Original Article

Serum Levels of Human Epididymis Protein 4 and Cancer Antigen 125 in Different Histological Types of Ovarian Cancer

Different Histological Types of Ovarian Cancer

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ABSTRACT

Objective: To determine level of HE4 and CA125 in patients of ovarian cancer, to determine diagnostic accuracy of HE4 alone and in combination with CA125 for ovarian carcinoma against histopathology report as the gold standard and compare the serum levels of HE4 and CA125 in different histological types of the tumor.

Study Design: Descriptive study.

Place and Duration of Study: This study was conducted at the Armed forces Institute of Pathology (AFIP), Rawalpindi in collaboration with North West School Of Medicine, Peshawar. Duration was one year from March 2015 to March 2016.

Materials and Methods: In this study a total of one hundred and twenty seven women (patients n=87, controls n=40), age greater than 18 years were enrolled. Women with suspected ovarian malignancy admitted in gynecology ward of North West School of Medicine, were included after written informed consent. Pregnant women, one receiving treatment of ovarian malignancy and those unable to give informed consent were not eligible. All patients underwent imaging by pelvic/abdominal ultrasound to document their presence of ovarian mass. Clinical information was retrieved from the patient's hospital notes. All patients were diagnosed preoperatively in laparoscopy/laparatomy and confirmed by histopathological evaluation. Ovarian cancer subjects were histologically typed according to WHO classification 2003 by specialized histo pathologists.

Results: Total number of patients were 127.Out of which 87 patients had ovarian cancer while 40 had benign disorders. The mean age of patients with benign tumors was 40 which were significantly lower than those with malignant tumors (58 years old, respectively, $p < .001$). The median range of CA125, 14 (12 -4140) and He4, 913 (58 – 2612) tumor markers were significantly elevated ($P <.001$) in ovarian cancer group compared to benign group i.e CA125 14 (4-241) and HE4 60 (37-151). From Receiver operator characteristic curve analysis, the area under the curve (AUC) was higher for HE4 at 0.934 (95% CI = 0.875 to 0.970) compared to CA125 0.904 (95% CI = 0.839 to 0.949). Serum HE4 at cut off value of 80pmol/L had higher sensitivity (90 percent) and specificity (64 percent) for ovarian cancer, compared to serum CA 125 at cut off value of 53.7U/m L, sensitivity 86% and specificity 59%. The combination of HE4 and CA125 gave the highest sensitivity 96% and specificity 97% respectively for detecting ovarian carcinoma than either marker alone. The bulk of the ovarian cancers (81/87 % or 93% were of the epithelial variety with the serous subtype predominant (76.5%). It is the serous subtye of epithelial ovarian cancer (EOC) that were often biomarker positive (87.1%) compared to other subtypes (mucinous, clear cell and endometroid). The few non-EOC cancers (n=6) were also biomarker positive.

Conclusion: HE4 had higher sensitivity and specificity for detecting ovarian carcinoma in women with pelvic mass compared to CA125. Dual marker combination of HE4 and CA125 was superior to either marker alone in predicting ovarian malignancy. Differential expression of biomarkers was noted among the various EOC varieties of ovarian cancer; serous EOC were more likely to be biomarker positive.

Key Words: Cancer Antigen (CA 125), Human Epididymis Protein 4 (HE4).Epithelial Ovarian Cancer (EOC)

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INTRODUCTION

Ovarian cancer is the leading cause of mortality from gynecological cancers World Wide. At present, the global incidence is approximately 165000 cases per year.[1] In Pakistan its incidence accounts for about 6%.[2] Presently over 80% of patients with ovarian cancer are diagnosed with advance stage 3 or 4 disease while only 20% are diagnosed with stage 1 or 2 disease.[3]Tradional clinical signs of ovarian cancer and routine tumor markers lack diagnostic accuracy for detection and monitoring of the tumor. At present the diagnostic work up for women with pelvic masses is preoperative ultra sonography and CA125 to identify risk of ovarian malignancy; high risk subjects are referred to a tertiary centre with gynecologic oncology for further management.

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CA125 is the most routinely used biomarker for detection of ovarian cancer, but it has been reported that CA125 is elevated in only 50% of clinically detectable early stage patients.[4] Its also been reported to be frequently elevated in women with benign gynecological conditions such as endometriosis, uterine fibroids and non ovarian malignancies.[5] CA125 limited predictive power has challenged the clinicians to reexamine whether there is need for another test that can be used with certainty for detection of ovarian cancer.

Human epididymis protein 4 is one such promising biomarker.[6] HE4 is found to be over expressed in ovarian cancers [7] and is not elevated in benign gynecological conditions. Only a few studies have been done in Asians regarding HE4 diagnostic role in predicting ovarian cancer in women with pelvic masses. No such study is available on the population in Pakistan. The purpose of this study is to determine the role of HE4 in the prediction of ovarian cancer in women with pelvic mass. The study determined blood levels of HE4 and CA125 in patients with ovarian cancer, and compare the diagnostic accuracy of HE4 alone or in combination with CA125 for ovarian carcinoma in women presenting with pelvic mass. The levels of HE4 and CA125 in different histopathological types of ovarian tumor were also compared.

MATERIALS AND METHODS

This descriptive study was carried out in the Chemical Pathology Department of Armed Forces Institute of Pathology (AFIP), Rawalpind in collaboration with North West School Of Medicine, Peshawar , after approval from the institutional ethical review committee. Research was carried out from March 2015-March 2016.A total of one hundred and twenty seven women (patients n=87, controls n=40) age greater than 18 years were enrolled. Women with suspected ovarian malignancy admitted in gynecology ward of North West School Of Medicine, Peshawar were included after written informed consent. All patients diagnosed with pelvic ,mass of suspected ovarian origin were scheduled for surgical intervention. Pregnant women, one receiving treatment of ovarian malignancy and those unable to give informed consent were not eligible. All patients underwent imaging by pelvic/abdominal ultrasound to document their presence of ovarian mass. Clinical information was retrieved from the patient's hospital notes. All patients were diagnosed preoperatively in laparoscopy/ laparatomy and confirmed by histopathological evaluation. Ovarian cancer subjects were histologically typed according to WHO classification 2003 by specialized histo-pathologist.

A blood sample (5ml) was obtained preoperatively into serum or serum separator tubes and centrifuged, aliquoted and frozen within 4 hours. The samples were stored at -20C until biochemical analysis. Blood samples were taken by trained personnel under strict hygienic conditions. Personal information of the participants were kept confidential and procedure of blood collection was explained to the patients in detail before taking the sample.

CA125 assay was performed on automated analyser VITROS. The reference range of CA125 is up to 35U/ml. HE4 assay was performed on automated analyser ARCHITCT. The reference range of HE4 is upto 1500pmol/L.

Data was entered and analyzed in SPSS (version 16.0). Mean ± SD was calculated for quantitative variables like age of patient. Frequencies and percentages were calculated for qualtitative variables like ovarian cancer and sensitivity and specificity of serum CA125, HE4 and its combination. The mean age for patients with ovarian cancer and benign group was compared using the student´s t-test. Tumor marker levels between groups were compared using Mann-whitney and Kruskal-Wallis test. Receiver Operating characteristic plots (ROC) were graphed and Area under the curve (AUC) was calculated for each marker. Sensitivity and specificity were compared using the McNemar test. We set the cut-off value at which the discrimination between the cases with positive diagnosis is optimal. The associations were quantified with 95% confidence intervals (95% CI). A two tailed value of <.05 was considered significant.

RESULTS

A total of 127 women with suspected ovarian cancer were admitted in gynecology ward of (North West School Of Medicine, hospital Peshawar). All women went through surgical intervention. Eighty seven were diagnosed histologically as ovarian carcinoma and forty as benign group. The mean age for patients with benign tumors was significantly lower than among patients with malignant tumors (40 vs 58 years old, respectively, $p< 0.001$).Of benign group, there were 4 (10%) serous & 4 (10%) mucinous cystadnoma, 4 (10%) endometriotic cyst, 13 (32.5%) leiomyoma, 15 (37.5%) benign gynaecological diseases, 2 (5 %) dermoid cyst. Along with these, two non ovarian malignant cases, abdominal cancer (2.5%) and vulval caner (2.5%) were also included in benign group.

Of malignancies, there were 81 (93.1%) epithelial ovarian tumors, out of which there were 62 serous tumors (71.3%), 14 mucinous tumors (16.1%), 3 endometroid tumors (3.4%) and 2 clear cell tumors (2.3%). There were 6(6.9%) cases in non-epithelial ovarian cancer group, out of which, there were 5(5.7%) germ cell tumors and 1(1.1%) sex-cord stromal tumor.

Table No.1: Histological type and distribution of benign disease

Histological type of benign disease	N	%
Cystadenoma	4	10
Endometriotic cyst	4	10
Dermoid cyst	2	5
Leiomyoma	13	32.5
Abdominal cancer	1	2.5
Vulval cancer	1	2.5
Other benign gynaecological diseases	15	37.5
Total	**40**	**100**

Table No.2: Histological type and distribution of malignant disease

	N	%
Histological type		
Epithelial	81	93.1
Serous tumor	62	71.3
Mucinous tumor	14	16.1
Endometroid tumor	3	3.4
Clear cell tumor	2	2.3
Non-epithelial	6	6.9
Germ cell tumor	5	5.7
Sex cord stromal cell tumor	**1**	**1.1**
Total	**87**	**100**

Table No.3: HE4 and CA125 levels in different Types of ovarian cancer

		HE4 (pmol/L)			CA125 (U/ml)		
Diagnosis	**N**	**Median**	**(range)**	**P-value***	**Median**	**(range)**	**P-value***
Benign ovarian disease	40	60	37-151	<0.001	14	4-241	<0.001
Epithelial ovarian tumor	81	953.5	58-2612	<0.001	1134	12-4140	<0.001
Germ cell tumor	5	442	60-790	<0.001	123	18-256	<0.001
Stromal cell tumor	1	378	-	<0.001	121	-	<0.001

Biomarker values were reported as median (range). P-values are evaluated by Kruskall-Wallis test. P-values between groups are evaluated by Mann-Whitney U test. This table indicates that HE4 is significantly better than CA125 in detection of Benign and Malignant (epithelial, germ cell and stromal cell tumor) as shown in Table 3.

Table No.4: Sensitivity, Specificity and AUC of CA125, HE4, Combined CA125 + HE4

Biomarkers	Sensitivity	Specificity	AUC
CA125	86%	59%	0.904
HE4	90%	64%	0.934
CA125+HE4	96%	97%	0.944

DISCUSSION

The commonest cause of gynecological cancer associated deaths are related to ovarian cancer. At an early stage, the symptoms of ovarian malignancy are not specific, therefore ultrasound is used to assess patients for ovarian carcinoma. Ultrasound has the ability to detect pelvic masses but has poor specificity in detecting, that whether the mass is benign or malignant. Doppler ultrasound and a morphology index can be used to improve specificity but performance varies among different operators.[8] Better detection of nature of pelvic mass will alleviate undue patient anxiety and will allow appropriate referrals to specialist centres for further assessment and treatment of patient. Improved outcomes have been seen in patients who are managed in specialized centres by gynecological oncologists[9]. The use of tumor markers to further characterize the mass has come into clinical use.CA125 is cancer marker most significantly used for following response to treatment and detecting disease recurrence in patients with ovarian cancer[10]. Most of the ovarian cancer patients with late stages have raised levels of CA125 while in 50% of cases who are detected earlier, there is no rise of CA125. [11]High false rate has been observed for CA125 among women with non-malignant disease and non-ovarian malignancies. [4,5] It has also been observed that expression of cancer antigen 125 has been lacked by about 20% of ovarian cancers. [12] Poor sensitivity and specificity of CA125 has hampered its use as a diagnostic test. [13]

In recent years, there has been search for new biomarkers and Human epididymis secretory protein 4 (HE4) is one of the most promising biomarkers for detecting ovarian carcinoma. It is expressed in reproductive and respiratory tracts[14,7] and is over expressed in epithelial ovarian cancer [15].HE4 gene product is an N-glycosylated protein which is secreted into extracellular environment and can be detected into the bloodstream of patients with ovarian cancer. It contains increase sensitivity for detecting ovarian malignancy at an early stage when compared with other markers that have been investigated earlier. [16,17] It is highly expressive in serous tumors but endometroid and clear cell ovarian tumors also show expression of HE4 on immuno-phenotyping. [18,7] HE4 protein are not specific to ovarian carcinoma, a strong HE4 immunoreactivity is also found in number of cancers like endometrial cancer, breast cancer, transitional bladder cancer, lung adenocarcinoma, pancreatic cancer

etc [19,20,7] (Bignotti et al.,2011;Kamei et al.,2010;Galgano et al.,2006)

In the present study, the diagnostic accuracy of HE4 and CA125 has been determined alone and in combination and we evaluated that whether HE4 can be used as a diagnostic tool for predicting ovarian malignancy.

According to our study the diagnostic sensitivity and specificity of CA125 was found to be (86% and 59%) while HE4 showed sensitivity of (90%) and specificity of (64%) which was higher than that of CA125.

The findings are consistent with other studies who also demonstrated high levels of HE4 in ovarian cancer patients. [6,17,21,22]

Specificity of CA125 is low for detecting ovarian tumors because it has the ability to produce high false rate in many benign conditions while HE4 is less frequently positive in these conditions therefore it has advantage over CA125 assay. [6] Moreover HE4 expression has found to become evident in half of tumors that lack expression of CA125 on immuno-phenotyping,[17] so sensitivity of CA125 can get improved by adding HE4 biomarker for diagnosis of ovarian malignancy.

Several multiple marker panels have been investigated to increase sensitivity and specificity of ovarian malignancy diagnosis. In this regard, CA125 and HE4 together with or without the addition of other biomarkers such as SMRP,[21,17] (MUC-1, Glycodelin, PAI-1, [16] CA72-4 and Osteopontin [17]) have been analyzed. According to reports when these markers were combined together they showed higher sensitivity and specificity than when they are used alone. Moore et al (2008)[17] investigated CA125, HE4 and seven other markers in patients presented with pelvic masses. In the study HE4 and CA125 combination was found to be best over other dual marker and triple marker combinations for detecting ovarian malignancy.

In our study, HE4 and CA125 combination has also been examined which demonstrated sensitivity and specificity of (96% and 97%) which is found to be higher than that achieved by HE4 (90% and 64%) and CA125 (86% and 59%) for predicting ovarian cancer.

Our study also demonstrated the cutoff value of HE4 and CA125 based on receiver operator characteristic curve. The cut-off value of HE4 was 80pM/L while that of CA125 was 53.7U/mL.

Three types of malignant ovarian tumors have been observed in the study i.e epithelial ovarian cancer, germ cell and stromal cell ovarian cancers. The frequent histological type found in epithelial ovarian cancer was serous followed by mucinous, endometroid and clear cell tumors. Same findings was found by Badgwell et al (2007)[22] who demonstrated that serous tumors are the common histo-type of epithelial ovarian cancer.

In the study, HE4 missed 1 out of 2 cases of clear cell carcinoma while CA125 misclassified both the two cases of this type.HE4 showed better diagnostic performance over CA125 by not missing clear cell ovarian cancer [18,7]

In non epithelial ovarian cancer, two major types, sex-cord and germ cell ovarian tumors have been observed.[23] Among the three types malignant groups, epithelial tumor group had the highest HE4 (953.5pmol/L) and CA125 (1134U/ml) median values while in other two groups the median values of HE4 and CA125 did not show significant increase i.e germ cell ovarian cancer (442pmol/l, 123U/ml) and sex-cord ovarian cancer (378pmol/L, 121U/ml).Similar findings were reported by Huhtinen et al (2009)[24] who showed raised levels of CA125 and HE4 in epithelial ovarian cancer group.

CA125 was found to be falsely elevated in 11 out of 40 benign cases. Among the 11 missed cases, two cases were of abdominal cancer and vulval cancer in whom CA125 were falsely raised.

The results were found to be in agreement with the studies demonstrating that CA25 is falsely elevated in non-malignant diseases as well as in non ovarian malignancies.[4,5] In case of HE4, it misclassified 8 out of 40 cases. It correctly classified abdominal and vulval cancer. By these findings HE4 showed its superiority over CA125 by less frequently raised in patients with non-malignant ovarian diseases and non ovarian malignancies.[7]

In summary the results of the present study demonstrate that HE4 is a valuable marker and is better than CA125 for detecting ovarian cancer. Improvement of sensitivity of CA125 can get achieved by the addition of HE4.CA125 and HE4 biomarkers complement each other and should be used in combination for diagnosing ovarian carcinoma. Future studies including larger clinical trials are needed to be undertaken to evaluate utility of HE4 biomarker for prediction of ovarian cancer.

CONCLUSION

HE4 is more reliable biomarker for diagnosis of ovarian cancer than CA125. Dual marker combination of HE4 and CA125 showed to be more accurate predictors of ovarian malignancy than either marker alone.

Author's Contribution:

Concept & Design of Study:	Sonia Aziz
Drafting:	Ejaz Hassan Khan
Data Analysis:	Mohsin Shaffi
Revisiting Critically:	Sonia Aziz, Ejaz Hassan Khan
Final Approval of version:	Sonia Aziz

Conflict of Interest: The study has no conflict of interest to declare by any author.

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