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Combined detection of sialic acid and hydroxyproline in diagnosis of ovarian cancer and its comparison with human epididymis protein 4 and carbohydrate antigen 125

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ABSTRACT

Background: Elevated serum sialic acid (SA) and hydroxyproline (Hyp) concentrations have been found in a variety of malignant cancers. We simultaneously detect serum concentrations of SA and Hyp (SA&Hyp) in ovarian cancer, and compare its diagnostic value with classic tumor markers—human epididymis protein 4 (HE4) and carbohydrate antigen 125 (CA125).

Methods: Serum concentrations of SA&Hyp, HE4 and CA125A were detected in a total of 767 serum samples collected from 484 patients with gynecologic diseases, 180 healthy individuals, 45 pregnant women and 58 patients with renal failure using chemical colorimetry and electrochemiluminescence immunoassay (ECLIA), respectively. Risk of ovarian malignancy algorithm (ROMA) was calculated based on HE4 and CA125 values.

Results: Serum SA&Hyp concentrations were influenced significantly by renal failure and pregnancy but not age and menopausal status. The median concentrations of SA&Hyp, HE4 and CA125 in patients with ovarian cancer were 119.0 U/ml, 190.2 pmol/l and 366.0 pmol/l, which were significantly higher than concentrations in patients with benign gynecologic diseases ($P < 0.001$). SA&Hyp showed a significantly higher AUC than HE4 and CA125 in the diagnosis of gynecologic malignancies ($P < 0.001$), while no significance was found when compared with ROMA. Specially, SA&Hyp in 48.3% subjects (29/60) diagnosed as positive before primary surgery showed negative after surgery.

Conclusions: Renal failure and pregnancy are the main source for increased false positive of SA and Hyp. Compared with HE4 and CA125, SA&Hyp shows a better diagnosis value and can be used in the diagnosis and dynamic monitoring of gynecologic pelvic malignancies, while no statistical significance was found compared with ROMA.

1. Introduction

Ovarian cancer is one of the major malignant diseases in women, accounting for about 4% of women malignant diseases. Its mortality ranks the fifth in women with cancer [1,2], because >75% of patients are diagnosed at the advanced stage. If patients could be diagnosed at stage I and II, its 5-y survival rate would be increased from current 10%–20% to more than 70% [3–6]. Therefore, early and accurate differential diagnosis of ovarian cancer is essential for improving prognosis and needs to be further investigated.

In current clinical practice, carbohydrate antigen 125 (CA125) has been partially used for the diagnosis of ovarian cancer. This glycoprotein is elevated in roughly 80% of patients with ovarian cancer, but only in <50% patients at stage I [6–8]. Moreover, its low positive predictive value (PPV) has limited the effectiveness [9]. Recently, human epididymis protein 4 (HE4), a proteinase inhibitor for sperm maturation and intrinsic immunity, has been identified as a more promising tumor marker in the detection of ovarian cancer. Although it has improved the specificity to ovarian cancer, its sensitivity is still low [10,11].

A hallmark of tumor phenotype is the altered glycosylation patterns such as increased size and number of N-linked glycan branches, additional terminal sialic acids and ultimate increase in global sialylation, due to up-regulation of sialyltransferase [12]. Thus, serum sialic acid (SA) concentration will increase in those tumors over-expressing glycoproteins and glycolipids and can be used as a diagnostic marker of cancer [13,14]. Studies found that serum SA was elevated in patients with colorectal tumor, prostate cancer and ovarian malignancies and approved it could be used as a marker for tumor diagnosis in clinical practice [15–17].

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Hydroxyproline (Hyp) is a specific ingredient of human collagen, and not in other animal proteins [18]. It has been reported that tumor invasion can destruct basement membrane, and cause an increase of endogenous Hyp in serum. Recently, some studies found serum Hyp was over-expressed in cancer patients and could be a useful marker for cancer screening [19,20].

During our clinical practice, a new kit was developed to simultaneously detect serum concentrations of SA and Hyp (SA&Hyp) using a specific polymer carrier composed of paradimethylaminobenzaldehyde (PDAB), ninhydrin and hydrochloric acid. Using this kit, SA&Hyp was detected in lung, liver, breast, esophageal, gastric, colorectal and pancreatic cancer patients in some previous studies, and demonstrated a good accordance with CEA, CA199, CA153, and AFP, which suggested it could be a surrogate for these conventional biomarkers [21,22]. In this study, for more accurate evaluation of this new kit in diagnosis of ovarian cancer, we firstly investigated the common influence factors on SA&Hyp detection, such as age, creatine concentration, menopausal and pregnancy status. Then, SA&Hyp concentrations were detected in patients with gynecologic diseases and compared with traditional tumor markers, HE4 and CA125, to systematically assess its diagnosis value for ovarian cancer.

2. Materials and methods

2.1. Patients

The study was approved by the Ethics Committee of Qilu Hospital, Shandong University and written informed consent was obtained from all participants after explanation. A total of 542 patients who admitted consecutively to Qilu Hospital of Shandong University from August 2012 to April 2014 were enrolled in the study. Among them, 155 had gynecologic malignancies, 329 had gynecologic benign diseases and 58 had renal failure. Among the patients with gynecologic malignancies, 77 were ovarian cancer (50 papillary serous carcinoma, 12 mucinous adenocarcinoma and 15 others), 37 were cervical malignancies, 22 were endometrium cancer, 10 were granulosa tumor and 9 were ovarian immature teratoma. Among the patients with gynecologic benign diseases, 48 were ovarian endometriosis, 67 were ovarian cyst, 35 were ovarian mature teratoma, 17 were ovarian cystadenoma, 21 were varies pelvic inflammatory diseases, 58 were uterine myoma, 48 were endometrial polyps, 12 were adenomyosis, 7 were uterus septum and 9 patients were diagnosed to have multiple gynecologic diseases. In addition, 180 healthy women (90 premenopausal women and 90 post menopausal women) and 45 pregnant women who admitted to Qilu Hospital of Shandong University for physical examination and had no any history of malignant diseases were enrolled in the study.

All patients with gynecologic diseases were excluded pregnant status within 3 months. Patients with renal failure were all at premenopausal status and selected randomly from nephrology department. Serum creatinine concentration was within the interval (<115 μmol/l) in all patients except those with renal failure. The diagnosis of patients with gynecologic diseases was confirmed pathologically and their information including definite diagnosis and disease stages were recorded through the patients' medical history. Patients who had received preoperative radiotherapy or chemotherapy were excluded.

2.2. Laboratory methods

Blood samples were collected by venous puncture from patients before surgery and healthy individuals at their first admission to the hospital, centrifuged and stored at −80 °C for further examination. All tests were conducted in strict accordance with the clinical operating requirements. HE4 and CA125 were determined on the Roche Cobas E601 analyzer using electrochemiluminescence immunoassay method with Elecsys HE4 kits (Roche Laboratories) and Elecsys CA125IIkits (Roche), respectively. The cut-off value of HE4 was 70 pmol/l in premenopausal women and 140 pmol/l in postmenopausal women, and that of CA125 was 35 U/ml in all population according to the kits. SA and Hyp were detected in accordance with SA&Hyp detection kit (Qingdao Bo-Xin Biotechnology Co. Ltd) and the values ≥95 U/ml were considered as positive according to the kits.

2.3. Statistical analysis

All statistical analyses were performed using SPSS statistical software (ver. 17.0). Kolmogorov–Smirnov test was used to define the distributions of markers' concentrations of each group. Concentrations of SA&Hyp, HE4 and CA125 were described as median (range), and their comparisons among multiple groups were determined by applying Kruskal–Wallis test or Mann–Whitney *U* test when appropriate. Correlation test was analyzed using Spearman test. Count data were described as frequency, and examined using χ^2 test. A $P < 0.05$ was considered to be statistically significant in all analyses.

3. Results

3.1. Influence of age, creatinine, menopausal and pregnancy status on diagnostic markers of ovarian cancer

3.1.1. Age and markers

Fig. 1 shows the serum concentrations of SA and Hyp, HE4 and CA125 in healthy individuals at different ages. The median concentration of serum SA and Hyp was 80.5 U/ml for patients at age of 20–30 y, 78.5 U/ml at age of 30–40 y, 80.5 U/ml at age of 40–50 y, 81.0 U/ml at age of 50–60 y, 83.5 U/ml at age of 60–70 y and 85.0 U/ml at age of >70 y. There was no significant correlation between serum SA and Hyp concentration and age (Spearman's $r = 0.023$, $P > 0.05$). Serum HE4 concentration was 40.9 pmol/l for patients at 20–30 y, 40.5 pmol/l at 30–40 y, 43.8 pmol/l at 40–50 y, 48.6 pmol/l at 50–60 y, 57.4 pmol/l at 60–70 y and 74.7 pmol/l at >70 y. Statistical analysis indicated that HE4 concentration increased with age increasing (Spearman's $r = 0.661$, $P < 0.001$). Serum CA125 concentration was 16.1, 14.2, 12.8, 11.7, 10.6, and 8.5 U/ml for patients at age of 20–30, 30–40, 40–50, 50–60, 60–70 and ≥70 y. By contrast, serum concentration of CA125 decreased along with age increasing (Spearman's $r = -0.330$, $P < 0.05$).

3.1.2. Creatinine and markers

High creatinine concentrations are associated with higher SA and Hyp, HE4 concentrations. The median HE4 concentration in patients with renal failure was 1157.0 pmol/l, 30 times higher than in healthy individuals (48.9 pmol/l, $P < 0.001$). Similarly, the median concentration of SA and Hyp in patients with renal failure was 110.0 U/ml, which was also significantly higher than in healthy individuals (81.0 U/ml,$P < 0.001$). Moreover, HE4 and SA and Hyp were found positive in 98.3% (57/58) and 77.6% (45/58) patients with renal failure, respectively, indicating that serum HE4 and SA and Hyp concentrations were extremely influenced by renal failure. By contrast, the median serum CA125 concentration in patients with renal failure was 17.8 U/ml, which was still under the cut-off values, and the false positive rate was 19.0% (11 of 58), revealing that serum CA125 was less influenced by renal failure.

3.1.3. Pregnancy and markers

We systematically analyzed the concentrations of the 3 serum markers in 45 pregnant women (Fig. 2). The results showed that the serum SA and Hyp and CA125 concentrations were 104.6 U/ml and 49.8 U/ml, respectively, both of which were significantly higher than in healthy individuals (both at $P < 0.001$). On the contrary, serum HE4 concentration in pregnant women was statistically lower than in healthy individuals (43.0 vs 48.9 pmol/l, $P = 0.007$).