Expression Immunohistochemical Study of CA-125 in Endometrial Hyperplasia in Correlation to its Grade and Progressive Endometrial Carcinoma.

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Abstract
CA-125 tumor marker is encountered in most patients with ovarian carcinoma and little has been reported about its role in endometrial carcinoma. This study was designed to assess the immunohistochemical expression of CA-125 in paraffin blocks of patients with endometrial hyperplasia and endometrial carcinoma and to clarify any correlation between these two conditions. Forty four patients with different grades of endometrial hyperplasia ranging from 38 to 56 years and 16 cases of endometrial carcinoma ranging from 48 to 69 years were included in this study and conducted in Department of Pathology, College of Medicine, Kufa University, during the period from December 2001 to November 2003. CA-125 tumor marker immunohistochemical overexpression was reported in 38.6% of endometrial hyperplasia and 62.5% of endometrial carcinoma, it looks to be more frequently reported in those with adenomatus and atypical types than simple cystic type, and well correlation has been reported between the intensity of expression and the grade of endometrial hyperplasia as well as endometrial carcinoma. We conclude that endometrial hyperplasia and endometrial carcinoma may share the same pathogenesis in some aspect. CA-125 tumor marker expression could be considered as an early predictive parameter for the diagnosis of endometrial carcinoma and could be used as a screening test for assessment of malignant potential of endometrial hyperplasia. Up to our knowledge, we didn’t find any published study regarding CA-125 immunohistochemistry in endometrial hyperplasia in Iraq and surrounding areas.
**Introduction**

Endometrial hyperplasia is a pre-malignant disease of endometrium. It is less well characterized because these lesions cannot be identified clinically and their detection is dependent on blind biopsy [1,2]. Numerous studies have since largely confirmed the malignant potential of certain endometrial hyperplasia, and the distinguishing feature from malignancy is the presence or absence of cytological atypia [3-5]. CA-125 is a glycoprotein of high molecular weight that was found in coelomic epithelium during embryogenesis and in patients with nonmucinous ovarian carcinoma. Adenocarcinomas arising in endometrium, endocervix, folla-pain tube and ovarian carcinomas react with monoclonal antibody OC125. This monoclonal antibody is directed against a glycoprotein found in non-mucinous ovarian carcinomas. CA-125 is not totally specific for ovarian carcinomas [6-7]. CA-125 is a substance that some endometrial cancer cells secrete into the bloodstream. If a woman's cancer appears to have cells that secrete CA-125, a CA-125 assay can provide some measures of whether the cancer has spread to other parts of the body. Serum CA-125 is elevated in some endometrial cancer patients [7]. CA-125 tumor marker is encountered in most of patients ovarian carcinoma and little has been reported about its role in both endometrial hyperplasia and endometrial carcinoma [8-10]. Endometrial hyperplasia is classified into [11]:

(I) Low grade hyperplasia includes simple hyperplasia and complex hyperplasia, simple hyperplasia also known as cystic hyperplasia or mild hyperplasia is characterized by cystic glandular alteration. Complex hyperplasia also known as adenomatous hyperplasia without atypia, less than 5% of these lesions evolve into carcinoma.

(II) Higher grade hyperplasias are usually termed atypical hyperplasia or adenomatous hyperplasia with atypia, 23% of patients with a typical hyperplasia eventually develop adenocarcinoma.

The main objectives of investigation of women found to have adenomatous or atypical endometrial hyperplasia are to exclude invasive endometrial cancer of ovarian cancer and to rule out an endogenous source of oestrogen secretion [12].

**Aims of the study**

This study was designed to assess the immunohistochemical expression of CA-125 in paraffin blocks of patients with endometrial hyperplasia and endometrial carcinoma and to clarify any correlation between these two conditions.

**Materials and Methods**

**Selection of patients**

1- **Study group** includes 44 patients with histologically confirmed endometrial hyperplasia, their ages were ranging from 38-55 years with a mean age of 43 years and 16 patients with histologically confirmed endometrial carcinoma, their ages were ranging from 42-75 years with a mean age of 53.5 years.

2- **Control group** includes 26 patients with normal looking endometrial tissue (normal D&C). **Positive Control** tissues was histologically confirmed ovarian edenocarcinoma known to be positive for CA-125. While **negative control** tissue was one case in each set of immunostaining slide not exposed to primary antibody.

**Histopathological examinations**

Endometrial tissue specimen were taken by D&C for patients. Tissue samples were fixed by 10% formation for at least 24hr and then processed automatically through series of alcohol and xylol, then tissues were embedded in paraffin block. Section 4-5 micron thickness from these formalin fixed, paraffin embedded tissues were taken and stained with hematoxyline and eosin (H&E) for routine histopathological examination. Endometrial hyperplasia is graded into low (simple cystic glandular...
hyperplasia and moderate adenomatous endometrial hyperplasia) and high grade (atypical adenomatous) glandular hyperplasia.

**Immunohistochemistry**[13]: Sections of 4-5 microns from formalin fixed paraffin embedded tissues were prepared for immunohistochemistry to assess the reactivity and expression of CA-125 using monoclonal anti- CA-125 antibody (biogen X Co) at dilution 1:50 for 30 minutes using avidin- biotin complex method and then staining procedure proceeds until final DAP (diamino benzedin) Step. The intensity of immunostaining was graded into; score+1 less than 10% of cells were positive, score +2 less than 25% of cells were positive, score +3 less than 50% of cells were positive, and score +4 more than 50% of cells were positive according to(Sophia K.)[14].

Statistical analysis of all results were proceeded by the help of SPSS statistical package using Chi square test at level of significance alpha = 0.05, Fisher’s exact test, and correlation test (r at a significant level of 0.3).

**Results**

Histopathological analysis revealed that 44 (78.6%) patients were found to have endometrial hyperplasia and 16 (21.4%) patients have endometrial carcinoma (Table.1). Low grade endometrial hyperplasia formed 90.9% (40 cases); of these, simple cystic glandular hyperplasia formed 67.5% (27 out 40 cases ) and adenomatous hyperplasia formed 32.5% (13 out 40 cases). While high grade endometrial hyperplasia (atypical endometrial hyperplasia ) formed 9.1% (only 4 patients) (Table.2).

**Immunohistochemistry of CA 125**

Immunohistochemical analysis of CA-125 expression in endometrial tissue revealed that 38.6% (17 out 44) of patients with endometrial hyperplasia were positive, and 62.5% (10 out 16) of endometrial carcinoma were positive. While only one case (3.8%) of normal control cases was positive, showing focal cytoplasmic immunostaining of the glandular endometrium (Fig.1). There was a significant difference among these groups (P value less than 0.05) (Table.1).

Immunohistochemical expression of CA-125 was reported more frequently in high grade endometrial hyperplasia than low grade endometrial hyperplasia (75% Vs 35%) with a significant difference between them (P value was less than 0.005)(Table.2)(Fig.2). Simple cystic hyperplasia revealed the lowest immunohistochemical expression rate of CA-125 (18.5%) (5 out 27), while adenomatous hyperplasia revealed higher rate of immunoreactivity for CA-125 (69.2%) (9 out 13). Also there was a high significant difference between these groups (P value was less than 0.005). There was no significant difference in CA-125 expression between atypical adenomatous hyperplasia and complex adenomatous hyperplasia (69.2% Vs 75%) (P value was more than 0.05) (Table.2).

All 5 cases of simple cystic hyperplasia were showing faint cytoplasmic staining of score 1 (Fig.3), while most cases of complex adenomatous endometrial hyperplasia and atypical showed intense immunostaining of score 3 and 4 (Fig.3). CA-125 was expressed more intensely in high grade than low grade endometrial hyperplasia. It looks that there was well correlation between the grade of endometrial hyperplasia and the intensity of CA 125 expression (r= 0.03) (Table.3).
Table 1 Immunohistochemical expression of CA-125 in control and study groups cancer in comparison with benign and normal tissue.

<table>
<thead>
<tr>
<th>Type of tissue</th>
<th>CA'125 Immunostaining</th>
<th>Total %</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive %</td>
<td>Negative %</td>
<td></td>
</tr>
<tr>
<td>Normal endometrial tissue</td>
<td>1 3.8</td>
<td>25 96.2</td>
<td></td>
</tr>
<tr>
<td>Endometrial Hyperplasia</td>
<td>17 38.6</td>
<td>27 61.4</td>
<td>44 51.2 &lt;0.005</td>
</tr>
<tr>
<td>Endometrial carcinoma</td>
<td>10 62.5</td>
<td>6 25</td>
<td>16 18.6</td>
</tr>
<tr>
<td>Total</td>
<td>28 32.5%</td>
<td>58 67.5%</td>
<td>86 100%</td>
</tr>
</tbody>
</table>

Table 2 Expression of CA 125 in different types of Endometrial Hyperplasia

<table>
<thead>
<tr>
<th>Histopathologic type Of Endometrial Hyperplasia</th>
<th>CA125 immunostaining</th>
<th>Total N. %</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low grade Simple cystic Adenomatous</td>
<td>14 14 (18.5%)</td>
<td>26 26 (100%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>High grade Atypical Hyperplasia</td>
<td>3 3 (75%)</td>
<td>1 1 (25%)</td>
<td>4 9.1</td>
</tr>
<tr>
<td>Total</td>
<td>17 39.7 %</td>
<td>27 61.3%</td>
<td>44 100%</td>
</tr>
</tbody>
</table>

Table 3 The intensity of P53 Expression in different types of Endometrial Hyperplasia.

<table>
<thead>
<tr>
<th>Histopathologic type Of Hyperplasia</th>
<th>CA125 immunostaining</th>
<th>Total</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low grade N=40 Simple cystic</td>
<td>5 0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Adenomatous</td>
<td>1 3</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>High grade N=4 Atypical Hyperplasia</td>
<td>0 0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Total N=44</td>
<td>6 3</td>
<td>6</td>
<td>2</td>
</tr>
</tbody>
</table>
**Figure 1** Focal CA-125 in the cytoplasm of glandular elements of normal endometrial glands (X40).

**Figure 2** Marked positive CA-125 in the cytoplasm of glandular elements of adenomatous endometrial hyperplasia (X40).

**Figure 3** Marked positive CA 125 in the cytoplasm of glandular elements of endometrial carcinoma (X40).
Discussion

Few studies concerning immunohistochemistry or serum assay of CA125 in endometrial hyperplasia were reported. It has noticed by Mylonas I, and his colleagues (2003) [15], that CA-125 was expressed in normal, hyperplastic and malignant endometrial tissue with a cyclical expression in premenopausal endometrial glandular cells. These findings are consistent to our study, we had found that immunohistochemical expression of CA125 in endometrial tissue was also reported in normal, benign and malignant endometrial tissue but more frequently in endometrial carcinoma rather than endometrial hyperplasia or normal control tissue (62.5%, 38.6% and 3.8% respectively) with a significant difference (P value less than 0.005).

These findings are in consistence with Neunteufel W, Breitenecker G. (1989) [16], they found that 65% of adenocarcinoma were positive, but it is in controversy to Mylonas I, and his colleagues (2003) [15], they had noticed that adenocarcinoma expressed CA-125 with a lower intensity, while our results has found that a higher intensity of CA-125 expression was reported in adenocarcinoma (score 3 & 4) (Fig.3).

It has been found that high grade (atypical) endometrial hyperplasia had expressed CA 125 more frequently than low grade endometrial hyperplasia (75% Vs 35%) with a significant difference between them (P value was less than 0.005). This finding was also consistent with Frauenklinik, et al [17], they found that highest CA-125 reaction was observed in atypical hyperplasia grade III and Mylonas I, and his colleagues (2003) [15] finding that a highest expression was observed in AH grade III in luminal and glandular cells, but in controversy to Neunteufel W, Breitenecker G (1989) [16], they had documented that CA-125 expression does not correlate with the degrees of differentiation or malignancy.

There was also no significant difference in CA 125 expression between the atypical adenomatous hyperplasia and complex adenomatous hyperplasia (69.2% Vs 75%) (P value was more than 0.05) (Table.3). This was really underlined by the malignant potential of high grade endometrial hyperplasia and reflects that possible sharing of some aspects of pathogenesis with endometrial carcinoma.

Simple cystic hyperplasia revealed the lowest immunohistochemical expression rate of CA 125 (18.5%), in comparison with adenomatous hyperplasia (69.2%) with a highly significant difference between these groups (P value was less than 0.005). This evidence is agreed with Frauenklinik, et al [17].

It has been found that there was well correlation between the grade of endometrial hyperplasia and the intensity of CA 125 expression (r = 0.03) (Table.3). This evidence was also agreed with Mylonas I, and his colleagues (2003) [15] finding. CA 125 was expressed more intensely in high grade than low grade endometrial hyperplasia as noticed that faint cytoplasmic staining of score 1 was reported in simple cystic hyperplasia and intense immunostaining of score 3 and 4 (Fig.3) was reported in complex adenomatous endometrial hyperplasia and atypical endometrial hyperplasia. There was no published paper concerning with CA-125 expression in simple cystic glandular hyperplasia.

Conclusions and recommendations:

It looks that CA125 immunostaining is more frequently reported in atypical adenomatous endometrial hyperplasia (grade III) rather than simple cystic adenomatous (grade I) or simple cystic glandular hyperplasia (grade II). There was a well correlation between the intensity and the grade of endometrial hyperplasia (r =0.3). There was no significant difference in CA125 expression in both atypical adenomatous endometrial hyperplasia and adenocarcinoma in both the rate and intensity of immunostaining.
We conclude that endometrial hyperplasia and endometrial carcinoma may share the same pathogenesis in some aspects. CA-125 tumor marker expression could be considered as an early predictive parameter for the diagnosis of endometrial carcinoma. It might be recommended that the use of CA-125 as a screening test may help the assessment of the malignant potential of endometrial hyperplasia.

References
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