RESEARCH ARTICLE

Clinical Applicability of Multi-Tumor Marker Protein Chips for Diagnosing Ovarian Cancer

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Abstract

Purpose: To assess the value of multi-tumor marker protein chips in the diagnosis and treatment of ovarian cancer. Materials and Methods: Twelve tumor markers (CA19-9, NSE, CEA, CA242, CK19, β-HCG, AFP, SCC, c-PSA, CA125, CA724 and CA15-3) were detected by protein biochip in 220 patients with ovarian carcinomas, 205 with benign ovarian tumors and 200 healthy subjects. Results: The positivity rate was obviously higher in ovarian cancer (77.7%), than that in the benign cases (26.3%, $p<0.01$) and healthy subjects (4.5%, $p<0.01$). Serum levels of tumor markers were furthermore significantly higher in cases with lymph node metastasis (86.8%) than those without metastasis (44.7%), $p<0.01$. Conclusions: Multi-tumor marker protein chips provide important assistance in the diagnosis and treatment evaluation in ovarian cancers.

Keywords: Protein chip - tumor markers - ovarian cancers - treatment evaluation

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Introduction

In human beings, a tumor can secrete several kinds of tumor marker antigens (Hourigan et al., 2011), so method of detecting single tumor marker has relatively low sensitivity and specificity. By using biochip technology and double-antibody sandwich principle, multi-tumor markers protein chip diagnose system can detect twelve tumor markers at the same time, it has the feature of high-speed, high-throughput and high-sensitivity (Sun et al., 2004; Yang et al., 2009; Liang et al., 2010; Hou et al., 2011; Sundar et al., 2012). In our test, we used protein chip to detect serum level of twelve tumor markers in ovarian cancer patients, benign ovarian disease patients and healthy controls, in order to explore the value of protein chip technique in diagnosing ovarian cancer.

Materials and Methods

Patient data were showed in Table 1. Design of experiment groups: Group I- Experimental group, 220 women diagnosed with ovarian cancer in First Affiliated Hospital of Zhengzhou University were consecutive collected during the period of June 2011 to October 2013. Clinical stages and histological classification were based on the criteria of the International Federation of Gynecology and Obstetrics (FIGO) and the World Health Organization (WHO) were established in all cases. The ovarian cancer histopathology was established in all cases by tissue biopsy of tumor or after surgery treatment from tumor cancer tissues. None of the patients had received pre-operative adjuvant chemotherapy or radiation therapy. The average age of cancer patients are 57. Group II- Benign ovarian diseases control, 205 patients diagnosed with benign ovarian tumor were collected in the study, all had to be confirmed benign or malignant by histology or cytology, and did not receive radio-chemotherapy. The average age of benign patients are 55 (this group selected postmenopausal and the exact age women as Group I). Group III- Healthy women control, 200 cases Healthy control are selected from Physical examination in our hospital women staff members, whose average age are 56 (this group selected postmenopausal and the exact age women as Group I).

According to protocol of multi-tumor markers protein chip diagnose system (Huzhou Shukang Technology co., LTD), serum samples were stored at -20°C until analysis.

The reference value of parameters respectively are- CA19-9<35 U/ml, NSE<13 ng/ml, CEA<5 ng/ml, CA242<20 U/ml, CK19<3.3 ng/ml, β-HCG<3Mi U/ml, AFP<20 ng/ml, SCC<2.5 ng/ml, c-PSA<4 ng/ml, CA125<35 U/ml, CA72-4<6.9 U/ml, CA15-3<35 U/ml. Computer can display positive automatically when the results have crossed the threshold.

The data were analyzed by χ^2 test, t test and ANOVA. Data analysis was performed with SPSS13.0 Statistical Analysis Software. $p<0.05$ was regarded as statistically significant for differences between groups.

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Results

Serum levels of twelve tumor markers in cancer group, benign group and healthy control were showed in Table 2. Positive rate of combine detection in cancer group was 77.7%, in benign group and healthy control respectively was 26.3% and 4.5%. Cancer group had a significantly higher serum level of tumor markers than that in benign group and healthy control (χ^2=112.47, p<0.01; χ^2=229.38, p<0.01). Comparing with benign group and healthy control, positive rate of CA19-9, CEA, CA242, AFP, SCC, CA125, CA724 and CA15-3 in cancer group were significantly higher (p<0.01), and positive rate of NSE, CK19, β-HCG and c-PSA had no significant difference (p>0.05). Positive rate of CA19-9, AFP, CA125, CA724 and CA15-3 in benign group also apparently higher than that in healthy control (p<0.05).

By using One-Way ANOVA Test (SNK was used between groups), Table 3 showed serum levels of CA19-9, CEA, CA242, AFP, SCC, CA125, CA724 and CA15-3 in cancer group were obviously higher than that in benign group and healthy control (p<0.01). Interestingly, serum levels of the twelve tumor markers had no statistical difference between benign group and healthy control (p>0.05).

Apparently, of the 115 cases ovarian cancer patients with surgery, as Table 4 showed, the expression of tumor markers were higher in lymph node metastasis positive patients (86.8%) than that in negative patients (44.7%, p<0.01), in the single index, expression of CA72-4, CA15-3 and CA125 were higher than in lymph node negative group (p<0.01), and expression of the other nine tumor markers had no significant difference (p>0.05).

Table 1. The Clinical Features of Ovarian Cancer Patients

Group	Number of patients
Ovarian cancer patients	220 (100%)
Median age (range)	57 (48-76)
FIGO stage	
IA	0
IB	12 (5.5%)
IC	0
IIA	0
IIB	33 (15%)
IIC	28 (12.7%)
IIIA	36 (16.4%)
IIIB	32 (14.5%)
IIIC	50 (22.7%)
IV	29 (13.2%)
Menopausal status:	
postmenopausal	220 (100%)
Benign ovarian tumor patients	205 (100%)
serous cystadenoma	90 (43.9%)
mucinous cystadenoma	50 (24.4%)
mature teratoma	65 (31.7%)
median age (range)	55 (47-72)
Menopausal status:	
postmenopausal	205 (100%)
Healthy control	200 (100%)
median age (range)	56 (45-68)
Menopausal status:	
postmenopausal	200 (100%)

Table 2. Positive Rate (%) of Twelve Tumor Markers in the Three Groups

Group	Ovarian cancer	Benign ovarian tumor	Healthy control	
n	220	205	200	
positive cases (n)	171	54	9	
Combined positive rate (%)	77.7	26.3	4.5	
Single positive rate (%)				
CA19-9	37.7	6.3	1	*△
NSE	2.7	1.5	0	
CEA	30.9	3.4	0.5	*
CA242	46	18	1.5	*
CK19	2.3	0	0	
β-HCG	2.7	0	0	
AFP	40	15.1	5	*△
SCC	35	5.4	3	*
c-PSA	0	0	0	
CA125	50	25.9	2	*△
CA724	55.9	20	3	*△
CA15-3	57.3	18	1	*△

* statistically significant when cancer group vs benigh group and healthy control (P<0.01); △statistically significant when benigh group vs healthy control (P<0.01)

Table 3. Serum Level of Twelve Tumor Markers in the Three Groups (mean±std)

Group	ovarian cancer	Benign ovarian tumor	Healthy control	F	P
n	220	205	200		
CA19-9 (U/ml) *	98.5±56.1	36.4±16.3	23.2±18.7	76.88	<0.01
NSE (ng/ml)	12.3±3.6	7.9±2.9	6.4±2.0	0.02	>0.05
CEA (ng/ml) *	26.3±10.2	8.9±2.3	3.6±1.6	79.37	<0.01
CA242 (U/ml) *	58.9±23.1	15.2±3.6	10.3±2.3	81.05	<0.01
CK19 (ng/ml)	5.0±3.3	3.2±1.0	3.0±1.3	0.51	>0.05
β-HCG (ml U/ml)	2.5±0.9	2.0±0.5	1.9±0.9	0.79	>0.05
AFP (ng/ml) *	29.6±15.9	10.2±9.6	8.3±6.9	101.02	<0.01
SCC (ng/ml) *	8.5±6.3	3.9±2.0	3.8±2.8	10.12	<0.01
c-PSA (ng/ml)	1.5±1.0	1.3±0.9	1.2±0.5	0.89	>0.05
CA125 (U/ml) *	210.8±78.6	56.3±39.7	39.8±19.6	360	<0.01
CA724 (U/ml) *	57.6±48.1	20.7±16.2	13.4±6.0	68.59	<0.01
CA15-3 (U/ml) *	49.9±12.0	29.6±16.3	20.9±18.9	79.23	<0.01

* statistically significant when cancer group vs benigh group and healthy control (P<0.01); △statistically significant when benigh group vs healthy control (P<0.01)

Table 4. Positive rate (%) of Twelve Tumor Markers in in Lymph Node Metastasis and Non-Metastasis Groups

Group	Metastasis Group	Non Group Metastasis	χ^2	P
n	68	47		
positive cases (n)	59	21		
Combined positive rate (%)	86.8	44.7	23.25	<0.01*
Single positive rate (%)				
CA19-9	54.4	42.6	1.563	>0.05
NSE	1.5	0	/	/
CEA	58.8	51.1	0.684	>0.05
CA242	16.2	13.8	0.257	>0.05
CK19	70.6	63.8	0.04	>0.05
β-HCG	5.9	4.3	0.149	>0.05
AFP	76.5	68.1	0.992	>0.05
SCC	80.9	85.1	0.345	>0.05
c-PSA	0	0	/	/
CA125	91.2	46.8	27.784	<0.01*
CA724	75	36.2	17.339	<0.01*
CA15-3	86.8	40.4	27.347	<0.01*

Discussion

Serum tumor markers, either produced by the tumor or in response to the tumor, can appear in cell, tissue or fluids, and reflect maintenance and growth of tumor (Meany et al., 2009). Ovarian cancer, as one of the most common and the most fateful cancer in women, has the slur of early symptom, the treatment of many patients was delayed because of the lateness of correct diagnosis (Zagouri et al., 2010; Khaider et al., 2012; Sundar et al., 2012). Besides diagnosing with imaging tests, pathology and cytology, detection of serum tumor markers also is a great part of diagnosis. Unfortunately, none of a tumor marker has the best specificity nowadays. So most experts agreed to combined detection of multi-tumor markers (Parikh et al., 2010; Rusling et al., 2010; Wesolowski et al., 2011; Zhu, et al., 2012).

In our test, multi-tumor markers protein chip diagnose system was used to detect twelve tumor markers in ovarian carcinoma patients, benign ovarian tumor group and healthy subjects, and further analyze based on the results. We found that the positivity rate in ovarian cancer was 77.7%, obviously higher than that in the benign cases (26.3%) and healthy subjects (4.5%). Serum levels of CA19-9, CEA, CA242, AFP, SCC, CA125, CA724 and CA15-3 in cancer group also were significantly higher than benign cases and healthy control. That indicated that CA19-9, CEA, CA242, AFP, SCC, CA125, CA724 and CA15-3 can play an important role in the process of diagnosis in ovarian cancer. Positive rate of CA19-9, AFP, CA125, CA724 and CA15-3 in benign group also apparently higher than that in healthy control, but had no difference in quantity of serum level. That means the elevated level of the five tumor markers had no statistical significance when compared with healthy population, so they can used as diagnostic tool distinguish ovarian cancer from other ovarian diseases and healthy women.

Our data also indicated that positive rate of tumor markers was higher in lymph node metastasis patients (86.8%) than that in non-metastasis patients (44.7%), and in single test, expression of CA72-4, CA15-3 and CA125 also were higher than in lymph node negative group, that further indicated that protein chip had value in progress monitoring of ovarian cancer.

As a whole, multi-tumor markers protein chip diagnose system in our test has high-sensitivity in diagnosing ovarian cancer. That may accompany with false negative. Besides this small flaws, protein chip diagnose system still play unique advantages in differential diagnosis of ovarian cancer. Consistent with our previous studies (Bian et al., 2013), CA72-4, CA15-3 and CA125 had the higher positive rate in ovarian cancer, and also had significant statistically significant between lymph node metastasis positive and negative groups, that indicated that combined assay of CA72-4, CA15-3 and CA125 can provide diagnosis value for ovarian cancer. HE4, recently was found up-regulated frequently in ovarian cancer (Lin et al., 2012; Devan et al., 2013), and has been proposed as a novel tumor marker for ovarian cancer, the sensitivity of combination with CA125 or other ovarian cancer markers may increase, and can be a new method to monitor the prognosis of ovarian cancer. For us, if HE4 can be implanted with CA72-4, CA15-3 and CA125 in the same protein chip, may further increase practical value of tumor markers in mass screening, diagnosis, progress, prognosis and therapeutic effect of ovarian cancer.

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