

RESEARCH ARTICLE

Pattern of Tissue Expression of CA-125 and HE4 in Primary Epithelial Ovarian Tumours and Correlation with Serum CA-125 Levels

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Abstract

The objective of this study is to assess tissue expression of CA-125 and HE4 protein in primary benign and malignant epithelial tumours of the ovary and correlate with serum CA-125 levels. A total of 100 formalin-fixed, paraffin embedded sections of ovarian tumours which included serous adenoma (11), mucinous adenoma (42), serous carcinoma (20), mucinous carcinoma (12) and endometrioid carcinoma (15), histologically diagnosed between 1st January 2004 to 31st December 2012 at the University Malaya Medical Centre, were stained for HE4 (rabbit polyclonal antibody, Abcam, UK) and CA-125 (mouse monoclonal antibody clone: OC125, Cell Marque Corporation, Rocklin, California, USA). Pre-operative serum CA-125 levels were obtained from the laboratory information system. Immunoscore (I score) for HE4 and CA-125 was given based on the intensity of staining and percentage of positive tumour cells and considered significant when it was >50 (intensity of staining multiplied by percentage of positive tumour cells). Serum CA-125 levels were compared with the I score of HE4 and CA-125 in tissues. We noted that the CA-125 levels in serum and tissues were significantly raised in malignant compared to benign ovarian tumours (p value<0.05). Tissue expression of HE4 protein was also significantly raised in malignant tumours compared to benign tumours (p value<0.05). We conclude that HE4 can be a useful tissue immunomarker in addition to CA-125.

Keywords: Immunohistochemistry - HE4 - CA-125 - serum CA-125 - ovarian tumours

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Introduction

Ovarian cancer is the fifth most common cause of cancer related deaths among women in the United States (Drapkin et al., 2005). In Malaysia, ovarian cancer is the fourth leading cause of cancer related deaths and the fifth commonest cause of cancer in women (Lim et al., 2008). It mainly affects those beyond the age of 40 years with the highest number of cases seen in those between the ages of 50-59 years (Lim et al., 2008). The risk factors for ovarian cancer are not as clear as for other genital tumours with general agreement on two: nulliparity and family history. At the time of diagnosis, three quarters of patients have locally advanced or disseminated disease that is characterized by diffuse intraperitoneal spread and in many cases, malignant ascites. Late diagnosis is most often due to the fact that patients generally do not have any symptoms until the disease has progressed to a more advanced stage with the survival rate being <20% despite aggressive surgery and chemotherapy (Hogdall et al., 2007).

There is no sensitive or specific test available to screen for the disease at an early stage which has a higher cure rate. Great emphasis is placed on early detection in trying to reduce ovarian cancer mortality (Hogdall et al.,

2007). Cancer antigen 125 (CA-125) was first identified in 1981 as a protein elevated in the serum of women diagnosed with ovarian cancer (Bast et al., 1981). CA-125 is one of the most widely used serum tumour marker for ovarian malignancy. Most oncology societies recommend the use of CA-125 for the differential diagnosis of a suspected pelvic mass, monitoring efficacy of treatment and detection of recurrence of ovarian cancer (Strugeon et al., 2008). However it is frequently elevated in other conditions such as endometriosis (Markman 1997) and liver cirrhosis (Bergman et al 1986). It is also not elevated in early stage of cancer (Terry et al., 2004; Paramasivam et al., 2005). A new serological marker which has been used for detection of ovarian cancer is Human Epididymal Protein 4 (HE4). It was first described in the male epididymis (Kirchhoff et al., 1991). However it was later found that HE4 protein was also expressed in the tissue of epithelial ovarian carcinomas (Drapkin et al., 2005). In normal tissues, HE4 expression is highly restricted to epithelia in the reproductive tracts (Galvano et al., 2006). Similar to CA-125, HE4 has also been found to be raised in the serum of individuals with epithelial ovarian tumours (Hogdall et al., 2007). Hence it is being used in combination with CA-125 in the identification and monitoring of patients with epithelial ovarian tumours.

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In the present study we assessed the tissue expression of CA-125 and HE4 in benign as well as malignant ovarian tumours of those cases who had serum CA-125 measurement prior to surgery. Serum HE4 test is not available in our centre at present.

Materials and Methods

Sample selection

All histologically diagnosed primary ovarian epithelial neoplasms between 1st January 2004 to 31st December 2012 at the Department of Pathology, University of Malaya Medical Centre (UMMC) were retrieved. The slides were reviewed. Serum CA-125 levels were obtained from the laboratory information system. Information on staging of tumours was obtained from the medical records. The histological slides of only those cases where there was preoperative serum measurement of CA-125 levels were chosen. The retrieved Hematoxylin-eosin stained slides were examined and one section containing tumour from each case was selected for immunohistochemical staining. Staining was performed on a Benchmark XT automated stainer (Ventana Medical Systems, Inc, Tucson AZ). Serum CA-125 levels done prior to surgery were used for analysis. The reference level used in the laboratory is <35U/ml for CA-125.

Immunohistochemistry

The selected parafinized archival tissue blocks are subjected to several steps of processing including sectioning with a microtome at room temperature to obtain 3µm sections. The ribbons of sections are then floated in a water-bath at a temperature of 50°C and ‘fished’ and then mounted onto the silanized slides. The Benchmark XT automated stainer includes online deparafinisation and antigen retrieval. Pre-diluted Anti-CA-125 antibody, (a mouse monoclonal antibody from Abcam) was incubated at 37°C for 16 minutes and Anti-HE4 antibody (a rabbit polyclonal antibody from Cell Marque) which was diluted to 1:50 was incubated at 37°C for 20 minutes. Antigen detection was performed using OptiView DAB IHC Detection Kit. Hematoxylin II was used as a counterstain (Ventana Medical Systems, Inc, Tucson AZ). Stained slides are evaluated for CA-125 and HE4 expression. HE4 and CA-125 staining was considered positive when there was cytoplasmic and apical staining of the epithelial lining and cytoplasmic and membranous staining of the cytoplasm respectively. Human epididymis was used as positive control for HE4 and a known ovarian serous adenocarcinoma was used as a positive control for CA-125. Negative controls for CA-125 and HE4 were performed by incubating samples without primary antibody. CA-125 and HE4 immunoscore was obtained by multiplying the percentage of positive cells with the numeric score. The percentage of positive cells was determined by estimating the number of cells which were positive for the respective immunohistochemical stain by histopathological examination. The numeric score was given as follows: score 0, no staining: cytoplasm and membrane blue; score 1, weak staining: cytoplasm and membrane blue-brown; score 2, cytoplasm and membrane

brown; score 3, cytoplasm and membrane deep brown or black (Figure 1). Scores including and above 50 were considered significantly raised.

Statistical analysis

SPSS for Windows 17.0 software package was used for statistical analysis. Spearman’s correlation was used to describe the correlation between serum CA-125 levels and CA-125 immunoscore in both benign and malignant tumours. Chi-square test was used to analyse the difference in the HE4 immunoscore of benign and

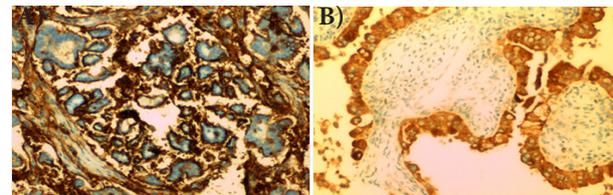


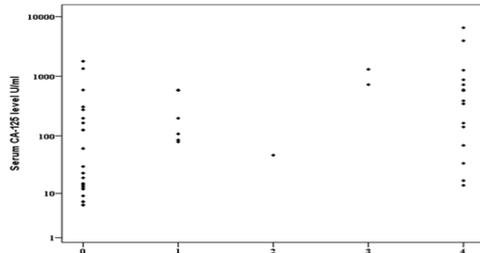
Figure 1. Strong Cytoplasmic and A) Membranous Staining for CA-125 in Endometrioid Adenocarcinoma; B) Apical Staining of HE4 in Serous Adenocarcinoma

Table 1. Tumour Type, Staging, Serum CA-125 Level and Immunohistochemical Expression of CA-125 and HE4 in Ovarian Tumour Tissue

Tumour type (No.)	Serum CA-125 U/ml		Tissue CA-125 I score	
	<35 (%)	>35 (%)	<50 (%)	>50 (%)
CA-125:				
Serous cystadenoma (11)	11 (100)	0 (0)	4 (36.4)	7 (63.4)
Mucinous cystadenoma (42)	36 (85.7)	6 (14.3)	38 (90.5)	4 (9.5)
Serous carcinoma (20)	3 (15)	17 (85)	4 (20)	16 (80)
Stage I (2)	1	1	1	1
Stage II (3)	1	2	0	3
Stage III (12)	1	11	2	10
Stage IV(3)	0	3	0	3
Mucinous carcinoma (12)	6 (50)	6 (50)	11 (91.7)	13 (8.3)
Stage I(9)	6	3	8	1
Stage II (1)	0	1	1	0
Stage III (1)	0	1	1	0
Stage IV (1)	0	1	1	0
Endometrioid carcinoma(15)	7 (46.7)	8 (53.3)	8 (53.3)	7 (46.7)
Stage I (4)	3	1	4	0
Stage II (0)	0	0	0	0
Stage III (10)	4	6	5	5
Stage IV (1)	0	1	0	1
Tumour type (No.)	Serum CA-125 U/ml		HE4 I score	
	<35 (%)	>35 (%)	<50 (%)	>50 (%)
HE4:				
Serous cystadenoma(11)	11 (100)	0 (0)	9 (81.8)	2 (18.2)
Mucinous cystadenoma (42)	36 (85.7)	6 (14.3)	23 (54.8)	19 (45.2)
Serous carcinoma(20)	3 (15)	17 (85)	3 (15)	17 (85)
Stage I(2)	1	1	1	1
Stage II (3)	1	2	1	2
Stage III (12)	1	11	0	12
Stage IV(3)	0	3	0	3
Mucinous carcinoma (12)	6 (50)	6 (50)	0	12(100)
Stage I(9)	6	3	0	9
Stage II (1)	0	1	0	1
Stage III (1)	0	1	0	1
Stage IV (1)	0	1	0	1
Endometrioid carcinoma(15)	7 (46.7)	8 (53.3)	2 (13.3)	13 (86.7)
Stage I(4)	3	1	0	3
Stage II (0)	0	0	0	0
Stage III (10)	4	6	1	9
Stage IV (1)	0	1	1	0

Table 2. Tumour Type and Immunohistochemical Expression of CA-125 and HE4 in Ovarian Tumour Tissue

Tumour type (number)	Serous cystadenoma(11)	Mucinous cystadenoma (42)	Serous carcinoma(20)	Mucinous carcinoma (12)	Endometrioid carcinoma(15)
Tissue CA-125 I score <50(%)	4 (36.4)	38 (90.5)	4 (20)	11 (91.7)	8 (53.3)
Tissue CA-125 I score >50(%)	7 (63.4)	4 (9.50)	16 (80)	13 (8.3)	7 (46.7)
HE4, I score <50(%)	9 (81.8)	23 (54.8)	3 (15)	0	2 (13.3)
HE4, I score >50(%)	2 (18.2)	19 (45.2)	17 (85)	12 (100)	13 (86.7)

**Figure 2. Graph Showing Serum CA-125 Levels and Tissue Expression of CA-125 (I Score) in Malignant Epithelial Ovarian Tumours**

malignant ovarian tumours. $p < 0.05$ was considered as significant difference.

Results

One hundred cases (11 serous adenomas, 42 mucinous adenomas, 20 serous carcinomas, 12 mucinous carcinomas and 15 endometrioid carcinomas) were included in the study. Borderline tumours were not included in the study due to the small numbers. The intensity of staining is shown in Figure 1. Table 1 shows the type of tumour, serum CA-125 level and immunoscore (I score) of tissue CA-125 and HE-4 protein. The malignant tumours (47 cases) were staged as follows: stage I (15 cases), stage II (4 cases), stage III (22 cases) and stage IV (6 cases). Significant correlation between serum and tissue expression of CA-125 in malignant ovarian tumours ($p < 0.05$) as shown in Figure 2. There was significant association between staging of the tumour and tissue and serum CA-125 ($p < 0.05$) and significant difference in the expression of HE4 in benign and malignant ovarian tumours ($p < 0.05$). No correlation was noted between serum CA-125 and tissue expression of either CA-125 or HE4 ($p > 0.05$).

Discussion

The findings of significantly increased serum and tissue expression of CA-125 in malignant epithelial ovarian tumours is similar to earlier study (Rosen et al., 2005). We noted that those 10 cases that had low serum CA 125 levels were in Stage 1 disease (62.5%). This is higher than reported by others (Montagnana et al., 2005; Paramasivam et al., 2005). On the other hand, no tissue expression of CA-125 was noted in 10 cases, despite high serum CA-125 levels and eight (80%) were diagnosed as mucinous carcinoma. The high percentage of mucinous carcinomas with loss of tissue expression of CA-125 in our study mirrors reports by others (Rosen et al., 2009). It was suggested CA 125 can be lost in tumour tissues during fixation or processing or due to insufficient sampling

(Breitenecker et al., 1989; Rosen et al., 2009).

In our study, we found no significant correlation between serum CA-125 levels and tissue expression of CA-125 in benign epithelial ovarian tumours. This means that, serum CA-125 levels are not raised in individuals with benign ovarian tumours and also there is no significant expression of CA-125 in the tissue. This is similar to findings in other studies (Rosen et al., 2009; Escudero et al., 2011). We also noted increased expression of CA-125 in four (9.5%) mucinous adenoma of the 42 cases. However, the intensity of staining was lower compared to malignant ovarian epithelial tumours. This minimal increase was also noted in another study (Rosen et al., 2005). Our findings show that CA-125 is a good marker both in the serum and also in the tissue to evaluate malignant epithelial ovarian tumours.

Among the genes most commonly over expressed in ovarian epithelial tumours relative to normal tissues is the gene for human epididymis protein 4. A number of publications suggest HE4 in combination with CA-125 is better than CA-125 alone for diagnosis of ovarian cancer (Escudero et al., 2011; Kim et al., 2011).

In our study, we found there was significant expression of HE4 immunomarker in malignant epithelial ovarian tumours compared to benign epithelial ovarian tumours. This is a similar finding in other studies (Drapkin et al., 2005; Galgano et al., 2006; Huhtinen et al., 2009). In fact, other studies have shown that HE4 protein is more specific than CA-125 in the diagnosis of malignant epithelial ovarian tumours (Drapkin et al., 2005; Galgano et al., 2006; Rosen et al., 2009).

However in our study, we found that all the cases of mucinous carcinomas showed increase expression of HE4 protein which was not in other studies. Loss of tissue expression was observed in serous carcinoma (15%) and endometrioid carcinoma. In contrast, earlier studies reported that HE4 is over expressed in serous and endometrioid carcinoma and not expressed in mucinous carcinoma (Drapkin et al., 2005; Galgano et al., 2006). This could be due to intratumoral variability of staining and probably warrants extensive sampling of the tumour (Galgano et al., 2006). The other factor that might be taken into consideration is the fact that not all tumours that express HE4 immunomarker also have increased serum levels of HE4. Hence, serum HE4 needs to be compared with tissue expression of HE4. However, we were not able to compare tissue expression of HE4 with serum HE4 level since this is a retrospective study.

In conclusion, our study reaffirms the usefulness of serum CA-125 and CA-125 immunomarker in the diagnosis of malignant epithelial ovarian tumours. We also found that apart from CA-125, HE4 protein is another useful marker that can be used in the diagnosis of

malignant epithelial ovarian tumours. Hence, we suggest the use of HE4 as an adjunct to CA-125 immunomarker in the diagnosis of malignant epithelial ovarian tumours.

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