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4 (50) 2018: 009-012 • REVIEW ARTICLE

Usefulness of serum HE4 assay in pregnant woman. A literature review

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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

The HE4 protein, human epididymis protein 4, is a new protein and one of the most promising markers to improve diagnostic efficiency in the detection of ovarian malignancies, particularly epithelial carcinoma, which accounts for 90% of all ovarian cancers in the population of pregnant women. Pregnant women are the population for which there are few reports on the concentration of the HE4 protein in the available literature. This knowledge is extremely important, because pregnancy coexists with an ovarian tumor in 1–2% of women. The paper presents an overview of available literature describing HE4 concentration changes in pregnant women. There is a certain regularity in this study, namely that HE4 concentrations are significantly higher in the third trimester of pregnancy than during the first two trimesters. HE4 concentrations have also been examined in the aspect of *in vitro* fertilization, where there was no change in its serum concentration at any of the stages of the procedure, and in the aspect of preterm delivery, where the effectiveness of this protein as a possible marker of this pathology has been confirmed. In conclusion, it should be noted that the HE4 protein in the population of pregnant women is of the greatest importance in women with a coexisting ovarian tumor and as a marker of preterm delivery, which has so far been confirmed only in women after *in vitro* fertilization. At the same time, the problem of the HE4 marker in the population of pregnant women requires further research on larger groups of women.

Key words: HE4; pregnancy

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Word count: 1638 **Tables:** 0 **Figures:** 0 **References:** 20

Received: 30.10.2018
Accepted: 06.11.2018
Published: 19.12.2018

INTRODUCTION

The HE4 protein, human epididymis protein 4, is a relatively new protein and one of the most promising markers to improve diagnostic efficiency in the detection of ovarian malignancies. The clinical usefulness of this protein concerns mainly epithelial carcinoma, which accounts for 90% of all ovarian cancers in pregnancy [1,2]. It is also invaluable in the prediction of ovarian cancer recurrences [3]. This protein is encoded by the *WFDC2* gene, located on the 20th chromosome [4]. It was initially identified as a secretory protein that plays a role in sperm maturation in men [1,4]. An analysis of HE4 and Ca-125 concentrations is used in the ROMA algorithm that serves for the differentiation of ovarian cancers [5]. Moreover, HE4 expression has been confirmed in the epithelia of the female respiratory organs, breasts, epididymis and vas deferens as well as in the respiratory epithelium, distal renal tubules, bowel and salivary glands [6]. Research has revealed that age, fertility, menopause, smoking, renal dysfunction and race may affect serum HE4 levels which may additionally increase in the course of certain gynecologic pathologies other than ovarian carcinoma [7].

HE4 IN PREGNANT WOMEN

Pregnant women are the population for which there are few reports on the concentration of the HE4 protein in the available literature. This knowledge, however, is extremely important, because pregnancy coexists with an ovarian tumor in 1–2% of women [8]. Although the histopathological picture is rarely malignant in pregnant patients, the risk of a malignant or borderline disease must always be considered. Moreover, surgically treated patients of childbearing age need a reliable marker to test for a potential relapse during pregnancy [8]. Moore et al. were the first to describe changes in serum HE4 levels in pregnant women [9]. They

have shown in their work that the concentration of this protein is lower in pregnant women compared with non-pregnant ones shortly before menopause ($p<0.001$), with mean levels amounting to 30.5 pmol/L and 46.6 pmol/L, respectively. However, due to a small number of samples from the studied group compared to the controls (the study was performed in only 67 pregnant women compared to a group 1,101 women), there were no correlations that would support other later studies displaying differences in the concentration of this glycoprotein depending on the trimester of pregnancy. Moore's study showed only that there are certain differences between the second and third trimester of pregnancy, but the level of statistical significance was exceeded ($p=0.059$). The authors could only show a trend rather than statistical significance. It was, however, clearly demonstrated that HE4 levels varied depending on age and menopausal status [9]. In 2017, Lu et al. [10] published the results of the study that investigated HE4 levels in a large group of 1,006 pregnant women where approximately 1/3 of the patients were in each trimester of physiological pregnancy. Mean HE4 concentrations measured in these groups were: 36.9 pmol/L, 39.8 pmol/L and 54.6 pmol/L, respectively for the three trimesters, with the upper referential limit reaching 50.3; 56.4 and 101.9 pmol/L, respectively [10]. There is a certain regularity in this research, namely that HE4 concentrations are significantly higher in the third trimester of pregnancy than during the first two trimesters. Moreover, approximately doubled reference intervals (RIs) for HE4 compared to those stated in Moore's work [9] suggest that other factors than age and pregnancy, e.g. also ethnic background, may affect its serum levels as Lu et al. conducted their study in the Asian population. However, the study did not reveal any significant RI increase for the ROMA index, particularly in the first and second trimesters of gestation, which suggests that it is useful for diagnosing cancerous lesions both in the general population and in pregnant women [10]. Our studies, conducted among pregnant women in various trimesters, have yielded similar results. We also argue that HE4 levels differ depending on the trimester, with the highest values noted in the third trimester. Our results clearly show that the general HE4 concentration in pregnant patients is higher in a statistically significant way than in non-pregnant ones [11].

Another research team that has studied HE4 concentrations in pregnant women were Park et al. [2]. They compared results obtained in 72 pregnant women to the results of 2,182 healthy non-pregnant ones and stated that, with the probability of $p=0.0098$, the HE4 level is higher among pregnant women and amounts to 22.8 pmol/L than in the general population where it was 21.2 pmol/L. Moreover, the authors argue that HE4 increases are more rarely false positive in pregnant women and are more specific for ovarian carcinoma than Ca-125 [2,12]. Tian et al. [1] are of a different opinion. They believe that pregnancy does not affect HE4 levels. Similar conclusions have been drawn by Gucer et al. [13] who argue that serum HE4 levels do not differ in pregnant women compared to non-pregnant ones ($p=0.510$) and that there are no differences in HE4 concentrations between different trimesters ($p=0.485$) [13].

The latest studies on HE4 levels in healthy pregnant women in each trimester of pregnancy and in women with threatened abortion between week 8 and 12 of pregnancy were conducted by Wang et al. [9]. They have observed no statistically significant difference in HE4 levels between non-pregnant women and women in the first and second trimesters of pregnancy. At the same time, HE4 expression was the highest in the third trimester of pregnancy. It was statistically significantly higher than in earlier trimesters or in non-pregnant women [9]. These conclusions are in line with our results [13]. Moreover, Wang et al. [9] found no differences in the concentration of the investigated protein between healthy women in the first trimester and women in the analogous gestational age with threatened abortion.

The subject matter of the clinical usefulness of HE4 assays in the population of pregnant women has also been discussed during several medical conferences. In 2016, Lisbon held the 16th Meeting of the International Society of Gynecologic Oncology. Two papers on HE4 in pregnant women were presented there. In the study conducted by Simsek et al. [14], blood was collected from 31 pregnant women three times, once in each trimester. It turned out that the concentration of HE4 in the first and second trimesters was significantly lower than in controls (40 age-matching healthy non-pregnant women). In the third trimester, however, there were no significant differences from the controls. Hallamaa et al. [15] compared HE4 concentrations in patients with tubal pregnancy,

spontaneous abortion and normal early pregnancy. They observed higher expression of this protein in tubal tissue than in healthy ovarian tissue. This entails higher levels of this protein in tubal and physiological pregnancies compared with spontaneous abortion [15]. During the 20th meeting of the European Society of Gynecologic Oncology in Vienna in 2017, Slovenian authors presented research on HE4 levels in each of the trimesters. Their observations are in line with the above quoted works; they state that, in physiological pregnancy, HE4 levels fall within referential ranges provided by the manufacturer of the reagents, but are higher in the third trimester, reaching 57 pmol/L, with a significance level of $p=0.001$ [16]. As early as in 2011 during the International Congress of Clinical Chemistry and Laboratory Medicine in Berlin, Hungarian authors presented a report proving that HE4 levels are higher in pregnant patients compared with non-pregnant controls. However, no differences were observed between individual trimesters. The authors also tested testosterone levels in these groups. While the concentration of testosterone was also higher in pregnant women, the authors found no correlations between this hormone and HE4 [17].

The literature also contains studies addressing HE4 levels in pregnant women after *in vitro* fertilization. In the study of Hallamaa et al. [18], serum samples for HE4 assay were collected from 20 patients several times during the entire *in vitro* procedure: ovarian stimulation, oocyte harvesting, embryo implantation and two weeks after an implantation attempt. It occurred that the levels of this protein did not vary substantially across the stages of the procedure. This means that the amount of circulating HE4 in women without an ovarian pathology is independent of stimulation with gonadotropin and that hormonal ovarian stimulation probably does not affect differential diagnosis of ovarian tumors using HE4 [18]. Kanninen et al. [19] on the other hand showed that HE4 may be a predictor of preterm delivery in women after *in vitro* fertilization. The authors investigated the levels of this protein on day 9 and 11 after embryo transfer, and subsequently analyzed the duration of pregnancy. Patients with preterm delivery had significantly lower HE4 levels ($p=0.001$), and the combination of HE4 and interleukin-13 assay was characterized by the greatest predictive value for preterm delivery. In cases of preterm delivery in women after *in vitro* procedures, the authors note decreased HE4 levels as a response to insufficient trophoblast invasion, which reduces the probability of maintaining proper mother–feus balance until term [19]. Moreover, Orfanelli et al. [20] retrospectively verified HE4 levels on day 24 and 28 after fertilization and analyzed the duration of pregnancy and the manner of its conclusion. The results confirmed the hypothesis that HE4 levels increase in physiological pregnancies on the first days of pregnancy and that the lack of this increase may be a predictor of miscarriage, preterm delivery or ectopic pregnancy. In non-physiological pregnancy groups, HE4 levels were significantly lower [20].

CONCLUSION

To conclude, it can be stated that pregnancy is a physiological condition that affects changes in cancer markers. HE4 expression assay may be found useful in pregnant women, especially when pregnancy coexists with an ovarian tumor. High hopes may be linked with the use of HE4 as a predictor of preterm delivery, which has been initially verified in pregnant patients, essentially those after *in vitro* fertilization. This, however, requires more extensive research on larger groups of patients and determination of reference limits for this marker in the population of pregnant women.

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