Original Research Article

Utility of serum HE4 level to diagnose epithelial ovarian cancer - A cross sectional study

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Abstract

Background: Epithelial Ovarian cancer is most common and lethal gynecological malignancy. Diagnosis of ovarian cancer at early stage remains in challenge because of unavailability of accurate tumor marker that detects cancer at early stage. Human epididymis protein 4 (HE4) level was significantly over expressed in the ovarian cancers compare to benign diseases and healthy controls therefore, it can be used to detect malignant ovarian cancer at early stage. **Objectives:** Indentify sensitivity and specificity of HE4 to diagnose epithelial ovarian cancer and its association with clinical variable of epithelia ovarian cancer. **Materials and Methods:** It is a cross sectional study. A total of 50 epithelial ovarian cancer (EOC) patients, 50 benign ovarian tumor and 50 age-matched healthy females were included in the study. The serum CA-125 was analyzed on ELECSYS 2010 (Roche diagnostics) by electrochemilumine scence immunoassay method. Serum HE4 was measured by using ELISA kit (Biovender R and D). Cut off values of CA-125 and HE4 was < 35 U/mL and<140 pmol/L according to manufacture instruction. **Results:** Both serum HE4 (P<0.001) and CA-125 (P<0.05) levels were high in epithelia ovarian cancer groups compared to benign tumor and controls. HE4 has an 82.0% sensitivity to detect ovarian cancer and 84.0% specificity to differentiate benign and ovarian cancer. Serum HE4 (> 140 pmol/l) detect more ovarian cancer compared to CA-125 (>35 U/ml) (76.1 % vs 71.4%) in reproductive age. HE4 detect more ovarian cancer compared to CA-125 in stage I and II (83.3% vs 58.3%). **Conclusion:** Serum HE4 levels was found more in epithelial ovarian cancer. HE4 has high specificity and positive predictive value to differentiate epithelial ovarian cancer from benign ovarian cases. HE4 has more sensitivity to detect ovarian cancer in premenopausal group and in early stage of cancer.

Key Words: Serum HE4.

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INTRODUCTION

Ovarian cancer is the 7th most common cancer and the 8th most common cause of death from cancer in women with overall life time risk is 1.6%.[1,2] Most of (>75 %) the ovarian cases were detected at late stage as early stage cancer mostly remains asymptomatic. Patients with advanced disease have a worst prognosis with the 5 year survival rate of only 10-20%.[3] Diagnosis of ovarian cancer at early stage remain in challenge because of unavailability of accurate tumor marker that detect cancer at early stage.[4] There is a need for diagnostic test that detect ovarian cancer at early stage. In current scenario generally CA 125 is used to detect ovarian cancer preoperatively.[5] But it has limited specificity as it also increased in cancer other than ovary,[6] endometriosis, pelvic inflammatory disease,[7] ovarian dysfunction, menstruation[8] and pregnancy.[9] Particularly it has a low specificity to detect ovarian cancer in reproductive age group and at early stage.[8] According to current research human epididymis protein 4 (HE4) has been reported as

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another tumor marker for ovarian cancer. HE4 is a precursor of the protein human epididymis protein, encoded by the *WFDC2* gene.[10,11] Serum HE4 level was significantly expressed in the ovarian cancers but not in benign diseases or health control, therefore, it can be used to detect malignant ovarian cancer at early stage.[12,13] It has been reported that HE4 has diagnostic sensitivity similar to CA125, but has more specificity to detect gynecological malignancy.[14,15] Based on above background, aim of the study is to indentify sensitivity and specificity of HE4 to diagnose epithelial ovarian cancer and its association with clinical variable of epithelia ovarian cancer in north Indian population.

MATERIALS AND METHODS

Study population: The study was conducted in Molecular Oncology Lab, Department of Biochemistry, Maulana Azad Medical College and Department of Obstetrics and Gynecology, Lok Nayak Hospital, New Delhi. It was a cross sectional observational study. A total of 50 epithelial ovarian cancer (EOC) patients, 50 benign ovarian tumor and 50 age-matched healthy females were included in the study. Patients with ages between 15-45 years were included in pre menopausal group and ages greater than 45 year were included in postmenopausal group. A detailed history about the onset of disease, any previous treatment and physical examination was carried out. The cancer was staged in according to the International Federation of Gynecology and Obstetrics surgical staging system (FIGO).

Selection of Patients: Patients admitted in obstetrics and gynecology department with complaints of bloating, abdominal or pelvic pain or discomfort, back pain, irregular menstruation, postmenopausal vaginal bleeding, pain or bleeding after or during sexual intercourse, disturbance in bowel and bladder habit and loss of appetite were main target of our study. Total 5 ml of venous blood was collected from target groups in plain vial after informed consent. Then patients were undergone surgery (unilateral salpingo-oophorectomy for reproductive age group and bilateral salpingo-oophorectomy for perimenopausal and postmenopausal women) and tissue was sent for histopathological analysis. Finally hisopatholically confirmed malignant and benign epithelial ovarian cancer patients samples were selected for analysis. Research study was approved by local ethical committee of Maulana Azad Medical College, New Delhi.

Exclusion Criteria: Patients with metastatic ovarian cancer, any other cancer, patients who has received radio/chemotherapy, pelvic inflammatory disease, and endometriosis were excluded from study.

Biochemical Analysis: After collection, blood allowed to clot and centrifuges at 3000 rpm for 15 min for separation of serum. Serum was used for analysis of CA-125 and HE4. The serum CA-125 was analyzed on ELECSYS 2010 (Roche diagnostics) by electrochemiluminescence immunoassay method. Serum HE4 was measured by using ELISA kit (Biovender R and D). Cut off values of CA-125 and HE4 was < 35 U/mL and < 140 pmol/L according to manufacture instruction.

Statistical Analysis: SPSS version 17 was used for statistical analysis. Serum level of HE4 and CA125 was expressed in mean and standard deviations. Difference of mean between study groups was analyzed by student's t-test. Association of high HE4 and CA125 level with different study groups and variable of ovarian cancer was analyzed by chi-square tests. P value <0.05 was considered statistically significant.

RESULTS ABD OBSERVATIONS

Baseline features of benign and malignant ovarian tumor were described in Table 1. Most of the malignant cases were in post menopausal group (68%) and in stage III (60%).

Table 1: Baseline features of benign ovarian tumor and epithelial ovarian cancer

Features	Benign ovarian tumor N (%)	Malignant ovarian tumor (%)
Age (year, mean ± SD)	42.7 ± 11.82	43.0 ± 8.04
Histopathology		
Serous	28 (56 %)	23 (46 %)
Mucinous	10 (20%)	23 (46 %)
Endometroid	8 (16 %)	2 (4 %)
Mixed	4 (8%)	-
Clear cell	-	2 (4 %)
Grading		
Poorly differentiated	-	8 (16 %)
Moderately differentiated	-	37 (74 %)
Well differentiated	-	5 (10 %)
Menopausal status		
Premenopausal	21 (42%)	16 (32 %)
Postmenopausal	29 (58%)	34 (68%)
Staging		
I	-	6 (12%)
II	-	6 (12%)
III	-	30(60%)
IV	-	8(16%)

There were significant differences of serum HE4 and CA-125 levels in benign and ovarian cancer groups. Both serum HE4 (P<0.001) and CA-125 (P<0.05) levels were high in ovarian cancer groups compared to benign tumor and controls (Table 2).

Table 2: Serum level of HE4 and CA 125 in study groups

Group	HE4 in pmol/L (Mean ± SD)	CA-125 in U/ml (Mean ± SD)
Ovarian Cancer	599.7 ± 398.5	426.0 ± 180.7
Benign tumor	144.1 ± 168.7	188.3 ± 51.5
Controls	61.6 ± 100.2	37.1 ± 33.8
	P<0.001*	P<0.001*

*Student's t-test

HE4 has an 82.0% sensitivity to detect ovarian cancer and 84.0% specificity to differentiate benign and ovarian cancer. There was significant difference of distribution of cases that is identified in benign and ovarian cancer group (P<0.001). Similarly sensitivity and specificity of CA-125 was 84.0% and 80.0 % respectively (Table 3).

Table 3: Diagnosis value of serum HE4 and CA125 levels in benign ovarian tumor and ovarian cancer

Group	Benign ovarian tumor	Ovarian cancer	Diagnosis value
HE4			
>140 pmol/L	8 (16%)	41 (82%)	Sensitivity-82.0% Specificity-84.0% PPV-83.67% NPV-82.35% Accuracy-83%
< 140 pmol/L	42 (84%)	9 (18%)	
	*p<0.001		
CA-125			Sensitivity-84.0% Specificity-80.0% PPV-80.76% NPV-83.34% Accuracy-82.0%
>35 U/ml	10 (20%)	42 (84%)	
< 35 U/ml	40 (80 %)	8 (16%)	
	*p<0.001		

*Chi-square test

Table 4: Diagnosis value of serum HE4 and CA125 levels according to menopausal status

Parameter	Premenopausal group N (%)	Post-menopausal group N (%)
HE4		
>140 pmol/L	16/21 (76.1)	25/29 (86.2)
< 140 pmol/L	5/21 (23.9)	4/29 (13.8)
	*p<0.001	
CA-125		
>35 U/ml	15/21 (71.4)	27/29 (93.1)
< 35 U/ml	6/21 (28.6)	2/29 (6.9)
	*p<0.001	

*Chi-square test

Serum HE4 (> 140 pmol/l) detect more ovarian cancer compared to CA-125 (>35 U/ml) (76.1 % vs 71.4) in reproductive age. CA-125 > 35 U/ml detect more ovarian cancer in post-menopausal compared to HE4 (93.1 vs 86.2) It suggests HE4 has more sensitivity to detect ovarian cancer in reproductive age group (Table 4).

Table 5: Diagnosis value of serum HE4 and CA125 levels according to staging

Parameter	Stage I and II	Stage III and IV
HE4		
>140 pmol/L	10/12 (83.3)	31/38 (81.5)
< 140 pmol/L	2/12 (16.4)	7/38 (18.5)
	*p<0.001	
CA-125		
>35 U/ml	7/12 (58.3)	35/38 (92.1)
< 35 U/ml	5/12 (41.7)	3/38 (7.9)
	*p<0.001	

*Chi-square test

HE4 detect more ovarian cancer compared to CA-125 in stage I and II (83.3% vs 58.3%). CA 125 detects more ovarian cancer compared to HE4 in stage III and IV (92.1 % vs 81.5%). It suggests HE4 has more sensitivity in early stage of cancer and CA-125 has more sensitivity in late stage of the cancer (Table 5). We found no any significant association of HE4 with type and grade of histopathology.

DISCUSSION

Ovarian cancer is most lethal gynecological malignancy due to late stage diagnosis, poor treatment response and lack of tumor marker that detect it at early stage. Early diagnosis is a crucial step to decrease survival rate in ovarian cancer management.[16] It has been found that HE4 is a emerging tumor marker for diagnosis and prognosis of ovarian cancer.[17] Overexpression of HE4 was found in ovarian cancer tissue compared to normal tissue results in entry of HE4 in blood and can used to detect ovarian cancer.[18] Aim of the study was to find utility of HE4 to diagnose ovarian cancer and its comparison with currently used tumor marker, CA-125. We found high level of HE4 in ovarian cancer cases compared to benign cases and control groups. A similar result was observed by Moore RG, *et al* (2010).[19] Sensitivity and specificity of HE4 to diagnose ovarian cancer was 82.0% and 84.0% respectively in our study. Sensitivity and specificity of CA-125 was 84.0% and 80.0% respectively. HE4 has more specificity and positive predictive value to detect ovarian cancer compared to CA-125. A similar result was reported by Lowe KA, *et al* (2008).[20] We found that HE4 was slight more accurate to differentiate ovarian cancer from benign cases compared to CA-125. HE4 also has more sensitivity to detect to detect ovarian cancer in premenopausal group and in early stage (Stage I and II). Shah CA, *et al* (2009)[21] and Montagnana M, *et al* (2009)[22] were also reported high sensitivity in reproductive age groups. In clinical practice, serum HE4 should be used along with CA-125 for better diagnosis of ovarian cancer.

Limitations: Study should be done on large population with ROMA index to validate the results

CONCLUSION

Serum HE4 levels was found more in epithelial ovarian cancer. HE4 has high specificity and positive predictive value to differentiate epithelial ovarian cancer from benign ovarian cases. HE4 has more sensitivity to detect ovarian cancer in premenopausal group and in early stage of cancer.

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