RESEARCH ARTICLE

Urinary Concentrations of Human Epididymis Secretory Protein 4 (He4) in The Diagnosis of Ovarian Cancer: A Case-Control Study

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Abstract

Objective: To analyze differential diagnostic accuracy of urinary human epididymis secretory protein 4 (HE4) in patients with ovarian tumors. Materials and methods: In the case-control study 23 patients with ovarian cancer, 37 patients with benign ovarian tumors and 18 women in the control group were included. Serum CA125 values and urinary concentrations of HE4were assessed quantitatively. Urinary creatinine concentrations and glomerular filtration rate were also determined and used to calculate ratios to HE4. Results: Higher urinary HE4 concentrations were observed in patients with late stage ovarian cancer (p=0.001) and also in patients with early stage ovarian cancer when compared to patients with benign ovarian tumors (p=0.044). On analysis where all ovarian cancer patients were included, higher diagnostic accuracy was observed with calculated ratio of HE4 to glomerular filtration rate (GFR) to unchanged urinary HE4 concentrations -AUC 0.861 vs. 0.858. When discriminatory accuracy was calculated for urinary HE4/GFR ratio and unchanged urinary HE4 concentrations, the last demonstrated a higher area under the curve - 0.701 vs. 0.602. The urinary HE4/creatinine ratio had lower discriminatory characteristics than unchanged concentrations of urinary HE4. However, HE4 serum concentration was more accurate for discrimination of patients with benign and malignant ovarian tumors when compared to urinary HE4 and CA125 in sera (AUCs were 0.868 for serum HE4 and 0.856 and 0.653 for urinary HE4 and CA125, respectively). Conclusions: Ovarian cancer patients have higher urinary concentrations of human epididymis secretory protein 4 than patients with benign ovarian tumors. Urinary HE4 has comparable discriminatory accuracy with serum HE4 for benign and malignant ovarian tumors and can be recommended as a non-invasive ovarian cancer risk assessment method.

Keywords: Urine - HE4 - ovarian cancer - diagnostic marker

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Introduction

Human epididymis secretory protein 4 is an 11-kDa protein, which is localized on chromosome 20q12 (Schummer et al., 1999). For the first it was identified in epididymis epithelium and its function was initially associated with the maturation of sperm involved in protease inhibition (Kirchhoff et al., 1998). Human epididymis secretory proteins in the cell can be found in the cytoplasm perinucleary as endoplasmatic network structure. There is glycosylation and release of the HE4 in extracellular environment from ovarian cancer cells (Drapkin et al., 2005). Ovarian cancer associated antigen CA125 is increased in 40-50% of early stage ovarian cancer patients (Rosen et al., 2005), but there have been documented that human epididymis secretory protein 4 is increased in 82.7-90.0% cases of early stage ovarian cancers (Havrilesky et al., 2008), moreover, HE4 is elevated in more than 50% of cases when the CA125 assay values are within the normal range (Moore et al., 2007). There are several explanations why human epididymis secretory protein 4 is more sensitive in comparison to CA125. HE4 is a high molecular weight glycoprotein with an approximate molecular mass 80 kDa, but its secreted form is a 11 kDa protein, that explains an earlier release of biomarker in the blood stream (Schummer et al., 1999). Earlier appearance of human epididymis secretory protein 4 in the serum in case of early stage ovarian cancer is likely to be associated with a simpler mechanism for its release (Boshell et al., 1992; Ligtenberg et al., 1992; Yin et al., 2001).

The role of HE4 in the human body is explained only partly. Human epididymis secretory protein 4 promotes tumor cell adhesion and motility. In cellular level HE4 contributes to epidermal growth factor receptor activation and adhesion molecule expression on the cell surface.

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HE4 expression is associated with cell proliferation and migration via mitogen activated protein kinase path (Guo et al., 2011). Hellström et al. was one of the first who have reported an increased concentration of HE4 in the sera of ovarian cancer patients (Hellström et al., 2003).

More expressed human epidydimis secretory protein 4 has been detected in tissues of serous and endometrioid ovarian cancers (Drapkin et al., 2005). Lower serum HE4 concentrations have been observed in patients with early stage ovarian cancer. Similarly, women with borderline ovarian tumors have lower serum HE4 concentrations than patients with invasive ovarian cancer. No differences have been identified in serum concentrations of HE4 in patients with various degrees of morphological grade ovarian cancers (van Gorp et al., 2011). Hellström et al. demonstrated that the combination of HE4 and CA125 assay provides higher ovarian cancer diagnostic sensitivity and specificity than each biomarker alone (Hellström et al., 2003). Relatively high diagnostic sensitivity and specificity of HE4 can be explained by the fact that the biomarker is not elevated in many benign gynecologic diseases when CA125 levels are increased (Hellström et al., 2003; Moore et al., 2008). Patients with endometrioid ovarian cysts and endometriosis have increased serum CA125 values, whereas the concentration of HE4 is not increased (Huhtinen et al., 2009).

Objective of the study was to analyze differential diagnostic accuracy of urinary human epidydimis secretory protein 4 in patients with benign and malignant ovarian tumors.

Materials and Methods

In the case-control study 23 patients with ovarian cancer, 37 patients with benign ovarian tumors and 18 women in the control group were included.

Blood samples were taken before surgery in two 6-ml vaccutainers with coagulation activator. Sera and urine for the women included in the control group were taken during outpatient visits. According to the manufacturer's recommendations vaccutainers were shaked, left to stay for 60 minutes in a vertical position and then centrifuged for 10 minutes with 1300 rpm. After serum separation, they were transferred to cold storage at -80 degrees Celsius. Before the anticipated surgery urine samples were collected in special, sterile urine collection containers. Urine samples were stored for 60-120 minutes at room temperature, then divided into four tubes of 0.5 ml each and transferred to the freezer for long-term storage at -80 degrees Celsius.Each defrost serum and urine sample was used only once.

Serum CA125 assay values were assessed using the automated Abbott Architect analyzer 2000 and the appropriate reagents Abbott ARCHITECT CA125 assay values II. ARCHITECT serum dilution buffer was used to determine the concentrations of CA125 assay values 0-10000 U/ml range. Human epidydimis secretory protein 4 quantitatively in urine was determined using automated analyzer Abbott Architect and appropriate reagent kits (Abbott Diagnostics). Urinary samples were diluted 1:100 before analysis of HE4 concentration. Urinary creatinine concentrations were determined and used to calculate human secretory protein epidydimis 4 and creatinine ratio in urine.

Statistical processing

To characterize the study groups descriptive statistics was applied. Compliance with the normal (Gaussian) distribution of the biomarker concentrations in the study groups were tested with the Shapiro-Wilk test. Data normalization was performed using the natural logarithm function in cases where the concentrations of biomarkers did not meet the normal distribution. Differences of mean biomarker concentrations in the study groups were determined using t-tests and 95% confidence interval (CI). Diagnostic accuracy of biomarkers was compared using area under the curve (AUC). Statistical and graphical data processing was performed using the professional statistical software SPSS 20.00 (SPSS Inc., USA).To evaluate sensitivity and specificity of urinary HE4 comparison to serum HE4 and Ca125 diagnostic accuracy was performed. Separately ovarian cancer diagnostic accuracy was calculated for HE4 concentration and glomerular filtration rate ratio and also to HE4 and urinary creatinine ratio. Glomerular filtration rate was calculated by the Cockcroft-Gault formula: GFR=[(140-Age)xWeight(kg) xConstant (1,04)]/[Serum creatinine(μmol/l)]

Results

Patients with ovarian cancer, especially, of late stage had the highest mean urinary concentrations of human epidydimis secretory protein 4 (Table 1.)

Higher urinary HE4 concentrations were observed for patients with late stage ovarian cancer and also for patients with early stage ovarian cancer when compared to patients with benign ovarian tumors (Table 2.).

Patients with malignant ovarian tumors had higher mean concentrations of urinary and also serum HE4 than the rest of the study group patients (Figure 1).

Glomerular filtration rate was similar (p=0.096) although patients with benign and particularly with malignant ovarian tumors had a tendency to lower filtration rate (77.06±28.07 mL/min and 64.82±12.95 mL/

Table 1. Mean Urinary Concentrations of Human Epidydimis Secretory Protein 4 in Study Groups

Study groups	Log transformed mean urinary HE4 concentrations (pmol/l±SD)
Late stage ovarian cancers (III/IV; n=12)	11.276 ± 1.145
Early stage ovarian cancers (I/II; n=11)	9.734 ± 1.053
Patients with benign ovarian tumors (n=37)	9.105 ± 0.970
Control group (n=18)	8.640 ± 0.732

Table 2. Mean Urinary Concentrations of Human Epidydimis Secretory Protein 4 in Study Groups

Study groups	*p (t-test)*
Late stage ovarian cancers (III/IV) vs. early stage ovarian cancers (I/II)	0.001
Early stage ovarian cancers (I/II) vs. benign ovarian tumor	0.044
Benign ovarian tumors vs. control	0.093

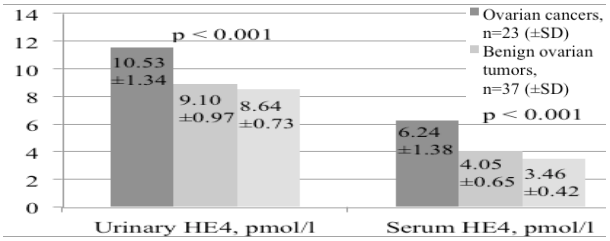

Figure 1. Mean Urinary and Serum HE4 Concentrations in the Study Groups.

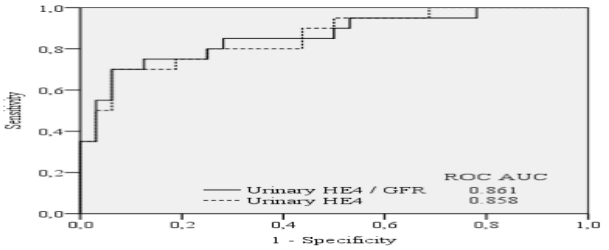

Figure 2. Glomerular Filtration Rate in the Ovarian Cancer Diagnostic Accuracy of Urinary He4 (All Ovarian Cancer Patients Included in the Analysis Irrespective of Stage).

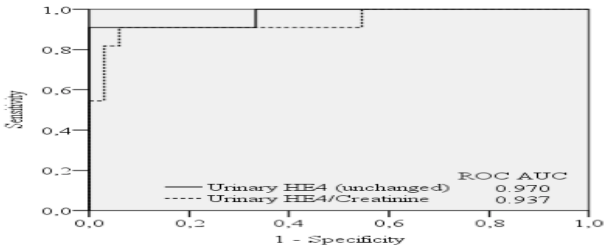

Figure 3. Comparison of Diagnostic Accuracy for Urinary He4 and Urinary He4/Creatinine Concentration in Discrimination of Ovarian Cancer Patients.

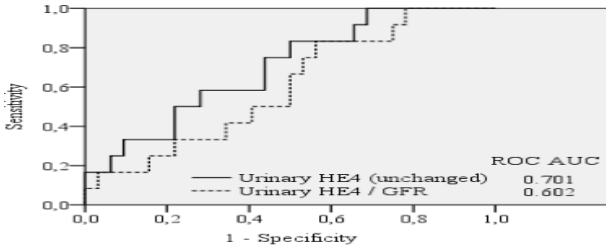

Figure 4. Accuracy of Urinary He4 Concentration and Urinary He4/Glomerular Filtration Rate Ratio in Early Stage Ovarian Cancer (Stage I/Ii) Diagnostic.

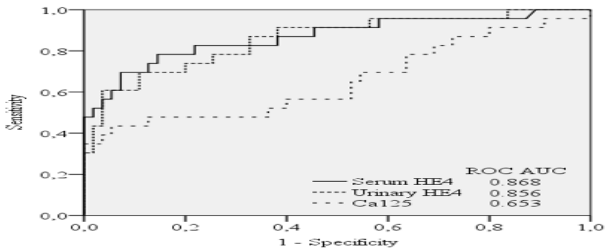

Figure 5. Ovarian Cancer Diagnostic Accuracy of Serum and Urinary He 4 in Comparsion to Ca125.

min, respectively) in comparison to women in the control group (81.86±20.60 mL/min).

Similarly, there was no difference in the mean urinary creatinine concentrations in (p=0.143), although there was tendency for higher urinary creatinine concentrations in ovarian cancer patients (9.57±0.82 pmol/L) when compared to patients with benign ovarian tumors (8.94±0.94 pmol/L) and women in the control group (9.08±0.77 pmol/L).

Higher ovarian cancer diagnostic accuracy was observed when there was calculated ratio between urinary human epididymis secretory protein 4 concentration and glomerular filtration rate (Figure 2).

Ovarian tumor discriminatory accuracy for unchanged concentrations of human epididymis secretory protein 4 and HE4 and urinary creatinine ratio was calculated only in 44 patients (11 ovarian cancer patients and 33 patients with benign ovarian tumors), therefore values for area under the curve are higher than in other ROC curves. Unchanged concentrations of urinary human epididymis secretory protein 4 revealed higher accuracy in discrimination of ovarian cancer patients in comparison to urinary HE4 and urinary creatinine ratio (Figure 3).

Higher diagnostic accuracy of early stage ovarian cancer (stage I/II) was observed for urinary HE4 concentration when compared to ratio of urinary HE4/ glomerular filtration rate (Figure 4).

Serum concentrations of human epididymis secretory protein 4 disclosed higher diagnostic accuracy when compared to urinary concentration of HE4, but both of them were higher when compared to discriminatory accuracy of CA125 (Figure 5). At fixed specificity of 75% unchanged urinary human epididymis secretory protein 4 had sensitivity of 78.3% (at cutoff level of 13,000 pmol/l), but sensitivity of CA125 was only 47.8%.

Discussion

Hellström et al. have described higher urinary concentrations of HE4 in patients with early and late stage ovarian cancers (Hellstrom et al., 2010). Higher mean urinary HE4 concentrations in ovarian cancer patients were found also in our study population.

It should be noted that urinary concentrations of human epididymis secretory protein 4 concentrations may be affected by renal function, because glomerular filtration rate decreases with age (Davies et al., 1950). For that reason, older women with reduced glomerular filtration rate are expected to have lower HE4 concentration in urine, compared with patients whose glomerular filtration rate is normal. In our study, ratio of urinary HE4 concentration and glomerular filtration rate revealed higher diagnostic accuracy when compared to unchanged urinary levels of HE4 - area under the curves were 86.1% and 85.8%, respectively. When HE4/GFR was applied to discriminate patients with early stage ovarian cancer, unchanged HE4 was more accurate and discriminatory power more pronounced – AUC for HE4/GFR 0.602 and 0.701 for unchanged concentrations of HE4. In the study of Hellström et al. ratio of urinary HE4 and urinary creatinine concentration was measured. They found that HE4/creatinine ratio had higher diagnostic accuracy in comparison to unchanged urinary HE4 concentration (Hellstrom et al., 2010). Findings of Hellstrom et al. study are conflicting to the data found in our study. Unchanged concentrations of urinary HE4 revealed higher accuracy

when compared to HE4/creatinine ratio – AUC 0.970 vs. 0.937. This can be explained by the model applied in the study of Hellstrom et al. – high accuracy was observed when a HE4/creatinine Combo model was applied. The particular HE4/creatinine Combo model has been calculated in the logistic regression analysis considering absolute concentration of HE4 and also HE4/creatinine ratio (Hellstrom et al., 2010). In the identification of early stage ovarian cancer Hellström et al. had higher diagnostic accuracy when compared to our study results - 70.1% vs. 96.9%. Diagnostic accuracy of urinary HE4 in the identification of early stage ovarian cancer patients discovered by Hellstrom et al. is also higher in comparison to previously described HE4 diagnostic characteristics when analysed in serum (Hellstrom et al., 2010; Partheen et al., 2011). This can be explained with the fact that HE4/creatinine Combo model has been analyzed in the validation setting consisting of only 32 ovarian cancer patients with advanced stage (stage III/IV) ovarian cancer (Hellstrom et al., 2010). Also higher diagnostic accuracy of early stage ovarian cancer comparatively to late stage ovarian cancer can be explained by using coefficients derived from logistic regression in the analysis of early stage ovarian cancer patients and healthy controls.

When the accuracy of urinary HE4 was analyzed in the context of serum HE4 and CA125, higher diagnostic accuracy was observed for HE4 analyzed in sera, but the difference was not statistically significant, and that gives promising options for noninvasive detection of biomarker levels in the future.

It should be noted that the diagnostic potential of urinary human epidydimis secretory protein 4 has been analyzed only in some studies; therefore further trials with larger sample size are necessary to reveal clear conclusions.

In conclusions, Ovarian cancer patients have higher urinary concentrations of human epidydimis secretory protein 4 than patients with benign ovarian tumors. Urinary HE4 has comparable discriminatory accuracy with serum HE4 of benign and malignant ovarian tumors and can be recommended as non-invasive ovarian cancer risk assessment method.

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