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Serum human epididymis protein 4 can be a useful tumor marker in the differential diagnosis of adnexal masses during pregnancy: a pilot study

F. Gucer[1], G. Kiran[1], E. Canaz[2], M. Kilinc[3], H.C. Ekerbicer[4], F. Avci[1], H. Kiran[1], A. Coskun[1], D.C. Arikan[1]

[1] Kahramanmaras Sutcu Imam University School of Medicine Department of Obstetrics and Gynecology, Kahramanmaras
[2] KSU School of Medicine Department of Obstetrics and Gynecology Division of Gynecologic Oncology, Kahramanmaras
[3] Kahramanmaras Sutcu Imam University School of Medicine Department of Biochemistry, Kahramanmaras
[4] Sakarya University School of Medicine Department of Public Health, Sakarya (Turkey)

Summary

Purpose: The purpose of this study was to evaluate serum concentrations of human epididymis protein 4 (HE4) and cancer antigen 125 (CA 125) in healthy women and their pregnant counterparts to determine the influence of pregnancy on these biomarkers. *Materials and Methods:* Serum concentrations of CA 125 and HE4 were measured in 27 healthy non-pregnant women and 26 healthy pregnant women in the first and second trimesters. *Results:* Higher concentration of CA 125 was found in pregnants than in non-pregnant women ($p = 0.002$). There was no difference in CA 125 concentrations between first and second trimesters ($p = 0.13$). Serum HE4 concentration was not different in pregnant group compared to non-pregnant women ($p = 0.510$). Likewise, no difference was found in HE4 levels between the trimesters ($p = 0.485$). There was a positive correlation between increasing parity and CA 125 ($p = 0.023$), but not HE4 ($p = 1.0$). *Conclusion:* HE4 serum biomarker is unaffected by pregnancy status and may be useful for evaluation of doubtful pelvic masses in pregnancy. Contrarily, increased serum levels of CA 125 could yield increased number of false-positive results.

Key words: Cancer antigen 125; Human epididymis protein 4; Tumor markers in pregnancy.

Introduction

Advances in the use of ultrasound for assessing pregnancy have led to an increase in the detection of adnexal masses in gravid women [1]. As the adnexal masses are detected at asymptomatic stage, the outcomes of these lesions have improved. Predictably, symptomatic or persistent masses into late gestation are usually associated with a higher rate of complications (torsion 1-22%, rupture 0-9%, obstruction of labor 2-17%) [2-4], and malignancy [3, 5] than those observed asymptomatically in early gestation [6, 7]. Management strategies for ovarian masses in pregnancy have not been well defined. Ultrasound assessment of masses can help to determine the risk of malignancy and guide the surgical management. In doubtful pelvic masses, it is necessary to utilize an approved biomarker for better characterization of the masses, but alterations in the levels of tumor biomarkers in pregnancy can render them useless in this period. Cancer antigen 125 (CA 125) was proposed as a serum biomarker for ovarian cancer in 1983 and approved for routine management of this disease. However, a major problem with CA 125 is its low diagnostic specificity. High concentrations found in benign gynecological conditions, particularly in premenopausal patients such as ovarian cysts, myomas, endometriosis, and non-gynecological conditions including effusions, liver or renal disease and also malignant diseases [8, 9]. Likewise, concentration of CA 125 alters during menstrual phase and pregnancy [10, 11]. Human epididymis protein 4 (HE4) has been proposed as a novel tumor marker to increase the diagnostic specificity of early stage ovarian cancer. United States Food and Drug Agency (FDA) approved the clinical use of HE4 in the monitoring of epithelial ovarian cancer in 2009. The aim of this study was to evaluate serum concentrations of HE4 and CA 125 in healthy patients and in their pregnant counterparts to determine the influence of pregnancy on the concentrations of these serum biomarkers and the reliability of HE4 as a tumor marker in pregnant population.

Materials and Methods

The authors enrolled 30 healthy pregnant women who admitted for antenatal follow up at the present department as a study group and 30 age-matched healthy non-pregnant women as a control group. Written informed consent was obtained from all participants and institutional ethics committee approved the study. All participants were subjected to an ultrasonographic examination to rule out the presence of any adnexal masses. Serum samples were obtained twice from the pregnant group: once at admission in the first

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Table 1. — *Characteristics of the samples.*

		Control group (n=27)	Study group (n=26)	p
Age	Median (min-max)	28.0 (18.0–37.0)	26.5 (18.0–39.0)	0.873
Parity	Median (min-max)	1.0 (0.0– 4.0)	1.5 (0.0–5.0)	0.011

Table 2. — *Serum concentrations of CA 125 in the control and the study group (U/ml).*

	Control group (n=27)	Study group (n=26) 1. trimester	2. trimester	p*
Median	10.3	16.6	16.3	0.002
Minimum	5.5	5.5	5.8	
Maximum	80.3	38.0	45.9	

* Serum concentration of CA 125 was significantly higher in the pregnant women than in the controls, while the levels were not statistically different between the first and second trimesters (p = 0.13).

Table 3. — *Serum concentrations of HE4 in the control and the study group (pmol/L).*

	Control group (n=27)	Study group (n=26) 1. trimester	2. trimester	p*
Median	53.3	59,3	56.3	0.510
Minimum	30.0	14.8	35.8	
Maximum	69.5	109.5	81.8	

* In pregnant group, serum concentration of HE4 was not statistically different form the control group. Likewise, there was no statistically significant difference in the concentration of HE4 between the first and second trimesters (p = 0.485).

trimester and again at the second trimester follow-up. Samples were obtained once from the control group when they attended the gynecology department for an annual check-up. Exclusion criteria were multifetal pregnancy and the existence of any systemic, gynecological, or non-gynecological disease that could elevate the serum CA 125 concentrations. Two women in the pregnant study group were excluded due to miscarriage, and two were excluded because they were lost to follow up in the second trimester. Three participants in the control group were excluded due to hemolyzed blood samples. The final study comprised 26 healthy pregnant women in the study group and 27 healthy nonpregnant women in the control group.

The blood samples were obtained by venous puncture in the present hospital, centrifuged, and stored at -80°C until assayed. The serum levels of CA 125 and HE4 were determined with a chemiluminescent enzyme immunoassay and an ELISA immunoassay, respectively. This solid-phase noncompetitive immunoassay was based on a direct sandwich technique using two mouse monoclonal antibodies (2H5 and 3D8) directed against two epitopes in the C-WFDC domain of HE4.

Data are reported herein as the median-min-max. All statistical analyses were performed using non-parametric tests (Wilcoxon's signed rank test, Mann–Whitney *U*-test). Correlations were evaluated with Spearman's rank correlation coefficients. Analyses were performed using SPSS software, version 9.0 for Windows (SPSS). The level of statistical significance was set at $p < 0.05$.

Results

This study included 79 serum samples from a study group of 26 healthy pregnant women with a median age of 28 years (18–37) and a control group of 27 healthy nonpregnant women with a median age of 26.5 years (18–39) (p = 0.873). The median parity in the study and the control group was 1.0 and 1.5, respectively (p = 0.011) (Table 1).

The results of the CA 125 measurements in the study and control groups are shown in Table 2. Higher serum concentration of CA 125 was found in the pregnant women than healthy nonpregnant women (p = 0.002). There was no statistically significant difference in the serum concentrations of CA 125 between the first and the second trimester of pregnancy (p = 0.13).

The results of the HE4 measurements in the study and control groups are shown in Table 3. No statistical differ-

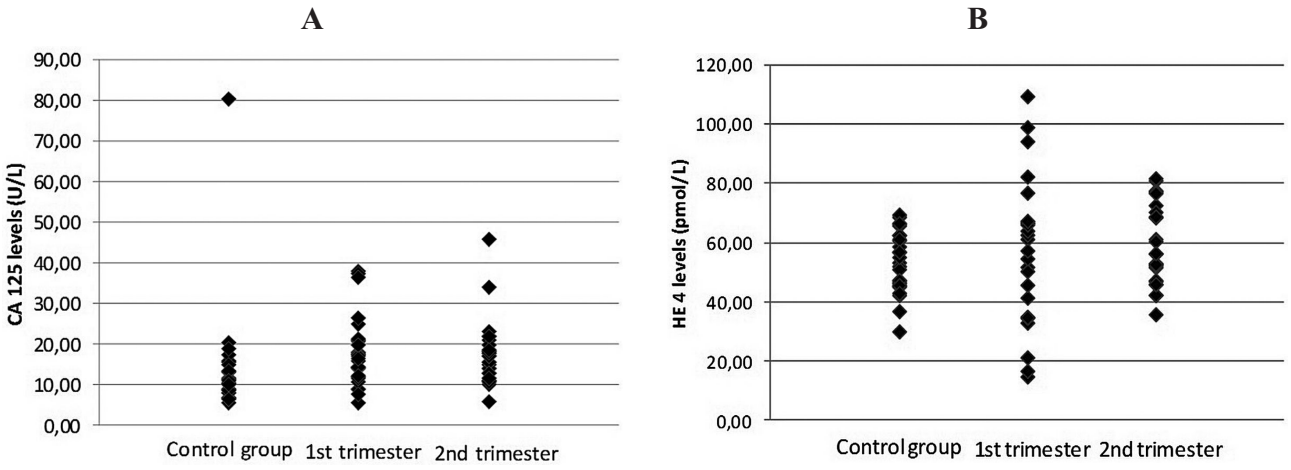

Figure 1. — Scatter plot of the serum CA125 (A) and HE4 (B) levels for healthy controls and pregnant women according to trimester.

ence was observed in sera HE4 levels between pregnant and non-pregnant groups ($p = 0.510$). Likewise, there was no statistically significant difference in HE4 levels between the first and second trimester of pregnancy ($p = 0.485$). Figure 1 displays a scatterplot of the serum CA 125 and HE4 levels for all subjects.

The serum concentrations of CA 125 and HE4 were compared with parity in all groups. A statistically significant elevation in the CA 125 concentration was found with increasing parity ($R = 0.31$, $p = 0.023$), but there was no relationship between the levels of HE4 and parity ($R = 0.00$ $p = 1.0$).

Discussion

The management strategy of ovarian masses in pregnancy is an unresolved issue among obstetricians. Although some propose elective removal in the second trimester, others argue that a conservative approach results in spontaneous resolution of most masses, which might provoke unnecessary surgery. Tumor markers, such as CA 125, have a restricted role in the discrimination of benign versus malignant lesions due to increased levels in pregnant sera [6, 11]. This rise in the levels of CA 125 begins 30–40 days after the last menstrual period, peaks between 35–60 days, and starts to decrease by the end of the first trimester [12]. The present results confirmed the elevation in serum CA 125 concentrations in pregnancy, but the authors found no difference between the first and second trimester. Another limitation of using CA 125 as a biomarker in the discrimination of adnexal masses in pregnancy is that up to 20% of ovarian cancers and almost 50% of early-stage disease do not express this antigen [8, 13, 14]. Surgical findings support that, the majority of ovarian cancers during pregnancy are diagnosed as Stage 1, with the disease confined to the ovaries [7, 15]. Therefore, it is necessary to combine CA 125 with novel markers that can provide better diagnostic efficiency.

Schummer *et al.* established that the HE4 gene, also known as WDFC2, is primarily overexpressed in patients with ovarian carcinomas [16]. This finding was later confirmed by gene-expression profiling studies [17, 18]. Furthermore, HE4 has a relatively subtype-specific expression pattern primarily restricted to the serous and endometrioid subclasses of epithelial ovarian carcinomas [19, 20]. Nonetheless, high or moderate HE4 expression can also be detected in adenocarcinomas of the lung, breast, transitional cell, endometrial and pancreatic carcinomas, but ovarian serous carcinomas have the highest expression [21]. Hellstrom *et al.* concluded that HE4 was less frequently positive in benign gynecological disease and may be more beneficial than CA125 [22]. Further studies showed that HE4 had the highest sensitivity (83%) as a single marker for ovarian cancer detection in patients with pelvic masses, particularly in those with early-stage disease [23]. Most serum HE4 studies have suggested that the sensitivity and the specificity of HE4 in gynecological diseases are better than those of CA 125 and that both tumor markers are complementary [9, 24]. Moore *et al.* proposed that the concentration of HE4 is lower in pregnant women compared with their premenopausal counterparts and that this was attributed to increased renal clearance in pregnancy [25]. They also reported that levels of HE4 did not change related with different trimesters of pregnancy. Consistent with their reports, a comparison of HE4 in the first and second trimesters revealed no statistical difference in the current study. This study also showed no significant difference in the serum concentration of HE4 in pregnancy, but elevated levels of CA125 when compared with nonpregnant controls. According to the literature, the elevation in CA125 in pregnancy occurs predominantly during the first trimester, probably because of its role in early fetal development [26, 27]. However, several studies also reported that this elevation persists throughout pregnancy [11, 28].

The present results revealed a positive correlation between increasing parity and CA 125 ($R = 0.31$, $p = 0.023$) but not HE4. There are several studies indicating elevated, decreased, or unaltered concentrations of CA 125 with increasing parity [29, 30]. One limitation of the present study was that the results are based on a small number of study subjects and controls. Large multicentric trials should be advocated to confirm these results. The findings of the current study suggest that HE4 is a credible marker, which does not fluctuate in pregnancy, and that it may be useful for the evaluation of ovarian cysts and doubtful pelvic masses in pregnancy. Contrarily, increased CA125 serum concentrations could yield an increased number of false-positive results.

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Address reprint requests to:
E. CANAZ, M.D.
Kahramanmaras Sutcu Imam Universitesi Tip Fakultesi
Kadin Hastaliklari ve Dogum Anabilim Dali
Avşar Kampüsü, 46000
Kahramanmaras (Turkey)
e-mail: emelcanaz@gmail.com