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Serum Biomarkers in Discrimination of Benign From Malignant Adnexal Mass Women

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ABSTRACT

Ovarian cancer has the highest mortality rate out of all types of gynecologic cancer and considered the fifth leading cause of cancer deaths among women. The main challenge for laboratory biomarkers of ovarian cancer diagnosis is to allow the accurate detection of malignancy as early as possible with a good screening test which must adequately address validity, reliability, yield, cost, acceptance and follow-up services to improve clinical outcome and survival of patients. This study aimed to evaluate the use of some serum biomarkers in discrimination of benign from malignant adnexal mass patients in the effort of early detection of ovarian cancer. A total of 174 gynecologic hospitalized patients presented with adnexal mass and planned for surgical management were included. Serum levels of human epididymis protein-4 (HE4), mesothelin and vascular endothelial growth factor (VEGF) were determined using ELISA, while cancer antigen-125 (CA125), cancer antigen 19.9 (CA19.9), cancer embryonic antigen (CEA) and alpha-fetoprotein (AFP) were determined by the electrochemiluminesence immunoassay technique. The present data revealed a significant statistical difference between studied groups as regard to HE4, mesothelin, VEGF and CA125. The present study demonstrated a significant positive correlation between studied markers (HE4 and VEGF) as regard to the stage of the tumor while mesothelin had a significant positive correlation with both stage and grade of the tumor. Using receiver operating characteristic curves (ROC) mesothelin, VEGF and HE4 revealed the prediction of malignancy in all included women donated by the area under the curves (AUC). It is concluded that using HE4, mesothelin and VEGF could be a useful tool in prediction and diagnosis of ovarian cancer.

Introduction

Ovarian cancer (OC) is the leading cause of death in women with gynecologic cancer [1]. Effective screening strategies have not been established and continue to be elusive. A good screening test must adequately address validity, reliability, yield, cost, acceptance and follow-up services [2]. An ideal screening test for ovarian cancer must have a high sensitivity in order to correctly diagnose all women with the disease and a high specificity to avoid false-positive results. The current screening modalities of bimanual examination, cancer antigen 125 (CA-125) and transvaginal ultrasonography together allow us to detect only 30-45% of women with early-stage disease [3].

The main challenge for laboratory biomarkers of OC diagnosis is to allow the accurate detection of malignancy as early as possible to improve clinical outcome and survival of patients [4]. The crude incidence rate of ovarian cancer changes from 4.7 per 100 000 in women <50 years of age to 29.6 per 100 000 in the age group of 50–64 years [5].

CA-125 is the established biomarker for detecting OC recurrence and monitoring therapeutic response. In addition, recent guidelines recommend its measurement in the primary care setting in women with suggestive symptoms or at high risk for OC, in combination with

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pelvic ultrasound [6]. Overall, CA-125 effectiveness in the identification of the malignancy is threatened by its low diagnostic specificity. In fact, this glycoprotein is widely distributed on the surface of cells in various benign and malignant conditions other than OC [4]. Therefore, considerable efforts are aimed to identify novel markers, which are more sensitive and specific compared to CA125 and may be used in combination with or instead of CA125 to improve the diagnosis of OC [7].

Human epididymis protein 4 (HE4) is highly restricted in normal human tissues specifically; it was expressed most highly in the epididymis and in the female reproductive tract (fallopian tubes, endometrium, and endocervix). HE4 expression was also present in the respiratory epithelium especially in the trachea [8]. Its expression in cortical ovarian cysts suggests the development of some types of epithelial ovarian cancer [4].

Mesothelin is a new tumor marker in patients with mesothelioma and ovarian cancers; it is a cell surface protein present on normal mesothelial cells lining the pleural, pericardium and peritoneum. It is highly expressed in several cancers including approximately 70% of ovarian cancers and 50% of lung adenocarcinomas [9].

Vascular endothelial growth factor (VEGF) is a potent angiogenic factor that also increases vascular permeability. It is present in the theca layer of the ovarian follicle and epithelia of the ovary and fallopian tube. High levels of VEGF are detected in ascites, cyst fluid and serum of patients with ovarian cancer. In some studies there was a correlation between VEGF, clinical outcome and prognosis in ovarian cancer patients [10].

Carcinoembryonic antigen (CEA) is a glycoprotein of 200 KD, excreted by certain embryonic and adult tissues in addition to adenocarcinoma of the digestive organs [11] .It is elevated approximately in 34-37% of patients with ovarian cancer [12].

Alpha-fetoprotein (AFP) is a major fetal serum globulin with a molecular weight of approximately 65,000. Its concentration in normal adults is below 15 ng/ml, the appearance of excess amount of serum AFP beyond 500 ng/ ml indicates underlying malignancy except in cases of pregnancies [13].

Cancer antigen 19.9 (CA19.9) is a carbohydrate tumor associated antigen of 210 KD; its levels are lower than 37 u /ml in 99.6% of healthy adults [14]. It is mainly increased in gastrointestinal system tumors but it could be detected in other malignancies such as ovarian dermoid cyst [15].

Subjects and Methods

The present study was carried out at Obstetric and Gynecology Hospital- Ain Shams University, during the period between **October 2013 and October 2014.** A total of 174 Gynecologic Hospitalized Patients presented with adnexal mass and planned for surgical management were included in this study (**Table 1**).

Subjects

All patients underwent imaging by pelvic ultrasound to document the presence of an ovarian mass. Clinical information was retrieved from the patients' hospital notes. All patients underwent surgical removal of the ovarian mass, and if a patient was diagnosed with an ovarian cancer, then surgical staging was performed.

Blood samples

Immediately before surgery, fasting blood samples were obtained and collected in vacutainer serum tubes and were centrifuged at 3000 r.p.m. for 15 min. Sera were separated and kept frozen at −80 °C until analysis.

Methods:

Serum levels of HE4, mesothelin and VEGF were determined using ELISA kit developed by Fujirebio Diagnostic, Inc. and was performed according to the manufacturer's specifications. While CA125, CA19.9, CEA and AFP were determined by the electrochemiluminesence immunoassay technique on Elecsys 2010 immunoassay analyzer-Hitachi Ltd- Japan.

BMI= weight (kg) / [height (m)]^2.

Statistical analysis:

All the data were analyzed using SPSS version 21.0 (SPSS Inc., Chicago, IL, USA). Data presented as range, median (interquartile range IQR); or number (percentage), evaluation of differences between analysed groups by using Mann-Whitney's U-Test and Chi-Squared Test. Correlation between measured markers and both staging and grading using Spearman's rank correlation coefficient was also studied. Receiver operating characteristic (ROC) curves were assessed for all serum markers in order to estimate the area under the curve for each marker (AUC).

Results

Of the included 174 women, 125 (71.8%) had a benign lesion, 46 (26.4%) had a malignant tumor, while 3 (1.7%) had borderline ovarian tumor (atypical proliferative ovarian tumor) (**Table-2**).

Of the included 174 women, 46 women had malignant tumors divided according to stage and grade of the tumor as shown in **Table 3**, which represents staging and grading of tumor in included women with malignant ovarian mass.

Correlation between the markers and both staging and grading showed a significant positive correlation between HE4, mesothelin and VEGF as regard to stage of the tumor while the mesothelin was the only marker which had a significant positive correlation as regard to grade of the tumor (**Table 4**).

A highly significant statistical difference as regard to the median values of age, menstrual status and duration of marriage between women who had benign and those with malignant tumors (**Table 5**).

The median values of all measured serum biomarkers (CA125, CA19.9, CEA, AFP, HE4, mesothelin and VEGF) were all significantly higher in women with malignant tumors when compared to women with benign lesions (**Table 6**).

Table 1: Demographic data of included women.

Age (years)	
Range	20 – 75
Median (IQR)	41 (30 – 50)
Duration of Marriage (years)	
Range	Less than 1 – 47
Median (IQR)	17 (6 – 27)
Menstrual Status	
Premenopausal	111 (63.8%)
Postmenopausal	63 (36.2%)
Parity	
Range	0 – 15
Median (IQR)	3 (1 – 4)
Weight (kg)	
Range	54 – 105
Median (IQR)	80 (69 – 88)
BMI (kg/m^2)	
Range	21.01 – 37.34
Median (IQR)	29.39 (25.71 – 32.03)

IQR: Interquartile range [central 50% of ascendingly-ordered set of data]
BMI: Body mass index [calculated as weight (in kilograms) divided by squared height (in meters)]
Data presented as range, median (IQR); or number (percentage).

Table 2: Histopathology of the removed ovarian masses in included women..

Benign Lesion (n)	**125 (71.8%)**
Serous Cystadenoma	37 (21.3%)
Mucinous Cystadenoma	15 (8.6%)
Mature Cystic Teratoma	33 (19%)
Endometrioma	14 (8%)
Fibroma/Thecoma	11 (6.3%)
Inflammatory Mass	4 (2.3%)
Functional Cyst	11 (6.3%)
Malignant Tumor (n)	**46 (26.4%)**
Serous Cystadenocarcinoma	14 (8%)
Mucinous Cystadenocarcinoma	10 (5.7%)
Endometrioid Adenocarcinoma	3 (1.7%)
Squamous Cell Carcinoma	3 (1.7%)
Clear Cell Carcinoma	5 (2.9%)
Mixed Epithelial Carcinoma	2 (1.1%)
Mixed Müllerian Tumor	1 (0.6%)
Granulosa Cell Tumor	2 (1.1%)
Immature Teratoma	1 (0.6%)
Undifferentiated Carcinoma	5 (2.9%)
Borderline Tumor (n)	**3 (1.7%)**

Data presented as number (percentage)

Table 3: Staging and grading of the malignant ovarian mass in included women.

Stage	
I	2 (4.3%)
II	19 (41.3%)
III	23 (50%)
IV	2 (4.3%)
Grade	
Grade 1	19 (41.3%)
Grade 2	22 (47.8%)
Grade 3	5 (10.9%)

Data presented as number (percentage)

Table 4: Correlation between measured markers and both staging and grading.

Markers		Staging	Grading
CA125	r_s ***P***	0.220 0.191 NS	0.278 0.095 NS
CA19.9	r_s ***P***	-0.153 0.365 NS	-0.067 0.692 NS
CEA	r_s ***P***	-0.163 0.335 NS	-0.155 0.358 NS
AFP	r_s ***P***	-0.165 0.329 NS	-0.040 0.814 NS
HE4	r_s ***P***	0.406 0.026 **S**	0.125 0.512 NS
Mesothelin	r_s ***P***	0.428 0.008 **S**	0.362 0.028 **S**
VEGF	r_s ***P***	0.512 0.009 **S**	0.112 0.498 NS

CA125, cancer antigen 125; CA19.9, cancer antigen 19.9; CEA, cancer embryonic antigen; AFP, alpha-fetoprotein; HE4, human epididymis protein-4; VEGF, vascular endothelial growth factor; r_s, Spearman's rank correlation coefficient; NS, non-significant; S, significant.

Table 5: Difference between women who had benign and those who had malignant lesions regarding demographic data.

Variable	Women with Benign Lesions (n=125)	Women with Malignant Tumors (n=46)	*P*
Age (years) **Range** **Median (IQR)**	 20 – 75 37 (28 – 48)	 20 – 69 50 (42.5 – 56)	 <0.001* **HS**
Duration of Marriage (years) **Range** **Median (IQR)**	 Less than 1 – 43 13 (5 – 24.5)	 Less than 1 – 47 23 (16.5 – 32)	 <0.001* **HS**
Menstrual Status **Premenopausal** **Postmenopausal**	 93 (74.4%) 32 (25.6%)	 17 (37%) 29 (63%)	 <0.001** **HS**
Parity **Range** **Median (IQR)**	 0 – 11 3 (1 – 4)	 0 – 15 3 (2 – 5)	 0.078* NS
Weight (kg) **Range** **Median (IQR)**	 54 – 102 80 (68.5 – 87.5)	 56 – 102 80 (70 – 90)	 0.279* NS
BMI (kg/m^2) **Range** **Median (IQR)**	 21.01 – 37.34 29.39 (25.59 – 31.91)	 22.04 – 37.34 29.4 (26.19 – 33.13)	 0.420* NS

IQR: interquartile range [central 50% of ascendingly-ordered set of data]
BMI: body mass index [calculated as weight (in kilograms) divided by squared height (in meters)]
Data presented as range, median (IQR); or number (percentage)
* Analysis using Mann-Whitney's U-Test
** Analysis using Chi-Squared Test
HS: highly significant – S: significant – NS: non-significant.

Table 6: Difference between women who had benign and those who had malignant lesions regarding serum biomarkers.

Markers	Women with Benign Lesions (n=125)	Women with Malignant Tumors (n=46)	*P**
CA125 (IU/ml)			
Range	2.25 – 812	8.48 – 3709	<0.001
Median (IQR)	24.25 (9.38 – 52.43)	266.8 (142.95 – 601.65)	**HS**
CA19.9 (IU/ml)			
Range	0 – 79.66	0.2 – 368	<0.001
Median (IQR)	4 (1.56 – 9.4)	23 (6.9 – 43.52)	**HS**
CEA (IU/ml)			
Range	0 – 198	0.1 – 472	0.002
Median (IQR)	2.22 (0.95 – 5.21)	4.45 (1.5 – 19.6)	S
AFP (ng/ml)			
Range	0 – 236	0 – 78.5	0.002
Median (IQR)	1 (0 – 2.4)	2 (0.96 – 5.7)	S
HE4 (pmol/L)			
Range	16.7 – 859.7	69.6 – 987	<0.001
Median (IQR)	146 (52.5 – 247.6)	634 (393.45 – 861)	**HS**
Mesothelin (nmol/L)			
Range	0.53 – 195.3	6.5 – 215.4	<0.001
Median (IQR)	11.4 (6.6 – 16.4)	94.8 (61.83 – 115.98)	**HS**
VEGF (pg/ml)			
Range	42.7 – 2862	193.6 – 2886	<0.001
Median (IQR)	429.5 (143.58 – 700.75)	1736.5 (1216 – 2470.5)	**HS**

Data presented as range, median (IQR). CA125, cancer antigen 125; CA19.9, cancer antigen 19.9; CEA, cancer embryonic antigen; AFP, alpha-fetoprotein; HE4, human epididymis protein-4; VEGF, vascular endothelial growth factor; HS, highly significant – S: significant.

* Analysis using Mann-Whitney's U-Test

ROC curves were constructed for measured serum biomarkers as predictors of malignancy in included women. All measured markers showed significant predictability as denoted by the significantly large area under the curves (AUCs); with serum mesothelin and serum VEGF being the most significant predictors followed by serum CA125 (**Figure 1, Table 7**).

Table 8 shows the sensitivity, specificity, positive and negative predictive values of the best cut-off values of the measured biomarkers according to the ROC curves.

Discussion

This study aimed to evaluate the using of some serum biomarkers in discrimination of benign from malignant adnexal mass women in the effort of early detection of ovarian cancer.

The present study revealed that the difference between women with benign and those with malignant lesions as regard the demographic data. The median values of age was significantly higher in women who had malignant lesions and those of benign lesion which agreed with Abdel-Azee *et al.* [16], who reported that the mean age for patients with malignant tumors was significantly higher than in patients with benign tumors. Furthermore, the study by Eisenhauer *et al.* [17] concluded that malignant neoplasms of the ovaries occur at all ages including infancy, childhood and adolescence but the incidence increases progressively with age. Also Cancer Research UK [18], reported that the ovarian cancer incidence strongly related to age, the highest incidence rates being in older women in UK and the average of 29% of cases were diagnosed in women aged 75 and over, and 75% were diagnosed in those aged 55 years and over.

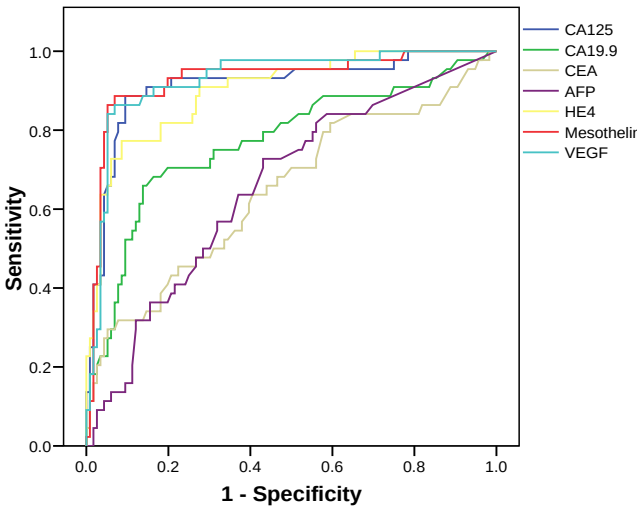

Fig. 1: ROC Curves for measured serum biomarkers as predictors of malignancy in included women.

Table 7: Area under ROC curves for measured serum biomarkers as predictors of malignancy in included women.

Markers	AUC (95% CI)	*P*
CA125	0.914 (0.858 to 0.970)	<0.001 (**HS**)
CA19.9	0.772 (0.683 to 0.861)	<0.001 (HS)
CEA	0.647 (0.546 to 0.748)	0.004 (S)
AFP	0.657 (0.564 to 0.751)	0.002 (S)
HE4	0.900 (0.847 to 0.954)	<0.001 (**HS**)
Mesothelin	0.929 (0.879 to 0.980)	<0.001 (**HS**)
VEGF	0.929 (0.883 to 0.974)	<0.001 (**HS**)

CA125, cancer antigen 125; CA19.9, cancer antigen 19.9; CEA, cancer embryonic antigen; AFP, alpha-fetoprotein; HE4, human epididymis protein-4; VEGF, vascular endothelial growth factor; AUC (95% CI) area under the ROC curve and its 95% confidence interval; HS, highly significant – S: significant.

Table 8: Accuracy of measured serum biomarkers as predictors of malignancy in included women.

Markers	Best Cutoff Value	Sensitivity	Specificity	PPV	NPV
CA125	≥ 35 IU/ml	93.5%	62.9%	48.3%	96.3%
CA125	≥ 58.7 IU/ml	93.5%	79%	62.3%	97%
CA19.9	≥ 7.4 IU/ml	75.6%	69.9%	47.9%	88.7%
CEA	≥ 3.17 IU/ml	65.2%	57.9%	37%	81.4%
AFP	≥ 1.35 ng/ml	68.9%	57.7%	37.3%	83.5%
HE4	≥ 285.4 pmol/L	82.2%	82.4%	62.7%	92.8%
Mesothelin	≥ 22.75 nmol/L	91.3%	82.3%	65.6%	96.2%
VEGF	≥ 872.5 pg/ml	89.1%	87.1%	71.9%	95.6%

CA125, cancer antigen 125; CA19.9, cancer antigen 19.9; CEA, cancer embryonic antigen; AFP, alpha-fetoprotein; HE4, human epididymis protein-4; VEGF, vascular endothelial growth factor; PPV, positive predictive value; NPV, negative predictive value.

The percentage of menopausal women of malignant lesion was (63%) and premenopausal of (37%) while for the benign lesion (74.4%) were premenopausal and (25.6%) of postmenopausal women. There was significant statistical difference between these percentages. This result agreed with Abdel-Azeez *et al.* [16], who concluded that the number of postmenopausal women was increased in patients with ovarian malignant tumors compared to women with benign ovarian tumors. Also Givens *et al.* [19], stated that in postmenopausal women, 30% of adnexal masses are malignant which is almost agreed with results of this study.

In regards to the median of the parity and weight, our results found no significant differences between women with benign ovarian lesions and those with malignant ovarian lesions which agreed with the results of Lowe *et al.* [20] and Kotsopoulosa *et al.* [21] who mentioned that the height, weight and parity were not significantly related to ovarian cancer.

The present study revealed that the serum HE4 correlated with the stage of the tumor while mesothelin had a significant statistical correlation with both the stage and grade of the tumor. This finding was in agreement with Havrilesky *et al.* [22] and Abdel-Azeez *et al.* [16], who reported that the mesothelin is significantly correlated with both stage and grade of the tumor in ovarian cancer so it can monitor the disease status. These results also agree with that of Huang *et al.* [23], who concluded that the mesothelin levels were higher in malignant cancer patients than in those with benign ovarian tumors and significantly increased from early to advanced stages. Abdel-Azeez *et al.* [16] reported that HE4 is significantly correlated with tumor stage and grade in ovarian cancer.

The median values of measured serum biomarkers CA125, HE4, Mesothelin and VEGF were all significantly higher in women with malignant tumors when compared to those with benign lesions. These results matched with that of Lin *et al.* [24] and Wu *et al.* [25], who mentioned that the measurement of serum HE4 is a useful for differential diagnosis between benign lesions and ovarian cancer. Ferraro *et al.* [4], concluded that HE4 measurement seemed to be superior to CA-125 in diagnostic performance for identification of ovarian cancer in women with suspected gynaecological disease, while Li *et al.* [26], concluded that HE4 is not better than CA125 for ovarian cancer prediction and Moore *et al.* [27], mentioned that HE4 is elevated less frequently than CA125 in benign disease. Also, Kalapotharakos *et al.* [28] concluded that high concentration of plasma HE4 is an independent preope-

rative marker of poor prognosis in patients with ovarian cancer, while Shah *et al.* [29], investigated the ability of CA125, HE4 and mesothelin to discriminate ovarian cancer from healthy controls and concluded that HE4 was the best marker. Yu *et al.* [30], evaluated the diagnostic value of HE4 for ovarian cancer and concluded that HE4 was found to be better than CA125 as an indicator for the diagnosis of ovarian cancer. The present results also agreed with Kadija *et al.* [31] and Kim *et al.* [32], who mentioned that the levels of HE4 and CA125 were significantly higher among the patients with malignant tumors, compared with patients with benign lesions, but Pitta *et al.* [33], concluded that in women with normal CA125 levels, neither mesothelin nor HE4 contributed to discriminate women with malignant ovarian tumors; however, for women with elevated CA125 levels HE4 may help in discriminating those who have a malignant ovarian tumor.

Also, this study showed that there was a significant correlation between VEGF and the stage of the tumor which agreed with Bandiera *et al.* [34], who found a statistically significant association between the level of sVEGF and both tumor stage, grade, and presence of ascites, while Osman *et al.* [35], concluded that in ovarian cancer the preoperative CA125 level did not correlate significantly with stage or tumor grade which agreed with our results.

Abou Seeda *et al.* [36] mentioned that preoperative serum VEGF revealed higher levels in malignant ovarian masses than benign conditions and normal controls. Also Ławicki *et al.* [37], suggested the usefulness of VEGF in the early diagnosis of ovarian cancer and advanced ovarian cancer without metastases, while Yu *et al.* [38], who evaluated the prognostic value of VEGF in ovarian cancer concluded that the association between high tissue VEGF level and poor prognosis exists in early stage patients, but not in advanced stage patients.

In the current study, the ROC curves were constructed for measured serum biomarkers as predictors of malignancy in included women. All measured markers showed significant predictability as denoted by the significantly large area under the curves (AUCs); level of mesothelin (0.929) and VEGF (0.929) followed by CA125 (0.914), HE4 (0.900) CA19.9 was (0.772), CEA (0.647) and AFP (0.657) these results did not agree with Abdel-Azeez *et al.* [16], who mentioned that as regard the area under ROC curve, CA125 had the highest AUC than mesothelin. A recent study by Ibrahim *et al.* [39], concluded that in ovarian cancer, mesothelin rather than CA125 was a significant predictor of early-stage ovarian cancer and that mesothelin is more specific than CA125.

This study revealed that the mesothelin had a lower sensitivity than CA125 91.3% and 93.5% respectively and higher specificity than CA125 82.3% and 62.9% respectively in detecting malignancy of included women, these results agreed with Abdel-Azeez *et al.* [16], Shah *et al.* [29], who mentioned that the level of mesothelin had a lower sensitivity than CA125 in detecting ovarian malignancy but was more specific than serum CA125 for discriminating ovarian cancer cases. Also Qiao and Li [40] concluded that serum mesothelin had high specificity in women with ovarian cancer, and can be used in the preoperative diagnostic evaluation for ovarian cancer. Jose *et al.* [41] concluded that in patients with benign gynecologic diseases the HE4 had significantly higher diagnostic specificity than CA 125, also Ławicki *et al.* [37], mentioned that VEGF has a diagnostic sensitivity, specificity and the area under the ROC curve in early stages of cancer tested groups.

Conclusion

The results of this study reported that serum HE4, mesothelin and VEGF can successfully differentiate benign from malignant ovarian masses, since the levels of serum HE4, mesothelin and VEGF were significantly elevated in malignant versus benign ovarian lesions. Also the studied tumor markers (mesothelin, VEGF and HE4) showed a high sensitivity and specificity in predicting ovarian malignancy in all included women, CA125 can do this mission but HE4, mesothelin and VEGF had a higher specificity than CA125. These findings demonstrated the usefulness of HE4, mesothelin and VEGF in supporting CA125 as a monitoring marker and the possibility of adding HE4, mesothelin and VEGF alongside CA125 in monitoring of ovarian cancer. It could be a promising new tumor marker which should be under investigation for clinical use.

References

1) **Stiekema, A., Lok, C., Kenter, G., van Driel, W. and Korse, M. (2014).** A predictive model combining human epididymal protein 4 and radiologic features for the diagnosis of ovarian cancer. Gynecol Oncol., 132**(3)**: 573-577.
2) **Nguyen, L., Segundo, J., Gordon, P., Curtin, C., Momeni, M., Chuang, L. and Fishman, D. (2013).** Biomarkers for early detection of ovarian cancer. Women's Health, 9**(2)**: 171-187.
3) **Cragun, J. (2011).** Screening for Ovarian Cancer. Cancer Control., 18**(1)**: 16-21.
4) **Ferraro, S., Braga, F., Lanzoni, M., Boracchi, P. and Panteghini, M. (2013).** Serum human epididymis protein 4 vs carbohydrate antigen 125 for ovarian cancer diagnosis: a systematic review. J Clin Pathol., 66**(4)**: 273-281.
5) **Ferlay, J., Steliarova-Foucher, E., Lortet-Tieulent, J. and Bray, F. (2013).** Cancer incidence and mortality patterns in Europe: Estimates for 40 countries in 2012. European Journal of Cancer., 49: 1374– 1403.
6) **National Institute for Health and Clinical Excellence (NICE) (2011).** Clinical guideline; 122.
7) **Cree, A. (2011):** Improved blood tests for cancer screening: general or specific? BMC Cancer., 11: 499-504.
8) **Galgano, M., Hampton, G. and Frierson, H. (2006).** Comprehensive analysis of HE4 expression in normal and malignant human tissues. Mod. Pathol., 19: 847–853.

9) **Hassan, R., Bera, T. and Pastan, I. (2004).** Mesothelin a new target for immunotherapy. Clin Cancer Res., 10: 3937–3942.
10) **Husseinzadeh, N. (2011).** Status of tumor markers in epithelial ovarian cancer has there been any progress? Gynecologic Oncology, 120**(1)**: 152–157.
11) **Gold, P. and Freedman, S. (1965).** Demonstration of tumor–specific antigens in human colonic carcinomata by immunological tolerance and absorption techniques. J Exp Med., 28**(3)**: 439–462.
12) **Tuxen, M. and Dombernowsky, P. (2001).** Serum tumour marker CA 125 in monitoring of ovarian cancer during first-line chemotherapy British J. of Cancer, 84**(10)**: 1301–1307.
13) **Malati, T., Saraswathi, A., Yadagiri, B., Kumar, A. and Dixit, V. (1995).** Alphafetoprotein in liver disorders. J Int Med Acad Sci, 8: 87-90.
14) **Koprowski, H., Steplewski, Z., Mitchell, K., Herlyn, M. and Herlyn, D. (1979).** Colorectal carcinoma antigens detected by hybridoma antibodies. Somatic Cell Genet, 5: 957–971.
15) **Ulkumen, B., Goker, A. and Pala, G. (2013).** Abnormal Elevated CA 19-9 in the Dermoid Cyst: A Sign of the Ovarian Torsion? Case Rep Obstet Gynecol., 2013: 860505.
16) **Abdel-Azeez, H., Labib, H., Sharaf, M. and Refaie, N. (2010).** HE4 and Mesothelin: Novel Biomarkers of Ovarian Carcinomain Patients with Pelvic Masses. Asian Pacific J Cancer Prev., 11: 111-116.
17) **Eisenhauer, L., Balani, R. and Copeland, J. (2012).** Epithelial ovarian cancer. Clinical Gynecologic Oncolcolgy, 8**(11)**: 286-328.
18) **Cancer Research UK, 2013.** Cancer statistics registration-England (No 43- 2013).
19) **Givens, V., Mitchell, G., Harraway-Smith, C. and Maness, D. (2009).** Diagnosis and management of adnexal masses. Am Fam Physician., 80**(8)**: 815-820.
20) **Lowe, K., Shah, C., Wallace, E., Anderson, G., Paley, P., Bergan, L., Jason, T. and Urban, N. (2008).** Effects of Personal Characteristics on Serum CA125, Mesothelin, and HE4 Levels in Healthy Postmenopausal Women at High-Risk for Ovarian Cancer. Cancer Epidemiol Biomarkers Prev, 17**(9)**: 2480–2487.
21) **Kotsopoulosa, J., Joel, R., Barry, R., Harvey, A. and Steven, A. (2012).** Height, weight, BMI and ovarian cancer survival. Gynecologic Oncology., 127**(1)**: 83–87.
22) **Havrilesky, J., lark, W., Jennifer, R., Robert, L. and John, J. (2008).** Evaluation of biomarker panels for early stage ovarian cancer detection and monitoring for disease recurrence. Gynecologic Oncology., 110: 374–382.
23) **Huang, C., Cheng, W., Lee, C., Su, Y., Chien, S. and Chen, A. (2006).** Serum mesothelin in epithelial ovarian carcinoma: a new screening marker and prognostic factor. Anticancer Res., 26**(6C)**: 4721-4728.
24) **Lin, J., Qin, J. and Sangvatanakul, V. (2013).** Human epididymis protein 4 for differential diagnosis between benign gynecologic disease and ovarian cancer: a systematic review and meta-analysis. Eur J Obstet Gynecol Reprod Biol., 167**(1)**: 81-85.
25) **Wu, D., Zhi-Yuan, Q., Yong-Hong, S. and Yang, C. (2012).** Diagnostic Value of Serum Human Epididymis Protein 4 (HE4) in Ovarian Carcinoma: A Systematic Review and Meta-Analysis. International Journal of Gynecological Cancer., 22**(7)**: 1106–1112.
26) **Li, F., Tie, R., Chang, K., Wang, F., Lu, W. and Chen, M. (2012).** Does risk for ovarian malignancy algorithm excel human epididymis protein-4 and CA125 in predicting epithelial ovarian cancer: A meta-analysis. BMC Cancer., 12: 258-266.
27) **Moore, G., Craig, M., Margaret, M., Skates, S. and Bast, J. (2012).** Serum HE4 levels are less frequently elevated than CA125 in women with benign gynecologic disorders. American Journal of Obstetrics and Gynecology., 206**(4)**: 351.e1–351.e8.
28) **Kalapotharakos, G., Asciutto, C., Henic, E., Casslén, B. and Borgfeldt, C., (2012).** High preoperative blood levels of HE4 predicts poor prognosis in patients with ovarian cancer. J Ovarian Res. 21, 5**(1)**: 20.
29) **Shah, A., Kimberly, L., Paley, P., Wallace, E., Garnet, A. and Drescher, C. (2009).** Influence of Ovarian Cancer Risk Status on the Diagnostic Performance of the Serum Biomarkers Mesothelin, HE4, and CA125. Cancer Epidemiol Biomarkers Prev., 18**(5)**: 1365–1372.
30) **Yu, S., Yang, H., Xie, S. and Bao, Y. (2012).** Diagnostic value of HE4 for ovarian cancer: a meta-analysis. Clin Chem Lab Med., 50**(8)**: 1439-1446.
31) **Kadija, S., Aleksandar, K., Milos, M. and Atanackovic, J. (2012).** The Utility of Human Epididymal Protein 4, Cancer Antigen 125, and Risk for Malignancy Algorithm in Ovarian Cancer and Endometriosis. International Journal of Gynecological Cancer, 22**(2)**: 238–244.
32) **Kim, Y., Whang, D. and Park, J. (2011).** Evaluation of the accuracy of serum human epididymis protein 4 in combination with CA 125 for detecting ovarian cancer: a prospective case-control study in a Korean population. Clin Chem., 49: 527-534.
33) **Pitta, R., Sarian, L., Barreta, A., Campos, E., Campbell, L. and Derchain, S. (2013).** Symptoms, CA125 and HE4 for the preoperative prediction of ovarian malignancy in Brazilian women with ovarian masses. BMC Cancer., 13: 423-428.
34) **Bandiera, E., Franceschini, R., Specchia, C. and Ravaggi, A. (2012).** Prognostic significance of vascular endothelial growth factor serum determination in women with ovarian cancer. ISRN Obstet Gynecol., 245-756.

35) **Osman, N., O'Leary, N., Mulcahy, E., Barrett, N. and Gupta, R. (2008).** Correlation of serum CA125 with stage, grade and survival of patients with epithelial ovarian cancer at a single centre. Ir Med J., 101**(8)**: 245–247.

36) **Abou Seeda, R., Mansour, G. and Ez-Elarab, S. (2014).** Preoperative serum vascular endothelial growth factor correlated to three dimensional power Doppler indices in ovarian masses. Journal of Obstetrics and Gynecology, 4: 112-119.

37) **Ławicki, S., Grażyna, E., Ewa, G. and Maciej, S. (2013).** The plasma concentration of VEGF, HE4 and CA125 as a new biomarkers panel in different stages and sub-types of epithelial ovarian tumors. Journal of Ovarian Research., 6:45.

38) **Yu, L., Deng, L., Li, J., Zhang, Y. and Hu, L. (2013).** The prognostic value of vascular endothelial growth factor in ovarian cancer: a systematic review and meta-analysis. Gynecologic oncology, 128**(2)**: 391-396.

39) **Ibrahim, M., Bahaa A., Ibrahim A., El Hakem A. and Abo-El Noor A. (2014).** Evaluation of serum mesothelin in malignant and benign ovarian masses. Arch Gynecol Obstet., 290**(1)**:107-113.

40) **Qiao, N. and Li, H. (2013).** The value of mesothelin in the diagnosis and follow-up of surgically treated ovarian cancer. Eur J Gynaecol Oncol., 34**(2)**: 163-165.

41) **Escudero, J. M., Auge, J. M., Filella, X., Torne, A. Pahisa, J. and Molina, R. (2011).** Comparison of serum human epididymis protein 4 with cancer antigen 125 as a tumor marker in patients with malignant and nonmalignant diseases. Clin Chem., 57**(11)**: 1534-1544.