ORIGINAL ARTICLE

Differential expression of Phophatase and Tensin Homologue in normal, hyperplastic and neoplastic endometrium

Summayya Shawana,[1] Shahnaz Imdad Kehar,[2] Fouzia Shaikh[3]

Abstract

Objectives: To observe the differential expression of phophatase and tensin homologue in normal proliferative, hyperplastic and malignant endometrial lesions.

Methods: .The retrospective study was based on the analysis of endometrial samples, both hysterectomies and curettage, received at the department of pathology Basic Medical Sciences Institute at the Jinnah Postgraduate Medical Centre, Karachi, from January 1, 2006 to December 31, 2010. A total of 55 endometrial samples were analysed for morphological features and results of immunohistochemical staining.

Results: Of the 55 samples, 25 (45.45%) were malignant endometrial lesions, 6 (10.9%) complex hyperplasias with atypia, 14 (25.45%) complex hyperplasias without atypia hyperplasia, 6 (10.9%) simple hyperplasias without atypia, and 4 (7.27%) normal proliferative endometrium. Among malignant endometrial lesions, 12 (48%) showed complete loss of phophotase and tensin homologue expression out of which majority were endometroid adenocarcinoma. Five (83.3%) cases of complex hyperplasias with atypia and 9 (64.28%) cases of complex hyperplasia without atypia showed complete loss of or diminished expression of phophotase and tensin homologue.

Conclusion: Loss of phophotase and tensin homologue expression was seen in a significant number of well differentiated endometrial adenocarcinomas and complex hyperplasias with atypia suggesting loss of PTEN expression as an early event in endometrial carcinogenesis.

Keywords: Endometrial carcinoma, Hyperplasia, PTEN expression, Atypia, Early event. (JPMA 64: 1103; 2014)

Introduction

Endometrial carcinoma is one of the most common invasive tumours of the female genital tract and accounts for about 7% of all invasive cancers in women.[1] It is the 5th most common cancer of women worldwide.[2]

An earlier retrospective statistical study showed that endometrial carcinoma with 86 out of 2223 cases formed 3.87% of all malignant neoplasms in females.[3] A collective Pakistani cancer registry (1994-2011) reported 707 cases of malignancies of corpus uteri contributing 3.02% of all neoplasms in females above 18 years of age.[4] A hospital-based registry showed that in 2010, out of 76 malignancies of the corpus uteri 65 (85.52%) were endometrial adenocarcinomas.[5]

Endometrial carcinomas have been classified into two main types; type 1 and type 2, on the basis of light microscopic appearance, clinical behaviour and epidemiology. Type 1 and type 2 tumours carry mutations of independent set of genes.

[1]Bahria University Medical and Dental College, [2]Basic Medical Sciences Institute, JPMC, [3]Ziauddin University Medical College, Karachi.

Correspondence: Summayya Shawana. Email: shawana75@yahoo.com

Endometroid (type 1) endometrial carcinoma is often preceded by a characteristic histopathologic lesion designated endometrial hyperplasia. Endometrial hyperplasia is usually associated with prolonged unopposed oestrogen stimulation and is characterised by increased gland-to-stroma ratio and abnormalities of epithelial growth relative to normal endometrium.

The much debated World Health Organisation (WHO) classification of endometrial hyperplasia divides it into 4 types depending on architectural changes and cellular atypia.[6] These are: Simple hyperplasia without atypia; Complex hyperplasia without atypia; Simple hyperplasia with atypia; and Complex hyperplasia with atypia.

Atypical hyperplasia has long been considered a precursor lesion for endometrial carcinoma. However endometrial hyperplasias are among the most commonly over-diagnosed lesions in surgical pathology.[7] Whether hyperplasia without atypia poses a potential risk for developing into endometrial carcinoma is still not clear. However, prolonged unopposed oestrogen exposure is seen to confer a 2-10 fold increased risk for endometrial carcinoma.[8-10]

In cases of non-atypical hyperplasias, the recommended treatment is cyclical progestins therapy,

whereas in patients with atypical hyperplasias hysterectomy is the recommendation. If, however, the patient is young and wishes to conceive, high-dose progestin therapy may be considered as an option. In one study, 16 out of 17 patients diagnosed as having atypical endometrial hyperplasia showed regression of the lesion with progestin therapy and concluded that treatment of atypical hyperplasia and well-differentiated carcinoma of the endometrium with progestins appears to be a safe alternative to hysterectomy in women under the age of 40.[11]

Common genetic changes in endometroid endometrial carcinoma include microsatellite instability and mutations of PTEN, K-ras and β-catenin genes along with others.

Phosphatase and tensin homologue deleted on chromosome 10 (PTEN) tumour suppressor gene is located at chromosome 10q23. It encodes a 55-KD protein with tyrosine kinase function. PTEN suppressor gene acts at the G1/S checkpoint of the cell cycle. The encoded protein has both lipid and protein phosphatase activity. It enables apoptosis through an AKT-dependent mechanism. PTEN acts in opposition to P13KCA to control levels of phosphorylated AKT. PTEN mutation results in increased P13KCA activity and thus leads to increased AKT phosphorylation. PTEN also phosphorylates FAK which plays a major role in a transcription regulatory signalling system. FAK is activated by integrin and growth factors and induces focal adhesions, cytoskeleton formation, cellular spreading, invasion and migration.

PTEN mutation is considered to be the most common and an early genetic defect in endometroid endometrial carcinoma. Inactivation of the PTEN gene has been seen in majority (about 83%) of cases of endometrial carcinoma preceded by a pre-malignant phase.[12] PTEN mutation has been documented in endometrial hyperplasia with and without atypia. Multiple studies have suggested loss of PTEN function as an early event in the pathogenesis of endometrial carcinoma.[12-15] However, a few studies showed contradicting results.[16,17] It should be emphasised, however, that different clones of antibody were used in these studies. PTEN mutations have been detected in both atypical hyperplasias associated with the development of adenocarcinoma and those that did not progress to carcinoma. It has been suggested that although PTEN null phenotype in endometrial hyperplasias does not necessarily predict an increase in the incidence of carcinoma in subsequent follow-ups, but the absence of PTEN null phenotype alone may predict a benign follow-up.[13] In the last two decades numerous researches have been carried out worldwide to find out ways for early diagnosis of endometrial carcinomas and proper therapeutic interventions. In Pakistan very limited researches have been carried out in this regard. It was therefore decided to carry out the current study to observe the differential expression of PTEN in different endometrial lesions ranging from normal proliferative endometrium to malignant endometrial tumours.

Materials and Methods

The retrospective analytical study was based on the analysis of endometrial samples, both hysterectomies and curettage, received at the Department of Pathology, Basic Medical Sciences Institute at the Jinnah Postgraduate Medical Centre, Karachi, from January 1, 2006 to December 31, 2010.

Over the five-year study period, there had been 303 endometrial lesions from which 55 were selected for the study which were analysed for morphological features and results of immunohistochemical staining. Poorly fixed tissue, inadequate material and samples of foreign nationals and Pakistanis living abroad for more than 10 years were excluded. Haematoxylin and eosin (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 was used in all immunohistochemical analysis. Antigen detection was done using HiDef detection horseradish peroxidase (HRP) polymer system kit (ready to use) procured from Cell Marque. Endometrial stroma was taken as internal positive control while phosphate buffer solution (PBS) substituted primary antibody for negative control. Sections of approximately 5µm were cut on to poly L-lysine coated slides and were deparrafinised 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 ultraviolet (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 counter-stained with haematoxylin, dehydrated and mounted. Between each step the slides were washed with PBS.

The intensity of staining was graded as no staining (0),