

Original Article

Diagnostic efficiency of KISS1 mRNA combined with CA-125 in detection of epithelial ovarian cancer

Liping Chen¹, Manhua Liu¹, Weiwei Lin², Jinlong Ji¹, Jing Xiao³, Hongbin Liu⁴, Bosheng He⁵

¹Department of Gynaecology and Obstetrics, The Second Affiliated Hospital of Nantong University, Nantong 226001, Jiangsu, China; ²Department of Histology and Embryology, Nantong University, Nantong 226001, Jiangsu, China; ³Department of Epidemiology and Statistics, Nantong University, Nantong 226001, Jiangsu, China; ⁴Department of Pathology, The Second Affiliated Hospital of Nantong University, Nantong 226001, Jiangsu, China; ⁵Department of Radiology, The Second Affiliated Hospital of Nantong University, Nantong 226001, Jiangsu, China

Received April 13, 2016; Accepted October 29, 2016; Epub April 15, 2017; Published April 30, 2017

Abstract: The aim of this study was to evaluate the sensitivity and specificity of KISS1 mRNA combined with CA-125 in detection of the epithelial ovarian cancer (EOC). The tissue and blood KISS1 mRNA expression levels and serum CA-125 level from EOC patients and control groups were measured by using RT-PCR method and chemiluminescence method. The four control groups were borderline ovarian tumor patients (control group 1), benign epithelial ovarian tumor patients (control group 2), leiomyoma of uterus patients (control group 3) and healthy people (control group 4). The tissue or blood KISS1 mRNA and serum CA-125 concentrations were significantly higher in EOC patients than that in healthy people ($P < 0.001$). However, there were no significant differences in tissue or blood KISS1 mRNA and serum CA-125 among control group 2, 3 and 4. The receiver-operator characteristic (ROC) analysis showed that the area under the curves (AUC) were 0.817, 0.696 and 0.853, respectively for tissue KISS1 mRNA, blood KISS1 mRNA and serum CA 125, while there was an increase in the AUC (0.925) for serum CA-125 combined with blood KISS1. KISS1 mRNA can be used as an additional tumor biomarker for diagnosis of EOC. Multiple biomarkers assay of combined blood KISS1 and serum CA-125 is superior to single biomarker alone in detection of EOC. KISS1 may exert a synergistic or additional effect on CA-125.

Keywords: KISS1 gene, CA-125, epithelial ovarian cancer, ROC curve, diagnosis

Introduction

Epithelial ovarian cancer (EOC) is one of the leading causes of death in women with gynecological cancers [1]. The origin and pathogenesis of EOC have been investigated for a long time but still poorly understood [2]. The surgical resection and chemotherapy have used in initial response to primary treatment, but most patients have already reached advanced stage at the time of diagnosis and most of them develop a recurrence [3]. EOC is a disease composed of several tumors that can be classified according to morphological characteristics or genetic features [4]. The severity and heterogeneity of this disease make the therapy of EOC a great challenge.

Thus, identification of some specific molecular markers contributing to the diagnosis of EOC is of great importance. CA-125 is suggested to

be the most common index in clinical diagnosis of ovarian cancer [5]. However, a recent study showed that CA-125 had only 67.89% sensitivity for type I and benign tumors differentiation [6]. Besides, the down-regulation of KISS1 (kisspeptin, a secreted protein) involved in metastases and poor prognosis in several cancers [7, 8]. KISS1 is one of the metastasis suppressor genes discovered in recent years [9]. Some studies showed that the KISS1 gene had diagnostic value in several cancers such as intestinal cancer [8], lung cancer [10] and breast cancer [11]. However, our understanding for KISS1 function in diagnosis of EOC is limited.

This study aimed to evaluate the sensitivity and specificity of blood KISS1 mRNA compared with serum CA-125 in diagnosis of EOC. KISS1 mRNA in the tumor tissue was examined to verify the results of blood KISS1 mRNA. In addition, the diagnostic efficiency of the combina-

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Table 1. Age, BMI, gravidity, parturition in case and control groups (mean \pm SD)

	Case group	Control group 1	Control group 2	Control group 3	Control group 4	F	P
N	40	20	20	20	20		
Age (year)	53.28 \pm 11.10	52.25 \pm 17.26	51.65 \pm 13.53	56.75 \pm 5.95	54.35 \pm 11.73	0.545	0.703
BMI (kg/m ²)	3.10 \pm 1.36	2.95 \pm 1.43	3.00 \pm 1.21	2.95 \pm 1.15	2.85 \pm 0.81	0.154	0.961
Gravidity times	1.33 \pm 0.47	1.40 \pm 0.68	1.20 \pm 0.52	1.30 \pm 0.47	1.35 \pm 0.59	0.379	0.823
Parturition times	23.20 \pm 2.88	23.21 \pm 2.90	23.31 \pm 2.62	23.64 \pm 2.67	24.05 \pm 3.09	0.372	0.828

Table 2. The expression of KISS1mRNA in peripheral blood and tumor tissue in the case and the control groups (x \pm s)

	Case group	Control group 1	Control group 2	Control group 3	Control group 4	F/ χ^2	P
N	40	20	20	20	20		
Tissue KISS1 mRNA	0.81 \pm 0.10 ^a	0.79 \pm 0.05 ^a	0.62 \pm 0.07 ^b	0.59 \pm 0.08 ^b	–	42.058	<0.001
Blood KISS1 mRNA	0.73 \pm 0.16 ^a	0.71 \pm 0.08 ^a	0.61 \pm 0.08 ^b	0.54 \pm 0.09 ^b	0.57 \pm 0.07 ^b	14.444	<0.001
Blood CA-125	216.40 (36.13, 430.48) ^a	51.86 (22.24, 89.92) ^b	12.03 (7.25, 19.12) ^b	16.49 (9.68, 23.74) ^b	19.50 (13.50, 22.85) ^b	59.394	<0.001

Case group: patients with EOC; Control group 1: patients with borderline ovarian tumor; Control group 2: patients with benign epithelial ovarian tumor; Control group 3: patients who had already resected normal ovarian because of fibroid; Control group 4: healthy people from physical examination center. *: no significant difference between case group and control groups; ^b: significant difference between case group and control groups.

tion of blood KISS1 mRNA and serum CA-125 was also estimated. Our results may play an important role in early detection of EOC.

Materials and methods

Subjects

This study was approved by the Ethics Committee of our hospital. The informed consents for all subjects were given.

Our study included patients admitted to our hospital during January, 2010 and January, 2014 if they met the following criteria. Inclusion criteria: (1) EOC patients have been finished their first tumor resection surgery in our hospital; (2) they have normal BMI (18-30 kg/m²) and aged more than 18 years old; (3) they have more than one time of gravidity and parturition; (4) patients had never received radiotherapy, chemotherapy or hormones treatment before surgery. Exclusion criteria: patients with other cancers were excluded.

The control subjects were selected from the in hospital patients at the same period in our hospital. There were totally four control groups: control group 1, borderline ovarian tumor patients (BOT, n=20); control group 2, benign epithelial ovarian tumor patients (n=20); control group 3, patients who had already resected normal ovarian because of leiomyoma of uterus (n=20); and control group 4, healthy people from physical examination center (n=20).

Peripheral blood samples and cancer tissues were collected before and during surgery in the case group and the control group 1-3, respectively. Furthermore, peripheral blood samples were obtained from patients in the control group 4 without tissue sampling.

Collection of blood and tumor tissue and detection of serum CA-125

Peripheral blood samples were collected in EDTA anticoagulative tubes before the surgery and were centrifuged at 2000 rpm for 30 min. The serum samples were collected and stored for CA-125 detection on a Roche Cobas 6000 Analyzer (Switzerland) according to methods described by Qiu et al. [12].

In addition, the liquid-liquid interface including nuclear cells was transferred into another centrifuge tube and was frozen in liquid nitrogen for KISS1 detection.

The tumor tissue samples (approximately 3 g) were collected during the resection surgery. Then, the tissue samples were washed and saved in liquid nitrogen for detection of tissue KISS1 mRNA.

Reverse transcription RNA (RT-PCR) for KISS1 mRNA detection

Total RNA from tissue samples and blood samples was isolated by using a TRIZOL Reagent (Sangon, Shanghai, China) according to the manufacturer's instructions. The RNA was

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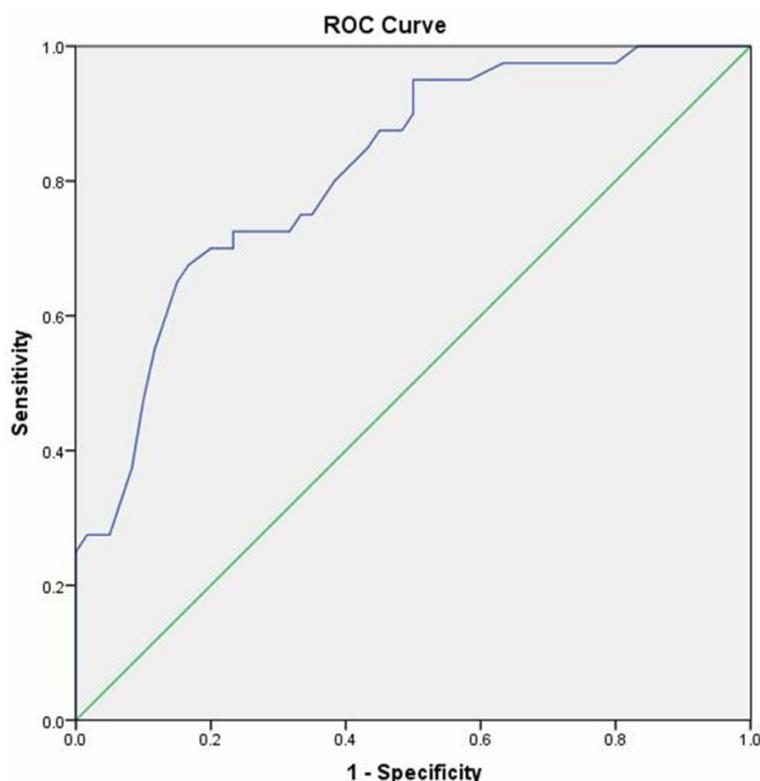


Figure 1. Receiver-operator characteristic (ROC) curves for KISS1 mRNA level in tumor tissue.

reverse transcribed and amplified by 40 cycles of PCR procedure by using specific primers. The RT-PCR was performed for 30 min at 42°C with 20 µl reaction system including 4 µl of RNA template, 4 µl of 10 × buffer, 1 µl of dNTP MIX (10 mmol/l), 1 µl of Oligo Primers, 0.5 µl of RNA inhibitor, 0.5 µl of AMV reverse transcriptase and 9 µl of DEPC water. Primer sequences used in this study were as follows: KISS1: F, CCA-CCCTCTGGACATTCA; R, GCCGAAGGAGTTCCAGTT; β-actin: F, ATCATGTTTGAGACCTTCAACA, R, CATCTCTTGCTCGAAGTCCA. Each cycle consisted of denaturation at 94°C for 10 min, annealing at 58°C for 31 s, extension at 72°C for 45 s.

The relative quantification of target gene = gene copy number of target gene/copy number of β-actin.

Statistical analysis

Data were expressed as mean ± SD (normally distributed data) or as median and interquartile range (data that deviated from the normal distribution). Student-Newman-Keuls test (equal variance) and Dunnett T3 test (unequal variance) were used for multiple comparisons. Pearson's chi-square test was used to assess

the statistical significance of the association between quantitative variables. All statistical analyses were conducted by SPSS 21.0, and *P* value <0.05 was regarded as statistically significant. Receiver-operator characteristic (ROC) analysis was used to assess the sensitivity and specificity of diagnostic efficiency.

Results

General information of the included subjects

Totally 40 EOC patients aged from 30-83 years old were included in our study. Basic characteristics of the included subjects were presented in **Table 1**. The case group and control groups were fairly well-matched for age, BMI index, gravidity and parturitiontimes (*P*>0.05). These patients were diagnosed as serous cystadenocarcinoma

(n=24), mucinous cystadenocarcinoma (n=6), endometrioid ovarian carcinoma (n=7) and clear-cell ovarian carcinoma (n=3). Surgical staging of EOC was established based on the International Federation of Gynecology and Obstetrics (FIGO) system. Cases were classified into stage I (n=7), stage II (n=5), stage III (n=24) and stage IV (n=4). Stage I and II were defined as early stage (n=12), and III and IV were defined as late stage (n=28). Pathological degrees were well differentiated (G1, n=12), moderately differentiated (G2, n=8) and poorly differentiated (G3, n=20).

The expression level of KISS1 mRNA and CA-125 in tumor tissue and blood in different groups

The expression levels of KISS1 and CA-125 were compared between case group and control groups (**Table 2**). There were significant differences in KISS1 and CA-125 expression levels among the five groups (*P*<0.001). Compared with control groups, the relative mRNA expressions of KISS1 in tissue and blood were all up-regulated. There were no significant differences of KISS1 mRNA between case group and con-

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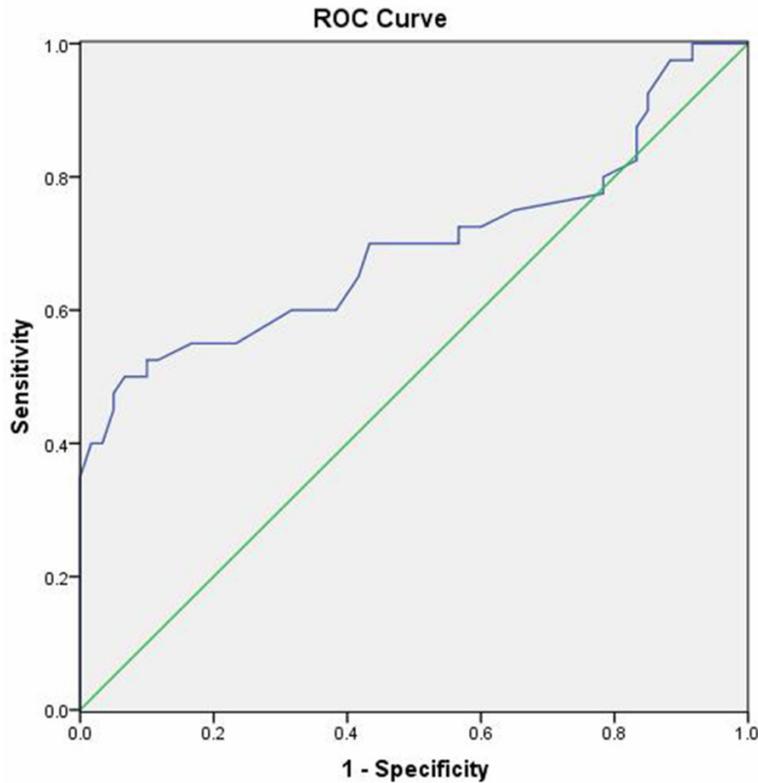


Figure 2. Receiver-operator characteristic (ROC) curves for KISS1 mRNA level in blood.

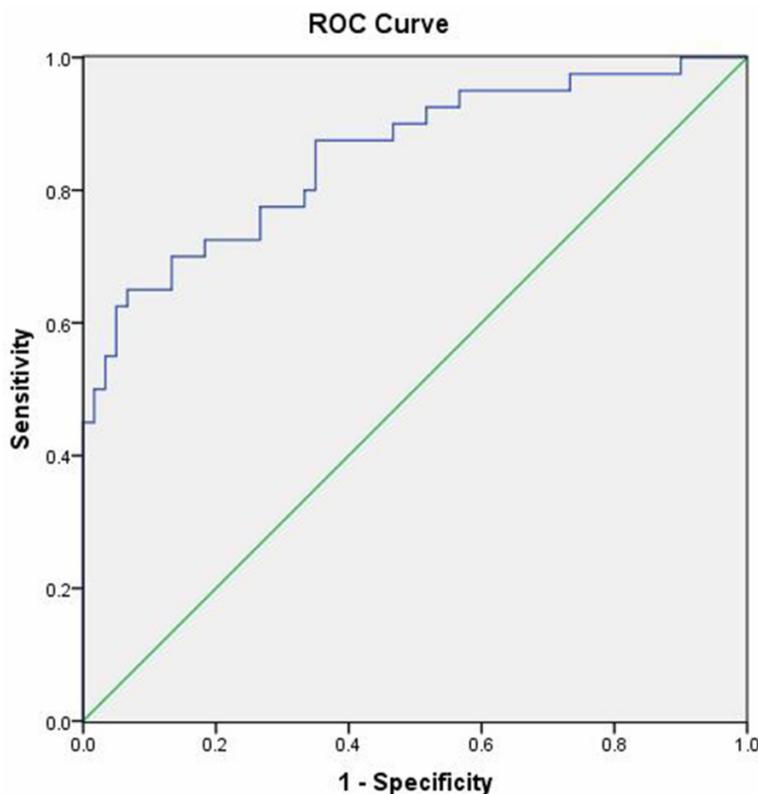


Figure 3. Receiver-operator characteristic (ROC) curves for serum CA 125 level.

trol 1 group both in tumor tissue and blood, but the KISS1 were much more expressed in the case group than that in control 2, 3 and 4 groups ($P < 0.05$). Furthermore, there was no significant difference of blood KISS1 mRNA between control group 3 and control group 4 ($P > 0.05$). With regard to blood CA-125, the expression level was significantly higher in the case group than that in four control groups ($P < 0.05$); however, there was no difference between control group 1 and control groups 2-4 ($P > 0.05$).

Diagnostic efficiency of KISS1 and CA-125 for EOC

In order to assess the diagnostic efficiency of KISS1 and CA-125 for EOC, the ROC analysis was performed.

For tissue KISS1 mRNA diagnostic efficiency, the area under the curve (AUC) was 0.817 with 95% CI of 0.735 to 0.900 ($P < 0.01$). The sensitivity and specificity at the optimal Youden index-based cutoff value (0.785) were 67.5% and 83.3% respectively (**Figure 1**).

For blood KISS1 mRNA diagnostic efficiency, the AUC was 0.696 with 95% CI of 0.580 to 0.812 ($P < 0.01$). According to the Youden's index, the optimal cutoff value was 0.775, and its corresponding sensitivity and specificity were 50% and 93.3% respectively (**Figure 2**).

For blood CA-125, the AUC was 0.853 (95% CI=0.775 to 0.932, $P < 0.01$). The sensi-

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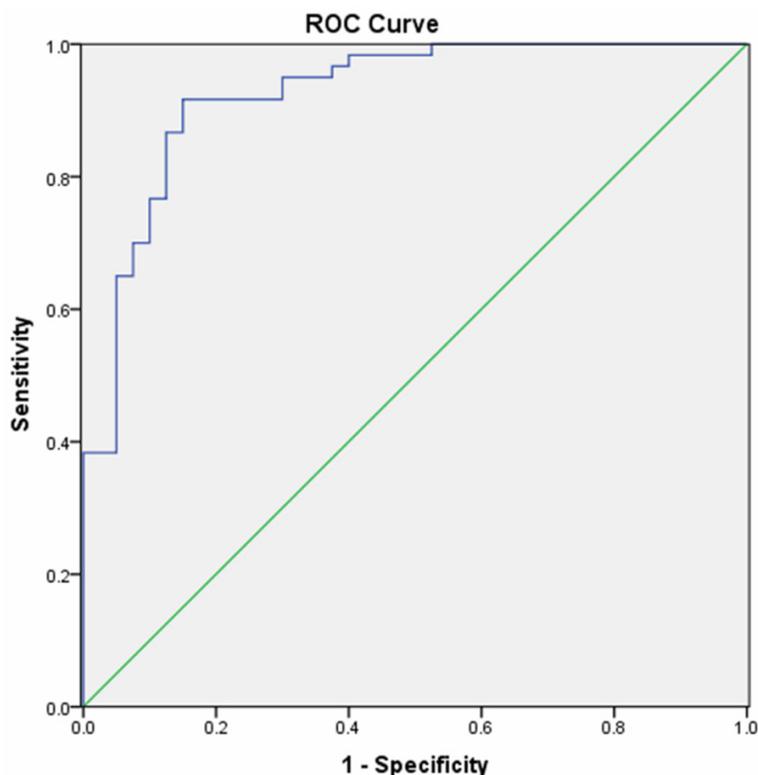


Figure 4. Receiver-operator characteristic (ROC) curves for combination of blood KISS1 mRNA level and serum CA 125 level.

Table 3. The positive predictive value (PPV) and negative predictive value (NPV) of the KISS1 and CA 125 for detection of EOC

	PPV (%)	NPV (%)
Tissue KISS1 mRNA	73.0	79.4
Blood KISS1 mRNA	83.3	73.7
Blood CA 125	86.7	80.0
Combination of blood KISS1 mRNA and CA 125	90.2	87.2

vity and specificity at the optimal Youden index-based cutoff point (105.18) were 65.0% and 93.35% respectively (**Figure 3**).

We then evaluated the diagnostic efficiency of combined blood KISS1 and serum CA-125. As shown in **Figure 4**, the AUC was 0.925 (95% CI: 0.871, 0.978; $P < 0.001$) and the sensitivity and specificity were 91.7% and 85.0% respectively.

The positive predictive value (PPV) and negative predictive value (NPV) of the KISS1 and CA-125 for detection of EOC were showed in **Table 3**. The PPV and NPV of the combination methods (blood KISS1 and serum CA-125)

were higher than single methods.

Discussion

EOC is one of the most important causes of death in cancer diseases of women. Unfortunately, once it is detected, about 75% of women are diagnosed with late-stage disease [13]. Therefore, early diagnosis is essential for improvement of prognosis.

CA-125, originated from coelomic epithelium, is an important biological indicator in monitoring EOC. However, because CA-125 is up-regulated in many other benign ovary tumors, the specificity of it is not so good. For example, the CA-125 sensitivity for type I and benign tumors differentiation does not show any statistical significance [6]. In our study, the serum CA-125 level was much higher in EOC patients than that in BOT patients and benign epithelial ovarian tumor patients as well as healthy control, while there was no significant difference in serum CA-125 level between BOT patients and normal controls. In addition, there was a lower sensitivity (65.0%) for EOC detection.

Thus we considered combining it with other markers for EOC diagnosis.

KISS1 was originally identified in melanoma [14]. Though more and more evidence has proven the association between KISS1 expression levels and increased tumor progression, the relationship between KISS1 and tumors of the reproductive system in women was not well understood. Studies showed that this gene was down-regulated in gastric cancer [15] and bladder cancer [16], but it contrasts with the reports of Ikeguchi et al. [17] and Papaoiconomou et al. [18] who found overexpression of KISS1 in liver cancer and breast cancer. Some other studies also suggested that the expression levels in

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EOC are still controversial [9, 19-22]. In addition, Isaksson and his partners [23] analyzed the expressions of 168 cancer- or metastasis-specific related genes from ovarian cancer patients by real-time qPCR analysis, and found that the KISS1 metastasis suppressor was significantly reduced in patients with residual tumor mass after primary surgery compared patients with macroscopically radically resected tumor. Therefore, they assumed that the KISS1 mRNA might relate to inhibition of ovarian cancer and improvement of prognosis.

To further evaluate the relationship between KISS1 mRNA and EOC, we compared the blood KISS1 mRNA levels in patients with EOC, BOT, benign epithelial ovarian tumor, leiomyoma of uterus, as well as normal controls. Our results showed that blood KISS1 mRNA and tissue KISS1 mRNA were much higher in EOC patients than that in patients with benign epithelial ovarian tumor or leiomyoma of uterus. However, there was no significant difference in KISS1 mRNA level between EOC and BOT, as well as between benign epithelial ovarian tumor and leiomyoma of uterus. We speculated from our data that the up-regulation of KISS1 mRNA in BOT might associate with EOC, and KISS1 mRNA was an effective diagnostic tool for EOC. The distinguishing of EOC and BOT need further examinations using other methods such as CA-125. Our results showed that the serum CA-125 was significantly higher in EOC than BOT, indicating that we could combine KISS1 mRNA and CA-125 for precise diagnosis of EOC.

Because normal ovarian tissues in healthy people could not be obtained, we designed the control group 3 to verify that tissue KISS1 mRNA expression levels in patients who had already resected normal ovarian because of leiomyoma of uterus could represent the tissues expression levels in healthy people. One study showed that KISS1 expression in ovarian tissues was positively correlated to that in the peripheral blood ($r=0.669$, $P<0.01$) [24]. Thus, blood KISS1 mRNA in ovarian cancer patients may mainly come from secretion of cancer tissues, and the KISS1 mRNA expression levels in ovarian cancer tissues can be reflected by blood KISS1 mRNA expression levels indirectly. In addition, there was no significant difference of blood KISS1 mRNA between control group 3 and control group 4 in our present study. Therefore, the effect of leiomyoma of uterus on blood KISS1 mRNA was excluded, and tissue KISS1 mRNA expression levels in control group 3

could represent the expression levels in healthy people.

In our present study, we detected the specificity and sensitivity of blood KISS1, tissue KISS1, and serum CA-125 respectively, as well as the specificity and sensitivity of the combined blood KISS1 and serum CA-125. The AUC was 0.817, 0.696 and 0.853 respectively for tissue KISS1, blood KISS1 and serum CA-125, suggesting that the diagnostic efficiency of tissue KISS1 and serum CA-125 was similar and blood KISS1 had a lower diagnostic efficiency.

The use of serial CA-125 combined with ultrasonography in asymptomatic women as an aid in the diagnosis of ovarian cancer has been reported [25, 26]. However, the major drawback of employing CA-125 as an initial step in ovarian cancer diagnosis is that more than 20% of this disease lack of antigen expression [27]. Thus, it is necessary to combine CA-125 with novel markers to provide a better diagnostic efficiency. In our study, an increase in the AUC (0.925) for serum CA-125 in combination with blood KISS1 was found when compared with blood KISS1 alone and serum CA-125 alone. Therefore, the sensitivity and specificity of multiple biomarkers assay was significantly higher than that achieved with a single biomarker alone. However, taking into consideration of the low power of KISS1 alone in distinguishing EOC from BOT, KISS1 may exert a synergistic or additional effect in the diagnosis of EOC.

The potential limitation of the present study was that the number of patients in the case group was twice the number of patients in each control group. Though the case group and control groups were fairly well-matched for age, BMI index, gravidity and parturition times, it still might introduce heterogeneity.

In conclusion, the blood KISS1 mRNA can be used as an additional tumor biomarker for diagnosis of EOC. Importantly, multiple biomarkers assay of combined blood KISS1 and serum CA-125 is superior to a single biomarker in detection of EOC. Furthermore, KISS1 may exert a synergistic or additional effect on CA-125.

Acknowledgements

Project supported by the Social Enterprise Technology Innovation and Demonstration Project of Nantong City (No. HS2013039).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Bosheng He, Department of Radiology, The Second Affiliated Hospital of Nantong University, No. 6 Hai Er Xiang North Road, Nantong 226001, Jiangsu, China. Tel: +86-0513-85061191; Fax: +86-0513-85061003; E-mail: doctorhe_ntu@163.com

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