



Evaluation of Human Epididymis Protein 4 Tumor Marker Level in the First Half of Pregnancy

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Abstract:

This study aims to understand the related factors and their effects on the HE4 Tumor markers and assess their values during pregnancy. In this cross-sectional study, 90 pregnant women referred to receive routine care in the first half of gestation were included. Demographic information was collected. The levels of the HE4 tumor marker in the mothers' blood samples were checked, and the results were recorded. The average level of HE4 was 41.27 ± 14.13 picomoles per liter, which was in the normal range of non-pregnant women in all subjects (less than 140). HE4 levels were not correlated with age, gestational age, gravida, and body mass index (P .value < 0.05). The mean HE4 in the abortion group was higher than the non-abortion group, which showed a statistically significant difference ($P = 0.001$). According to the results of this study, pregnancy has no significant impact on this marker. Hence, it can be used as a tumor marker to evaluate adnexal masses during pregnancy.

Keywords: Tumor Marker, HE4, Pregnancy, Adnexal mass, Ovarian mass.

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Introduction:

According to ultrasonography, the prevalence of adnexal masses in the first trimester of pregnancy ranges from 0.1% to 10%. (1, 10). Approximately 2 to 6% of the tumors found by ultrasonography in pregnant women are malignant and necessitate surgery during the pregnancy (1). Despite significant advances in treating numerous malignancies, early detection remains the most critical aspect of the disease's treatment and elimination (9).

Ultrasound is the safest diagnostic procedure approved during pregnancy. However, in other situations, it does not provide enough reliable information regarding pathological findings to allow the physician to make an accurate diagnosis and make a definite medical decision (8). Therefore, there is a requirement to utilize other diagnostic methods during pregnancy to achieve higher diagnostic accuracy (5).

Assessment of serum levels of tumor markers in the patient's blood is one of the standard diagnostic tests to evaluate pelvic/adnexal masses (7). Cancer antigen 125 (CA125) is the most classic and widely utilized tumor marker for assessing adnexal/pelvic tumors suspected of being epithelial malignancies (7). In addition to CA125, the FDA approved the Human Epididymis Protein 4 (HE4) tumor marker in 2008 to monitor women with ovarian epithelial tumors for the same

Impact statement:

- **What is already known on this subject?**

The HE4 is a protein that is produced by most, but not all, epithelial ovarian cancer cells and is a valuable tumor marker to monitor women with epithelial ovarian cancers with the same indications as previously used for CA125.

- **What do the results of this study add?**

The average level of HE4 in pregnant women was 41.27 ± 14.13 picomoles per liter, which was in the normal range of non-pregnant women. HE4 levels were not correlated with age, gestational age, gravida, and body mass index. The mean HE4 in the abortion group was 13.65 and in the non-abortion group was 5.37 picomol / L, which showed a statistically significant difference. In conclusion, pregnancy has no significant impact on this marker, and it can be used as a tumor marker to evaluate adnexal masses during pregnancy.

- **What are the implications of these findings for clinical practice and further research?**

Pregnancy has no significant impact on this marker and can be used as a tumor marker to evaluate adnexal masses during pregnancy. For further studies, a cut-off must be set for this marker to use as a diagnostic tool in adnexal gatherings during pregnancy.



data. Demographic information, maternal age, and gestational age were recorded. Then, when requesting routine pregnancy tests for the mother, the level of the HE4 tumor marker in the mother's blood sample was checked, and the results were recorded. To increase the results' reliability and compare models, all models were sent to the same laboratory. The necessary coordination was made with the reference laboratory to utilize the same protocol for taking, maintaining, and analyzing the patient's blood sample. The used kit was manufactured by the American company Biocompare, and HE4 serum levels of less than 140 picomoles per liter were considered normal. Blood samples were taken from patients and poured into a clot sample test tube. The non-centrifuged selection could be stored outdoors for 1-2 hours. The centrifuged sample could be stored in the freezer for up to a week and sent through the cold chain.

Inclusion criteria

Pregnant women went to the perinatal clinic at Mahdih Hospital for routine pregnancy care in the first half of their pregnancy.

Exclusion criteria

- Pregnant women whose routine ultrasound showed any adnexal mass or other gynecological pathology such as uterine myoma.
- Pregnant women who have previously been diagnosed with endometriosis or adenomyosis.
- Pregnant women with a history of ovarian cysts or benign ovarian masses treated in the year before pregnancy
- Pregnant women with a history of cancer, including breast cancer, etc.
- Dissatisfaction with participating in the study

Ethical considerations

The ethical committee of Shahid Beheshti University of Medical Sciences registered this study. After a full explanation of the benefits of participating in the study, signed consent and informed permission were obtained from all participating patients. All patients' personal information was preserved as confidential in

indications as CA125 (3,7). The HE4 antigen is a tumor marker encoded by the WFDC2 gene that has been linked to ovarian and possibly other adenocarcinomas (3). This marker, which is easy to measure and available in most laboratory centers, can be an ideal method to evaluate pelvic masses because of its acceptable efficacy and safety for screening pregnant women (3).

The HE4 tumor marker is a relatively new tumor marker that has recently found an important place in diagnosing adnexal masses. Nonetheless, the varied growing and decreasing patterns of HE4 in adnexal and other cancers have yet to be entirely understood. Assessing tumor markers in the body is a non-invasive and safe diagnostic approach during pregnancy. Suppose it is determined that the serum level of this marker is not affected by physiological changes that occur during pregnancy and follows the same pattern of increase and decrease as in non-pregnant women. In that case, it can be used as a diagnostic method to detect possible adnexal malignancies in pregnant women. As a result, this study aims to assess HE4 levels and establish the prevalence of pathological HE4 levels (above 140 pmol/L) in pregnant women.

Material and methods:

This cross-sectional observational study was conducted in Mahdih Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran, in 2019 after obtaining the Ethical Committee's approval (IR.SBMU.MSP.REC.1398.456). Ninety pregnant women in the first half of their pregnancy (the first 20 weeks) were referred to the perinatal clinic of Mahdih Hospital for routine pregnancy care and for whom no adnexal mass was reported in their first-trimester ultrasound examinations entered the study. The sampling method was convenient and available. A researcher-made checklist, laboratory tests, and clinical assessments of patients were used to collect



mean level of HE4 in all pregnant women was average (less than 140 pmol/L). According to Moore et al., the middle story of HE4 in pregnant women in the first trimester was 34.3 ± 9.9 pmol/L (10), close to the 41 ± 14.12 pmol/L presented in our study.

In a pilot study on HE4 levels in pregnant women, Gucer and colleagues measured and compared CA125 and HE4 in 27 healthy non-pregnant women and 26 healthy pregnant women in the first and second trimesters of pregnancy. This study demonstrated that the concentration of CA125 in pregnant women is higher than in non-pregnant women. The concentration of this marker did not differ in the first and second trimesters of pregnancy. Serum HE4 levels were not statistically significant in pregnant or non-pregnant women or the first and second-trimester pregnancy. There was a positive correlation between the increase in parties and the CA125 level. In contrast, the HE4 level did not significantly relate to several parties. In addition, common tumor markers such as CA125 can have many false positives (6). In our study, the results were quite similar to those of the mentioned study.

This study illustrated that serum HE4 levels are not affected by pregnancy and can be used as a diagnostic laboratory test to detect malignant ovarian masses during pregnancy. HE4 levels were not correlated with age, gestational age, gravidity, or body mass index. In the study by Moore et al. (10), serum HE4 levels were measured and compared in 1,101 healthy and 67 pregnant women. This study demonstrated that in the age range older than 40, HE4 has a significant relationship with age, where serum levels of HE4 increase with age. The mean level of HE4 in pre-menopausal women was approximately 55.4 ± 52.9 pmol/L; in postmenopausal women, it was 67.6 ± 45.6 pmol/L. Moore's study revealed that the serum HE4 level had a statistically significant relationship with the patient's age. Therefore, when interpreting the serum level of HE4, its relationship with the patient's age should be

the datasheets. Performing the HE4 test did not interfere with patients' diagnostic and therapeutic processes. Conducting this research did not impose additional costs on the patient, and the researchers covered the price of this project.

Data analysis

Statistical tests used in this study included the Kolmogorov-Smirnov test, independent t-test, and Pearson linear regression. The significance level was considered less than 0.05 to interpret the relationships between variables. Statistical software, SPSS version 26, was used for statistical analysis.

Results:

The mean age of the cases was 30.79 ± 6.96 years. Also, the mean gravida was 2.22 ± 0.99 , and the mean gestational age was 12.42 ± 3.74 weeks. The average body mass index (BMI) was $28.16 \text{ kg/m}^2 \pm 5.10$. The mean level of HE4 was 41.265 picomoles per liter with a standard deviation of 14.12, and it was normal in all subjects (less than 140 pmol/L). (See table 1). Based on Pearson linear regression test, between HE4 level and age ($P = 0.871$); Gravida ($P = 0.482$); Body mass index ($P = 0.258$); and gestational age ($P = 0.117$), there was no significant correlation. (See table 2 and Fig.1).

The mean of HE4 in the abortion group was 65.13 and in the non-abortion group was 37.05 picomole per liter, demonstrating a statistically significant difference based on an independent t-test ($P = 0.001$). (See table 2 and Figure 1).

Discussion:

The HE4 titer was first used around 2011 to screen suspected women with ovarian epithelial cancers. According to the US Food and Drug Administration's standard serum HE4 measurement kit brochure, the highest limit of an average serum level of HE4 for premenopausal and postmenopausal women is 140 picomol per liter, which is the cutting point in our study. This study found that the



HE4 in our pregnant women was double that of the mentioned study, but overall, it was lower than the 140pmol/L boundary range.

In a study by Uslu et al., 46 pregnant women were compared with 40 pre-menopausal women. This study revealed that HE4 levels in the first and second trimesters of pregnancy were slightly lower than in the control group. However, there was no such difference between the control group and third-trimester pregnant women (12). Our study had no significant relationship between HE4 and gestational age, but there was a substantial difference between the abortion and non-abortion groups in the pregnancy outcome. A survey by Delic et al., performed on 229 uncomplicated pregnant women and compared them with those who had complications, revealed that the HE4 level in the first trimester of pregnancy averaged 42 pmol/L, which gradually increased to the third trimester. Its average value was less than 140 picomoles per liter and illustrated higher levels in pregnant women with complications (2). Their results are consistent with the findings of our study, which state that the mean of HE4 in the aborted and non-aborted groups showed a statistically significant difference based on an independent t-test. The standards of HE4 in the aborted and non-aborted groups are 65.127 pmol/L and 37.054 pmol/L, respectively.

In an analytical study conducted by Wang et al. (13) in China and published in 2018, 167 healthy pregnant women and 46 women who had abortions were compared, and the researchers realized that HE4 levels were significantly higher in abortions, which is in agreement with the findings of our study.

Conclusion:

According to the results of this study, the level of tumor marker HE4 in the first half of pregnancy in pregnant women under prenatal care is within the normal range. Still, higher levels are seen in cases leading to miscarriage compared to other pregnancies. Also, serum

considered (4). Nevertheless, in our research, there was no significant relationship between age, BMI, gestational age, and gravida with serum HE4 levels. This can be attributed to the age of our study group, all of whom were in the premenopausal range.

In a study of healthy Korean women, the highest average level of HE4 in these women was about 82 pmol/L, and the border between normal and increased levels was about 65 pmol/L (15). In the South Korea study, 1,809 women were studied, including 140 patients with an ovarian mass and 123 with a benign ovarian group. Other specimens belonged to healthy women. This study showed that HE4 levels were higher in those with mild or malignant ovarian masses than in women without. Also, the increase of HE4 in malignant masses was more significant than in benign masses. Hence, we included cases of the adnexal group in the exclusion criteria. As the patient ages, the serum level of HE4 in the patient's blood increases (15). However, in our study, there was no correlation between age and HE4 status.

A large study in the Asian population aimed to define the normal and abnormal boundaries for the tumor marker HE4 to evaluate the average level of HE4 and its increase in gynecological pathologies. In this study, serum HE4 levels were measured in 2182 women whose health had been proven in clinical and paraclinical surveys. It was shown that the average serum concentration of HE4 in these women was 21.2 pmol/L. Also, in this study, 72 pregnant women without gynecological pathology were investigated, and the results demonstrated that the mean serum level of HE4 in them was 22.8 pmol/L. According to this study's findings, the mean levels of HE4 in women with uterine myoma, ovarian cyst, adenomyosis, and ovarian malignancies were 24, 31, 39, and 64, respectively, and the difference was statistically significant in comparison with healthy individuals (11). In our study, according to Park et al. study (11), these cases were excluded. Also, the level of



The authors declare that there were no competing interests.

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levels of this index in pregnant women are in the normal range of HE4 that previously was determined (140pmol/L). Therefore, pregnancy has no significant impact on this marker, and it can be used as a tumor marker to evaluate adnexal masses during pregnancy.

Study Limitations

One of the significant limitations of this study was the lack of comparison with pregnant women with ovarian malignancies and determining the limit of HE4 in normal and malignant pregnant patients. Also, conducting a study in one center reduces the ability to generalize the results.

Recommendations:

It is recommended that more multicenter studies with malignant ovarian tumor patients be performed to confirm the findings of this study with a larger sample size and determine the HE4 normal range in pregnancy. Besides, further studies on other existing tumor markers may be considered for future analytical studies.

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Author Contributions

A.M. and S.H. contributed to the study concept. Z.N. and A.M. participated in planning the study. Also, S.H. analyzed the data. S.H, A.B, E.P, F.R, A.K, and S.A helped with the literature review and recruitment. All the authors cooperated in interpreting the results, drafting, critical thinking, and approving the final version of this manuscript.

Data Availability:

All relevant data are included in the article. Additional information is available from the corresponding author upon reasonable request.

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Competing Interests



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Tables

Table 1- Frequency distribution of demographic variables and HE4 in the participants

Variables	Mean	Std. Error of Mean	Median	SD	Variance
Age	30.79	0.697	32.00	6.969	48.572
Gravid	2.22	0.099	2.00	0.991	0.981
BMI	28.16	0.511	29.90	5.109	26.099
GA	12.42	0.374	11.00	3.745	14.024
HE4	41.265	1.4127	37.00	14.1271	199.575

Table 2 - Comparison of HE4 in abortion and non-abortion cases

Variable	Abortion	Mean	SD
HE4	Positive	65.127	19.0029
	Negative	37.054	7.4439



Figures

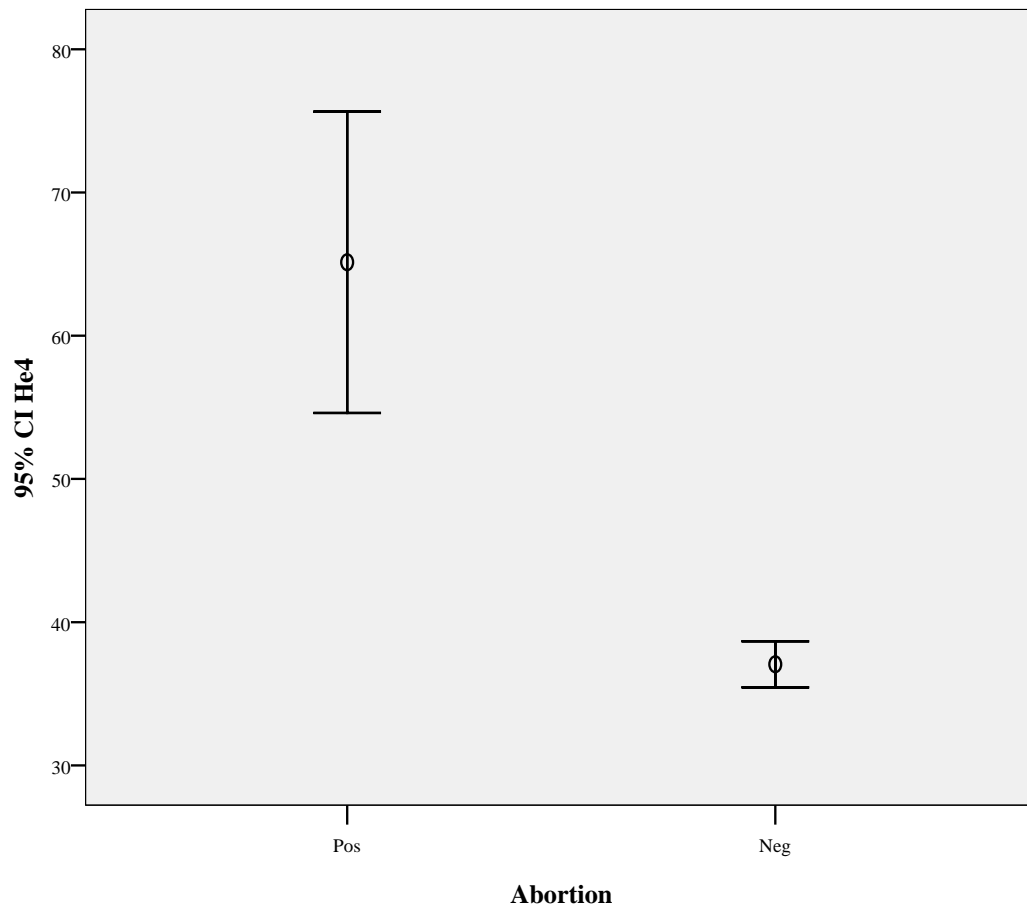


Figure 1. Comparison of HE4 in abortion and non-abortion cases.

