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HE4 values in individual trimesters of physiological pregnancy

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AUTHORS' CONTRIBUTION: **(A)** Study Design · **(B)** Data Collection · **(C)** Statistical Analysis · **(D)** Data Interpretation · **(E)** Manuscript Preparation · **(F)** Literature Search · **(G)** Funds Collection

SUMMARY

Introduction. HE4 is a protein currently used as a tumor marker for ovarian carcinoma with markedly higher specificity than CA125. To date, normal values of this protein have not been determined for pregnant women. The aim of the study was to evaluate HE4 values in individual trimesters of pregnancy and compare them with levels found in non-pregnant healthy women.
Material and methods. The study included 59 women in individual trimesters of physiological pregnancy and 20 non-pregnant healthy controls. Blood for evaluation was taken during a routine clinical examination. Assays were made using the chemiluminescence phenomenon in the Architect system by Abbott (USA). The results were presented using basic descriptive statistics.
Results. The mean age of pregnant women in physiological pregnancy was similar to the age of controls. The average HE4 concentration in controls (non-pregnant healthy women) was lower in a statistically significant way than in the whole group of pregnant women and in individual trimesters. HE4 values in the third trimester were higher in a statistically significant way than in the first and second trimesters.
Conclusions. HE4 levels increase with the development of pregnancy and are generally higher than in non-pregnant healthy women. An increase in HE4 values in pregnant women should be taken into consideration while diagnosing adnexal tumors during pregnancy. HE4 may be a factor influencing prediction of the severity of diseases during pregnancy. The role of this protein requires further investigation.
Key words: HE4; pregnancy; tumor marker

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INTRODUCTION

HE4 protein (*human epididymis protein 4*) is a small, soluble glycoprotein that takes part in physiological and pathological processes in the human organism. It was first identified in 1991 by Kirchhoff et al. [1] in epithelial cells of the distal epididymis. Initially, it was thought to be merely an inhibitor of proteases engaged in spermatogenesis [2–4]. HE4 is produced by healthy tissues of the trachea, salivary glands, lungs, thyroid, kidneys, pituitary, colon, glandular tissue of the female genital epithelium, breast, epididymis, vas deferens and prostate [5–8]. It is expressed in numerous pathological cell lines, e.g. neoplastic cell lines of the endometrium, lungs, colon, breast, kidneys, stomach, pancreas and in primary hepatic carcinoma. Authors concur that the greatest expression is observed in epithelial ovarian carcinoma. HE4 is thought to be a good tumor marker for ovarian carcinoma with markedly higher specificity than CA125 and higher sensitivity for differentiating malignant from benign tumors [7,9,10–13]. Its superiority over CA125 also results from the fact that it presents fewer age-related differences [14,15]. Not only is this marker more sensitive in detecting ovarian carcinoma than CA125 [16], but also indicates disease recurrence. During a 20-month observation, its elevated values indicating a relapse occurred earlier than increased CA125 levels [6,17]. Moreover, HE4 concentration can be a predictor of patient survival. Survival in advanced ovarian carcinoma with markedly elevated HE4 levels was shorter than in patients with normal values of this protein [18]. To date, normal HE4 values have not been determined for pregnant women without malignant and/or benign gynecologic disorders. Moreover, the available literature does not contain reports about a possible role of this protein in etiopathogenesis, prediction and prognosis of gestational pathology.

The aim of the study was to evaluate HE4 values in pregnant women in individual trimesters and compare them with non-pregnant healthy controls.

MATERIAL AND METHODS

The study was conducted from January 1 to March 31 2015 in the Department and Clinical Unit of Gynecology, Obstetrics and Gynecologic Oncology in Bytom, Silesian University of Medicine. In total, 59 women in different trimesters of physiological pregnancy were enrolled (20 women in the first trimester, 19 in the second trimester and 20 in the third trimester). The control group consisted of 20 age-matching non-pregnant healthy women. The participants were recruited from among patients of outpatient clinics. Blood for evaluation was taken during a routine clinical examination. Each time, blood (approximately 5 ml) was taken from the cubital fossa in the morning with the fasting patient. All controls had blood drawn in identical conditions. After clot formation, the blood was centrifuged and the obtained serum was stored in -70°C until testing. All women were informed about the purpose of the study and expressed consent to participate in writing. The study was approved by the Ethics Committee of the Medical University of Silesia.

Tests were conducted using the chemiluminescence phenomenon in the Architect system by Abbott (USA). This was done in the Department of Clinical Immunodiagnosis and Tumor Marker Analysis of No 5 St Barbara Regional Specialist Hospital in Sosnowiec.

The results were presented using basic descriptive statistics. The agreement with normal distribution was checked with the Shapiro–Wilk test. Differences between groups were compared using non-parametric Kolmogorov–Smirnov and Mann-Whitney U tests whilst in-group variability was tested with the Kruskal–Wallis ANOVA test. The significance level was $p<0.05$. The calculations were made in the STATISTICA program.

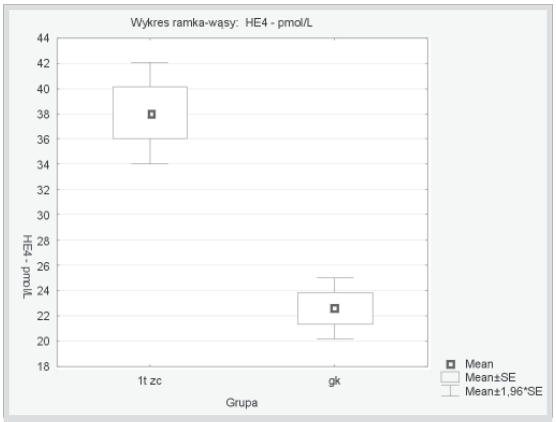

Fig. 1. Average HE4 values [pmol/l] in women in trimester I and controls (p=0.0000001)

RESULTS

The mean age of pregnant women in physiological pregnancy (29.5 ± 6.5 years) was similar to the age of controls (27.9 ± 7.3 years). Table 1 presents average HE4 values for individual trimesters of pregnancy and in controls. The average HE4 concentration in controls (non-pregnant healthy women) was 22.5 ± 11.8 pmol/l and was lower in a statistically significant way than in the whole group of pregnant women and in individual trimesters (Tab. 1). The HE4 level in the third trimester was 48.9 ± 12.3 pmol/l and was higher in

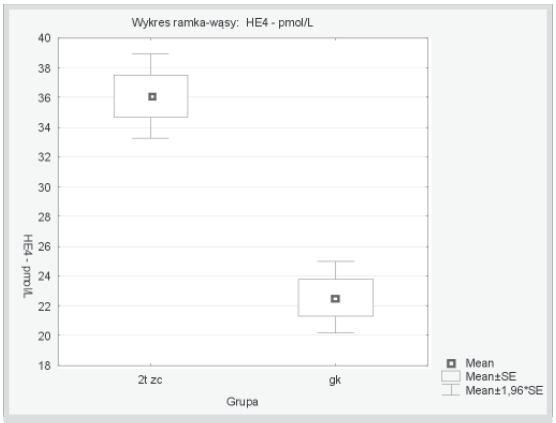

Fig. 2. Average HE4 values [pmol/l] in women in trimester II and controls (p=0.0000001)

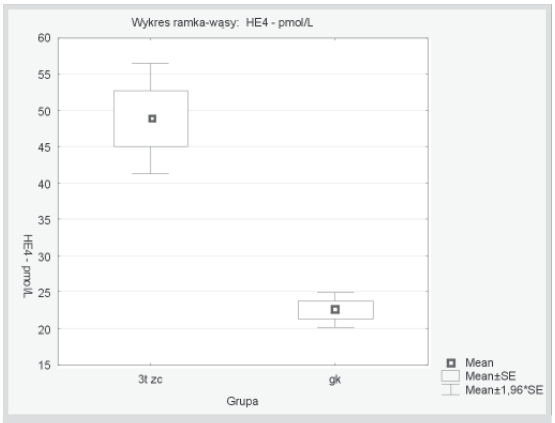

Fig. 3. Average HE4 values [pmol/l] in women in trimester III and controls (p=0.0000001)

a statistically significant way than in the first (38.0 ± 9.2 pmol/l) and second trimesters (36.1 ± 6.1 pmol/l). There were no differences in HE4 levels between healthy women in the first and second trimesters of pregnancy. The relationships are presented in figures 1–5.

DISCUSSION

HE4 has been shown to be a useful marker to detect and monitor ovarian carcinoma. In particular, its combination with CA125 is currently thought to be the gold standard in biochemical diagnosis of this cancer. It also improves the detectability of various histological types and can be used at all stages of the disease [9,10]. To date, normal values of this protein have not been determined in pregnant women without malignant and/or benign gynecologic disorders. Moore et al. [19] investigated HE4 values in individual trimesters of pregnancy and did not note any statistically significant differences in mean concentrations depending on the trimester and between trimesters. However, the difference between the second and third trimesters was nearing statistical significance (p=0.059) – HE4 values were found to increase between the second and third trimesters. This trend is in agreement with our study where a marked increase in HE4 levels was seen in the third trimester compared with the second trimester. The same authors also noted that HE4 values were considerably lower in women at all stages of pregnancy compared with non-pregnant premenopausal women. The upper limit of normal HE4 value in pregnant women was established by Moore et al. [19] at 49.6 pmol/l for trimester 1, 35.1 pmol/l for trimester 2 and 50.2 pmol/l for trimester 3. The average concentration in all pregnant women irrespective of the stage of pregnancy was 49.7 pmol/l. Similar findings were published by Li et al. [20] who found that serum HE4 levels in pregnant women were statistically lower than in healthy non-pregnant women (43.0 vs 48.9 pmol/l, respectively; p=0.007). However, these authors did not investigate HE4 levels in individual trimesters [19]. Different conclusions were drawn by Park et al. [14] after a study in 72 healthy pregnant women. The average HE4 concentration was 22.8 pmol/l (range 20.7–25.6 pmol/l) and it was higher in a statistically significant way than in healthy non-pregnant women (21.2; range 18.7–24.3 pmol/l; p=0.0098). These authors did not investigate HE4 levels in individual trimesters either. The results obtained by Park et al. [14] are in line with our findings. We have shown that the average HE4 concentration is markedly higher in pregnancy and in its individual trimesters compared with levels found in non-pregnant women (Tab. 1).

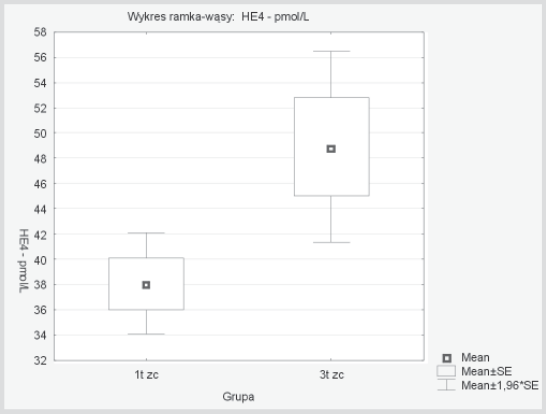

Fig. 4. Average HE4 values [pmol/l] in women in trimester I and trimester III of physiological pregnancy (p=0.01)

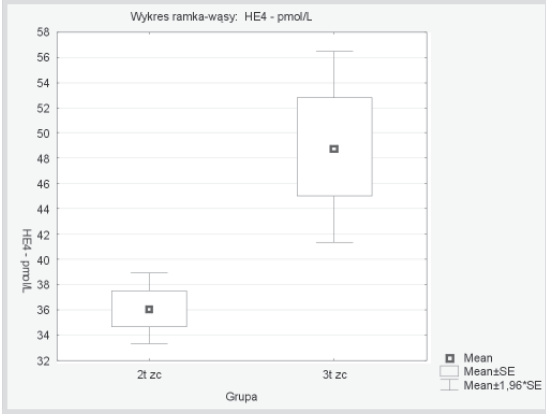

Fig. 5. Average HE4 values [pmol/l] in women in trimester II and trimester III of physiological pregnancy (p=0.001)

Tab. 1. Average HE4 values in pregnant women in individual trimesters and in controls

Mean ± SD [pmol/l]	All pregnant women	Trimester I (n=20)	Trimester II (n=19)	Trimester III (n=20)	Controls (n=20)
HE4	39,6 ± 10,0[a)]	38,0 ± 9,2[a)]	36,1 ± 6,1[a)]	48,9 ± 12,3[a) b) c)]	22,5 ± 11,8

[a)] p<0.001 compared with controls; [b)] p=0.01 compared with trimester I; [c)] p<0.001 compared with trimester II

As HE4, certain other tumor markers also increase during pregnancy (CA19-9, CA15-3, carcinoembryonic antigen, squamous cell carcinoma antigen and mucin-like cancer-associated antigen) [21, 22]. CA125 levels rise, particularly in the first trimester, perhaps because it plays a role in early fetal development and due to increased renal clearance in pregnancy [14,19, 22,23]. During pregnancy, CA125 is found in relatively high levels in decidual cells, amniotic fluid and amniotic cells. Significantly lower levels are found in umbilical blood. This suggests that the decidua and amniotic cells, rather than the fetus, produce and secrete this glycoprotein into the amniotic fluid [22,24]. By contrast with CA125, other tumor markers tended to decrease their concentrations during pregnancy (inhibin B, lactate dehydrogenase LDH) whereas anti-Müllerian hormone values were comparable to those found in non-pregnant women in the follicular phase and tended to decrease as pregnancy progressed [22].

Being familiar with normal levels of tumor markers during pregnancy is of paramount importance. Since the 1960s, the incidence of female cancers has been increasing continuously [25]. The most common cancers are breast cancer, cervical cancer and blood neoplasms [26]. Pregnancy after oncological treatment is becoming more and more common mainly due to the development of fertility-sparing treatment methods and improving prognosis in patients with malignancies [22]. A decrease in HE4 suggested by Moore et al. [19] makes this protein a reliable ovarian carcinoma marker that can be used during pregnancy in the diagnosis of pelvic cysts and tumors. CA125 can give false positive results in these women. Literature reports state that CA125 levels are higher in one fourth of pregnant women [14,27]. HE4 values were obtained by this author [18] using HE4 EIA tests (*Fujirebio Diagnostics Inc*). However, the author himself [19] and others [11] admit that currently the only reliable tools to measure HE4 levels are the Roche test and Abbott Diagnostics. Results obtained using these tests are perhaps more reliable and should constitute a reference. In our own assays, as in the study by Park et al. [14], who also noted an HE4 increase during pregnancy, Abbott tests (USA) were applied.

There are single publications about the role of selected tumor markers in detecting certain gestational pathologies. Fiegler et al. [28] analyzed 200 women with signs of threatened abortion between weeks 5 and 12 of pregnancy and noted an increase in CA125 values. In other studies, He et al. [29] found increased LDH values in pregnant women with symptoms of pre-eclampsia and HELLP syndrome. However, there are no reports about the role of HE4 in gestational pathologies. According to the current state of knowledge, the role of HE4 has not been fully explored. Our preliminary results, even if they are difficult to compare due to the lack of similar published studies, are interesting and suggest a potential role of this protein as a factor that can affect prediction of the severity of diseases during pregnancy.

CONCLUSIONS

HE4 levels increase with the development of pregnancy and are higher than in non-pregnant healthy women. An increase in HE4 values in pregnant women should be taken into consideration while diagnosing adnexal tumors during pregnancy. HE4 may be a factor influencing prediction of the severity of obstetric conditions. The role of this protein during pregnancy needs further investigation.

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