Original Article

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

Wei Zhang[1*], Xiao-xia Hu[2*], Xing-zhi Yang[1], Qi Wang[1], Hong Cheng[1], Shu-mei Wang[1], Yan-ling Hu[1], Zhi-jun Yang[1], Li Li[1**]

[1]*Department of Gynecologic Oncology, Cancer Institute and Hospital, Guangxi Medical University, Nanning 530021, China*
[2]*Department of Gynecology and Obstetrics, the People's Hospital of Guangxi Province, Nanning 530021, China*

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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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*Contributed equally to this study.
**Corresponding author.
E-mail: lili@gxmu.edu.cn

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].

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

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

and histological grade and stage.

Effect of Serum Levels of CL, Hpa and MMP-9 in Diagnosis of Ovarian Malignant Tumor

The serum CA125 was also detected in patients with ovarian malignant tumor in order to compare the diagnostic performance. The ROC curve was performed (Figure 1) and areas under curve (AUC) was obtained (Table 3). MMP-9 had greater AUC (0.843) than others. The sensitivity from high to low for predicting ovarian malignant tumor was CL, Hpa, CA125, and MMP-9, and the specificity was MMP-9, CA125, CL, and Hpa. Comprehensive analysis showed MMP-9 and CA125 had higher positive likelihood ratio and lower negative likelihood ratio compared to CL and Hpa. Comparison of the diagnosis prefermence of four serum markers, showed that MMP-9 had higher diagnostic value than others (Table 4).

Evaluation of Combined Detection of CL, Hpa and MMP-9 in Diagnosis of Ovarian Malignant Tumor

The mathematic model was established by using step by step screening on their diagnostic performance for ovarian malignant tumor. Finally, we got the model: Logit(*P*)=14.90–43.24×Hpa–33.12×h1–43.80×MMP-9+71.0

Table 1. Serum levels of CL, Hpa and MMP-9 in each group

Groups	*n*	CL (ng/ml)	Hpa (ng/ml)	MMP-9 (ng/ml)
Control	100	5.59±1.75 (2.55–10.21)	2.77±0.80 (1.54–5.82)	57.99±11.42 (39.04–107.18)
Benign	101	10.97±3.84 (5.10–23.03)*	4.86±1.37 (2.11–9.15)	143.66±28.47 (70.53–207.13)
Malignant	217	21.23±8.17 (5.92–52.81)**	7.68±2.34 (2.39–13.31)**	193.95±42.49 (69.35–316.20)**

*Compared to normal control, P=0.000; **compared to benign group and normal control, *P*=0.000.

Table 2. Serum levels of CL, Hpa and MMP-9 and clinicopathologic variables in patients with ovarian cancer

Indices	*n*	CL (ng/ml)	Hpa (ng/ml)	MMP-9 (ng/ml)
Histological type				
Epithelial	177	21.598±8.249†	8.054±2.050	195.743±41.667
Serous	109	21.620±8.520	8.100±2.020	194.250±43.470
Mucinous	54	20.280±7.440	7.970±2.140	195.840±37.830
Others*	14	26.490±7.640	8.060±2.170	207.040±42.620
Non-epithelial	40	19.565±7.698	6.020±2.270	186.019±45.671
Histological grade				
G1–G2	29	18.540±7.300‡	7.200±2.510‡	173.430±39.370‡
G3	148	23.040±7.670	8.220±1.920	200.120±40.820
FIGO stage				
I–II	62	19.660±7.830§	7.210±2.050§	182.630±42.300§
III–IV	115	22.640±8.310	8.510±1.920	202.810±39.740

*Including endometrioid, clear cell, and undifferentiated cancer. †Comparison between epithelial and non-epithelial, *P*<0.05; ‡comparison between histological grades, *P*<0.05; §comparison between stages, *P*<0.05.

Table 3. AUC of four markers

Markers	AUC	Standard error	*P*	95% CI
CL	0.708	0.040	0.000	0.630–0.786
Hpa	0.761	0.031	0.000	0.700–0.823
MMP-9	0.843	0.025	0.000	0.794–0.892
CA125	0.776	0.030	0.000	0.717–0.836

95% CI: 95% confidence interval; AUC: areas under curve.

Table 4. Comparison of diagnosis performance of markers

Markers	AUC	Sensitivity (%)	Specificity (%)	False negative rate (%)	False positive rate (%)	Positive LR	Negative LR
CL	0.708	77.4	55.9	22.6	44.1	1.8	0.4
Hpa	0.761	76.3	55.9	23.7	44.1	1.7	0.4
MMP-9	0.843	74.6	81.4	25.4	19.6	3.6	0.3
CA125	0.776	75.7	69.5	24.3	30.5	2.5	0.3

LR: likelihood ratio; AUC: areas under curve.

Table 5. Comparison of diagnosis property of markers and diagnosis model

Markers	AUC	Sensitivity (%)	Specificity (%)	False negative rate (%)	False positive rate (%)	Positive LR	Negative LR
CL	0.708	77.4	55.9	22.6	44.1	1.8	0.4
Hpa	0.761	76.3	55.9	23.7	44.1	1.7	0.4
MMP-9	0.843	74.6	81.4	25.4	19.6	3.6	0.3
CA125	0.776	75.7	69.5	24.3	30.5	2.5	0.3
Diagnosis model	0.935	86.4	82.1	13.6	17.9	4.8	0.2

LR: likelihood ratio; AUC: areas under curve.

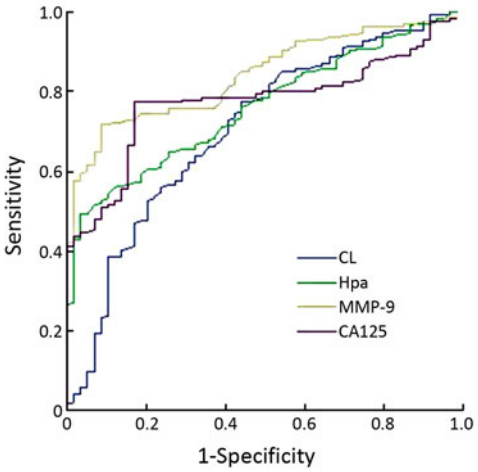

Figure 1. ROC curve of CL, Hpa, MMP-9 and CA125.

8×(CL×Hpa)+55.83×(CL×MMP-9), then R^2_{SS}=0.866. To verify this model, the values of CL, Hpa and MMP-9 were filled in the model, and then logit(*P*) was obtained. The diagnosis performance is showed in Table 5. The diagnosis model had greater areas of under ROC curve (0.935) and higher sensitivity (86.4%) and specificity (82.1%) than single marker (*P*=0.000). Overall null hypothesis (β=0) test showed that there were significant differences for likelihood ratio, score and Wald (*P*<0.0001).

DISCUSSION

Our studies showed that the serum levels of MMP-9, CL and Hpa were higher in patients with ovarian malignant tumor than in ovarian benign tumor and healthy controls, and the level of CL was higher in ovarian benign tumor than in healthy controls. Our results were consistent with some researches[10-12]. Manenti, et al.[13] showed the serum level of MMP-9 was higher in patients with ovarian malignant tumor than in benign tumor and normal group (*P*=0.01 and *P*<0.0002), and had a great value in diagnosis of ovarian malignancies. Recently, Karihtala, et al.[14] revealed that serum MMP in patients with ovarian cancer can be a marker of monitoring chemotherapy effect. Ralph, et al.[15] reported serum Hpa in patients with ovarian cancer was significantly higher than those with benign tumor and normal control, which decreased after tumor was removed and increased when tumor recurred. Their results also showed serum Hpa level correlated with the size and growth of tumor. Siewinski, et al.[16] reported serum level of CL was higher in malignant tumor than benign tumor and normal control. These results revealed serum MMP-9, CL and Hpa increased in patients with ovarian cancer, and they may be the useful serum markers for diagnosis of ovarian cancer.

Our studies also showed that the serum levels of MMP-9, CL and Hpa were relevant to clinical pathological stage, histological type and degree of differentiation, which were higher in patients with advanced-stage, epithelial and low-grade tumor than in early-stage, non-epithelial and high-grade tumor. These results are consistent with previous studies[17, 18], and revealed that MMP-9, CL and Hpa are relevant to ovarian cancer development , invasion and metastasis.

The development of cancer is a complex process and associated with multiple factors. At present, no single serum marker can be satisfying for diagnosis of cancer. Researchers have been looking for a multi-marker panel to improve diagnosis performance[19, 20]. Nolen, et al.[3] discovered the combined detection of HE4 and CA125 had higher diagnosis performance than single marker by multiple regression analysis on 65 tumor markers. Our study showed serum MMP-9, CL and Hpa were significantly higher in ovarian cancer and relevant to the development of cancer. ROC curve and analysis showed each of these markers had certain extent of diagnosis performance, but they were not very satisfying biomarker of ovarian cancer when single marker was used.

We combined serum MMP-9, CL and Hpa, and established a mathematic diagnosis model by using logistic regression analysis, in which R^2_{SS}=0.866, close to 1. By using this model, the diagnosis performance for ovarian malignant tumor was enhanced compared to single marker and CA125. The sensitivity, specificity

and positive likelihood ratio increased, and negative likelihood ratio decreased. The overall null hypothesis (β=0) test showed that there were significant differences for likelihood ratio, score and Wald. Therefore, we suggest united detection of MMP-9, CL and Hpa because united detection has higher value in diagnosis of ovarian malignant tumor by using the united diagnosis model. However, much work should be done to improve the diagnosis model so as to assist clinical diagnosis. The united diagnosis model is expected to be a new assistant diagnosis model in diagnosis of malignancies.

REFERENCES

1. Shaaban A, Rezvani M. Ovarian cancer: detection and radiologic staging. Clin Obstet Gynecol 2009; 52:73–93.
2. Chen WQ, Zhang SW, Zou XN, et al. Cancer incidence and mortality in China, 2006. Chin J Cancer Res 2011; 23:3–9.
3. Nolen B, Velikokhatnaya L, Marrangoni A, et al. Serum biomarker panels for the discrimination of benign from malignant cases in patients with an adnexal mass. Gynecol Oncol 2010; 117:440–5.
4. Wang SM, Li L, Zhang W, et al. Clinical value of detection of serum cathepsin L on diagnosis of ovarian cancer. Zhong Guo Zhong Liu Lin Chuang (in Chinese) 2008; 35:167–8.
5. Zhou J, Li L, Wang SM, et al. Elevated Serum Level of MMP-9 in malignant ovarian tumor and its clinical significance. Shi Yong Fu Chan Ke Za Zhi (in Chinese) 2008; 24:346–9.
6. Kolwijck E, Massuger LF, Thomas CM, et al. Cathepsins B, L and cystatin C in cyst fluid of ovarian tumors. J Cancer Res Clin Oncol 2010; 136:771–8.
7. de Moura JP Jr, Nicolau SM, Stávale JN, et al. Heparanase-2 expression in normal ovarian epithelium and in benign and malignant ovarian tumors. Int J Gynecol Cancer 2009; 19:1494–500.
8. Gershtein ES, Levkina NV, Digayeva MA, et al. Matrix metalloproteinases 2, 7, and 9 and tissue inhibitor of metalo-proteinases-1 in tumors and serum of patients with ovarian neoplasms. Bull Exp Biol Med 2010; 149:628–31.
9. Mittlböck M, Schemper M. Explained variation for logistic regression. Stat Med 1996; 15:1987–97.
10. Arvatz G, Shafat I, Levy-Adam F, et al. The heparanase system and tumor metastasis: is heparanase the seed and soil? Cancer Metastasis Rev 2011; 30:253–68.
11. Matarrese P, Ascione B, Ciarlo L, et al. Cathepsin B inhibition interferes with metastatic potential of human melanoma: an *in vitro* and *in vivo* study. Mol Cancer 2010; 9:207.
12. Alshenawy HA. Immunohistochemical expression of epidermal growth factor receptor, E-cadherin, and matrix metalloproteinase-9 in ovarian epithelial cancer and relation to patient deaths. Ann Diagn Pathol 2010; 14:387–95.
13. Manenti L, Paganoni P, Floriani I, et al. Expression levels of vascular endothelial growth factor, matrix metalloproteinases 2 and 9 and tissue inhibitor of metalloproteinases 1 and 2 in the plasma of patients with ovarian carcinoma. Eur J Cancer. 2003; 39:1948–56.
14. Karihtala P, Mäenpää J, Turpeenniemi-Hujanen T, et al. Front-line bevacizumab in serous epithelial ovarian cancer: biomarker analysis of the FINAVAST trial. Anticancer Res 2010; 30:100–6.
15. Ralph S, Brenchley PE, Summers A, et al. Heparanase gene haplotype (CGC) is associated with stage of disease in patients with ovarian carcinoma. Cancer Sci 2007; 98:844–9.
16. Siewinski M, Saleh Y, Gryboc M, et al. Determination of cysteine peptidases-like activity and their inhibitors in the serum of patients with ovarian cancer treated by conventional chemotherapy and vitamin E. J Exp Ther Oncol. 2004; 4:189–93.
17. Gershtein ES, Levkina NV, Digayeva MA, et al. Matrix metalloproteinases 2, 7, and 9 and tissue inhibitor of metallo-proteinases-1 in tumors and serum of patients with ovarian neoplasms. Bull Exp Biol Med 2010; 149:628–31.
18. Fredriksson S, Horecka J, Brustugun OT, et al. Multiplexed proximity ligation assays to profile putative plasma biomarkers relevant to pancreatic and ovarian cancer. Clin Chem 2008; 54:582–9.
19. Yurkovetsky Z, Ta'asan S, Skates S, et al. Development of multimarker panel for early detection of endometrial cancer. High diagnostic power of prolactin. Gynecol Oncol 2007; 107:58–65.
20. Kanat-Pektas M, Yenicesu O, Gungor T, et al. Predictive power of sexual hormones and tumor markers in endometrial cancer. Arch Gynecol Obstet 2010; 281:709–15.130