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Diagnostic accuracy of peripheral blood Kisspeptin mRNA and plasma CA125 protein for detection of epithelial ovarian cancer in patients who have ever been pregnant

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To evaluate the diagnostic accuracy of peripheral blood kisspeptin (KISS1) mRNA and plasma cancer antigen 125 (CA125) protein of epithelial ovarian cancer (EOC) in previously pregnant patients, we prospectively enrolled 40 EOC patients as cases and 20 uterine fibroids patients with normal ovary as controls. Levels of peripheral blood KISS1 mRNA and plasma CA125 protein was respectively measured by RT-PCR and electrochemiluminescent method, respectively. Receiver operating characteristic curves with area under curve (AUC) were used to evaluate the diagnostic accuracy. Logistic regression analysis was used to obtain a prediction model for combined diagnosis of KISS1 mRNA and CA125 protein. Both KISS1 mRNA and CA125 protein and had good diagnostic accuracy for EOC, early EOC and advanced EOC (AUC > 0.5, P < 0.05). The CA125 protein had higher diagnostic accuracy than KISS1 mRNA for advanced EOC (P = 0.0009). Moreover, the combination of KISS1 mRNA and CA125 protein had higher diagnostic accuracy for EOC than them alone (P < 0.05). However, this combined diagnosis was more effective than KISS1 mRNA alone for the diagnosis of advanced EOC (P = 0.0001), but similar with CA125 protein alone (P = 0.3125). In addition, there was similar diagnostic accuracy among KISS1 mRNA, CA125 protein and prediction model for early EOC (P > 0.05). Peripheral blood KISS1 mRNA was a novel biomarker for detecting EOC in previously pregnant patients. Combination application of KISS1 mRNA and CA125 protein was recommended for the diagnosis of EOC, but not for advanced and early EOC.

Key words: epithelial ovarian cancer, biomarker, diagnosis, KISS1 mRNA, cancer antigen 125

Ovarian cancer (OC), as the most lethal gynecologic malignancy and itd incidence is highest among women worldwide [1]. In China, the crude incidence of OC was 7.91/100,000 during the period 1999-2010 [2]. Almost 80% of human ovarian malignant neoplasms originate from epithelium, with the rest originating from granulosa cells, stroma or germ cells [3]. Previous studies reviewed the possible pathology and pharmaceutical therapy of OC [4-6]; however, the problem at early stage diagnose remain [7]. Most diagnosed OC are in advanced stage, with a poor five-year survival rate of less than 15% [8], whereas the patients with early stage OC had more than 80% survival rate [9]. The lack of specific symptoms is the principal reason for the delayed diagnostic of OC [7], thus, seeking for a diagnosis method for early EOC was currently an important task.

Cancer antigen 125 (CA125), one member of mucin family glycoprotein, has been known as a serum marker for OC and epithelium OC (EOC) [10-12]. However, it has been reported that serum CA125 is a poor biomarker for detecting the early EOC [13]. Thus, it is necessary to develop a novel marker for the detection of early EOC. Kisspeptin, a 54-amino acid peptide that is encoded by the KiSS-1 metastasis-suppressor gene (KISS1), is an essential gatekeeper in control of reproduction and plays a crucial role in puberty period and fertility [14, 15]. It has reported that plasma kisspeptin could be a novel tumor marker in women with malignant gestational trophoblastic neoplasia due to its raised expression [16]. Moreover, kisspeptin is also known as a metastasis suppressor in OC [17]. In addition, Zhang et al found a higher expression of KISS1 mRNA in EOC than in normal ovary [18]. However, it is still

unknown whether the level of KISS1 mRNA was associated with the stage of OC and whether it can be used as a biomarker for the detection of EOC, especially for the early EOC. In addition, no study has investigated the diagnostic accuracy of plasma CA125 protein for EOC. Therefore, we performed this study to evaluate the diagnostic accuracy of peripheral blood KISS1 mRNA and plasma CA125 protein for EOC.

In addition, previous studies have reported that pregnancy could affect the risk of OC [19, 20]. Although patients who have ever been pregnant had less risk of OC than that who have never been pregnant [20], there were still approximately 80% of previously pregnant patients in total OC patients [21, 22]. Thus, we specifically investigated the diagnostic accuracy of peripheral blood KISS1 mRNA and plasma CA125 protein in EOC patients who have ever been pregnant. Meanwhile, the combined diagnosis model of KISS1 mRNA and CA125 protein for EOC was established and evaluated in this study.

Patients and methods

The study has been approved by the Ethics Committee of First People's Hospital of Nantong, and written informed consent was obtained from each participant.

Patients. Total 40 patients with EOC, who received cytoreductive surgery at First People's Hospital of Nantong from January 2010 to January 2014, were prospectively recruited in the case group of this study. The inclusion criteria were (1) Chinese Han people aged over than 18 years old, (2) no consanguinity with each other (to avoid the influence of genetic factors on the results of this study), (3) body mass index (BMI) from 18 kg/m^2 to 30 kg/m^2, (4) patients experiencing pregnancy 1-5 times and parturition 1-3 times, (5) no history of radiotherapy, chemotherapy and sex hormone drugs before operation, (6) patients receiving platinum-based chemotherapy after operation. In addition, patients had pregnancy during this study or had tumors in other tissues or organs were excluded in this study.

All the patients were diagnosed with EOC by two experienced doctors using the pathological examination. According to the criteria of World Health Organization (WHO) [23], there were 7 cases with endometrioid carcinoma, 24 cases with serous cystadenocarcinoma, 6 cases with mucinous cystadenoma and 3 clear cell carcinoma. Meanwhile, the staging of EOC was performed based on the FIGO (International Federation of Obstetrics and Gynecology) staging system [24]. Among the 40 patients, 12 cases were at FIGO stage I or II (early EOC), 28 cases were at FIGO stage III or IV (advanced EOC).

In addition, 20 patients with uterine fibroids and normal ovaries, who had voluntarily received the oophorectomy, were recruited in the control group.

After operation, the follow up was performed by telephone or letter in all the 40 patients with EOC. The follow up duration ranged from 11.2 months to 62.1 months.

Measurement of the peripheral blood markers. The peripheral blood samples were collected from the elbow vein into EDTA anticoagulated tubes before surgery for all the participants (40 patients with EOC and 20 controls). Plasma was immediately separated by centrifuging anticoagulated blood samples at 1000 g for 10 min and stored at −20°C until CA125 detection. The nuclear cells including leukocytes and few circulating ovarian cancer cells were isolated by Ficoll density gradient centrifugation, transferred into another centrifuge tube and frozen in liquid nitrogen until KISS1 mRNA extraction. The total RNA was extracted from samples using the Trizol method (Invitrogen, Carlsbad, CA, USA). The cDNA was synthesized from the total RNA using a TaqMan Reverse Transcription Kit (Perkin Elmer/Roche Molecular Systems, Inc, Branchburg, NJ, USA). The reaction was conducted at 25°C for 10 min, 50°C for 60 min and 85°C for 5 min. Subsequently, the cDNA was amplified using PCR with the following conditions: 35 cycles of 94°C for 45 s, 58°C for 60 s and 72°C for 60 s, and a final extension at 72°C for 10 min. The primers of KISS1 (forward primer: CCACCCTCTGGACATTCA, reverse primer: GCCGAAGGAGTTCCAGTT) and *β-actin* (forward pimer: ATCATGTTTGAGACCTTCAACA, reverse primer: CATCTCTTGCTCGAAGTCCA) were used. The sizes and quantities of PCR products were determined by 2% agarose gel electrophoresis. The bands in gels were visualized and analyzed using the Chemi Imager 5500 gel image analysis system (Alpha Innotech, San Leandro, CA). The relative expression of KISS1 mRNA was calculated using the *β-actin* as internal reference.

In addition, the level of CA125 protein in plasma was detected by the automated electrochemiluminescence (ECL) analyzer E170 (Roche Diagnostics, Germany) using the ruthenium bipyridine as ECL label.

Statistics analysis. The analyses were performed using the SPSS 19.0 and Stata 11.0. Data were presented as mean ± standard deviation [SD] when the data meet the normal distribution using Shapiro-Wilk W test. Then, the comparison between two groups was tested by two sample independent t-test; the one-way analysis of variance (ANOVA) was used to test the differences among three or more groups and Tukey's honestly significant difference (HSD) or Dunnett's T3 tests were used for post hoc comparisons. When data were not in normal distribution, the Mann-Whitney U test was used for comparison between two groups; the Kruskal-Wallis test was used for comparison among multiple groups. Meanwhile, due to the non-normal distribution of data of CA125 protein, the correlation between KISS1 mRNA and CA125 protein was assessed using the Spearman'rho correlation coefficient. The diagnostic accuracy of KISS1 mRNA and CA125 protein for EOC was evaluated using the receiver operating characteristic (ROC) curves. An area under curve (AUC) close to 1 represents good diagnostic accuracy, while poor diagnostic accuracy will have an AUC as low as 0.5. The comparison between AUCs was evaluated using Stata software. The prediction model of diagnosis was obtained by the logistic regression analysis. The Kaplan-Meier method was conducted to assess the survival of patients with EOC. The prognostic roles of age, BMI, times of pregnancy and parturition, KISS1

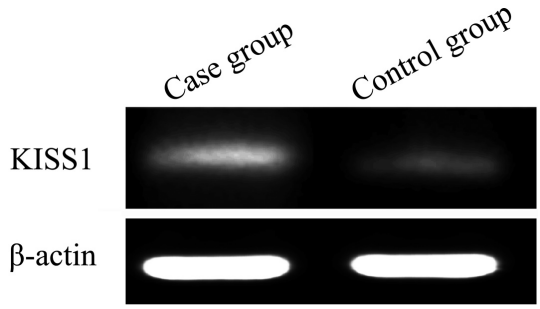

Figure 1. Agarose gel electrophoresis of KISS1 mRNA.

mRNA, CA125 protein, FIGO stage, residual tumor size, differentiation and metastasis for the survival of patients with EOC was evaluated using the Cox regression analysis (forward likelihood ratio method).

Results

Characteristics of participants. As shown in Table 1, there was no significant difference between EOC cases and controls in terms of age, BMI and times of pregnancy and parturition. However, patients with EOC had higher levels of KISS1 mRNA (case group: 0.73 ± 0.16; control group: 0.57 ± 0.07; P < 0.001; Figure 1) and CA125 protein (case group: 216.40 [36.13, 430.48]; control group: 19.50 [13.50, 22.85]; P < 0.001) than the uterine fibroids patients with normal ovaries.

Levels of peripheral blood KISS1 mRNA and plasma CA125 protein. The EOC patients in this study were further grouped according to the basic and clinical characteristics (age, times of parturition and pregnancy, BMI, pathological type, FIGO stage, differentiation, residual tumor size and metastasis). Results showed that the levels of KISS1 mRNA were similar between groups based on age, metastasis, BMI and times of parturition and pregnancy (P > 0.05). However, the significant differences in the levels of KISS1 mRNA were found among groups based on pathological type (P = 0.018), FIGO stage (P = 0.041, early vs. advanced cancer: P = 0.009), differentiation (P = 0.002) and residual tumor size (P = 0.002). Meanwhile, the CA125 protein levels were significantly different among the patients with different FIGO stages (P < 0.001, early vs. advanced cancer: P < 0.001), different grade of differentiation (P = 0.047) and between patients with and without metastasis (P = 0.003). Similar levels of CA125 protein were observed among groups based on age, times of parturition and pregnancy, BMI, pathological type and residual tumor size (P > 0.05, Table 2).

In addition, the results also showed that there was a negative correlation between the levels of KISS1 mRNA and CA125 protein (r = -0.538, P < 0.001) in patients with EOC.

Diagnostic reliability of KISS1 mRNA and CA125 protein for EOC. The ROC analysis of KISS1 mRNA determined that AUC was 0.773 (95% confidence interval [CI] = 0.656 – 0.890; P = 0.001, Figure 2A) with an optimal cut-off value of 0.685, yielding a sensitivity of 0.600 and a specificity of 1.000. The ROC curve of CA125 protein revealed that the AUC was 0.902 (95% CI = 0.823 – 0.982; P < 0.001, Figure 2B) with an optimal cut-off value of 33.55 U/ml, yielding a sensitivity of 0.775 and a specificity of 1.000. Besides, the diagnostic accuracy of KISS1 mRNA and CA125 protein was similar (P = 0.5241). Combined with KISS1 mRNA and CA125 protein, the prediction model ($P = 1/[1+e^{-(19.476-23.772\times KISS1\ mRNA-0.131\times CA125\ protein)}]$) was constructed by logistic regression analysis. The AUC based on this prediction model (AUC = 0.989, 95% CI = 0.969 -1.000, P < 0.001) was more close to 1 than that based on KISS1 mRNA (P =0.0063) or CA125 protein alone (P = 0.0039).

In addition, the KISS1 mRNA and CA125 protein were of good diagnostic accuracy for the diagnosis of early EOC (KISS1 mRNA: AUC = 0.879, 95% CI = 0.721 -1.000, P < 0.001; CA125 protein: AUC = 0.779, 95% CI = 0.583 – 0.976, P = 0.009; Figure 3A) and advanced EOC (KISS1 mRNA: AUC = 0.728, 95% CI = 0.583 – 0.872, P =0.008; CA125 protein: AUC = 0.955, 95% CI = 0.893 – 1.000, P < 0.001, Figure 3C). Meanwhile, the diagnostic accuracy of CA125 protein was significantly higher than that of KISS1 mRNA for advanced cancer (P = 0.0009), whereas no significant difference was found between the diagnostic accuracy of CA125 protein and KISS1 mRNA for early cancer. Moreover, there were no significantly difference between the diagnostic accuracy

Table 1. Characteristics of participants in this study

Factors	Case group (n = 40)	Control group (n =20)	P-value
Age (year)	53.28 ± 11.10	56.75 ± 5.95	0.112
Pregnancy (time)	3.10 ± 1.36	2.95 ± 1.15	0.674
Parturition (time)	1.33 ± 0.47	1.30 ± 0.47	0.817
BMI (kg/m^2)	23.20 ± 2.88	23.64 ± 2.67	0.570
Level of KISS1 mRNA (relative to *β-actin*)	0.73 ± 0.16	0.57 ± 0.07	< 0.001
Level of CA125 protein (U/ml)*	216.40 (36.13, 430.48)	19.50 (13.50, 22.85)	< 0.001

Abbreviations: BMI, body mass index; KISS1, KiSS-1 metastasis-suppressor gene; CA125, carcinoma antigen 125.

*: Mann-Whitney U test was used for the comparison between two groups due to the data of CA125 protein level with non-normal distribution. Two sample independent t-test was used to test the differences between cases (patients with epithelial ovarian cancer) and controls with regard to other parameters.

Table 2. Levels of KISS1 mRNA and CA125 protein in patients with epithelial ovarian cancer

Group	Sample size (n)	KISS1 mRNA (relative to β-actin)	CA125 protein (U/ml)*
Age			
≤ 60 years	27	0.73 ± 0.15	184.30 (30.11, 422.30)
> 60 years	13	0.73 ± 0.17	363.10 (36.65, 751.14)
P-value		0.929	0.411
Pathological type			
Endometrioid carcinoma	7	0.69 ± 0.15**ab**	401.30 (360.92, 738.00)
Serous cystadenocarcinoma	24	0.73 ± 0.16**a**	253.18 (42.82, 677.70)
Mucinous cystadenoma	6	0.87 ± 0.09**a**	70.42 (17.98, 246.53)
Clear cell carcinoma	3	0.54 ± 0.04**b**	78.19 (25.93, 140.50)
P-value		0.018	0.085
FIGO stage			
I	7	0.81 ± 0.20**a**	30.11 (20.24, 78.19)**a**
II	5	0.87 ± 0.07**a**	27.30 (16.70, 85.65)**a**
III	24	0.70 ± 0.14**a**	340.91 (173.95, 723.55)**b**
IV	4	0.65 ± 0.16**a**	535.75 (372.65, 858.30)**b**
P-value		0.041	< 0.001
Early vs. advanced cancer			
Early cancer (FIGO stage I-II)	12	0.84 ± 0.16	28.71(21.66, 68.07)
Advanced cancer (FIGO stage III-IV)	28	0.70 ± 0.14	373.25 (184.38, 723.55)
P-value		0.009	< 0.001
Differentiation			
G1	12	0.86 ± 0.12**a**	36.65 (18.96, 538.78)**a**
G2	9	0.70 ± 0.14**b**	383.40 (227.25, 976.70)**b**
G3	19	0.67 ± 0.15**b**	248.20 (78.19, 401.30)**ab**
P-value		0.002	0.047
Residual tumor size			
≤ 1 cm	28	0.78 ± 0.15	137.05 (28.00, 412.58)
> 1 cm	12	0.63 ± 0.12	381.10 (202.77, 784.11)
P-value		0.004	0.067
Metastasis			
No	7	0.81 ± 0.20	30.11 (20.24, 78.19)
Yes	33	0.72 ± 0.15	320.90 (124.05, 675.20)
P-value		0.148	0.003
Parturition			
1 time	27	0.72 ± 0.15	248.20 (58.16, 433.20)
2 times	13	0.76 ± 0.17	133.60 (26.62, 568.55)
P-value		0.452	0.554
Pregnancy			
1 – 2 times	15	0.71 ± 0.17	320.90 (30.11, 670.20)
3 – 6 times	25	0.74 ± 0.15	184.30 (36.62, 410.70)
P-value		0.564	0.567
BMI			
< 25 kg/m^2	29	0.71 ± 0.15	248.20 (68.18, 709.10)
≥ 25 kg/m^2	11	0.81 ± 0.14	133.60 (27.30, 360.92)
P-value		0.063	0.256

*: Mann-Whitney U test was used for the comparison between two groups and Kruskal-Wallis test was used for the comparison among multiple groups due to the data of CA125 protein level with non-normal distribution.

For data of KISS1 mRNA, two sample independent t-test was used to test the differences between two groups, and the one-way analysis of variance (ANOVA) was used to test the differences among three or more groups.

Different letters (**a** and **b)** show significant differences (P < 0.05) between two levels**.**

Abbreviations: KISS1, KiSS-1 metastasis-suppressor gene; CA125, carcinoma antigen 125; FIGO, International Federation of Obstetrics and Gynecology; BMI, body mass index.

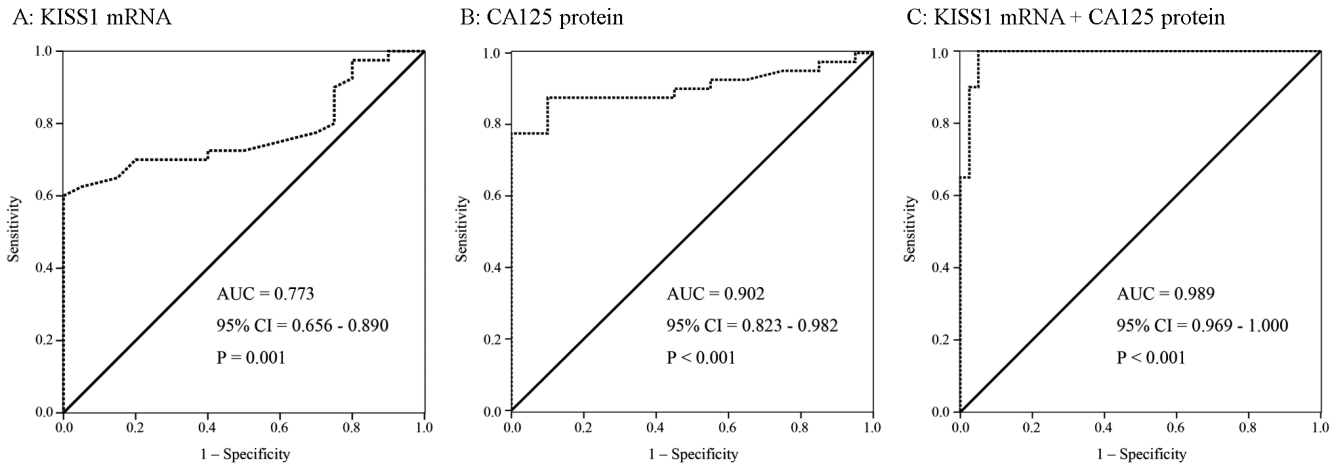

Figure 2. The receiver operating characteristic (ROC) curves of KISS1 mRNA and CA125 protein for epithelial ovarian cancer.
A: ROC curve based on KISS1 mRNA; B: ROC curve based on CA125 protein; C: ROC curve based on the prediction model. Abbreviations: KISS1, KiSS-1 metastasis-suppressor gene; CA125, carcinoma antigen 125.

of the combined prediction model (AUC = 0.971, 95% CI = 0.911 – 1.000, P < 0.001, Figure 3B) and KISS1 mRNA or CA125 protein alone for early cancer (P > 0.05). However, the prediction model had significantly higher diagnostic accuracy for advanced EOC (AUC = 0.996, 95% CI = 0.987 – 1.000, P < 0.001, Figure 3D) than the KISS1 mRNA alone (P = 0.0001), but had similar with CA125 protein alone (P = 0.3125).

Prognostic reliability of KISS1 mRNA level for survival of patients with EOC. Among the 40 EOC patients, two cases were lost to follow up due to the wrong telephone number and address. In addition, 27 cases were dead during follow up. The mean survival time were 38.5 months (95% CI: 32.8 months to 43.9 months).

According to the results of Cox regression analysis, the KISS1 mRNA level (hazard radio [HR] = 0.022, 95% CI = 0.001 – 0.444, P = 0.013) was a prognostic factor of survival of EOC patients undergoing cytoreductive surgery, independent of the FIGO stage (Table 3).

Discussion

The CA125 protein is one of the known serum biomarker for OC and EOC. In this study, we found the peripheral blood KISS1 mRNA and plasma CA125 protein levels could also be used as a biomarker for the diagnosis of EOC in patients who have ever been pregnant. Moreover, the combined diagnostic model of KISS1 mRNA and CA125 protein had higher diagnostic accuracy for EOC than KISS1 mRNA or CA125 protein alone.

In the present study, the patients with EOC had significantly higher levels of peripheral blood KISS1 mRNA and plasma CA125 protein than uterine fibroids patients with normal ovaries. Moreover, significant negative correlation was found between the levels of peripheral blood KISS1 mRNA and plasma CA125 protein in EOC patients. The CA125 is encoded by the mucin 16 gene (*MUC16*) [25]. However, the *MUC16* gene polymorphisms is not associated with the CA125 in EOC [26]. It was reported that the CA125 played a critical role in tumor cell growth, tumorigenesis and metastases in EOC [27], and the role of CA125 in the metastasis of OC was to mediate cell adhesion via binding to mesothelin [28]. This was further supported by our results that the EOC patients with metastases had significantly higher levels of CA125 than those without, which might also be the main reason contributing to the high levels of CA125 in EOC patients relative to uterine fibroids patients with normal ovaries considering the high proportion of EOC patients with metastases (n = 33) than those without (n = 7). Moreover, previous studies have showed that the kisspeptin was a metastasis suppressor in OC [17, 29, 30], which may be responsible for the negative correlation between the levels of plasma CA125 protein and peripheral blood KISS1 mRNA in this study. However, there was no significant difference in the level of peripheral blood KISS1 mRNA between patients with metastasis and without metastasis in this study. The inconsistent results between our study and previous studies may be attributed to the different sources of samples (blood or tissue) and the disproportion of EOC patients with and without metastases. Further study

Table 3. Prognostic analysis of KISS1 mRNA level for the survival of patients with epithelial ovarian cancer using Cox regression analysis.

Factor	HR	95% CI	P-value
KISS1 mRNA	0.022	0.001 – 0.444	0.013
FIGO stage	3.448	0.984 – 12.078	0.053

Abbreviations: KISS1, KiSS-1 metastasis-suppressor gene; FIGO, International Federation of Obstetrics and Gynecology; HR, hazard radio; CI, confidence interval.

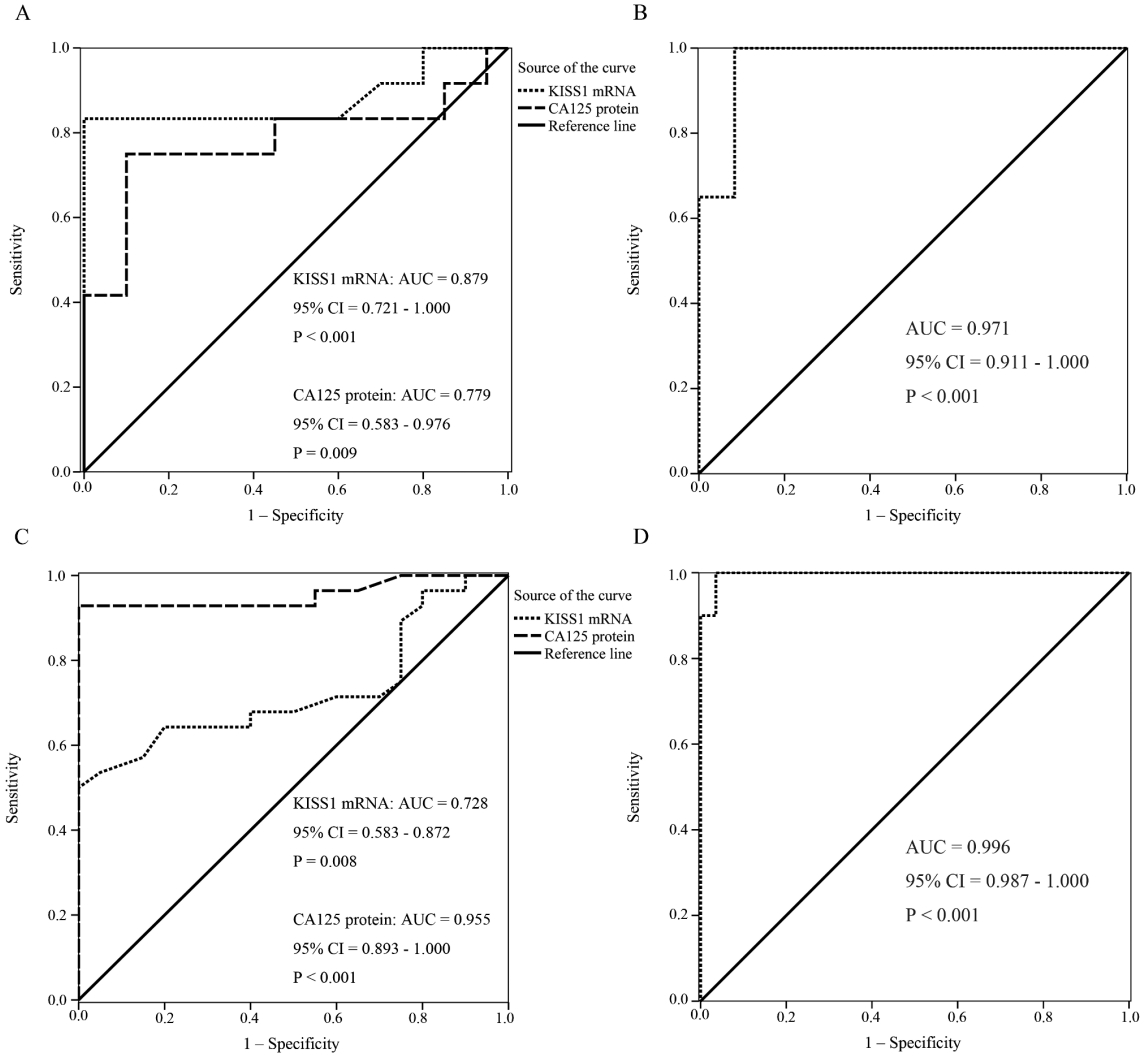

Figure 3. The receiver operating characteristic (ROC) curves of KISS1 mRNA and CA125 protein for early and advanced epithelial ovarian cancer. A: the ROC curves based on KISS1 mRNA and CA125 protein for early stage epithelial ovarian cancer; B the ROC curves based on prediction model for early stage epithelial ovarian cancer; C: the ROC curves based on KISS1 mRNA and CA125 protein for advanced epithelial ovarian cancer; D: the ROC curves based on prediction model for advanced epithelial ovarian cancer.

was needed to investigate the relationship between peripheral blood KISS1 mRNA level and the metastases of EOC.

In addition, the presented results showed also that the level of peripheral blood KISS1 mRNA was significantly associated with the pathological type, FIGO stage and differentiation. This might be explained by the combined role of GPR54 and kisspeptin, as an previous study have proved that kisspeptin and GPR54 immunoreactivity was associated with the pathological type of EOC [31]. Furthermore, it was reported that GPR54 could trans-activate the epidermal growth factor receptor (EGFR) by kisspeptin-10 (Kp-10) to promote the invasiveness of breast cancer cells [32]. Moreover, the EGFR overexpression was also found in EOC [33], and the EGFR-transactivated Akt signaling mediated the OC progression through the upregulating of proinflammatory chemokines [34]. Thus, the overexpression of KISS1 mRNA was associated with the progression of EOC.

The CA125 protein with higher diagnostic accuracy for advanced EOC than KISS1 mRNA was also witnessed in the presented study, suggesting that the plasma CA125 protein may be more appropriate in detecting advanced EOC than early EOC, which was consistent with the serum CA125 [13, 35]. In addition, the present study also revealed that the prediction model combining the KISS1 mRNA and CA125 protein had higher diagnostic accuracy for EOC than KISS1 mRNA or CA125 protein alone. However, the diagnostic accuracy of this combined prediction model was more effective than KISS1 mRNA for the diagnosis of advanced EOC, but

similar with CA125 alone. Moreover, no significant difference was found among the diagnostic accuracy of the combined prediction model, KISS1 mRNA and CA125 protein for detecting the early cancer. Consequently, the clinical application of the combination was still unclear. More studies should be performed for further exploration.

Besides, the results of Cox regression analysis showed that the prognostic role of KISS1 mRNA for the survival of EOC patients was significant and independent of the FIGO stage. More studies were needed to further investigate the prognostic role of KISS1 mRNA for EOC patients.

Notably, there were some limitations in this study. Firstly, the sample size of this study was small (40 cancers and 20 benign controls), especially for early EOC patients, there were only 12 cases. Secondly, no healthy controls and no patients with benign adnexal masses were included. There might be wide statistical bounds surrounding normal values of KISS1 mRNA. Thirdly, the combination of an mRNA biomarker and a protein biomarker requires two very different assay methods that will complicate clinical application, but this could still be accomplished if the combination was sufficiently robust. In addition, we only specially investigated the diagnostic accuracy of peripheral blood KISS1 mRNA and plasma CA125 protein in patients who have ever been pregnant. Further studies with large samples of both cases and control were required to confirm the diagnostic accuracy of peripheral blood KISS1 mRNA and plasma CA125 protein in EOC patients who have ever and never been pregnant, and to verify the robust diagnostic accuracy of this combined prediction model.

In conclusion, both the peripheral blood KISS1 mRNA and plasma CA125 protein had good diagnostic accuracy for EOC, early EOC and advanced EOC in patients who have ever been pregnant. Moreover, the combined diagnosis of KISS1 mRNA and CA125 protein had higher diagnostic accuracy than them alone in detecting EOC, although, the clinical application of this combined diagnosis in detecting early and advanced EOC still need further investigation and exploration. In addition, this study developed a novel biomarker (peripheral blood KISS1 mRNA) for the diagnosis of early EOC and provided more evidences for the clinical application of the biomarkers in detecting EOC.

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