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ORIGINAL ARTICLE

Changes in tumor markers, coagulation function and serum VEGF in patients with ovarian cancer and benign ovarian disease

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Summary

Purpose: *To investigate the changes in tumor markers (TMs), coagulation function and vascular endothelial growth factor (VEGF) in patients with ovarian cancer (OC) and benign ovarian disease (BOD).*

Methods: *A total of 68 OC patients admitted to and treated in our hospital were selected (OC group), and another 68 BOD patients in the same time period were enrolled (BOD group). The variations in TMs, coagulation function and VEGF in OC and BOD patients were explored by analyzing the TMs, coagulation function and expression levels of serum VEGF and D-dimer in OC group and BOD group as well as the differences in TMs and coagulation function in patients in different stages.*

Results: *The values of TMs such as cancer antigen 125 (CA125), carbohydrate antigen 19.9 (CA19.9) and human epididymis protein 4 (HE4) in OC group were remarkably higher than those in BOD group, with significant differences (p<0.05). The values of those TMs were relatively low in the patients in stage I-II but relatively high in the patients in stage III-IV, and the patients in stage I-II had evidently lower values of those TMs than those in stage III-IV (p<0.05). The coagulation function was similar in both groups (p>0.05), while OC group exhibited a notably higher serum fibrinogen (FIB) level than BOD group (p<0.05). The levels of coagulation function indexes [prothrombin time (PT), thrombin time (TT) and activated partial thromboplastin time (APTT)] in the patients in stage I-II were comparable to those in stage III-IV, showing no differences (p>0.05), but the serum FIB level was markedly higher in the patients in stage III-IV than that in in stage I-II (p<0.05). The expression level of serum VEGF was increased distinctly in OC group compared with that in BOD group [(378.15±94.45) pg/mL vs. (164.02±67.38) pg/mL, p<0.05]. Moreover, OC group manifested obviously elevated expression level of serum D-dimer in comparison with BOD group [(4.58±1.48) μg/mL vs. (0.67±0.12) μg/mL, p<0.05].*

Conclusions: *TMs, coagulation function indexes and serum VEGF and D-dimer are highly expressed in OC patients, and the combined detection of TMs, coagulation function and serum VEGF can serve as an important method of diagnosing OC.*

Key words: *ovarian cancer, tumor markers, coagulation function, VEGF*

Introduction

Ovarian cancer (OC) is a relatively common gynecological cancer in the clinic [1]. It usually cannot be diagnosed clinically in time due to lack of special symptomes such as ambiguity and inconspicuous pain sensation in the early stage, so that the patients have been in the intermediate and advanced stage when definitely diagnosed, with a very low cure rate. Therefore, OC is a major cause of death in females with an increasing morbidity rate [2]. There are many causes of OC that are generally associated with the genetics of the patients themselves, environment and other factors [3]. No great achievements have been made in the research on the pathogenesis of OC so far, and its

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pathogenicity cannot be clarified, thus making the in-depth study on the prevention and treatment of the disease impossible [4]. Currently, there are no specially targeted treatment protocols for OC, and only some conventional therapeutic methods, including surgery and postoperative chemotherapy can be applied [5]. Nevertheless, such conventional methods are unable to control the proliferation and differentiation of tumor cells. Therefore, finding the drugs controlling the cancer cell growth is the primary method to lower the mortality rate of OC patients [6]. Vascular endothelial growth factor (VEGF), a central player in vascularization in organisms, can enhance the permeability and promote the migration of endothelial cells. It participates in various activities in the body [7]. Multiple studies have illustrated that the abnormal expression of VEGF is detected in OC patients and plays a crucial role in the progression of the disease. Tumor markers (TMs) can directly and effectively reflect the growth cycle and differentiation grade of tumors since they are produced and released into the blood by the tumor cells [8]. There are 5 kinds of serum TMs related to OC, including cancer antigen 125 (CA125), human epididymis protein 4 (HE4), carbohydrate antigen 19.9 (CA19.9), AFP and hCG, at present [9]. In this experiment, the OC indexes were observed through the serum TMs (HE4, CA125 and CA19.9) in two groups of patients. Hence, this paper aimed to investigate the changes in TMs, coagulation function and VEGF in patients with OC and benign ovarian disease (BOD) by analyzing the TMs, coagulation function and expression levels of serum VEGF and D-dimer in OC group and BOD group, as well as the differences in TMs and coagulation function in patients in different stages

Methods

General data

A total of 68 OC patients admitted to and treated in our hospital from January 2016 to December 2018 composed the OC group, and another 68 patients with BOD that did not develop into malignant lesion in the same time period composed the BOD group. OC group and BOD group had no difference in general data (Table 1). This study was approved by the Ethics Committee of the affiliated Hospital of School of Medicine of Ningbo University. Signed informed consents were obtained from all participants before the study entry.

Inclusion and exclusion criteria

Inclusion criteria: (1) patients meeting the diagnostic criteria for OC [9]; (2) those who were informed of and agreed to participate in this research; and (3) those with complete data, normal consciousness and ability to cooperate in the treatment. *Exclusion criteria:* (1) patients who voluntarily withdrew from the study during the research; (2) those accompanied with other gynecologic diseases or malignancies; or (3) those with liver disease, blood disorders or peripheral vascular disease.

TMs

Fasting venous blood was drawn from the patients in both OC group and BOD group after admission to hospital, and the serum was obtained via conventional methods for detection of TMs using AXS YM automated chemiluminescence immunoassay analyzer and supporting reagents (Abbott, Abbott Park, IL, USA). The automated chemiluminescence immunoassay analyzer can not only measure the content of objects to be detected at varying wavelengths to the greatest extent, but also automatically dilute the serum. After detection, the final results were calculated via microparticle enzyme immunoassay.

Table 1. General data (n)

Item	*OC group (n=68)*	*BOD group (n=68)*	x^2	*p*
Age (years)			0.074	0.785
18-36	26	28		
37-76	42	40		
Pathological stage			-	-
Stage I-II	38	-		
Stage III-IV	30	-		
Type			0.273	0.601
Clear cell carcinoma	15	18		
Borderline ovarian tumor	17	20		
Granulosa cell tumor	21	23		
Serous adenocarcinoma	15	7		

Staging criteria of OC: Stage I: The tumor only grows in the ovaries and does not invade into the abdominal cavity or other sites, and the capsule is intact. Stage II: The tumor grows in the ovaries and spreads to the abdominal cavity, oviducts and other sites. Stage III: Abdominal metastasis or lymph node metastasis occurs. Stage IV: Distant metastasis exceeding the abdominal cavity occurs, and cancer cells are detected in the lungs, liver, etc.

Coagulation function

After admission to hospital, fasting venous blood was collected from the patients in OC group and BOD group, and the serum was taken using conventional methods for detection. The centrifuged serum was mixed with sodium citrate at 1:9 for anticoagulation and then sent for coagulation test. STR-R automatic coagulation analyzer was employed to determine prothrombin time (PT), thrombin time (TT), activated partial thromboplastin time (APTT) and fibrinogen (FIB). After that, the results were calculated using BCD6EF GHI7JBK machine.

Serum VEGF

The expression of serum VEGF in OC group and BOD group was measured through ELISA using kits purchased from R&D, USA in strict accordance with the instructions. Finally, the standard curves were plotted by OriginLab software.

D-dimer

Fasting peripheral blood in OC group and BOD group was drawn into vacuum blood collection tubes containing anticoagulant, and then the expression level of serum D-dimer in both groups was examined by means of immune turbidimetry according to the specific steps of CA7000 automatic coagulation analyzer.

Statistics

SPSS 22.0 software (IBM, Armonk, NY, USA) was used for sorting of results. T-test was applied to analyze the differences in TMs, coagulation function, VEGF and D-dimer between OC group and BOD group, and $p<0.05$ suggested that the difference was statistically significant.

Results

Comparisons of TMs between OC group and BOD group

The values of TMs in OC group were remarkably higher than those in BOD group ($p<0.05$) (Table 2).

Table 2. Comparisons of mean TMs values between OC group and BOD group

Group	*n*	*CA125 (KU/L)*	*CA19.9 (KU/L)*	*HE4 (pmol/L)*
OC group	68	385.35±93.57	23.94±5.35	204.75±68.32
BOD group	68	32.84±4.25	10.34±3.83	47.83±12.84
t		31.031	17.042	18.615
p		<0.001	<0.001	<0.001

Table 3. Comparisons of mean TMs values among different stages

Stage	*CA125 (KU/L)*	*CA19.9 (KU/L)*	*HE4*
Stage I-II	245.34±57.21	15.34±4.85	137.15±24.56
Stage III-IV	394.71±112.08	25.37±5.36	231.08±71.09
t	9.788	10.915	10.302
p	<0.001	<0.001	<0.001

Table 4. Comparison of mean coagulation values function between OC group and BOD group

Group	*n*	*PT (s)*	*TT (s)*	*APTT (s)*	*FIB (g/L)*
OC group	68	13.45±1.54	16.35±2.46	37.25±8.36	4.63±2.04
BOD group	68	13.03±1.62	16.84±2.53	37.26±8.29	2.43±1.76
t		1.550	1.145	0.007	6.733
p		0.123	0.254	0.994	<0.001

Table 5. Comparison of mean coagulation values function among different stages

Stage	*PT (s)*	*TT (s)*	*APTT (s)*	*FIB (g/L)*
Stage I-II	13.58±1.34	18.98±3.57	38.47±6.94	3.21±1.31
Stage III-IV	13.97±1.21	18.34±3.26	38.31±6.17	5.24±2.65
t	1.781	1.092	0.142	5.663
p	0.077	0.276	0.887	<0.001

Comparisons of TMs among different stages in OC group

The results indicated that the values of the TMs were relatively low in the stage I-II patients but relatively high in the stage III-IV patients, and the stage III-IV patients had evidently higher values of TMs such as CA125, CA19.9 and HE4 than those in stage I-II ($p<0.05$) (Table 3).

Comparison of coagulation function between OC group and BOD group

The numerical values of PT, TT and APTT in the serum in OC group were close to those in BOD group, and the differences were not significant ($p>0.05$), while OC group exhibited a notably higher FIB level than BOD group ($p<0.05$) (Table 4).

Comparison of coagulation function among different stages in OC group

According to the results, the numerical value of PT, TT and APTT in the stage I-II patients were similar with those in stage III-IV, showing no differences ($p>0.05$), but the serum FIB level was markedly higher in the patients in stage III-IV than that in stage I-II ($p<0.05$) (Table 5).

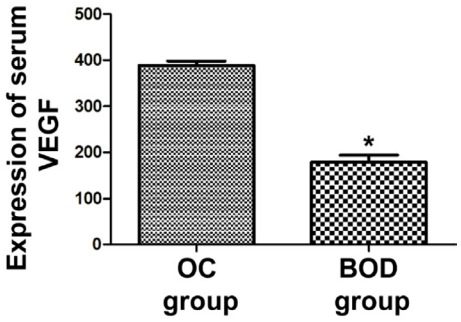

Figure 1. Comparison of expression of serum VEGF between OC group and BOD group (*$p<0.05$ *vs.* OC group).

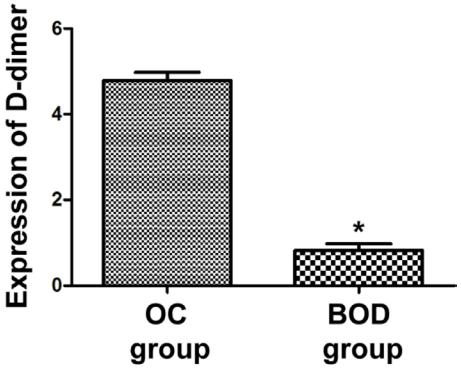

Figure 2. Comparison of expression of D-dimer between OC group and BOD group (*$p<0.05$ *vs.* OC group).

Comparison of expression of serum VEGF between OC group and BOD group

The mean expression level of serum VEGF was 378.15±94.45 pg/mL in OC group and 164.02±67.38 pg/mL in BOD group. It was increased distinctly in OC group compared with that in BOD group ($t=15.223$, $p<0.05$) (Figure 1).

Comparison of expression of D-dimer between OC group and BOD group

The mean expression level of serum D-dimer was 4.58±1.48 μg/mL and 0.67±0.12 μg/mL in OC group and BOD group, respectively. OC group showed an obviously elevated level in comparison with BOD group ($t=21.71$, $p<0.05$) (Figure 2).

Discussion

The incidence rate of OC, one of the most important malignant tumors in women, tends to increase in younger ages in recent years [10]. The majority of OC cases have been in the end stage at the time of diagnosis because of the severe shortage of early diagnostic methods, leading to a fairly low cure rate of the OC patients and causing certain impacts on the physical health and psychology of women [11]. Up until today, no favorable achievements have been made in the investigation of the pathogenesis of OC, and its pathogenicity has not been defined yet, thus making the further research on the prevention and treatment of the disease practically impossible [12]. Therefore, deeply studying the pathogenesis of OC can facilitate the search of methods repressing the proliferation of OC cells and reduce the mortality rate of OC patients at the same time [13].

The patients in OC group showed prominently higher levels of TMs including CA125, CA19.9 and HE4 than those in BOD group, and the differences were significant. It has also been shown that the levels of TMs in the stage III-IV patients were markedly higher than those in stage I-II. According to literature reports in China and foreign countries, CA125, HE4 and HE4 are typical TMs of OC, which are highly expressed in patients with malignant ovarian tumor, and their expression levels are remarkably higher than those in healthy people and patients with benign tumor. Besides, it is also concluded that the expression levels of TMs are positively correlated with cancer stages, that is, the later the stage, the poorer the cell differentiation, and the more apparent the increases in the expression levels of TMs [14,15].

Coagulation function indexes such as PT, TT and APTT were similar in numerical values be-

tween the two groups, without differences. However, the serum FIB level was notably elevated in OC group in comparison with BOD group. The results also revealed that the patients in both stage I-II and stage III-IV had similar numerical values of coagulation function indexes PT, TT and APTT, and there were no differences. Moreover, the stage III-IV patients had an obviously higher serum FIB level than those in stage I-II. Large numbers of studies have demonstrated that FIB is a coagulating substance with the highest expression in the blood and has a prominent relation with the coagulation function in the body. When the expression level of FIB is altered in patients, coagulation disorders are triggered. According to experiments, the FIB level in OC patients is raised markedly compared with that in healthy people and patients with benign tumor, while it displays no significant difference between healthy people and BOD patients [16-18]. It has been elaborated that the level of serum FIB is correlated with the stage of OC, and the patients in a later stage have a higher level of FIB than those in an earlier stage, suggesting that the FIB level may reflect the growth status of OC cells [19].

OC group exhibited distinctly higher expression levels of VEGF and D-dimer than BOD group (see relevant Figures). The research results indicate that the expression of serum VEGF in the patients with ovarian serous adenocarcinoma is evidently increased compared with that in normal people and people with ovarian lesions, proving that VEGF is highly expressed in the blood of patients with ovarian serous adenocarcinoma. VEGF can be taken as one of the key indicators of identifying the malignancy of ovarian lesions in clinical practice [20]. In a study [20] it was revealed that the level of serum D-dimer is raised clearly in patients with epithelial ovarian cancer in comparison with that in BOD patients. Therefore, the development of the disease can be judged by measuring the expression level of D-dimer in the patients.

Conclusions

In conclusion, the TMs, coagulation function indexes and serum VEGF and D-dimer are highly expressed in OC patients, and the combined detection of TMs, coagulation function and serum VEGF can serve as an important method of diagnosing OC.

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Conflict of interests

The authors declare no conflict of interests.

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