The Value of Immunoexpression of Phosphate and Tensin Homolog, Glucose Transporter-1 and β-Catenin in Endometrioid Carcinoma and its Precursor Lesions

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Abstract

Background: Endometrial carcinoma is the most common malignancy in the female genital tract and associated with considerable degree of morbidity. Endometrial carcinoma has traditionally been divided into type I and type II cancers. Type I tumors are low grade endometrioid carcinoma that frequently arise in a setting of excess estrogen and associated with good clinical outcome while type II are high grade which have aggressive clinical course and hormone-independent pathogenesis.

Aim of Work: To study the value of expression of phosphate and tensin homolog (PTEN), glucose transporter-1 (GLUT-1) and β-catenin in endometrioid carcinoma and its precancerous lesions as predictive markers for endometrioid carcinoma.

Methods: PTEN, Glut-1 and β-catenin expressions were evaluated using Immunohistochemical staining in 25 cases of endometrial hyperplasia and 25 cases of endometrioid carcinoma.

Results: Positive PTEN immunoexpression was found in all cases of normal proliferative endometrium, 21 cases (84%) of endometrial hyperplasia, and 13 cases (52%) of endometrioid carcinoma with a highly statistically significant correlation between PTEN immunoexpression in endometrial hyperplasia and endometrioid carcinoma (p.value=0.013). All cases of normal proliferative endometrium, 19 cases (76%) of endometrial hyperplasia were negative Glut-1 immunoexpression while positive Glut-1 immunoexpression was found in 22 cases (88%) of endometrioid carcinoma with a highly statistically significant correlation between Glut-1 immunoexpression in endometrial hyperplasia and endometrioid carcinoma (p.value <0.001). Positive membranous β-catenin immunoexpression was found in all cases of normal proliferative endometrium and 23 cases (92%) of endometrial hyperplasia while no immunoexpression was found in endometrioid carcinoma. Positive cytoplasmic β-catenin immunoexpression was found in 13 cases (52%) of endometrioid carcinoma which was higher than found in endometrial hyperplasia and these findings were statistically significant (Mean: 41 vs 0; p<0.001). Positive nuclear β-catenin immunoexpression was found in 12 cases (48%) of endometrioid carcinoma which was higher than endometrial hyperplasia and these findings were statistically significant (Mean: 3 vs 0; p<0.001).

Conclusions: Loss of PTEN expression as detected by immunohistochemistry is an informative biomarker for endometrial neoplasia. Lack of PTEN expression and GLUT1 overexpression are early events in tumorgensis of endometrioid carcinoma and the different patterns of expression of PTEN and GLUT1 were helped in distinguishing endometrial hyperplasia from endometrioid carcinoma. Cytoplasmic and nuclear β-catenin immunoexpression may be useful for a correct early diagnosis of endometrioid carcinoma.


Introduction

ENDO METRIAL Cancinoma (EC) remains the most common gynaecological malignancy in women across the globe. IT represents the sixth most common cancer in women worldwide [1]. EC is more common in developed countries, where the risk of endometrial cancer is 1.6%, compared to 0.6% in developing countries [2]. In Egypt, endometrial cancer accounted 2.6% of total female neoplasm [3]. The peak incidence is in the 55- to 65-years old age group for endometrioid types [4].

Endometrioid carcinoma accounts for 75% of the endometrial cancers and is thought to develop after a continuum of premalignant lesions ranging from endometrial hyperplasia without atypia to hyperplasia with atypia and finally to well-differentiated carcinoma [8].

Phosphatase and tensin homolog (PTEN) a tumor suppressor gene located on chromosome 10 (10q23.3) is a dual protein/lipid phosphatase [6]. Overexpression of PTEN induces growth inhibition by supporting cell cycle arrest, which needs lipid
phosphatase activity of PTEN, overexpression of PTEN also correlates with decreased levels and nuclear localization of cyclin D1, a key cell cycle molecule regulated by AKT kinase [7]. One of the mechanisms by which PTEN induces cell cycle arrest is by regulating AKT function so that levels of the cell cycle inhibitor p27kip1 is increased [8]. Immunohistochemical detection of PTEN in cycling endometrium reveals high levels of protein expression in all different cell types during the proliferative phase, with diminution or absence of PTEN protein expression in mid secretory glands. It has been shown that PTEN plays several roles in tumor suppression including cell arrest and promotion of apoptosis. Loss of PTEN function predispose endometrial cells to neoplastic transformation particularly in high estrogenic state [9].

GLUT-1 (Glucose Transporter-1) is a basic, high-affinity glucose transporter that normally expressed in erythrocytes, the perineurium of peripheral nerves, renal tubules, germinal center of reactive lymphoid tissue and the blood-brain barrier [10]. Glucose uptake and glycolytic metabolism are enhanced in cancer cells compared to normal cells [11]. It was found that GLUT-1 immunostaining is preferentially expressed in complex hyperplasia with atypia and in adenocarcinoma and it is useful in distinguishing benign hyperplasia from hyperplasia strongly associated with malignancy and the expression of GLUT-1 may be closely related to malignant transformation of endometrial tumors which could be of significant diagnostic as well as therapeutic value in the future [12].

β-catenin is a subunit of the cadherin protein complex and acts as an intracellular signal transducer in the Wnt signaling pathway, it is a member of the catenin protein family and homologous to γ-catenin [13]. Beta-catenin is a proto-oncogene. Mutations and overexpression of β-catenin are associated with many cancers, including hepatocellular carcinoma, colorectal carcinoma, lung cancer, malignant breast tumors, ovarian and endometrial cancer [14].

The aim of this work is to study the value of expression of phosphate and tensin homolog (PTEN), glucose transporter-1 (GLUT-1) and β-catenin in endometrioid carcinoma and its precancerous lesions as predictive markers for endometrioid carcinoma.

**Patients and Methods**

**Patients and tissue Specimens**:  
This retrospective study was carried out on 50 cases of endometrial lesions included 25 cases of endometrial hyperplasia (8 cases simple endometrial hyperplasia, 11 cases complex endometrial hyperplasia without atypia and 6 cases of atypical endometrial hyperplasia) and 25 cases of endometrioid carcinoma. In addition five cases of proliferative endometrium as control group. The specimens were selected from Department of Pathology Faculty of Medicine Zagazig University in the period from September 2012 to September 2014. Specimens were obtained by Dilatation & Curettage (D&C) 49/50 (25 cases of endometrial hyperplasia and 24 cases of endometrioid carcinoma) and Total Abdominal Hysterectomy (TAH) (one case of endometrioid carcinoma) fixed immediately in 10% formalin for 18-24 hours. The exclusion criteria used in current study were (Chronic nonspecific endometritis, endometrial polyp, secretory change or progesterone effect and other types of endometrial carcinoma rather than endometrioid carcinoma) were excluded.

Clinicopathological informations were abstracted from archive files of the corresponding departments. Informed consent was obtained from each patient and the study was approved by the local ethics committee. Tissue specimens were fixed in 10% buffered formalin and embedded in paraffin. Consecutive 4 μm sections were prepared and stained with hematoxylin & eosin (H&E) for histopathological examination, Hyperplastic specimens were evaluated according to the WHO histological evaluation [14].

Endometrioid carcinoma is graded using a 3-tiered system based primarily on architecture and secondarily on cytologic atypia. According to International Federation of Gynecology and Obstetrics (FIGO) system [15].

**Immunohistochemistry**:  
Immunohistochemical staining was carried out using streptavidin-biotin immunoperoxidase technique. 3-5 μm thick sections were cut from formalin-fixed, paraffin-embedded blocks and mounted on positive charged slides. They were deparaffinized in xylene and rehydrated in graded alcohol. The mounted sections were immersed and boiled in a ready to use Dako target retrieval solution (PH 6.0) for 20min, and then washed in phosphate buffer saline (PBS). Thereafter, blocking of endogenous peroxidase activity with 6% H2O2 in methanol was carried out. The slides were then incubated for 60 minutes at room temperature using: Phosphate and tensin homolog (PTEN) antibody, a mouse monoclonal antibody, (Clone: 28H6, dilution 1:100; Thermo Scientific/Lab Vision Corporation, FERMONT, USA); Glucose transporter-1 (GLUT1):
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Rabbit Polyclonal antibody, (dilution 1:200; Thermo Scientific/Lab Vision Corporation, Fermont, USA); Beta-catenin (CTNNB): Rabbit Polyclonal antibody (Clone:17C2, dilution 1:100; Thermo Scientific/Lab Vision Corporation, Fermont, USA). Incubation with a secondary antibody and product visualization were performed (Dako, Glostrup, Denmark) with diaminobenzidine substrate as the chromogen. The slides were finally counterstained with Mayer’s haematoxylin, and washed with distilled water and PBS. Normal prostatic tissue was used as positive control for PTEN, RBCs and perineurium of nerve were used as positive controls for GLUT 1 and tissue from breast cancer was taken as positive controls for Beta-catenin. Their negative controls were obtained by omission of the primary antibody.

**Immunohistochemical evaluation of immunostaining markers:**

PTEN immune-reactivity was graded semi-quantitatively by considering the percentage of staining on the whole section. The immunoreactivity was regarded as positive when brown staining was localized in the nuclei of the normal endometrial glands (an internal positive control) or tumor cells. Extension of PTEN staining of cells was scored as: Negative if <10%; + (10%-50%) and ++ (>50%) of slide’s area was stained positive [16].

GLUT 1 expression was considered positive only if distinct linear membrane staining was present. The percentage of staining ranged from: Negative; Focal if confluent foci of positive cells separated by a significant non-stained area; Diffuse if extensive or most of the cells are stained. The intensity of immunoreactivity ranged from mild, moderate to strong intensity [12].

For evaluation of β-catenin immunoreactivity, a staining reaction of brown granules in the cells was considered positive and loss of staining was considered negative. Positive immunoreactivity was classified as a membranous, cytoplasmic and nuclear. If only a membranous was stained it was classified as membranous positive. If both the membrane and the cytoplasm were stained it was classified as cytoplasmic positive. If both the cytoplasm and the nucleus were stained then it was classified as nuclear positive. Five hundred tumor cells were counted in randomly selected fields in each cases and the proportion of positive cells clumps was evaluated [17].

**Statistical analysis:**

Data were coded and entered to EPI-INFO file using EPI-INFO version 6.1 computer package. The sample mean, standard deviation (SD), and the range were obtained for numerical variables; the frequency, distribution and percentage were calculated for categorized variables. The statistical analysis was done using the Chi-square ($X^2$) test as the data were qualitative data and The ANOVA test was used as it is done on quantitative data. $p<0.05$ was considered statistically significant (S), $p<0.01$ was considered highly statistically significant (HS), and $p>0.05$ was considered none statistically significant (NS).

**Results**

**Clinicopathological characters:**

The current study was conducted on formalin fixed paraffin embedded tissue samples from 50 cases of endometrial lesions. They include 25 cases (50%) of endometrial hyperplasia and 25 cases (50%) of endometrioid carcinoma. The demographic data of our patients were shown in Table (1). The age of studied cases ranged from 0 to >60 year, 68% cases of endometrial hyperplasia was below the age of 40 years while 64% of endometrioid carcinoma was above age of 50 years. This result was statistically highly significant ($p<0.001$).

**I- Phosphate and Tensin homolog (PTEN) immunoeexpression results:**

Positive PTEN immunoeexpression was found in all cases of normal proliferative endometrium in all nuclei of glandular and stromal cells whereas 4 cases (16%) were negative with no statistically significant correlation between PTEN immunoeexpression and different types of endometrial hyperplasia ($p$ value=0. 126). Positive PTEN immunoeexpression was found in 13 cases (52%) of endometrioid carcinoma of which 7 cases (58.3%) were of Grade 1 endometrioid CA, 4 cases (57.1%) were of Grade 2 endometrioid CA and 2 cases (33.3%) were of Grade 3 endometrioid CA ($p$ value =0.575) whereas 12 cases (48%) were negative (Table 3) with a highly statistically significant correlation between PTEN immunoeexpression in endometrial hyperplasia and endometrioid carcinoma ($p$ value=0.013) (Table 2).

**II- Glucose transporter-1 (Glut-1) immunoeexpression results:**

Negative Glut-1 was found in all cases of normal proliferative endometrium, 19 cases (76%) of endometrial hyperplasia while positive membranous Glut-1 immunoeexpression was found in 6 cases (24%) of endometrial hyperplasia, mostly with focal pattern expression in approximately 20% of positive studied cases. There was a statistically significant correlation between Glut-1 immunoex-
pression and different types of endometrial hyperplasia ($p\text{-value}=0.012$). Positive Glut-1 immunoexpression was found in 22 cases (88%) of endometrioid carcinoma of which 10 cases (83.3%) were of Grade 1 endometrioid CA, 6 cases (85.7%) were of Grade 2 endometrioid CA and all cases of Grade 3 endometrioid CA mostly with diffuse strong pattern expression (Table 3). There was a highly statistically significant correlation between Glut-1 expression in the studied cases of endometrial hyperplasia and endometrioid carcinoma (Table 2) ($p\text{-value} <0.001$). There was significant inverse correlation between PTEN & Glut-1 immunoexpression in all studied subjects ($p=0.022$).

**Beta-catenin ($\beta$-catenin) immunoexpression results:**

Positive cytoplasmic $\beta$-catenin immunoexpression was found in 13 cases (52%) of endometrioid carcinoma while all cases of endometrial hyperplasia showed membranous immunostaining these findings were statistically significant (Mean: 41 vs 0; $p<0.001$) (Table 2). Positive nuclear $\beta$-catenin immunoexpression was found in 12 cases (48%) of endometrioid carcinoma which was higher than endometrial hyperplasia and these findings were statistically significant (Mean: 3 vs 0; $p<0.001$) (Table 2).

There was no significant association between membranous or cytoplasmic $\beta$-catenin immunoexpression in different types of endometrial hyperplasia ($p=1.000$) (Table 4). Nuclear $\beta$-catenin immunoexpression was found in complex endometrial hyperplasia with atypia in approximately 33.3% [Mean 0.5 (0-1)] while simple endometrial hyperplasia and complex endometrial hyperplasia without atypia were negative and these findings were highly statistically significant ($p=0.006$) (Table 4). Positive cytoplasmic $\beta$-catenin immunoexpression was higher in Grade 2 endometrioid carcinoma (Mean 50.28) compared with Grade 1 (Mean 40.83) and Grade 3 (Mean 32.00). These findings were not statistically significant ($p=0.227$). Positive Nuclear $\beta$-catenin immunoexpression was higher in Grade 3 endometrioid carcinoma (Mean 10.16) compared with Grade 2 (Mean 3.42) and Grade 1 (Mean 1.17) and these findings were highly statistically significant ($p=0.001$) (Table 4).

**Table (1):** Histopathological diagnosis of the studied cases (N=50).

<table>
<thead>
<tr>
<th>Histological diagnosis</th>
<th>Studied cases (N=50)</th>
<th>No</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Endometrial hyperplasia:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Simple endometrial hyperplasia</td>
<td>(25) (50)</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>Complex endometrial hyperplasia without atypia</td>
<td>11 22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complex endometrial hyperplasia with atypia</td>
<td>6 12</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>FIGO grading system of Endometrioid carcinoma:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 1</td>
<td>12 24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 2</td>
<td>7 14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 3</td>
<td>6 12</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data were expressed as a number (percentage).

**Table (2):** PTEN, Glut-1 and $\beta$-catenin immunoexpression in the studied cases of endometrial hyperplasia & endometrioid carcinoma.

<table>
<thead>
<tr>
<th>PTEN Immunoreactivity</th>
<th>Endometrial hyperplasia (n=25)</th>
<th>Endometrioid carcinoma (n=25)</th>
<th>$\chi^2$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PTEN Immunoreactivity:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative ($&lt;10%$)</td>
<td>4</td>
<td>12</td>
<td>4.504</td>
<td>0.033</td>
</tr>
<tr>
<td>Positive ($\geq10%$)</td>
<td>21</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Glut-1 Immunoreactivity:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>19</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>6</td>
<td>22</td>
<td>16.092</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>$\beta$-catenin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Membranous</td>
<td>(23) 92%</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage of positivity:</td>
<td>100</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean $\pm$ SD</td>
<td>0</td>
<td>0</td>
<td>-7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Median (Range)</td>
<td>0</td>
<td>15-90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytoplasmic</td>
<td>0</td>
<td>41.36±18.94</td>
<td>-6.485</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Percentage of positivity:</td>
<td>0</td>
<td>40 (15-90)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean $\pm$ SD</td>
<td>0</td>
<td>3.96±4.74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (Range)</td>
<td>0 (0-1)</td>
<td>2 (0-18)</td>
<td>-5.680</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nuclear</td>
<td>(2) 8%</td>
<td>(12) 48%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage of positivity:</td>
<td>0.12±0.33</td>
<td>3.96±4.74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean $\pm$ SD</td>
<td>0 (0-1)</td>
<td>2 (0-18)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (Range)</td>
<td>0</td>
<td>2 (0-18)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$p\text{-value} <0.05$ is significant.
Table (3): Immunoreactivity of PTEN and Glut-1 in the studied cases of endometrial hyperplasia & endometrioid carcinoma.

<table>
<thead>
<tr>
<th>PTEN Immunoreactivity</th>
<th>N</th>
<th>PTEN immunoreactivity</th>
<th>GLUT-1 immunoreactivity</th>
<th>$\chi^2$</th>
<th>$p$</th>
<th>$\chi^2$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Negative</td>
<td>Positive (≥10%)</td>
<td>$&lt;1%$</td>
<td>$10-50%$</td>
<td>$&gt;50%$</td>
<td>Focal</td>
</tr>
<tr>
<td>Endometrial hyperplasia:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Simple endometrial hyperplasia</td>
<td>25</td>
<td>4 (16%)</td>
<td>4 (16%)</td>
<td>17 (68%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Complex endometrial hyperplasia without atypia</td>
<td>11</td>
<td>2 (18.2%)</td>
<td>2 (18.2%)</td>
<td>7 (63.6%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Atypical endometrial hyperplasia</td>
<td>6</td>
<td>2 (33.3%)</td>
<td>2 (33.3%)</td>
<td>2 (33.3%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Endometrioid carcinoma:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 1</td>
<td>25</td>
<td>12 (48%)</td>
<td>6 (24%)</td>
<td>7 (28%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Grade 2</td>
<td>7</td>
<td>3 (43%)</td>
<td>2 (28.5%)</td>
<td>2 (28.5%)</td>
<td>3.603</td>
<td>0.462</td>
<td></td>
</tr>
<tr>
<td>Grade 3</td>
<td>6</td>
<td>4 (66.7%)</td>
<td>2 (33.3%)</td>
<td>0 (0%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

$p$-value <0.05 is significant.

Table (4): $\beta$-catenin immunoreactivity in the studied cases of endometrial hyperplasia & endometrioid carcinoma.

<table>
<thead>
<tr>
<th>$\beta$-catenin Immunoreactivity</th>
<th>PTEN immunoreactivity</th>
<th>GLUT-1 immunoreactivity</th>
<th>KW</th>
<th>$p$</th>
<th>Grade 1 (n=12)</th>
<th>Grade 2 (n=7)</th>
<th>Grade 3 (n=6)</th>
<th>F</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membranous:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Grade 1 (n=12)</td>
<td>Grade 2 (n=7)</td>
<td>Grade 3 (n=6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage of positivity:</td>
<td>100%</td>
<td>100%</td>
<td>4 (66.7%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>0.000</td>
<td>(NS)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (Range)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytoplasmic:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Grade 1 (n=12)</td>
<td>Grade 2 (n=7)</td>
<td>Grade 3 (n=6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage of positivity:</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5 (41.7%)</td>
<td>4 (57.1%)</td>
<td>4 (66.7%)</td>
<td>1.588</td>
<td>0.227</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>40.83 ± 15.20</td>
<td>50.28 ± 26.56</td>
<td>32 ± 12.24</td>
<td>42.5 (15-60)</td>
<td>40 (22 – 90)</td>
<td>31.5 (20-46)</td>
<td>(NS)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (Range)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nuclear:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Grade 1 (n=12)</td>
<td>Grade 2 (n=7)</td>
<td>Grade 3 (n=6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage of positivity:</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2 (33.3%)</td>
<td>7 (58.3%)</td>
<td>3 (42.9%)</td>
<td>2 (33.4%)</td>
<td>16.713 &lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>0.5 ± 0.54</td>
<td>3.42 ± 1.61</td>
<td>10.16</td>
<td>0.006</td>
<td>1.17±0.86</td>
<td>5 (41.7%)</td>
<td>4 (57.1%)</td>
<td>4 (66.7%)</td>
<td>16.713 &lt;0.001</td>
</tr>
<tr>
<td>Median (Range)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td>5 (0-1)</td>
<td>4 (0-1)</td>
<td>4 (0-1)</td>
<td>16.713 &lt;0.001</td>
</tr>
</tbody>
</table>

$p$-value <0.05 is significant.

Table (5): Association & correlation between PTEN and Glut-1 immunoreactivity in all studied cases (N=50).

<table>
<thead>
<tr>
<th>PTEN Immunoreactivity</th>
<th>Glut-1 Immunoreactivity</th>
<th>C</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-ve (n=16)</td>
<td>+ve (n=34)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>-ve (n=21)</td>
<td>3</td>
<td>18.7</td>
<td>18</td>
</tr>
<tr>
<td>+ve (n=29)</td>
<td>13</td>
<td>81.3</td>
<td>16</td>
</tr>
</tbody>
</table>

C: Contingency Coefficient. $p$<0.05 is significant.
Fig. (1): Simple endometrial hyperplasia showing marked and diffuse PTEN immunoexpression in almost all glandular and stromal cells (ABC, M.H counter stain X 100).

Fig. (2): Complex endometrial hyperplasia showing positive severe PTEN expression in almost all nuclei of glandular and stromal cells ((ABC, May- er’s Hematoxylin counter stain X 400).

Fig. (3): Complex endometrial hyperplasia with atypia showing positive heterogeneous PTEN immunoexpression pattern in which PTEN-negative hyperplastic cells are adjacent to PTEN-positive hyperplastic cells (ABC, Mayer’s Hematoxylin counter stain X 400).

Fig. (4): Low grade endometrioid carcinoma showing positive nuclear PTEN immunoexpression (ABC, Mayer’s Hematoxylin counter stain X 200).

Fig. (5): High grade endometrioid carcinoma showing negative PTEN immunoexpression in malignant endometrial cells (ABC, Mayer’s Hematoxylin counter stain X 100).

Fig. (6): Simple endometrial hyperplasia showing negative GLUT-1 immunoexpression of endometrial glandular and stromal cells and positive staining of erythrocytes as an internal positive control (ABC, Mayer’s Hematoxylin counter stain X 400).

Fig. (7): Complex endometrial hyperplasia without atypia showing negative membranous GLUT-1 immunoexpression of endometrial glandular and stromal cells and positive staining of erythrocytes as an internal positive control (ABC, Mayer’s Hematoxylin counter stain X 400).

Fig. (8): Complex endometrial hyperplasia with atypia showing positive strong focal membranous GLUT-1 immunoexpression of atypical endometrial glandular epithelial cells (ABC, Mayer’s Hematoxylin counter stain X 400).

Fig. (9): Low grade endometrioid carcinoma showing positive strong diffuse membranous GLUT-1 immunoexpression of malignant endometrial glandular cells (ABC, Mayer’s Hematoxylin counter stain X 400).
Discussion

The pathogenesis of endometrial carcinoma and its precursor lesions is complex and involves many molecular disturbances. The most common altered gene in endometrial carcinoma especially endometrioid carcinoma is PTEN inactivation.

Several studies have found that PTEN inactivation is correlated with clonal growth patterns detected in endometrial hyperplasia and carcinoma [18,19].

It has been shown that PTEN plays several roles in tumor suppression including cell arrest and promotion of apoptosis. Loss of PTEN function
predispose endometrial cells to neoplastic transformation particularly in high estrogenic state [9].

In the present study positive PTEN immunoexpression was observed in all cases of simple endometrial hyperplasia, 81.8% cases of complex endometrial hyperplasia without atypia and 66.7% cases of complex endometrial hyperplasia with atypia with no statistical difference.

This finding that agrees with Sarmadi et al. [16], Rao et al. [20] and Lee et al. [21] who observed that lowered PTEN immunoreexpression in complex endometrial hyperplasia with atypia compared to PTEN immunoreexpression in simple endometrial hyperplasia. Tantbirojin et al. [22], observed that PTEN immunoreexpression was found in 76% of typical endometrial hyperplasia and 40% of atypical endometrial hyperplasia.

These finding is incompatible with Hassab El-Naby et al. [23], who observed PTEN immunoreexpression in 60% of simple endometrial hyperplasia, 100% complex endometrial hyperplasia without atypia and 46.6% complex endometrial hyperplasia with atypia.

Elnashar et al. [9], also observed PTEN immunoreexpression in 37.5% cases of simple endometrial hyperplasia, 58.3% cases of complex endometrial hyperplasia without atypia and 50% of complex endometrial hyperplasia with atypia. These differences in results may be due to differences in number of cases, different technique of staining, the use of different type of primary monoclonal antibodies and the different method of calculating the IHC scores.

In the current study, positive PTEN immunoreexpression was found in 52% of endometrioid carcinoma indicating that loss of PTEN function by mutation or other mechanisms found to be early events in endometrial tumorigensis [24].

These findings are agreed with Sarmadi et al. [16] and Hassab El-Naby et al. [23] who observed that PTEN immunoreexpression in endometrioid carcinoma was 40%, 51.4%, 47.8% and 37.5% respectively.

Elnashar et al. [9] observed marked reduction of PTEN immunoreexpression in approximately 28.5% of endometrioid carcinoma.

In the present study, there is decreasing in PTEN immunoreexpression with increasing the FIGO grades as follow positive PTEN immunoreexpression was found in 58.3% cases of Grade 1, 57.1% cases of Grade 2 and 33.3% cases of Grade 3 endometrioid CA (p.value=0.575). These finding was in agreement with Elnashar et al. [9] who found PTEN immunoreexpression 33.3% Grade 1, 33.3% Grade 2 and 16.6% Grade 3. Hassab El-Naby et al. [23] reported that PTEN immunoreexpression in well differentiated endometrioid carcinoma were representing than that found in less differentiated endometrioid carcinoma 83.3%, 16.7% respectively and this suggesting a higher expression in well differentiated tumors may be due to defects in PTEN gene that associated with loss of the ability of endometrial cells to differentiate and thus increasing its malignancy.

Similar studies obtained by Kawamata et al. [25], Rao et al. [20] found reduction of PTEN positive glands along with increase the grade of endometrioid carcinoma. An et al. [26] also found higher PTEN immunoreexpression in Grade 1 and Grade 2 endometrioid carcinoma compared with Grade 3 but this difference with no statistically significance.

On the contrary Kimura et al. [27] reported PTEN immunoreexpression scores are increased with grades of endometrioid carcinomas as follow 7.6 /± 5.2 in Grade 1, 9.6 /± 5.2 in Grade 2, and 11.9 /± 3.7 in Grade 3 and this finding may be due to PTEN protein might have been induced to inhibit the aggressiveness growth of poorly differentiated carcinoma whereas in well differentiated carcinoma PTEN might have been expressed in a low level.

There was a statistically significant correlation between the immunoreexpression of PTEN in endometrial hyperplasia and endometrioid carcinoma (p=0.033). This finding is consistent with Tantbirojin et al. [22] who found that PTEN immunoreexpression was detected in 40% of endometrial carcinoma, 40% of atypical endometrial hyperplasia, and 76% of typical endometrial hyperplasia. There is a significant statistical difference of PTEN immunoreexpression among proliferative endometrium, endometrial hyperplasia and endometrioid carcinoma group (p=0.004).

The existence of PTEN mutation in endometrial hyperplasia is thought to be a link to an initiating event in endometrial carcinoma of endometrioid type and precede the development of cytological atypia. PTEN mutation was reflected as loss of PTEN protein so anti-PTEN antibody is positive for normal endometrial tissue including glandular epithelium but glands may become negative if mutation toward carcinogenesis occurs and therefore the immunohistochemistry of PTEN expression may be an effective screening methods that can demonstrate the absence of PTEN protein [17].
PTEN immunoeexpression could provide a pathologic tool of importance to seek out precancerous clones of endometrial hyperplasia, signaling a development of malignancy [28].

PTEN inactivation is frequently seen in endometrial tumor particularly endometrioid type suggestive of the PTEN-PI3K-AKT pathway dysregulation. So PTEN immunohistochemistry (IHC) provides an effective tool and easy method to detect PTEN inactivation with potential response to these inhibitors that are targeteting the PI3K-AKT-mTOR pathway [29].

Glucose metabolism governs many functions, because the oxidation of glucose generates a major source of metabolic energy in eukaryotic cells [30]. Thus, glucose regulates transcription, enzymatic activity, hormone secretion and the activity of glucoregulatory neurons. These functions typically are secondary to glucose uptake, which is controlled primarily by the glucose transporter family (Glut 1-14) [31].

Glut-1, is the first member of the Glut family identified [32] that mediate basal glucose transport in cancer cells and regulate the maintenance of energy metabolism in the cells located in limited supply tissue regions [31]. The expression of Glut-1 is absent in most types of normal epithelial cells [12,33].

Increases in glucose consumption help supply the energy that is necessary for tumor cell proliferation and reflect adaptation to the adverse conditions of the tumoral environment as hypoxia is a hallmark of various cancers and is often associated with disease progression which occurred when tumors outgrow the existing vasculature. Thus, tumors respond to hypoxic conditions by activating genes that regulate glycolysis and glucose transport [34]. In cancer-induced starvation, Glut-1 overexpression governed mechanisms that favor tumor growth at the expense of host tissues [35]. Glut-1 is overexpressed in many tumors, including hepatic, pancreatic, breast, esophageal, brain, renal, lung, and cutaneous, colorectal, endometrial, ovarian and cervical cancers. So Glut-1 immunoeexpression in malignant cells indicates increased proliferative activity, energy requirements and aggressive behavior [31]. So higher levels of Glut-1 in cancer indicate a poor prognosis [36].

These metabolic changes have prognostic and diagnostic value [37].

In the present study positive membranous Glut-1 immunoeexpression was found in 18.1% cases of complex endometrial hyperplasia without atypia and 66.7% cases of atypical complex endometrial hyperplasia mostly with strong focal expression pattern, while all cases of simple endometrial hyperplasia were negative (p.value <0.001).

These finding was in agreement with Ashton-Sagar et al. [12] who observed Glut-1 was expressed in 24% cases of complex endometrial hyperplasia without atypia and in 71% cases of complex endometrial hyperplasia with atypia while all cases of simple endometrial hyperplasia were negative. Similar observations were detected in ovarian cancers in which Glut-1 staining was absent in benign ovarian epithelial tumors with gradually increased in expression in invasive tumors compared with borderline tumors [38].

Wang et al. [10] also found that all cases of simple endometrial hyperplasia and complex endometrial hyperplasia without atypia were negative for Glut-1 immunoeexpression, while all cases of complex endometrial hyperplasia with atypia were positive for Glut-1 immunoeexpression ranging from scattered minute foci to several or multiple foci. Thus, Glut-1 immunoeexpression in endometrial hyperplasia appears to be a useful indicators of high risk for development of endometrial carcinoma and it helps to make the distinction of the new WHO classification of the precursor lesions of endometrial carcinoma into two categories; atypical and non atypical hyperplasia [39]. Glut-1 should be helpful to make this distinction which is the hallmark in surgical pathology [12].

In the present study, positive membranous Glut-1 immunoeexpression was observed to be increased with increasing FIGO grading system of endometrioid carcinoma as follows 83.3% cases were Grade 1, 85.7% cases were Grade 2 and all cases of Grade 3 mostly with strong diffuse pattern (p.value= 0.477).

Similar studies obtained by Horree et al. [40]. Jia et al. [41] and Xiong et al. [42] found that all cases of endometrioid carcinoma were positive for Glut-1 immunoeexpression mostly strong diffuse expression as tumor grades increase.

Sadlecki et al. [43] also observed cytoplasmic-membranous Glut-1 immunoeexpression was found in all cases of endometrial adenocarcinoma.

A stepwise increased in Glut-1 immunoeexpression as the tumor grade become poorly differentiated may be due to increased the need of glucose uptake in the tumor cells and So Glut-1 may be important markers in tumor differentiation, as well
as providing energy to rapidly dividing tumor cells [44].

The gradual increase of Glut-1 immunoexpression from hyperplasia to frankly malignant tumors suggest that Glut-1 may be closely related to malignant transformation of epithelial endometrial tumors by supporting their increased need for glucose metabolism.

Glut-1 immunoexpression may be an independent prognostic factor for response of chemotherapy as tumors that overexpressed Glut-1 had more chance of responding completely to chemotherapy, so it may play a role not only in early diagnosis but also helped in the treatment of patients with this disease [12].

There was a statistically weak indirect correlation between PTEN and GLUT 1 immunoexpression in all studied subjects and that is support the molecular pathway of PTEN and GLUT 1 upregulation in the different studied subjects (p=0.022) as these finding agreed with Shackelford et al. [45] and Sadlecki et al. [43] who observed mutation in PTEN led to overactivation of PI3K/AKT/mTOR pathway that leads to uncontrolled growth of the tumor which lead to stabilization of HIF-1 α that change metabolism of glucose from aerobic to anaerobic process and so expression of GLIT1 increase under anerobic conditions.

There was also a growing evidence of a complex interplay between the AKT/PI3K/mTOR pathway and hypoxia [45,46,47].

These finding supported by Wahl et al. [48] who found that GLUT1 was strongly expressed in type I endometrioid carcinoma which has mutant PTEN and when these cases treated with glucose analog (2D), it induced apoptotic cell death. It may suggest that alteration in PTEN pathway may identify the patients who may be benefit from adjuvant treatment with glucose analogs.

We concluded that lack of PTEN immunoexpression and GLUT1 overexpression are early events in tumorigensis of endometrioid carcinoma and the different patterns of expression of PTEN and GLUT1 were helped in distinguishing endometrial hyperplasia from endometrioid carcinoma.

The β-catenin protein encoded by the CTNNB1 gene located in 3p2 1 is very unusual protein with multiple functions depending on cellular localization [49].

β-catenin plays a critical role in maintenance of epithelial cell-cell adhesion and normal tissue architecture [50].

At the membrane β-catenin is a part of the cadherin complex involved in cell-cell adhesion. In the cytoplasm β-catenin mediates Wnt signaling interacting with several different partners (APC, GSK-3b, Axin) and so participating in several cellular processes. In the nucleus B-catenin interact with Lef/Tcf factors that regulates variety of target genes [51].

Normal cells lack the Wnt signal, aberration in Wnt signaling pathway have been implicated in human tumorgenesis. Both the cell adhesion and the transcriptional activating role of β-catenin have been found to be deregulated in human malignancies [49].

In our study, positive membranous β-catenin immunoexpression was found in 23 cases (92%) of endometrial hyperplasia with no expression was observed in endometrioid carcinoma. However positive cytoplasmic and nuclear β-catenin immunoexpression in 13 cases (52%) and 12 cases (48%) of endometrioid carcinoma respectively were found with significant statistics for endometrial hyperplasia (p<0.001).

These findings were in agreement with Norimatsu et al. [50] who found an increase in cytoplasmic and nuclear β-catenin immunoexpression and a loss of membranous β-catenin immunoexpression. This pattern appeared to be useful for the correct diagnosis of EC in endometrial cytology and may aid in the stratification of EC into low grade and high grade EC.

In the current study, all cases of simple endometrial hyperplasia, complex endometrial hyperplasia and 66.7% cases of atypical endometrial hyperplasia showed positive membranous β-catenin immunoexpression while only 33.3% cases of atypical endometrial hyperplasia showed positive nuclear β-catenin immunoexpression and these findings were statistically highly significant (p=0.006).

Hassab El-Naby et al. [23] observed β-catenin immunoexpression in the membrane and the cytoplasm in cases of proliferative endometrium, secretory endometrium, simple endometrial hyperplasia and complex typical hyperplasia, 60% of atypical endometrial hyperplasia, while nuclear staining was found in 33.3% of atypical endometrial hyperplasia.

Norimatsu et al. [50] showed positive membranous β-catenin immunoexpression in all cases of proliferative endometrium and atypical endometrial hyperplasia.
In the present study, Positive cytoplasmic β-catenin immunoexpression was found approximately in 13 cases (52%) of endometrioid carcinoma; 41.7% of cases were Grade 1, 57.1% of cases were Grade 2 and 66.7% of cases were Grade 3 while positive nuclear β-catenin immunoexpression was found in 13 cases (48%) of endometrioid carcinoma; 58.3% of cases were Grade 1, 42.9% of cases were Grade 2 and 33.4% of cases were Grade 3. These findings were highly statistically significant \( p=0.001 \).

These results were in agreement with Fukuchi et al. [52] and Hassab El-Naby et al. [23] who observed β-catenin immunoexpression in the membrane and the cytoplasm of 81.25% of endometrioid carcinoma. Nuclear staining was noted in 37.5% of endometrioid carcinoma; of which 83.3% were well differentiated and 16.7% were poorly differentiated endometrioid carcinoma.

Norimatsu et al. [50] also observed cytoplasmic β-catenin immunoexpression in 49.5% of endometrioid carcinoma of which 31.3% were well differentiated and 46.1% were poorly differentiated endometrioid carcinoma while nuclear staining was noted in 6% of endometrioid carcinoma mostly were in poorly differentiated groups.

Similar results obtained by Shih et al. [53] who found positive cytoplasmic β-catenin immunoexpression in 40% cases of Grade 1, 34.6% cases of Grade 2 and 32% cases of Grade 3 endometrioid carcinoma while the frequency of positive nuclear β-catenin immunoexpression was found in 5.3% cases of Grade 1, 5.5% cases of Grade 2 and 6.1% cases of Grade 3 endometrioid carcinoma.

Schlosshauer et al. [54] observed nuclear expression of β-catenin in 47% endometrioid carcinoma but no expression was found in serous carcinomas.

In contrast, Schelton et al. [55] who reported negative β-catenin immunoexpression in 33% of endometrial carcinomas especially with Grade 3, Norimatsu et al. [50] also reported negative β-catenin immunoexpression in high grade endometrioid carcinoma than in low grade and these supported that β-catenin immunoexpression in the cytoplasm and nucleus has been related to well differentiated ECs and loss of β-catenin expression has been related to invasive ECs.

Cytoplasmic β-catenin is a key element in the Wnt signal transduction pathway and its nuclear translocation has been linked to the induction of proto-oncogene such as c-myc, cyclin D1, c-jun and fra1 among others [54].

Saegusa et al. [56] noticed that nuclear β-catenin immunoexpression seemed to be resulted from abnormality of Wnt/B-catenin/Lef-1 signaling which is characteristic for type 1 endometrioid carcinoma. These abnormalities probably due to mutation in exon 3 of β-catenin gene or alteration of other genes involved in Wnt/ B-catenin/Lef-1 signaling pathway lead to stabilization of the protein, cytoplasmic and nuclear accumulation and signal transduction and transcriptional activation [50].

β-catenin immunoexpression is characteristic of the cytoplasm and the nucleus and may be useful for a correct early diagnosis of endometrioid carcinoma [50].

Conclusions:

Loss of PTEN expression as detected by immunohistochemistry is an informative biomarker for endometrial neoplasia including precancerous lesions. Lack of PTEN expression and GLUT1 overexpression are early events in tumorigenesis of endometrioid carcinoma and the different patterns of expression of PTEN and GLUT1 were helped in distinguishing endometrial hyperplasia from endometrioid carcinoma. β-catenin immunoexpression is characteristic of the cytoplasm and the nucleus and may be useful for a correct early diagnosis of of endometrioid carcinoma.

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الملخص العربي

يعتبر سرطان ببطانة الرحم الأكثر الأورام الخبيثة شيوعاً في الجهاز التناسلي للإناث، وتتطلب درجة كبيرة من الاعتقال. ينقسم سرطان ببطانة الرحم إلى ثلاثة أنواع أساسية.

الهدف من العمل: هو إجراء العملية ودراسة قياس التفارقة في الفص الوعائي من الأورام اللوزية الليفي من الأورام الخبيثة الغينية من الأورام الرحمية لقياس التفاضل بين الأمراض الخبيثة والمرضية الساقية للسرطان بوصفها علامات تنبؤية للمضاعفات المرضية.

الطريقة: أشتقت الدراسة الحالية على 25 حالة من حالات تضخيم ببطانة الرحم و65 حالة سرطان ببطانة الرحم وتتم معالجتها بالصفقات المسموعة من الاستفادة من استخدام التلقيح المناعي.

النتائج: تم العثور على الرياح المناعية لـ (الجول-1) في جميع حالات ببطانة الرحم الخبيثية الطبيعية، 41 حالة (82%) من سرطانات ببطانة الرحم ووجود علاقة ذات دلالة إحصائية بين الرياح المناعية (الجول-1) وتضخم ببطانة الرحم وسرطانات ببطانة الرحم من قيمة (p<0.001). وقد أظهرت الرياح المناعية لـ (الجول-1) في جميع حالات ببطانة الرحم الخبيثية الطبيعية، 19 حالة (78%) من فرط تنسج ببطانة الرحم في حين تم العثور على نتائج إيجابية للرياح المناعية لـ (الجول-1) في 22 حالة (88%) من سرطانات ببطانة الرحم ووجود علاقة ذات دلالة إحصائية بين الرياح المناعية في جميع حالات ببطانة الرحم الخبيثية الطبيعية.

و(23) من فرط تنسج ببطانة الرحم في حين تم العثور على أي آثار مناعية في سرطانات ببطانة الرحم. تم العثور على الرياح المناعية لـ (الجول-1) في 12 حالة (24%) من سرطانات ببطانة الرحم التي كانت ضمنت ببطانة الرحم وفوات ذلك ذات دلالة إحصائية (قيمة: 3 مقابل 0.001) في العثور على الآثار المناعية لـ (الجول-1) في جميع حالات ببطانة الرحم التي كانت ضمنت ببطانة الرحم والمطابقة ذات دلالة إحصائية (قيمة: 3 مقابل 0.001).

الاستنتاجات: فردان الرياح المناعية لـ (الجول-1) هو من العلامات البيولوجية للكشف عن سرطانات ببطانة الرحم والانماط المختلفة من الأورام المناعية لـ (الجول-1) يساعد على تمييز تضخيم ببطانة الرحم عن سرطانات ببطانة الرحم والانماط المختلفة من الأورام المناعية لـ (الجول-1) ويساعد على التشخيص المبكر لسرطانات ببطانة الرحم ومراقبة الأورام المناعية والتأثيرات على سرطانات ببطانة الرحم.