INTRODUCTION

Endometrial carcinoma is one of the most common invasive tumors of the female genital tract. A recent cancer registry report (1994 - 2011) reported that malignancies of the Corpus uteri comprised 3.02% of all neoplasms in adult females. Based on light microscopic appearance, clinical behavior, and epidemiology, endometrial carcinomas have been classified into type 1 and type 2 tumors, both carrying mutations of an independent set of genes. Type 1 or endometrioid endometrial carcinomas are usually preceded by endometrial hyperplasia (EH). A prolonged unopposed estrogen exposure is seen to confer a 2 - 10 fold increased risk for endometrial carcinoma. Currently, 2 systems for classifying endometrial pre-cancers are in use. The WHO classification is based on architectural complexity and nuclear atypia and comprises of 4 categories: simple hyperplasia, complex hyperplasia, simple hyperplasia with atypia and complex hyperplasia with atypia. The EH-EIN-CA classification developed by “The Endometrial Collaborative Group” proposes terms endometrial hyperplasia, endometrial intraepithelial neoplasia and adenocarcinoma to define distinct sub-groups relevant to the clinical management of patients with endometrial disease. The terms endometrial hyperplasia (EH) or benign endometrial hyperplasia apply to diffuse architectural and proliferative changes due to excess estrogen stimulation. EIN is defined as a clonal proliferation of architecturally and cytologically altered pre-malignant endometrial glands which are prone to malignant transformation to endometrioid (type 1) endometrial carcinoma.

Mutations of PTEN, K-ras and β-catenin genes and microsatellite instability along with others are common genetic changes observed in endometrial carcinomas. PTEN is a tumor suppressor gene located at chromosome 10q23 and it encodes a 55-KD protein with tyrosine kinase function. It acts at the G1/S checkpoint of the cell cycle and enables apoptosis through an AKT-dependent mechanism. PTEN acts in opposition to PI3KCA to control levels of phosphorylated AKT and its mutation results in increased PI3KCA activity leading to increased AKT phosphorylation. PTEN mutations have also been documented in endometrial hyperplasia with and without atypia and multiple studies have suggested loss of PTEN function as an early event in the

ORIGINAL ARTICLE

Immunoexpression of Cyclin D1 and PTEN in Various Endometrial Pathologies

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ABSTRACT

Objective: To determine the expression of cyclin D1 and PTEN (phosphatase and tensin homolog) in endometrial hyperplasias and neoplasias.

Study Design: Analytical study.

Place and Duration of Study: The study was conducted at BMSI, JPMC, Karachi, from January 2008 to December 2012.

Methodology: Analysis of endometrial samples, comprising of hysterectomies and curettage, was carried out. Immunohistochemical staining was done for PTEN and cyclin D1 expression.

Results: Fifty-three endometrial samples including 23 endometrial carcinomas, 6 complex hyperplasias with atypia, 14 complex hyperplasias without atypia, 6 simple hyperplasias without atypia and 4 normal proliferative endometrium were analyzed. Fifty-two percent (12 out of 23) and 48% (11 out of 23) cases of endometrial carcinomas showed complete loss of PTEN expression and cyclin D1 overexpression, respectively. Five (5 out of 6) cases of complex hyperplasias with atypia and 64.28% (9 out of 14) cases of complex hyperplasia without atypia showed complete loss of or diminished expression of PTEN whereas 66.66% (4 out of 6) cases of endometrial hyperplasia with atypia and 50% (7 out of 14) cases of endometrial hyperplasia without atypia showed cyclin D1 overexpression (p < 0.001).

Conclusion: Loss of PTEN, expression and cyclin D1 overexpression was seen in a significant number of well differentiated endometrial adenocarcinomas and complex hyperplasias with atypia, suggesting both as an early event in endometrial carcinogenesis.


INTRODUCTION

Endometrial carcinoma is one of the most common invasive tumors of the female genital tract. A recent cancer registry report (1994 - 2011) reported that malignancies of the Corpus uteri comprised 3.02% of all neoplasms in adult females. Based on light microscopic appearance, clinical behavior, and epidemiology, endometrial carcinomas have been classified into type 1 and type 2 tumors, both carrying mutations of an independent set of genes. Type 1 or endometrioid endometrial carcinomas are usually preceded by endometrial hyperplasia (EH). A prolonged unopposed estrogen exposure is seen to confer a 2 - 10 fold increased risk for endometrial carcinoma. Currently, 2 systems for classifying endometrial pre-cancers are in use. The WHO classification is based on architectural complexity and nuclear atypia and comprises of...
pathogenesis of endometrial carcinoma. Although it does not necessarily predict an increase in the incidence of carcinoma in subsequent follow-ups, absence of PTEN null phenotype alone, however, may predict a benign follow-up.7 Cyclin D1 is a member of the cyclin G1 family and controls the transition from G1 to S phase in the cell cycle. The cyclin D1 protein is encoded by the CCND1 proto-oncogene localized on chromosome 11q13. Binding of cyclin D1 to the cyclin dependent kinases 4 and 6 (CDK4/6) results in the formation of active complexes that phosphorylate the retinoblastoma tumor suppressor gene during the G1 phase. Multiple studies have shown cyclin D1 overexpression as a potential biomarker for precancerous and cancerous endometrial lesions, however, whether it participates in a causative or incidental manner in tumor progression is still to be determined. PTEN and cyclin D1 influence the cell cycle at the same stage, i.e. G1/S phase. Studies assessing the correlation between PTEN and cyclin D1 expression have shown cyclin D1 overexpression in moderate to high grade endometrial carcinomas resulting in loss of PTEN expression.9

However, very limited researches have been carried out in Pakistan in this regard. Therefore, it was decided to carry out a study to observe the differential expression of PTEN and cyclin D1 and their association in different endometrial samples ranging from normal proliferative endometrium to malignant endometrial tumors.

**METHODOLOGY**

This is a retrospective analytical study based on the analysis of endometrial samples comprising of both hysterectomies and curettage, received at the Department of Pathology, BMSI, JPMC, Karachi, over a 5-year period from January 2008 to December 2012. Samples were selected for immunohistochemical analysis on the basis of clinical presentation and histological diagnosis. Sample size was calculated using the survey system sample size calculator. Poorly fixed tissue and inadequate material were excluded. Samples of foreign nationals and Pakistanis living abroad for more than 10 years were also excluded as the environmental influences are different and hence would not be representative of Pakistani data. H&E stained slides were reviewed to confirm the diagnosis. The most representative section was used for immunohistochemical analysis.

Anti-PTEN (clone 6H2.1), mouse monoclonal antibody procured from Millipore and Anti-Cyclin D1, rabbit monoclonal antibody procured from Cell Marque, were used in all immunohistochemical analysis. Antigen detection was done using HiDef detection HRP polymer system kit (ready to use) procured from Cell Marque. Endometrial stroma was taken as internal positive control for PTEN while ductal carcinoma breast was taken as positive control for cyclin D1. PBS substituted primary antibody for negative control. Sections of approx. 5 µm were cut on to poly L-lysine coated slides and were deparaffinized and rehydrated. Antigen retrieval was achieved by steamer method using citrate buffer. Slides were allowed to cool for 20 minutes and were then placed in UV block for 5 minutes. Tissues were covered with primary antibody at dilution 1:50 and were incubated for 1 hour at room temperature. Slides were then incubated first with Amplifier and then with HRP polymer for 10 minutes. Chromogen was applied for 20 minutes and all the slides were counterstained with Hematoxylin, dehydrated and mounted. Between each step, the slides were washed with phosphate buffer solution (PBS).

The intensity of staining was graded as no nuclear staining (0), weak nuclear staining (1+), moderate nuclear staining (2+) and strong nuclear staining (3+). No staining (0) and weak nuclear staining (1+) was taken as diminished staining. The extent of staining was estimated in percentage by counting at least 50 nuclei, calculating the ratio of reactive nuclei to total number of nuclei and multiplying it by 100. A score of 0 was used when less than 10% cells were positive, 10 - 30% immunoreactive cells were scored as 1, 31 - 60% positive cells were scored as 2; and more than 60% immunoreactive cells were scored as 3. After observing cyclin D1 immunostaining in normal proliferative endometrium, strong staining in more than 30% nuclei was taken as overexpression of cyclin D1.

Relevant data was collected on specially designed proformas. Statistical analysis was performed using SPSS version 21. Mean and standard deviation were calculated for quantitative variables while percentages and frequencies were calculated for qualitative variables. Wherever appropriate, Fisher’s exact test was applied and p-value of less than 0.05 was considered significant at 95% confidence interval.

**RESULTS**

Out of a total of 294 endometrial lesions observed, 53 were selected for immunohistochemistry, including 4 normal proliferative endometrium, 6 simple hyperplasia without atypia, 14 complex hyperplasias without atypia, 6 complex hyperplasias with atypia, and 23 endometrial carcinomas.

Table I shows the distribution of different endometrial lesions according to age groups along with the mean age for every lesion. Table II shows the nuclear intensity and extent of immunoreexpression of PTEN in normal proliferative, hyperplastic and neoplastic endometrial samples. Complete loss of PTEN expression was observed in 12 out of 23 cases of endometrial...
canceromas. Four out of 6 cases of complex hyperplasia with atypia showed complete loss of PTEN expression. In hyperplasias without atypia, 5 out of 14 cases of complex and 3 out of 6 cases of simple hyperplasia showed moderate to strong PTEN staining while 9 cases of complex and 3 cases of simple hyperplasia showed diminished staining with PTEN. Three out of 4 cases of normal proliferative endometrium showed moderate while 1 showed strong nuclear staining for PTEN. Eleven cases of endometrial carcinomas showed positive staining for PTEN. However, the intensity of staining was strong (3+) in only one of these cases while the remaining 10 cases showed weak to moderate staining with PTEN. These cases included 2 poorly differentiated adenocarcinomas and 2 papillary serous carcinomas.

Table III shows the extent and intensity of nuclear staining of cyclin D1 in different endometrial samples. Eleven out of 23 cases of endometrial carcinomas showed strong staining for cyclin D1 with 9 cases showing strong reactivity in > 60% of neoplastic cells while 2 showed strong reaction in approx 40% of cells. Four out of 6 cases of atypical complex hyperplasia showed strong expression of cyclin D1 with 2 cases showing expression in > 60% of cells and 2 cases showing strong reaction in 50% of cells. Complex hyperplasias without atypia showed strong nuclear staining for cyclin D1 in 7 out of 14 cases with all 7 cases showing positive staining in > 60% of cells. Two out of 6 cases of simple non-atypical hyperplasias showed strong nuclear staining for cyclin D1 with > 60% of cells showing positive staining in both cases. Two out of 4 cases of normal proliferative endometrium showed moderate staining with cyclin D1 while 2 showed no staining at all. A strong association (p < 0.001) was seen between loss of PTEN expression and cyclin D1 overexpression in cases of endometrioid endometrial carcinoma and complex hyperplasia with atypia.

Table IV: Loss of PTEN expression and cyclin D1 overexpression in cases of endometrioid carcinoma and complex hyperplasia with atypia.

Table I: Distribution of different endometrial lesions according to age (n=294).

Table II: Nuclear intensity and extent of PTEN immunoreactivity in normal and hyperplastic endometrium and endometrial carcinoma.

Table III: Nuclear intensity and extent of cyclin D1 immuno-reactivity in normal and hyperplastic endometrium and endometrial carcinoma.
DISCUSSION

In this study, it was attempted to determine the frequency of endometrial carcinomas and the differential expression of PTEN and cyclin D1 in various endometrial morphologies.

Out of 35 malignant endometrial tumors, 2 (5.4%) were malignant spindle cell tumors, 2 (5.4%) were papillary serous carcinomas, 6 (18.9%) were poorly differentiated carcinoma, 1 (27%) moderately differentiated carcinoma, and the remaining 24 (67.5%) were endometroid adenocarcinomas. The mean age for endometrial carcinomas was found to be 57 years and majority of the patients (67.5%) belonged to the 6th and 7th decade of life. Similar findings were observed in a study done at AKUH; where out of 86 cases of endometrial carcinomas, 53 (61.5%) belonged to the age range of 51 - 70 years.10

In the present study, all cases of well differentiated (endometrioid) endometrial adenocarcinoma showed either loss of or diminished expression of PTEN. About 61% cases of endometrioid carcinoma showed complete loss of staining with PTEN. These findings are in accordance with different studies conducted worldwide.11,12 Six cases of endometrioid endometrial carcinoma with complete loss of PTEN expression also showed, in the adjacent areas, foci of potential premalignant lesions in the form of complex hyperplasias with and without atypia with loss of PTEN expression in more than 50% of nuclei. Robbe et al. in 1 study concluded that loss of PTEN expression in complex endometrial hyperplasia could predict the presence of a coexisting carcinoma.13 Cyclin D1 immunostaining revealed strong staining in > 30% of nuclei in 8 (44.4%) cases of endometrioid endometrial carcinoma. Our findings correspond to those observed in studies by Stewart et al.14 and Liang et al.15, where strong expression of cyclin D1 was seen in endometrial adenocarcinomas.

The two papillary serous carcinomas in this study showed moderate to intense expression of PTEN. This is an expected finding as non-endometrioid (type 2) endometrial carcinomas have been shown to be associated with p53 rather than PTEN mutation16 and intense PTEN staining has been observed in non-endometrioid endometrial carcinomas.17 Both the cases of papillary serous carcinomas in this study showed strong staining with cyclin D1 in 60% of nuclei. Balan et al. showed moderate to strong expression of cyclin D1 in 2 out of 2 cases of papillary serous carcinomas.18

Immunohistochemistry was done on the 6 cases of complex hyperplasia with atypia, out of which 4 (66.6%) showed complete loss of PTEN expression, 1 (16.6%) showed moderate and 1 (16.6%) showed weak staining with PTEN. These findings correspond to those quoted by Quddus et al. and Abd El-Maqsood et al., who observed loss of PTEN in majority of cases of complex hyperplasia with atypia.7,11 In case of atypical complex hyperplasia, 66.6% i.e. 4 out of 6 showed cyclin D1 overexpression. Similar higher figures were seen by Balan et al., who observed strong cyclin D1 staining in 3 out of 3 cases of atypical hyperplasias.18

In cases of complex hyperplasia without atypia, 85.7%, i.e. 12 out of 14 cases showed weak to moderate PTEN staining while 1 (7.1%) showed strong staining with PTEN. These findings correspond to those of different studies done worldwide.11,19 One case in the present study showed foci of extensively crowded glands revealing loss of PTEN expression in almost 75% of nuclei and the same case also showed overexpression on cyclin D1 immunostaining, strongly suggesting this lesion as potentially premalignant and also representing an example of a strong relationship between PTEN loss and cyclin D1 overexpression (Figure 1 and 2). However, available clinical data was inadequate and the patient could not be followed-up to assess progression to atypical or malignant phase. On cyclin D1 staining, 50% i.e. 7 out of 14 cases of complex hyperplasia
without atypia showed overexpression. These findings correspond to those quoted by Sherva et al.20 The reason for this overexpression can be explained by the fact that as normal proliferating cells enter the cell cycle from G0 phase, cyclin D1 is localized in the nucleus early in the G1 phase, and exits as cell progresses into the S phase while PTEN and other CK inhibitors enter late into the G1 phase, resulting in down regulation of cyclin D1.

Out of 6 cases of simple hyperplasias without atypia, all 6 showed weak to moderate staining with PTEN and this finding is in accordance with those by Tantbirojn et al.19 Out of these 6 cases, 33.3% i.e. 2, showed strong cyclin D1 expression, corresponding to those observed by Liang et al., who showed reactivity of cyclin D1 in 30% cases of simple hyperplasias.15

All 4 normal proliferative endometrial samples showed moderate to strong PTEN staining. Mutter et al.21, while studying the expression of PTEN during different phases of menstrual cycle, found that with the regeneration of the functional layer of endometrium during the proliferative phase, PTEN signals became wide spread in epithelial and stromal compartments. With cyclin D1, 2 cases of normal proliferative endometrium showed moderate staining in less than 30% of nuclei, while 2 showed no staining with cyclin D1. Similar findings were observed by Sherva et al.20

**CONCLUSION**

A strong correlation was seen between loss of PTEN expression and cyclin D1 overexpression in cases of well differentiated (endometrioid) endometrial carcinoma and complex hyperplasia with atypia where 8 out of 12 and 4 out of 4 cases, respectively showing PTEN loss, also showed cyclin D1 overexpression. Progressively diminishing PTEN expression and increase in cyclin D1 overexpression were seen from normal proliferative endometrium to complex hyperplasia with atypia. This observation further supports the importance not only of hyperplasias as significant pre-cancerous lesions, but also of lack of PTEN expression and cyclin D1 overexpression as early events in endometrial carcinogenesis. Despite multiple studies done worldwide, utility of PTEN and cyclin D1 expression as sensitive and specific diagnostic markers for early detection of endometrial pre-cancers is yet to be established. Further, preferably prospective, studies with proper long-term follow-up of patients is, therefore, recommended to assess the final outcomes of lesions morphologically and immunohistochemically, suggestive of being potentially pre-malignant.

**REFERENCES**

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