

# The Utility of Serum HE4 in Predicting Myometrial Invasion in Patients with Endometrial Cancer

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## Abstract

**Background:** Human epididymis protein 4 (HE4) is a novel tumor marker that has shown a strong potential in diagnosing and predicting the prognosis of endometrial cancer. In this study, we aimed to assess the association of tumor size and stage, ovarian involvement, lymph node metastasis, lymphovascular space invasion and deep myometrial invasion with preoperative serum levels of HE4 in endometrial cancer patients.

**Method:** This cross-sectional study included patients with endometrial cancer awaiting surgery in Ghaem Hospital, Mashhad, Iran, from May 2016 to May 2017. We measured the HE4 levels preoperatively and gathered other required information postoperatively; collected data were analyzed using SPSS version 16.

**Results:** We enrolled 104 endometrial cancer patients. Mean serum HE4 was  $149.6 \pm 186.2$  pmol/l. Level of serum HE4 had significant positive correlations with tumor size and stage, lymph node metastasis, ovary involvement, lymphovascular space invasion and profound myometrial invasion ( $P < 0.001$ ). There was no association between age and myometrial invasion. Moreover, using ROC curve, we calculated a serum HE4 cut-off of 104 pmol/l to be 91% sensitive and 89.6% specific for the detection of deep myometrial invasion.

**Conclusion:** HE4 is a novel biomarker capable of preoperatively predicting the depth of myometrial invasion with high specificity and sensitivity. This marker can be utilized in guiding the surgical plan of patients with endometrial cancer.

**Keywords:** Endometrial cancer, HE4, Myometrial invasion, Lymphadenectomy, Surgical staging

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## Introduction

Endometrial cancer is the second most prevalent gynecological malignancy worldwide, and 151700 new cases are estimated to occur

annually in developing countries.<sup>1,2</sup> Fortunately, most cases are diagnosed at an early stage, resulting in a favorable prognosis. The gold standard of treatment is curative

surgery based on the stage of cancer. Main treatment consists of hysterectomy alongside salpingo-oophorectomy. Moreover, lymphadenectomy may be required to reduce the rate of recurrence and increase survival. However, lymphadenectomy may cause serious complications such as symptomatic lymphocyst, lymphedema, deep vein thrombosis, vascular and neurologic injuries, and the need for blood transfusion.<sup>3,4</sup>

Recent data did not support the need for lymphadenectomy in low-risk patients at early stages of the disease. Lymphadenectomy led to moderate-to-severe sequela in 13% of patients with stage one disease.<sup>5</sup> Furthermore, a meta-analysis of 1851 endometrial cancer patients showed that lymphadenectomies for stage one patients not only did not increase the overall and recurrence-free survival rates but also increased the risk of the formation of associated morbidities such as lymphedema and lymphocysts.<sup>6</sup>

Risk of lymph node metastasis is based on the extent of myometrial and capillary-lymphatic invasion and the histologic grade of the tumor.<sup>7</sup> Therefore, a reliable preoperative test, able to distinguish between different stages of endometrial cancer, is essential in guiding the decision to perform lymphadenectomy in these patients. Current preoperative strategies for predicting the likelihood of lymph node metastasis include computed tomography, magnetic resonance imaging, and positron emission tomography, which only offer sensitivities between 50% and 60%.<sup>8-10</sup> Human epididymis protein 4 (HE4) is a novel circulatory tumor marker, which is highly expressed in patients with endometrial cancer.<sup>11</sup> Some recent studies have proposed the ability of HE4 in predicting the endometrial cancer stage and prognosis.<sup>12-15</sup> Moreover, HE4 showed higher sensitivity for detection and staging of endometrial cancer compared with the long known cancer antigen 125 (CA-125).<sup>11</sup> However, further studies are required for HE4 to become widely acceptable in predicting the chance of lymph node metastasis in the endometrial cancer patient, thereby guiding the decision to perform lymphadenectomy without causing more harm.

In this study, we aimed to evaluate the

association of different prognostic factors such as tumor size and stage, ovarian involvement, lymph node metastasis, lymphovascular space invasion (LVSI), and deep myometrial invasion (more than 50%) with preoperative serum levels of HE4 in endometrial cancer patients presenting to Ghaem hospital, Mashhad, Iran, awaiting surgery. Moreover, we measured the specificity and sensitivity of HE4 in predicting deep myometrial invasion.

## Materials and Methods

This prospective cross-sectional study was conducted on all endometrial cancer patients (endometrioid type), who scheduled for surgery in Ghaem Hospital (an academic hospital of Mashhad University of Medical Sciences, Mashhad, Iran), from May 2016 to May 2017. After obtaining written informed consents, we preoperatively collected venous blood samples using standard serum sampling tubes. Each sample was centrifuged and stored at -80 C for 4 hours. Afterwards, we sent the samples to Dr. Moayed's Laboratory for measurement of serum HE4 using the electrochemiluminescence immunoassay 'ECLIA' on the Cobas<sup>®</sup>6000 analyzer (Roche Diagnostics GmbH, Mannheim, Germany).

All patients underwent a surgery, which consisted of Hysterectomy and Salpingo-oophorectomy; if the sentinel lymph node biopsy was positive, we also performed pelvic and paraaortic lymphadenectomy and peritoneal washing; biopsies were further taken from inside pelvis and abdomen. A single pathologist studied the biopsy samples. Prior to surgery, we recorded the information pertaining to age, menopause and parity status, tumor size, grade and FIGO stage, depth of myometrial invasion (more or less than 50%), ovaries or lymph node involvement, LVSI and level of HE4. Patients were excluded if clinical data were unavailable or if there was an impaired renal function at the time of diagnosis because the latter is known to influence serum HE4 concentration.

### Sample size

We determined the sample size using the type I error at 0.05 with a power of 80%. Moore et al.<sup>12</sup> study was used in the following formula for sample size calculation:

$$n = \frac{(Z^2 * P(1-P))}{d^2}$$

$$N = \frac{n}{\text{prevalence}}$$

Z=1.96

P=85.4%

d=0.13

Prevalence=57%

The sample size was determined to be 104 in total.

### Statistical analysis

One-Sample Kolmogorov-Smirnov Test confirmed the normality of HE4 level distribution.

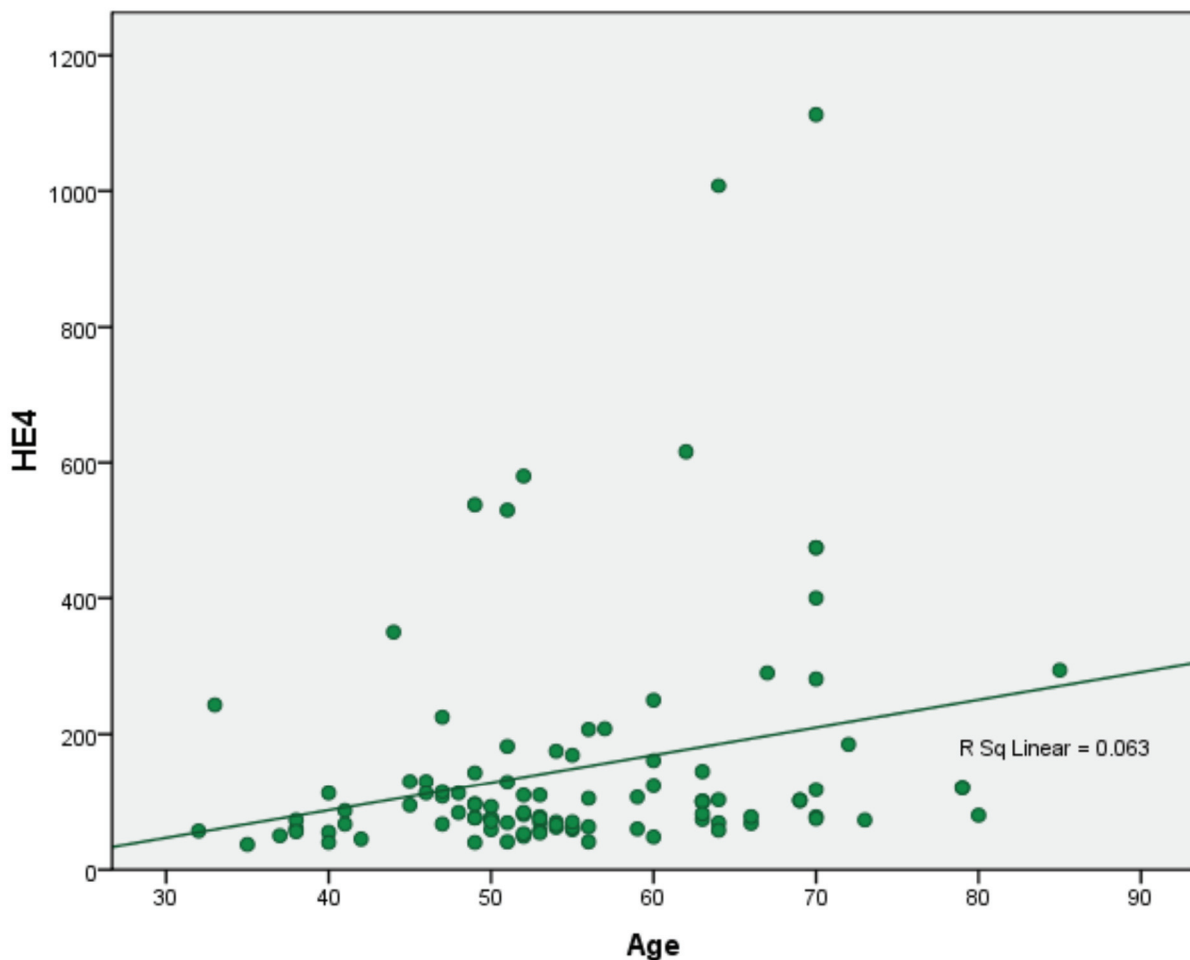
We compared the mean HE4 values between groups via appropriate statistical tests based on each variable characteristics. The ROC curve was drawn to predict deep myometrial invasion using serum HE4; based on the curve, we identified the value with the highest combined specificity and sensitivity.  $P < 0.05$  was considered statistically significant. All analyses were carried out using SPSS version 16.

### Ethical consideration

The Ethics Committee of the Mashhad University of Medical Sciences approved this study (No.: IR.MUMS.fmREC.1395.273).

### Results

We included 104 pathologically confirmed endometrial cancer patients aged 32-85 years. Mean age of the patients was  $55.22 \pm 10.8$  years.



**Figure 1.** This figure shows the relationship between age and serum HE4.

The lowest serum HE4 was 37, whereas the highest was 1113 pmol/l. Mean serum HE4 was  $149.62 \pm 176.29$  pmol/l. 72 of the patients were menopausal, and seven were nulliparous. 39 patients had grade 2 and above cancer, and only five patients had lymph node involvement. A deep myometrial invasion was observed in 69 patients (Table 1).

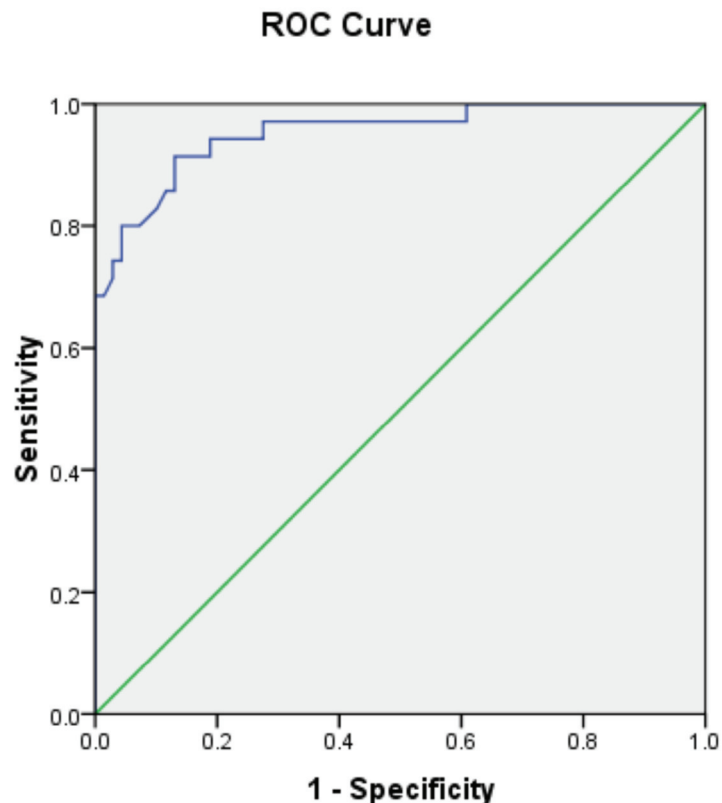
In our multivariate analysis, HE4 level had significant positive associations with tumor grade, LVSI, tumor Stage, tumor size, ovary involvement, lymph metastasis, and myometrial invasion (Table 1) ( $P < 0.05$ ). Moreover, there was no significant relationship between HE4 level and menopause status and parity ( $P$  values were 0.57 and 0.34, respectively). Initially, myometrial invasion was correlated with age and serum HE4 using t-test (Figure 1). Subsequent logistic regression denied the statistically significant relationship between

age and depth of myometrial invasion ( $P = 0.489$ ); however, it confirmed the significant association between serum HE4 and deep myometrial invasion ( $P < 0.001$ ).

We calculated the sensitivity and specificity of HE4 in predicting the myometrial invasion of more than 50%. The results suggested that serum HE4 values more than 104 pmol/L has sensitivity and specificity of 91% and 89.6%, respectively (Figure 2).

## Discussion

We conducted a cross-sectional study on 104 patients with endometrial cancer, awaiting surgery. Our results showed that the level of serum HE4 was significantly associated with tumor size and stage, lymph node, ovary, lymphovascular space, and myometrial invasion. No association existed between age and myometrial invasion. Moreover,



Diagonal segments are produced by ties.

**Figure 2.** This figure shows the ROC curve for serum HE4 in predicting deep myometrial invasion.

**Table 1.** Demographic characteristics and their association with preoperative serum HE4

		Count	Percent	HE4 (mean ± standard deviation)	P value
<b>Menopause</b>	No	32	30.8%	100±66	0.57*
	Yes	72	69.2%	172±204	
<b>Parity</b>	0	7	6.7%	298±386	0.34**
	1	26	25.0%	181±154	
	2	32	30.8%	148±188	
	3	39	37.5%	103±96	
<b>Tumor Size</b>	<2cm	83	79.8%	88±40	<0.001**
	≥2cm	21	20.2%	394±273	
<b>Tumor Grade</b>	1	65	62.5%	97±76	<0.001**
	2	29	27.9%	198±229	
	3	10	9.6%	353±279	
<b>Tumor Stage</b>	Ia	64	61.5%	75±23	<0.001**
	Ib	11	10.6%	108±40	
	II	21	20.2%	158±57	
	III	8	7.7%	434±275	
<b>Lymph node metastasis</b>	No	99	95.2%	137±167	<0.001***
	Yes	5	4.8%	406±172	
<b>Ovary involvement</b>	No	99	95.2%	118±95	<0.001*
	Yes	5	4.8%	771±268	
<b>LVSI</b>	No	95	91.3%	125±136	<0.001***
	Yes	9	8.7%	413±314	
<b>Myometrial invasion</b>	<50%	69	66.3%	76±24	<0.001*
	≥50%	35	33.7%	294±246	

\*: T-test; \*\*: Spearman correlation coefficient; \*\*\*: Mann-Whitney test; LVSI: lymphovascular space invasion

using ROC curve, we calculated the serum HE4 cut-off of 104 pmol/l to be 91% sensitive and 89.6% specific regarding the detection of deep myometrial invasion.

In 1988, the International Federation of Gynecology and Obstetrics (FIGO) proposed a new staging system for endometrial cancer.<sup>16</sup> The new system comprises three stages with the first one further divided into stages 1a and 1b. The difference between stage 1a and 1b is the depth of myometrial invasion (more or less than 50%). This staging system guides the surgical treatment of endometrial cancer. Stage 1b and above patients require lymphadenectomy as well as conventional hysterectomy and salpingo-oophorectomy applied

to stage 1a patients. Lymphadenectomy can ensue multiple complications, including symptomatic lymphocyst, lymphedema, deep vein thrombosis, vascular and neurologic injuries and the need for blood transfusion.<sup>3,4</sup>

HE4 was first discovered by Kirchoff et al.<sup>17</sup> Later, it was demonstrated that HE4 is highly expressed in cancer tissues, particularly endometrial and ovarian cancers. Moreover, several studies have reported the higher efficacy of HE4 compared with CA-125 in differentiating between benign gynecologic lesions and cancers.<sup>18-20</sup> Therefore, HE4 emerged as a promising tumor marker in endometrial cancers.

Angioli and colleagues compared the levels



of HE4 and CA-125 in the serum of endometrial cancer patient and patients with benign uterus diseases. Their results showed that HE4 levels of more than 70 pmol/L had a positive predictive value of 100% in differentiating these two groups; furthermore, HE4 had higher specificity and sensitivity in comparison with CA-125. They also showed that CA-125 combined with HE4 did not result in a significantly higher sensitivity in detecting endometrial cancer.<sup>13</sup> Our results suggested that serum HE4 levels higher than 104 pmol/l had a sensitivity and specificity of 91% and 89.6%, respectively, in detecting deep myometrial invasion.

There is a need for an easily accessible tool for differentiating high-risk patients requiring lymphadenectomy from low-risk patients; therefore, researchers have sought to examine the application of HE4 to the prediction of lymph node metastasis and tumor invasion level. In 2012, Kalogera et al. reported that serum HE4 significantly increased in patients with more than 50% myometrial invasion and a tumor size of more than 2cm. They further showed that HE4 with a cut-off value of 8 MFI (mean fluorescence intensity) was more sensitive than CA-125 in detecting advanced disease stages (sensitivity 71% vs. 58%).<sup>14</sup> However, our results revealed that serum HE4 with a cut-off value of 104 pmol/l was 91% sensitive and 89.6% specific in detecting deep myometrial invasion. Additionally, they found a positive correlation between age and level of serum HE4, which is in contrast to the results of our logistic regression

Bignotti et al. suggested that HE4 is an independent prognostic factor, highly expressed in patients with aggressive endometrial cancer phenotype.<sup>15</sup> Brennan and colleagues conducted a study on 373 patients with endometrial cancer and showed that serum HE4 could be utilized as a novel biomarker for identifying high-risk patients who might require lymphadenectomy. Moreover, they showed that HE4 was a better predictor of myometrial invasion compared with CA-125.<sup>21</sup> Antonsen et al. reported that the combination of HE4 and CA125 was significantly related to the histological grade of the tumor, depth of

myometrial invasion, and lymph node involvement.<sup>22</sup> They further proposed using combined CA-125 and HE4 in clinical settings to improve the prediction of prognostic factors. Similarly, another study also reported HE4 as an independent prognostic factor and indicated that combination of HE4 with CA-125 yielded a higher hazard ratio for overall survival compared to HE4 alone.<sup>23</sup> Our results are in line with previous studies, suggesting that HE4 can be employed as a novel tumor marker in endometrial cancer for the prediction of tumor stage, especially lymph node metastasis.

Several limitations existed in our study, including the limited sample size, lack of a control group, and no comparison between serum He4 and other such biomarkers as CA-125. However, our results suggest a significant correlation between HE4 and endometrial cancer stage.

## Conclusion

Our results suggested that HE4 could be utilized as a novel biomarker for distinguishing deep myometrial invasion, thereby guiding the decision for lymphadenectomy.

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## Conflict of Interest

None declared.

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