Current Problems in Cancer 43 (2019) 450–460

Contents lists available at ScienceDirect

Current Problems in Cancer

journal homepage: www.elsevier.com/locate/cpcancer

Diagnostic accuracy of CA125 and HE4 in ovarian carcinoma patients and the effect of confounders on their serum levels

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A B S T R A C T

Objectives: To evaluate the diagnostic accuracy of serum cancer antigen 125 (CA125) and human epididymis protein 4 (HE4) in the prediction of malignant ovarian masses then to analyze the effect of personal criteria and medical diseases on this accuracy.
Study Design: This prospective study was performed in Zagazig University Hospital. The eligibility criteria for inclusion were; consecutive women, at any age ≥18 years, with established diagnosis of ovarian mass based on symptoms, signs, and imaging techniques. All patients underwent personal and medical history taking, preoperative serum CA125 and HE4 (cutoff 35 IU/mL and 150 pmol/L, respectively) assessment then postoperative histopathologic examination of lesions as a reference standard.
Results: Among the included 140 patients, 62 were confirmed to have ovarian malignancy and 78 had benign lesions. Serum CA125 ≥35 IU/mL was associated with ovarian malignancy at sensitivity 91.9%, specificity 53.8%, and accuracy 70.7%. Raising its cutoff to 67.5 IU/mL decreased the sensitivity 83.9%, increased the specificity 80.7% with accuracy 82.1%. The combination of HE4 and CA125 showed sensitivity 75.8%, specificity 93.5%, and accuracy 85.7%. Women suffering from both diabetes mellitus and hypertension showed a significant decrease in CA125 concentration $P = 0.02$ with false negative results in (5/11) of them, making its sensitivity 54.5% in this condition.
Conclusions: The performance of CA125 in cancer ovary prediction can be improved by increasing its cutoff or by combining CA125 with HE4. Diabetes mellitus and hypertension can influence CA125 performance while HE4 is independent on these factors. This can be an additional value of the introduction of HE4 in cancer ovary prediction protocols.

☆ Conflict of interest: Authors have no conflict of interest to declare.
☆☆ Funding sources: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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https://doi.org/10.1016/j.currproblcancer.2018.12.004
0147-0272/

ARTICLE INFO

Keywords: CA125; HE4; Ovarian carcinoma; Tumor markers

Introduction

Ovarian carcinoma represents 4.5% of cancers in Egyptian women.[1] It is the most common malignancy in female's reproductive system and the most lethal one because of the advanced stage at presentation. Here comes the importance of tumor markers estimation, to aid in the early diagnosis and hence increase the survival rate of patients.[2] In patients presenting with ovarian mass, efficient methods for the prediction of malignancy are required, as an early referral of these patients to the appropriate center and surgeon improves the disease outcome,[3] on the other side, to protect women with uncertain diagnosis of malignancy from unnecessary extensive surgical staging and consequently decrease the postoperative morbidity.[4]

Efficient tumor markers are both specific and sensitive for malignancy. Most of the ovarian tumor markers are of poor sensitivity and specificity. This limits their use in the differentiation between malignant and benign ovarian masses.[5] In addition, tumor markers should be away from the effect of any confounding factors that can influence their serum levels.[6-9]

Cancer antigen 125 (CA125) is not only the most widely used tumor marker for the diagnosis of epithelial ovarian cancers but also it is used in monitoring the success of treatment.[5] CA125 inherits limitations in sensitivity and specificity, as it is elevated in only 50% of women with early stage disease and is detected in other benign gynecological conditions such as endometriosis and pregnancy as well as nongynecological conditions as cirrhosis and congestive heart diseases.[10]

Human Epididymis protein 4 (HE4) is specific and sensitive as CA125. Additionally, it has a superior sensitivity in detecting stage I ovarian cancer.[11] Serum level of HE4 is elevated in association with preoperative CA125, advanced stage, grade, and existence of residual tumors. Moreover, it correlates with prognosis and patients overall survival.[12]

Research hypothesis

We hypothesized that, the diagnostic accuracy of CA125 will be improved by combining HE4 values and considering personal and medical factors which could influence the serum levels of these biomarkers.

Aim of this work

To participate in the improvement of CA125 performance in cancer ovary diagnosis and to draw attention to the confounding factors that should be considered carefully during the interpretation of CA125 and HE4 results.

Objectives of this work

To evaluate the diagnostic accuracy of CA125 and HE4 "each one alone and in combination" in the prediction of malignant ovarian mass then to analyze the effect of personal criteria and medical diseases on this accuracy.

Patients and methods

This prospective study was carried out at the obstetrics and gynecology department of Zagazig University Hospital in Zagazig city, Al Sharqia Governorate, Egypt, from January 2016 to June 2017. Eligibility criteria for included patient were as follow, consecutive women at any age ≥18 years, admitted to hospital with ovarian masses diagnosed by clinical examination and confirmed by imaging techniques (US and/or CT and/or MRI) in the absence of other causes of adnexal masses (eg, fibroid). In addition, patients should have normal kidney function tests and accept to give an informed consent to participate in this study. Patients with masses originating from other pelvic organs, having medical conditions that may affect the results, eg, renal problems, cases have been subjected to preoperative chemotherapy, patients who refused or unable to provide informed consent were excluded from this study.

Data collection

After obtaining written informed consent from each patient, personal and medical history was taken and data from investigation reports were collected.

Preoperative serum tumor markers estimation

Along with the routine preoperative laboratory investigations, 3 mL of blood, withdrawn in BD Vacutainer serum separation tubes, were referred to Zagazig University Hospital Lab for separation of serum then tumor marker estimation. CA125 was measured using Elycsys 2010 (Roche diagnostics, Switzerland) based on electrochemiluminescence immunoassay, using the universal cut-off point (35 IU/mL). Then, sera were aliquoted and stored at −20°**C** for further estimation of HE4 within 12 weeks. HE4 was estimated with "Fujirebio Diagnostics, Inc, Malvern PA" kit, it uses sandwich ELISA technique according to manufacturer guidelines with cutoff (150 pmol/L) as assigned by the Fujirebio Diagnostics AB kit catalogue. ELISA procedure was semi-automated, as samples and reagents pipetting was manual, strip washing, and reading were performed using Tecan Hydroflex microplate washer and Tecan Sunrise absorbance microplate reader respectively. Four qualified well-trained staff members of Zagazig University Hospital Lab "chemistry and research units" performed the laboratory investigations of this study under supervision of the first author. Results equal to or exceeding the assigned cutoffs were considered as positive test and patients were suspected to be at high risk of malignancy while results less than the cutoffs were considered negative (low risk of malignancy). Tumor markers performers were blinded to the clinical information and histopathology results of patients. Heamolyzed and lipemic samples were rejected and collected again, with 12hs fasting for lipemic patients.

Assessment after surgical intervention

All patients were subjected to exploratory laparotomy and were properly staged according to the International Federation of Gynecology and Obstetrics (2014). Followed by histopathologic examination of masses "as a gold standard for the diagnosis of malignancy"[13] by qualified 2 pathologists in the pathology department of faculty of medicine Zagazig University and a third senior one to confirm or to re-evaluate results with fallacies between the 2 opinions. After clinical, surgical, and histopathologic evaluation, all patients were categorized in to 2 groups, group I (benign) includes benign neoplastic and non-neoplastic lesions, and group II (malignant) includes borderline, nonepithelial ovarian carcinoma and epithelial ovarian carcinoma. Pathologists were blinded to the tumor markers results.

Statistical analysis

Data were analyzed using SPSS version 20 software. The following tests were used; *t* test for comparing means. Mann Whitney and Krusskal Wallis test for medians. Chi square and Fisher's exact test for frequencies. Receiver operating characteristic (ROC) curve was constructed to estimate the area under curve (AUC) at 95% confidence interval (CI). From the output data of the ROC curve, Youden's index ($J = \text{sensitivity} + \text{specificity} - 1$) for each observed cutoff was calculated; a point that retains the maximum Youden's index ie the maximum diagnostic accuracy was considered to be the best cutoff. Spearman's Rank correlation coefficient was used to assess the correlation between lesion size and tumor markers level. *P* value was set at <0.05 for significant results.

Sample size calculation

Sample size of this study was calculated using Buderer N 1996 formula, the expected prevalence was 49%[14] and sensitivity was 95%, at 95% CI.

Ethical consideration

Each patient signed an informed consent prior to inclusion in this study. In addition, Zagazig University-Institutional Research Board approved this study prior to initiation of the work. Approval number (IRB#4726/26-9-2015).

Results

From January 2016 to June 2017, 164 patients were eligible for inclusion in this study. Nine of them refused to participate and 6 moved out to another hospital. Of the included 149 cases, 9 were excluded from the analysis; 5 of them were excluded due to missed tumor markers results and 4 due to controversy histopathologic diagnosis. The remainders were 140. Fifty-six patients (40.1%) were established to have malignant ovarian masses (48 epithelial ovarian carcinoma and 8 nonepithelial ovarian carcinoma), 6 (4.3%) had borderline lesions all were included in group II. While group I included, 76 women (54.2%) who had benign ovarian neoplasms and 2 (1.4%) had inflammatory masses.

Demographic features and medical history of patients participated in this study are summarized in Table 1. The mean age of group II women as well as the frequency of postmenopausal status were higher in comparison with those in group I, $P < 0.01$. Parity, weight and body mass index did not differ significantly. Medical diseases were more frequent among group II patients $P < 0.01$.

Family history of cancers, presenting complaints and sonographic features are summarized in Table 2. Abdominal distention and gastrointestinal tract symptoms appeared more frequently in group II, $P = 0.01$ and $P < 0.01$, respectively. While the proportion of cases discovered accidentally during abdominal US was higher in group I patients $P < 0.01$. There were no significant differences regarding laterality and side of the mass. Highly significant differences regarding the size, consistency, and the presence of ascites between the 2 groups appeared $P < 0.01$.

The median serum level of CA125 was higher in group II patients (541 IU/mL, IQR 105-887 IU/mL) when compared to group I (32.5 IU/mL, IQR 15-57 IU/mL). As well as, HE4 which was (624 pmol/L, IQR 209-890 pmol/L) in group II patients and (95 pmol/L, IQR 65-211 pmol/L) in group I, $P < 0.01$ for both markers.

Of the 78 cases with benign ovarian lesions, HE4 was positive in 23 (29.5%), while CA125 was positive in 36 (46.2%) $P = 0.03$, distribution of these patients are described in Supplementary Table (I).

Table 1
Demographic features and medical history of participated patients.

	Criteria	Group I Females with benign lesions n = 78	Group II Females with malignant lesions n = 62	*P* value
Age (year)	Range	21-69	44-74	<0.01
	Mean ± SD	36.4 ± 14.1	61.6 ± 6.8	
Menstrual status	Premenopausal	65 (83.3%)	5 (8.1%)	<0.01
	Postmenopausal	13 (16.7%)	57 (91.9%)	
Parity	Range	0-5	0-7	0.13
	Median (IQR)	2(1-3)	3(2-4)	
Weight (Kg)	Range	54-102	54-105	0.99
	Mean ± SD	69.0 ± 102.0	59.0 ± 105.0	
BMI (Kg/m^2)	Range	28.1-36.8	22.2-37.3	0.75
	Mean ± SD	31.5 ± 2.2	31.3 ± 3.1	
Past history of a disease	Free	76 (97.4%)	39 (62.9%)	<0.01
	Diabetes mellitus	0 (0.0%)	5 (8.1%)	
	Chronic hypertension	1 (1.3%)	5 (8.1%)	
	Diabetes mellitus and chronic hypertension	0 (0.0%)	11 (17.7%)	
	Chronic liver diseases	0 (0.0%)	2 (3.2%)	
	Ischemic heart diseases	1 (1.3%)	0 (0.0%)	

BMI, body mass index.
t test was used for the comparison of the means, chi square and Fisher's exact tests were used for comparing frequencies and Mann Whitney test was used for the comparison of medians.

Table 2
Family history of cancers, presenting complaints and sonographic features of the ovarian masses of the included cases.

	Criteria	Group I Females with benign lesions n = 78	Group II Females with malignant lesions n = 62	*P* value
Family history of malignancy	Breast cancer	0 (0.0%)	3 (4.8%)	0.09
Presenting complaint	Abdominal pain/discomfort	55 (70.5%)	36 (58.1%)	0.08
	Abnormal genital bleeding	11 (14.1%)	5 (8.1%)	0.2
	GIT symptoms*	0 (0.0%)	13 (20.9%)	<0.01*
	Accidental finding of Ultrasound	12 (15.4%)	0 (0.0%)	<0.01*
	Abdominal distention	0 (0.0%)	5 (8.1%)	0.01
	Abdominal enlargement	0 (0.0%)	3 (4.8%)	0.08
Sonographic features of the ovarian mass	Largest dimension of the mass(cm)			
	Range	4-14	6-25	<0.01*
	Mean ± SD	7 ± 2.7	12.4 ± 4.6	
	Laterality of the mass			
	Unilateral	62 (79.5%)	44 (70.9%)	0.2
	Bilateral	16 (20.5%)	18 (29.1%)	
	Right sided	49/62 (79.0%)	28/44 (63.6%)	0.07
	Left sided	13/62 (21.0%)	16/44 (36.4%)	
	Consistency of the mass			
	Cystic	67 (85.8%)	26 (41.9%)	<0.01*
	Heterogeneous	11 (14.2%)	36 (58.1%)	
	Unilocular	59/67 (88.0%)	3/26 (11.5%)	<0.01*
	Multiloculer	8/67 (12.0%)	23/26 (88.5%)	
	Presence of ascites	0(0.0%)	7 (11.3%)	<0.01

Chi square and Fisher's exact tests were used for comparing frequencies and *t* test was used in the comparison of the size of lesions.
* GIT symptoms, Gastrointestinal tract symptoms. Including loss of appetite, vomiting, diarrhea, or constipation.

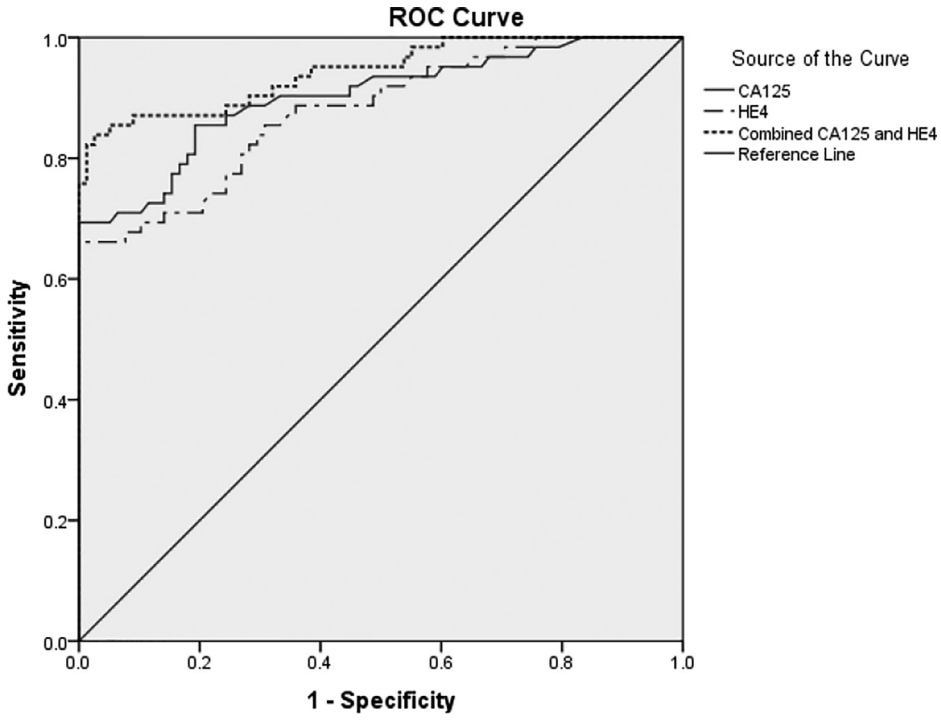

Fig. 1. ROC curves for CA125, HE4 individually and in combination in patients presented with ovarian mass. CA125, cancer antigen 125; HE4, human epididymis protein 4; ROC, receiver operating characteristic.

Measured tumor markers were significant predictors as noted by the large AUC; serum CA125 AUC = 0.90 (0.85-0.95), serum HE4 AUC = 0.88 (0.82-0.94) while combined serum CA125 and HE4 AUC = 0.94 (0.90-0.98) at 95% CI. The constructed ROC curves for the 2 markers individually and in combination are shown in Figure 1. AUC for both markers in pre- and postmenopausal women are illustrated in Supplementary Table (II).

Cut-off points obtained from the best accuracy results were; 156 pmol/L for HE4 "which approaches to the established cutoff by Fujirebio Diagnostics AB kit catalogue" and 67.5 IU/mL for CA125. The accuracy of measured serum tumor markers as predictors of ovarian malignancy in included women is illustrated in Table 3. Contingency tables instructed to show the performance of CA125 and HE4 as predictors of cancer ovary are gathered in Supplementary Table (III). Sensitivity and accuracy at 98% specificity for CA125 and HE4 are illustrated in Supplementary Table (IV).

Serum CA125 concentration above the universally accepted cutoff was associated with ovarian malignancy at sensitivity 91.9%, specificity 53.8%, while serum HE4 concentration ≥150 pmol/L was associated with ovarian malignancy at sensitivity 83.9% and specificity 70.5%. HE4 was more accurate than CA125 (76.4% vs 70.7%), but when serum CA125 cut-off level was raised to 67.5 IU/mL the test was more specific 80.7% and less sensitive 83.9%, with better accuracy over HE4 (82.1% vs 76.4%). When combined together, HE4 ≥150 pmol/L and serum CA125 ≥ 35 IU/mL was accompanied by sensitivity 75.8% and specificity 93.5% with accuracy 85.7%. While the combination between HE4 ≥150 pmol/L and serum CA125 at cutoff 67.5 IU/mL made the sensitivity 67.7%, the specificity 96.1% and accuracy became 83.6%.

Variations of serum level of both markers in relation to stage, grade, histopathologic type of malignant lesions, and the presence of ascites were evaluated. Results are summarized in Table 4.

Table 3
The accuracy of measured serum tumor markers as predictors of ovarian carcinoma.

Tumor Marker	Sensitivity	Specificity	PPV	NPV	Accuracy
Serum CA125 ≥35I U/mL	91.9% (95% CI 81.4, 96.9)	53.8% (95% CI 42.2, 65.1)	61.2% (95% CI 51.0, 71.0)	89.3% (95% CI 76.1, 96.0)	70.7%
Serum CA125 ≥67.5 IU/mL	83.9% (95% CI 71.9, 91.6)	80.7% (95% CI 70.0, 88.5)	77.6% (95% CI 65.5, 86.5)	86.3% (95% CI 75.8, 92.9)	82.1%
Serum HE4 ≥150 pmol/L	83.9% (95% CI 71.9, 91.6)	70.5% (95% CI 59.0, 80.0)	69.3% (95% CI 57.5, 79.2)	84.6% (95% CI 73.0, 92.0)	76.4%
Combined serum CA125 ≥35 IU/mL and Serum HE4 ≥150 pmol/L	75.8% (95% CI 63.0, 85.4)	93.5% (95% CI 85.0, 97.6)	90.3% (95% CI 78.2, 96.4)	82.9% (95% CI 73.1, 89.8)	85.7%
Combined serum CA125 ≥67.5 IU/mL and Serum HE4 ≥150 pmol/L	67.7% (95% CI 54.5, 78.7)	96.1% (95% CI 88.4, 99.0)	93.3% (95% CI 80.6, 98.2)	78.9% (95% CI 69.1, 86.3)	83.6%

CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.

Table 4
Variations of serum levels of both markers according to the biological behavior of malignant ovarian lesions (n = 62).

Factors	Subgroups	n (%)	CA125 (Median)	*P* value	HE4 (Median)	*P* value
Stage	I, II	5 (8.1%), 28 (45.2%)	169.5, 605	$P=0.01$	531, 690.5	$P=0.9$
	III	23 (37.1%)	764		477	
Grade	1	28 (45.2%)	541		624	
	2, 3	23 (37.1%), 5(8.1%)	474.5, 416	$P=0.8$	554.5, 449	$P=0.7$
Histopathology of malignant lesions	Serous	20 (32.2%)	572		724	
	Mucinous	15 (24.2%)	616.5	$P=0.15$	630.5	$P=0.08$
	Endometrioid	10 (16.1%)	815.5		471.5	
Ascites	Others*	17 (27.5%)	115.5		198	$P=0.3$
	Present	7 (11.3%)	836	$P=0.2$	558	
	Absent	55 (88.7%)	478		684	

Mann Whitney and Krusskal Wallis tests were used.

* Others group includes 6 borderline, 3 clear cell carcinoma and 8 non-EOC patients. The latest includes "5 immature teratoma, 2 dysgerminoma, and 1 Krukenburg tumor."

Grade, histopathology of malignant lesions, and the presence of ascites did not show significant effects on the serum levels of both markers. In advanced lesions (stage III) CA125 increased significantly $P=0.01$ while HE4 did not $P=0.9$. There were no correlation between the size of the tumor and the serum level of both markers in malignant and benign conditions.

Variations of serum level of CA125 and HE4 were analyzed in relation to personal characteristics of patients and the past history of medical disease. Results are summarized in Table 5.

In patients having benign ovarian masses, CA125 increased with aging $P < 0.01$ while HE4 decreased with obesity $P=0.01$. In group II patients HE4 increased in postmenopausal females in comparison with premenopausal ones $P=0.03$. Parity showed no significant effects on serum levels of both markers.

Among group II patients, the median serum level of CA125 in women who had diabetes mellitus (DM) or chronic hypertension was lower than that in cases free from both diseases, but difference was not significant. However, in patients who suffer from both diseases, the median serum level of CA125 (36 IU/mL) was significantly lower, compared with that in women free from DM and hypertension, $P=0.02$. In other words; 5 patients of 11 had false negative results, making the sensitivity of CA125 in these patients 54.5%. HE4 levels are not influenced by these diseases.

Table 5
Variations of serum levels of CA125 and HE4 in relation to personal factors and medical history of patients.

Factors	Malignancy status	Subgroups	n (%)	CA125 (Median)	*P* value	HE4 (Median)	*P* value
Age	Malignant (62 cases)	<60	18(29.0%)	203	$P=0.17$	202	$P=0.2$
		≥60	44(71.0%)	629		684	
	Benign (78 cases)	<50	62(79.5%)	25.5	$P<$	114	$P=0.1$
		≥50	16(20.5%)	103	0.01**	71	
BMI	Malignant (62 cases)	<30	13 (21.0%)	905	$P=0.4$	345	$P=0.3$
		≥30 (obese)	49 (79.0%)	478		684	
	Benign (78 cases)	<30	23 (29.5%)	31	$P=0.2$	209	$P=0.01$**
		≥30 (obese)	55(70.5%)	39		82	
Menstrual status	Malignant (62 cases)	Premenopausal	5(8.1%)	307	$P=0.1$	139	$P=0.03$**
		Postmenopausal	57 (91.9%)	616.5		690.5	
	Benign (78 cases)	Premenopausal	65 (83.3%)	129	$P=0.5$	98	$0=0.2$
		Postmenopausal	13(16.7%)	115		78	
Parity	Malignant (62 cases)	Nulliparous	5(8.1%)	491	$P=0.6$	76	$P=0.1$
		1-2	23(37.1%)	283		769	
		3-4	23(37.1%)	629		627.5	
		≥5	11(17.7%)	640		748.5	
	Benign (78 cases)	Nulliparous	8(10.2%)	16	$P=0.8$	112	$P=0.4$
		1-2	44(56.5%)	32		98	
		3-4	23(29.5%)	48		84	
		≥5	3(3.8′%)	41		77	
Past history of a disease*	Malignant (62 cases)†	Free	39 (62.9%)	764	$P=0.02$**	564	$P=0.4$
		Diabetes mellitus and Hypertension	11(17.7%)	36		577	
		Diabetes mellitus or Hypertension	10(16.1)	465.5		579.5	

Benign = Group I, Malignant = group II; BMI, body mass index; Mann Whitney and Krusskal Wallis tests were used.

* Patients with benign lesion are not included as only 2 of them had history of medical diseases(1 had chronic hypertension and the other had ischemic heart disease).

† The remaining 2 patients had chronic liver diseases are not included in the comparison.

Discussion

This study included 140 women, 78 of them had benign ovarian lesions (group I), while 56 had ovarian malignancy and 6 had borderline lesions, both were enrolled in one group (group II). CA125 and HE4 were significant predictors of ovarian malignancy as concluded from the ROC-AUC. When used as a single marker, serum CA125 with cut-off point 35IU/mL was more sensitive and serum HE4 was more specific as it was positive less frequently in case of benign lesions than CA125 (29.5% vs 46.2%). This agrees with the results of Holcomb et al[15] and Moore et al.[16] On the contrary, Montagnana et al[17] concluded that HE4 had higher AUC than CA125.

After raising its cutoff to 67.5 IU/mL, CA125 performed better, it had a superior specificity over HE4 with modest decrease in sensitivity than CA125 at 35 IU/mL and the overall accuracy was better. This cutoff approximates to that in Van Gorp et al study[3] who considered 62.5 IU/mL as the ideal cutoff for CA125 for cancer ovary prediction. This signifies the recommendation of Benjapibal et al[14] that, the reliability of clinical use of this level (35 IU/mL) in patients with ovarian mass needs to be evaluated. Combination of the 2 markers "HE4 and CA125 ≥35 IU/mL" was more accurate in predicting cancer ovary. This agrees with Kristjansdottir et al[18], Zhao and Hu[19] who found that the combination of both markers had the best diagnostic power in patients with cancer ovary.

After all, based on the low prevalence of ovarian carcinoma in postmenopausal women (1:2500), increasing the specificity of the diagnostic tools to 99.6% with sensitivity ≥75% are required to obtain positive predictive value ≥10% for early detection of ovarian malignancy.[20] This can be achieved either by the introduction of a third marker or using algorithms.

Risk of ovarian malignancy algorithm, is an algorithm that utilize HE4 and CA125 with the menopausal status to provide a numeric risk stratification of ovarian masses. Moore et al reported that it performs better than Risk malignancy index in ovarian malignancy prediction.[21-23] Many publications have approved the usefulness of the introduction of this model,[24,25] and others have not.[26,27]

Evaluating tumor marker performance in isolation of the influence of other factors is less informative, and the detection of the effect of these factors on the serum levels and hence the diagnostic accuracy of this marker will be more beneficial.[28]

In this study, among cases with benign lesions, CA125 increased significantly with aging. Most of the prior studies reported that CA125 decreases with age[29-31] and others showed no correlation.[32] Our results came in accordance with other studies where the CA125 levels showed a positive correlation with age.[33,34] Dehaghani et al[33] attributed their results to the variation in the studied population, while Johnson et al[34] attributed theirs to the aging process of their cases. Pauler et al[35] explained that the history of having cancer could attenuate the decrease of CA125 with age. As well, our result can be attributed to the effect of the existing lesions themselves.

In this work, obese patients with benign lesions had significant decrease in HE4 serum levels; this could be explained by the effect of increased plasma volume in obese patients (hemodilution).[36] In group II postmenopausal patients HE4 increased significantly, this finding agrees with Moore et al[37] who illustrated that specified thresholds depending on age or menopause are needed.

In this study CA125 showed a decrease in patients having DM or hypertension, but when both condition occurred together CA125 decreased significantly $P = 0.02$ with median serum level (36 IU/mL). In other words, 45.5% of patients suffering from both DM and hypertension had false negative results of CA125, decreasing its sensitivity from 91.9% to 54.5%. So, DM with hypertension can be real confounding factors affecting its serum level and this should be taken in consideration during the interpretation of CA125 results. HE4 was independent on these diseases; this can be considered as an additional benefit of the introduction of HE4, as it can replace CA125 in this particular condition. More investigations with larger sample size are required to confirm and explain this finding. To our knowledge, few articles concerned with the evaluation of serum levels of CA125 in association diabetes mellitus and hypertension, Esteghamati et al[7] concluded that CA125 is inversely correlated with diabetes, metabolic syndrome components and the associated systolic hypertension. Joo et al,[38] showed that, CA125 decreases in association with all components of metabolic syndromes including elevated blood sugar and hypertension.

Limitations

Sample size is relatively small, future studies with larger sample size are recommended. Full data about the personal characteristics of patients including smoking, oral contraceptive pills administration, age of menarche, and age of menopause were not available. In addition, in patients with diabetes mellitus and hypertension, information about type, medications, age of onset, and whether they are part of metabolic syndrome or not were not obtained.

Conclusions

The accuracy of CA125 in risk stratification of ovarian masses can be improved either by raising its cutoff or better by combining it with HE4 serum level. An additional benefit of introducing HE4 is being independent on diabetes mellitus and hypertension, as in the presence of both diseases the sensitivity of CA125 markedly decreased. DM with hypertension can be real confounding factors affecting CA125 performance.

Acknowledgment

Gratitude to Dr. Heba A. Ahmed, assistant professor in Faculty of Veterinary Medicine Zagazig University for her advices in writing and publishing this work.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.currproblcancer.2018.12.004.

References

1. Ibrahim AS, Khaled HM, Mikhail NN, et al. Cancer incidence in Egypt: results of the national population-based cancer registry program. *J Cancer Epidemiol*. 2014;2014:18.
2. Huang J, Hu W, Sood AK. Prognostic biomarkers in ovarian cancer. *Cancer Biomark*. 2010;8:231–251.
3. Van Gorp T, Cadron I, Despierre E, et al. HE4 and CA125 as a diagnostic test in ovarian cancer: prospective validation of the risk of ovarian malignancy algorithm. *Br J Cancer*. 2011;104:863–870.
4. Partheen K, Kristjansdottir B, Sundfeldt K. Evaluation of ovarian cancer biomarkers HE4 and CA-125 in women presenting with a suspicious cystic ovarian mass. *J Gynecol Oncol*. 2011;22:244–252.
5. Nolen B, Velikokhatnaya L, Marrangoni A, et al. Serum biomarker panels for the discrimination of benign from malignant cases in patients with an adnexal mass. *Gynecol Oncol*. 2010;117:440–445.
6. Bolstad N, Øijordsbakken M, Nustad K, et al. Human epididymis protein 4 reference limits and natural variation in a Nordic reference population. *Tumour Biol*. 2012;33:141–148.
7. Esteghamati A, Seyedahmadinejad S, Zandieh A, et al. The inverse relation of CA-125 to diabetes, metabolic syndrome, and associated clinical variables. *Metab Syndr Relat Disord*. 2013;11:256–261.
8. Urban N, Thorpe J, Karlan BY, et al. Interpretation of single and serial measures of HE4 and CA125 in asymptomatic women at high risk for ovarian cancer. *Cancer Epidemiol Biomarkers Prev*. 2012;21:2087–2094.
9. Cramer DW, Vitonis AF, Welch WR, et al. Correlates of the preoperative level of CA125 at presentation of ovarian cancer. *Gynecol Oncol*. 2010;119:462–468.
10. Maggino T, Gadducci A, D'Addario V, et al. Prospective multicenter study on CA 125 in postmenopausal pelvic masses. *Gynecol Oncol*. 1994;54:117–123.
11. Moore RG, Brown AK, Miller MC, et al. The use of multiple novel tumor biomarkers for the detection of ovarian carcinoma in patients with a pelvic mass. *Gynecol Oncol*. 2008;108:402–408.
12. Simmons AR, Baggerly K, Bast RC. The emerging role of HE4 in the evaluation of advanced epithelial ovarian and endometrial carcinomas. *Oncology*. 2013;27:548–556.
13. Dora SK, Dandapat AB, Pande B, et al. A prospective study to evaluate the risk malignancy index and its diagnostic implication in patients with suspected ovarian mass. *J Ovarian Res*. 2017;10:55–76.
14. Benjapibal M, Neungton C. Pre-operative prediction of serum CA125 level in women with ovarian masses. *J Med Assoc Thai*. 2007;90:1986–1991.
15. Holcomb K, Vucetic Z, Miller MC, et al. Human epididymis protein 4 offers superior specificity in the differentiation of benign and malignant adnexal masses in premenopausal women. *Am J Obstet Gynecol*. 2011;205:358 e1-6.
16. Moore RG, Miller MC, Steinhoff MM, et al. Serum HE4 levels are less frequently elevated than CA125 in women with benign gynecologic disorders. *Am J Obstet Gynecol*. 2012;206:351 e1-8.
17. Montagnana M, Lippi G, Ruzzenente O, et al. The utility of serum human epididymis protein 4 (HE4) in patients with a pelvic mass. *J Clin Lab Anal*. 2009;23:331–335.
18. Kristjansdottir B, Levan K, Partheen K, et al. Diagnostic performance of the biomarkers HE4 and CA125 in type I and type II epithelial ovarian cancer. *Gynecol Oncol*. 2013;131:52–58.
19. Zhao T, Hu W. CA125 and HE4: measurement tools for ovarian cancer. *Gynecol Obstet Investig*. 2016;81:430–435.
20. Das PM, Bast RC. Early detection of ovarian cancer. *Biomark Med*. 2008;2:291–303.
21. Moore RG, McMeekin DS, Brown AK, et al. A novel multiple marker bioassay utilizing HE4 and CA125 for the prediction of ovarian cancer in patients with a pelvic mass. *Gynecol Oncol*. 2009;112:40–46.
22. Moore RG, Jabre-Raughley M, Brown AK, et al. Comparison of a novel multiple marker assay vs the risk of malignancy index for the prediction of epithelial ovarian cancer in patients with a pelvic mass. *Am J Obstet Gynecol*. 2010;203:228 e1-6.
23. Moore RG, Miller MC, Disilvestro P, et al. Evaluation of the diagnostic accuracy of the risk of ovarian malignancy algorithm in women with a pelvic mass. *Obstet Gynecol*. 2011;118:280–288.
24. Bandiera E, Romani C, Specchia C, et al. Serum human epididymis protein 4 and risk for ovarian malignancy algorithm as new diagnostic and prognostic tools for epithelial ovarian cancer management. *Cancer Epidemiol Biomark Prev*. 2011;20:2496–2506.
25. Wang J, Gao J, Yao H, et al. Diagnostic accuracy of serum HE4, CA125 and ROMA in patients with ovarian cancer: a meta-analysis. *Tumor Biol*. 2014;35:6127–6138.
26. Kaijser J, Van Gorp T, Van Hoorde K, et al. A comparison between an ultrasound based prediction model (LR2) and the Risk of Ovarian Malignancy Algorithm (ROMA) to assess the risk of malignancy in women with an adnexal mass. *Gynecol Oncol*. 2013;129:377–383.

27. Kaijser J, Van Gorp T, Smet M-E, et al. Are serum HE4 or ROMA scores useful to experienced examiners for improving characterization of adnexal masses after transvaginal ultrasonography? *Ultrasound Obstet Gynecol*. 2014;43:89–97.
28. Lowe KA, Shah C, Wallace E, et al. Effects of personal characteristics on serum CA125, mesothelin, and HE4 levels in healthy post-menopausal women at high-risk for ovarian cancer.. *Cancer Epidemiol Biomark Prev*. 2008;17:2480–2487.
29. Crump C, McIntosh MW, Urban N, et al. Ovarian cancer tumor marker behavior in asymptomatic healthy women: implications for screening. *Cancer Epidemiol Biomark Prev*. 2000;9:1107–1111.
30. Grover S, Quinn MA, Weideman P, et al. Factors influencing serum CA 125 levels in normal women. *Obstet Gynecol*. 1992;79:511–514.
31. Koper NP, Thomas CMG, Massuger LFAG, et al. Serum CA 125 concentrations in women of different ages, hormonal statuses, orclinical conditions. *Int J Gynecol Cancer*. 1997;7:405–411.
32. Cengiz B, Atabekoglu C, Cetinkaya E, et al. Effect of hormone replacement therapy on serum levels of tumor markers in healthy postmenopausal women. *Maturitas*. 2003;46:301–306.
33. Dehaghani AS, Ghiam AF, Hosseini M, et al. Factors influencing serum concentration of CA125 and CA15-3 in Iranian healthy postmenopausal women. *Pathol Oncol Res*. 2007;13:360–364.
34. Johnson CC, Kessel B, Riley TL, et al. The epidemiology of CA-125 in women without evidence of ovarian cancer in the prostate, lung, colorectal and ovarian cancer (PLCO) screening trial. *Gynecol Oncol*. 2008;110:383–389.
35. Pauler DK, Menon U, McIntosh M, et al. Factors influencing serum CA125II levels in healthy postmenopausal women. *Cancer Epidemiol Biomarkers Prev*. 2001;10:489–493.
36. Park M, Chang IH, Kang H, et al. Effect of obesity-related plasma hemodilution on serum tumor marker concentration in women. *J Obstet Gynaecol Res*. 2015;41:784–789.
37. Moore RG, Miller MC, Eklund EE, et al. Serum levels of the ovarian cancer biomarker HE4 are decreased in pregnancy and increase with age. *Am J Obstet Gynecol*. 2012;206:349 e1-7.
38. Joo NS, Kim KN, Kim KS. Serum CA125 concentration has inverse correlation with metabolic syndrome. *J Korean Med Sci*. 2011;26:1328–1332.