tensin homolog) immunohistochemistry staining among different types of studied endometrioid carcinomas cases

Cancer type	PTEN		P*
	Positive (n = 10) n (%)	Negative (n = 30) n (%)	
Endometrioid adenocarcinoma (n = 33)	9 (22.5)	24 (60.0)	0.078
Serous adenocarcinoma (n = 6)	0 (0)	6 (15.0)	
Clear cell adenocarcinoma (n = 1)	1 (2.5)	0 (0)	

*Chi-square test, PTEN: Phosphatase and tensin homolog

Table 3. p53 immunoreactivity among different studied grades of endometrial cancer cases

Grade	p53		P*
	Positive (n = 9) n (%)	Negative (n = 31) n (%)	
Grad I (n = 5)	0 (0)	5 (100)	0.010
Grad II (n = 16)	1 (6.2)	15 (93.8)	
Grade III (n = 19)	8 (42.1)	11 (57.9)	

*Chi-square test

grade III endometrial cancer was 63.3% (19 samples were studied). Sensitivity of PTEN IHC for overall endometrial cancer was 80.0% (40 samples were studied), for endometrioid carcinoma was 78.6% (33 samples were studied), for serous carcinoma was 100% (six samples were studied), for grade I endometrial cancer was 83.3% (five samples were studied), for grade II endometrial cancer was 76.2% (16 samples were studied), and for grade III endometrial cancer was 82.6% (19 samples were studied).

Table 4. PTEN (Phosphatase and tensin homolog) immunoreactivity among different studied grades of endometrial cancer cases

Grade	PTEN		P*
	Positive (n = 10) n (%)	Negative (n = 30) n (%)	
Grad I (n = 5)	1 (20.0)	4 (80.0)	0.757
Grad II (n = 16)	5 (31.2)	11 (68.8)	
Grade III (n = 19)	4 (21.1)	15 (78.9)	

*Chi-square test, PTEN: Phosphatase and tensin homolog

Discussion

The present study aimed to evaluate the applicability of p53 and PTEN expression using IHC method for diagnostic and prognostic purposes. The results did not show the promising results regarding the diagnostic values of p53 and PTEN biomarkers for such a purpose because the IHC results were significantly different from that in histopathology. Besides, p53 showed encouraging results regarding the diagnosis of serous adenocarcinoma, in which all 6 samples were detected by the p53 IHC. This finding is supporting the results of previously performed studies.[14-17]

Mutations in p53 gene are common in uterine serous carcinoma and mostly occur early in the pathogenesis;[16] thus, p53 genotyping can be a good prognostic tool for early detection of this type of cancer. Tashiro et al. observed p53 immunoreactivity in the majority of their studied uterine serous carcinoma and endometrial intraepithelial carcinoma cases. They also proposed that the lack of immunoreactivity did not always indicate the absence of a gene mutation. They also observed the loss of heterozygosity of chromosome 17p in almost 100% of serous carcinoma and in 43% of endometrial intraepithelial carcinoma cases. This observation indicates that loss of the wild-type p53 allele, mostly occurs in the early development of serous carcinoma.[16] In the present study, the sensitivity of p53 for diagnosis of serous carcinoma was 100%, whereas the sample size was very small and cannot be regarded as a definite result.

The biomarkers, p53 and PTEN, showed very low sensitivity for detection of endometrioid adenocarcinoma, which was 51.5% and 80.0%, respectively. This shows the poor diagnostic values of P53, while PTEN showed better results. Mao et al. studied the expression of AT rich interactive domain 1A (ARID1A), p53, PTEN and mismatch repair (MMR) proteins in high-grade endometrioid carcinomas with or without concurrent low-grade endometrioid carcinomas, using IHC method. Their results revealed PTEN loss, ARID1A loss, MMR deficiency or MSI and aberrant p53 expression in 37%, 58%, 37% and 47% in high-grade tumors, and 45%, 77%, 55% and 32% in high-grade tumors with concurrent low-grade components, respectively. They reported that the high-grade tumors showed a higher frequency of type II endometrial cancers (positive for PTEN, ARID1A and MMR proteins, p53 aberrant expression) than high-grade tumors with concurrent low-grade components.[18] Their results are

relatively similar to the findings of the present study. In the present study, p53 expression was observed in 42.1% of high-grade (grade II) endometrial cancers, while none of the grade I cases showed the p53 positive IHC result. On the other hand, PTEN loss was observed at the highest rate in grade III cancers. Moreover, the PTEN IHC result showed the positive and negative staining in 9 (27.27%) and 24 (72.73%) out of 33 endometrioid adenocarcinoma samples, respectively. It was positive only in two (6.06%) out of 33 endometrioid carcinoma samples regarding the p53 biomarker.

Holtz et al. studied PTEN expression in tamoxifen-associated endometrial cancers by IHC method. Tamoxifen is reported to be associated with increased rate of endometrial adenocarcinoma. They examined 28 endometrial carcinoma specimens from patients with a history of breast cancer with (15 samples) and without (13 samples) tamoxifen administration. Their results showed that 4 (27%) out of 15 tamoxifen-treated cases were immunoreactive for PTEN compared to 2 (15%) out of 13 of non-treated ones. They also concluded that the tamoxifen-associated endometrial cancers are not different from sporadic endometrial cancer, regarding the PTEN IHC expression.[19] Moreover, they reported PTEN IHC positivity in endometrial cancer, regardless of tamoxifen-treated and non-treated, is very close to the findings of the present study, 21.4% versus 22.5%.

Most of the published studies on PTEN and p53 markers with IHC method reported the very similar results compared to the present study. Similar to our findings, other studies have claimed that p53 and PTEN IHC tests show heterogeneous results and are not compatible with the histopathological findings, albeit p53 can be a good biomarker for endometrial serous carcinoma.[20-22] PTEN also showed roughly promising but not accurate results regarding endometrial cancer diagnosis.

Conclusion

The IHC markers, p53 and PTEN, show heterogeneous results as diagnostic and prognostic markers of endometrial carcinoma and are suggested to be used along with other markers for such purposes.

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Authors' Contribution

All of the authors contributed equally.

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Conflict of Interest

Authors have no conflict of interest.

Ethical Approval

This study was approved by the Medical Ethics Committee of Tabriz University of Medical Sciences.

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