Original Article

Combined Detection of Serum Matrix Metalloproteinase 9, Acetyl Heparinase and Cathepsin L in Diagnosis of Ovarian Cancer

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ABSTRACT

Objective: To investigate the clinic values of combining test of serum matrix metalloproteinase 9 (MMP-9), acetyl heparinase (Hpa) and Cathepsin L (CL) in diagnosis of ovarian cancer.

Methods: Serum levels of MMP-9, Hpa and CL were detected in a total of 418 cases, including 217 cases with ovarian malignant tumor, 100 cases with ovarian benign tumor and 101 healthy controls, by using enzyme-linked immunosorbent assay (ELISA). Their correlation with clinicopathologic feature of ovarian malignant tumor was analyzed and their diagnosis performance was evaluated by receiver operating characteristic (ROC). The combined diagnosis model was established by logistic regression analysis.

Results: The serum levels of MMP-9, Hpa and CL were significantly higher in patients with ovarian malignant tumor than in benign tumor and healthy control, the serum levels of CL and Hpa were higher in epithelial cancer than in non-epithelial tumor, and MMP-9, Hpa and CL were elevated in low grade and advanced stage compared to high grade and early stage. The sensitivity for diagnosis of ovarian malignant tumor from high to low was CL, Hpa and MMP-9, and the specificity was MMP-9, CL and Hpa. The united diagnosis model was established and showed the sensitivity and specificity of combined detection were 84.6% and 82.1%, respectively, which were significantly higher than a single tumor marker.

Conclusion: Serum MMP-9, Hpa and CL were correlated with ovarian malignant tumor and the combined detection of which may be valuable for clinical diagnosis of ovarian malignant tumor.

Key words: Ovarian cancer; Matrix metalloproteinase 9; Acetyl heparinase; Cathepsin; Diagnosis

INTRODUCTION

Ovarian cancer is the 4th most frequent cause of cancer death in woman and also is hard to be diagnosed in the early stage due to lack of special symptom and physical sign. About 60%–70% patients with ovarian cancer were diagnosed in their advanced stage and overall 5-year survival is poor. Ideal primary cytoreductive surgery and combination chemotherapy with platinum have improved the prognosis of patients with advanced ovarian cancer, but the 5-year survive rate is still about 40%[1, 2]. At present, the major method of early diagnosis is detection of serum tumor markers, such as carbohydrate antigen 125 (CA125) and human epididymis protein 4 (HE4), which have been used in clinic for diagnosis and monitoring of ovarian cancer. However, there is limitation for CA125 or HE4 in clinic practice. More and better tumor markers are needed to help early diagnosis of ovarian cancer. At present, serum biomarker panels are used to enhance the diagnosis rate[3]. Our and other studies have shown that the overexpression of cathepsin L (CL), matrix metalloproteinase 9 (MMP-9) and acetyl heparinase (Hpa) were relative to the development of malignant tumor, and the serum levels of CL, MMP-9 and Hpa were significantly high in patients with ovarian malignant tumor[4-8]. CL, MMP-9 and Hpa may be hopeful serum tumor markers. In this study, we tested

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the serum levels of CL, MMP-9 and Hpa, analyzed their correlation with pathology and established a math diagnostic model in order to evaluate the value of combined detection of CL, MMP-9 and Hpa in diagnosis of ovarian cancer.

MATERIALS AND METHODS

Human serum samples

Serums were obtained from patients who received surgery in the Department of Gynecologic Oncology, Affiliated Tumor Hospital of Guangxi Medical University, Nanning, Guangxi. This research included the data of 217 patients with malignant ovarian tumor (109 serous, 54 mucus, 14 undifferentiated, 19 gonad mesenchymoma, 21 malignant germ cell tumor), and 100 benign tumor (62 serous, 24 mucus, 14 benign teratoma). In malignant ovarian tumor group, the patients' median age was 44.6 (range 16–67) years. The disease was staged according to the International Federation of Gynecology and Obstetrics (FIGO) classification. Eighty-three patients had stage I–II tumor, and 134 patients had III–IV. In benign group, the median age was 35.6 (range 14–64) years. Serums of normal control were obtained from 101 healthy women. This study was endorsed by the Ethics Committee of the Guangxi Medical University. All subjects received an explanation of the aims of the study and signed written informed consent. All subjects understood that they could withdraw from the study at any time without influencing their oncological or general medical treatment.

Enzyme-Linked Immunosorbent Assay (ELISA) Detection

Two milliliters of peripheral blood was obtained from patients before any treatment was taken. Sera were collected and stored at –80°C. ELISA for MMP-9, Hpa, CL and CA125 were performed with immunoassay kit (Boatman, China) according to the manufacturer's instructions. Goat polyclonal antibody against MMP-9, goat polyclonal antibody against CL, goat polyclonal antibody against Hpa-1 and standard substance were purchased from Santa Cruz (USA). The optical density (OD) at 450 nm was determined. The standard curves were established with OD_{450} as Y axle and the concentration of standard substance as X axle. The level of protein was obtained through standard curve. The equation of curve was $Y=a(1-e^{-bx})$. a=1,096.1137, b=0.0416, r=0.8578 for CL; a=1,165.6651, b=0.0259, r=0.8398 for Hpa; and a=678.2558, b=0.9717, r=0.6769 for MMP-9.

Establishment of Combined Diagnosis Model

The serum levels of CL, MMP-9 and Hpa were detected in 250 cases of ovarian malignant, benign tumor and normal control. Logistic regression analysis was performed with Proc logistic module in SAS software (SAS Inc., Cary, USA) to screen the markers for malignant tumor, and determine the weight factor parameters according to their correlation and influence on diagnosis for ovarian tumor[9]. After parameter estimation of logistic regression was obtained, another 168 samples were used to validate this model. Model verification was presented as R2[6].

$$R^2_{SS}=1-SSE/SST,$$
$$SST=\Sigma (y_i-\bar{p})^2,\ SSE=\Sigma (y_i-\hat{p}_i)^2,\ 1\le i\le n,$$
$$\hat{p}_i=\exp(\hat{\beta}x_i)/[1+(\exp \hat{\beta}x_j)],$$
$\hat{\beta}$ is an estimation of a parameter vector,
$$\bar{p}=\Sigma y_i/n.$$

Statistical Analyses

Statistical analyses were performed by using the SPSS software (SPSS Inc., Chicago, IL, USA). Data of ELISA were presented as $\bar{x}\pm s$. $P<0.05$ was considered statistically significant. The *t* test and one-way analysis of variance (ANOVA) were used for statistical evaluation of measurement data. The threshold of sensitivity and specificity was identified by receiver operating characteristic (ROC) curves.

RESULTS

Serum Levels of CL, Hpa and MMP-9 in Each Group

The serum levels of CL, Hpa and MMP-9 were significantly higher in patients with malignant ovarian tumors than in patients with benign ovarian tumor and healthy controls ($P=0.000$). The level of CL in benign group was significantly higher than that in normal control, but there was no significant difference in Hpa and MMP-9 between benign tumor and healthy controls (Table 1).

Serum Levels of CL, Hpa and MMP-9 in Patients with Malignant Ovarian Tumor and its Correlation with Clinicopathologic Variables

The levels of serum CL, Hpa and MMP-9 and clinicopathologic variables in patients with ovarian cancer are shown in Table 2. There were significant differences between histological grade and FIGO stage for serum CL, Hpa and MMP-9. The difference between epithelial and non-epithelial tumor was observed only for serum CL. The levels of serum CL, Hpa and MMP-9 were higher in patients with low histological grade and advanced stage than in high grade and early stage. These results showed that there was correlation between serum CL, Hpa and MMP-9