**Relation between Human Epididymis Protein 4 and endometrial pathology in women with postmenopausal bleeding**

**Ahmed L AboulNasr MD 1 Ghada A AbdelMoety MD1 Mostafa S. Salem MD2 Marwa M Elsharkawy MD3 Nada Kamal MD1 Ahmed M Maged MD1**

\*Corresponding author at: Department of Obstetrics and Gynecology, 27 Nafezet Sheem El Shafaey St Kasr Al Ainy Faculty of Medicine, Cairo University, Cairo, Egypt. Tel.: +20 1011322138. E-mail address: ndakamal@gmail.com.

1Department of Obstetrics &Gynecology, Faculty of Medicine, Cairo University, Egypt.

2Department of pathology Cairo University, Cairo, Egypt.

3Department of clinical pathology Cairo University, Cairo, Egypt.

**Abstract**

*Objective*

To evaluate the value of human epididymis protein 4 (HE4) in predicting endometrial pathology in women with postmenopausal bleeding (PMB).

*Methods*

A cohort study included 100 women with PMB. Women with endometrial thickness (ET) >5mm were subjected to hysteroscopic guided fractional curettage (FC) followed by total abdominal hysterectomy and bilateral salpingo-oophorectomy with or without pelvic lymphadenectomy.

After exclusion of 10 patients, the value of serum HE4 was tested in 90 patients for the ability to predict endometrial pathology based on hysterectomy specimen.

Results

Level of HE4 showed a significant difference among women with different endometrial pathologies. HE4 showed a significant positive correlation with the severity of the endometrial lesion, with mean values of 38.33±27 pmol/L for atrophic endometrium (11 cases), 51.26±28.59 pmol/L for simple endometrial hyperplasia (SEH,51 cases), 148.4 ±67.34 pmol/L for atypical endometrial hyperplasia (AEH ,16 cases) and 390.9±351.72 pmol/L for endometrial carcinoma (EC,12 cases) Using the cut-off value of 69.5 pmol/L for preoperative HE4 yielded a sensitivity of 75% and a specificity of 88.5% in prediction of EC.

*Conclusion*

HE4 can predict endometrial pathology in women with PMB with a high specificity and a fair sensitivity.

*Keywords*

Human epididymis protien4 (HE4); postmenopausal bleeding; endometrial carcinoma; endometrial pathology.

**Introduction**

Endometrial carcinoma accounts for 20% to 30% of malignant tumors in the female reproductive system. As a consequence to increased obesity, hypertension, diabetes, and prolonged life expectancy, the incidence and mortality of endometrial carcinoma have risen lately, with a tendency for onset at a younger age [1]. The prognosis is closely related to the disease stage. If the diagnosis is during stage I, then the survival rate is about 90% [2].

There are no specific tumor markers for endometrial carcinoma. CA-125 was detected in 1983 by Bast et al. [3] as the epithelial ovarian carcinoma antigen. However, CA-125 is less effective in the diagnosis of EC compared with the diagnosis of other gynecological carcinomas. CA-125 can only produce obvious effect in diagnosing some common tumors in advanced stage [4] .

HE4 biomarker has been recently studied. It was identified in the epithelium of the distal epididymis and was predicted to be a protease inhibitor involved in sperm maturation [5]. In 2003, HE4 was approved by the FDA as a serum tumor marker for ovarian carcinoma and attracted great attention [6]. Recent studies indicate that HE4 is highly expressed in ovarian and endometrial carcinoma tissues with increased serum level in these patients as well [7].

**Materials and methods**

This prospective cohort study included 100 women with PMB who were recruited from Kasr Al Aini Hospital, Cairo University, Egypt between June 2014 and August 2016. An informed written consent was obtained from all participants prior to inclusion.

All patients included in the study had single or multiple episodes of PMB with an ET of more than 5mm. Exclusion criteria were having history of other malignancies, history of intake of chemotherapy or radiotherapy, the use of hormone replacement therapy, and being unfit for surgical intervention.

Full history was taken (including the duration of menopause, the number of episodes of PMB, and previous investigations and current medications), general examination was performed (including blood pressure measurement, calculation of body mass index (BMI= weight (kg)/ [height (m)]2, and the presence of any signs of systemic diseases), and local examination was performed for all patients.

Transvaginal ultrasound (TVS) done by the same observer to nullify the effect of interobserver variability.

For the level of HE4: 5 ml of venous blood were withdrawn from all patients. The samples were left to clot. The separated sera were stored at -20˚ until all samples were obtained. Frozen samples were allowed to reach room temperature prior to use. Samples were then mixed thoroughly by gently inverting multiple times before analysis. HE4 was quantitatively assayed using the enzyme immunoassay (EIA) method (Fujirebio Diagnostics, Inc. Göteborg, Sweden). The functional sensitivity of the HE4 EIA is ≤ 25pM. The analytical specificity is 100 ± 15%.

All patients were then submitted to hysteroscopy under general anesthesia and guided endometrial biopsy.

Definitive management was later performed in the form of total abdominal hysterectomy, bilateral salpingo oophrectomy, with or without pelvic lymph nodal dissection and histopathological examination.

**Results**

The consort flow chart is described in figure 1.

Women with malignancy had significantly older age, lower parity, higher BMI and longer duration of menopause when compared to those with non-malignant lesion (table 1).

ET of the malignant group was significantly higher than that of the non-malignant group (20.33 ± 7.4 versus 12.68 ± 4.22mm, p: <0.001), level of preoperative serum HE4 was significantly higher in the malignant group as compared to the non-malignant group (390.92 ± 351.72 versus 61.25 ± 31.65pmol/L, p: <0.001) (table 1).

The level of HE4 in different endometrial pathologies of the cases group is presented in (table 2).

A scale was proposed in which the endometrial pathologies were arranged in a descending manner according to the severity of the lesion, where malignancy was the severest, followed by AEH, then SEH, and atrophic endometrium being the least severe form. Hence, correlation between the preoperative HE4 level and the severity of the endometrial lesion could be evaluated. This study showed that there was a significant strong positive correlation between the preoperative level of HE4 and the severity of the endometrial pathology (r= 0.735, p: <0.001).

ROC curve was generated to evaluate the performance of the preoperative level of HE4 in distinguishing malignant from non-malignant endometrium (figure 2).

Using the cut-off value of 69.5 pmol/L for preoperative HE4 yielded a sensitivity of 75%, a specificity of 88.5% and an AUC-ROC of 0.933 (table 3).

All malignant cases (12 cases) were of the endometrioid type, 5 were stage Ia, 5 were stage Ib, and 2 were stage II. All were operable and a total abdominal hysterectomy and bilateral salpingo-oophorectomy with pelvic lymphadenectomy was performed for all.

For the degree of differentiation, 2 cases were grade 1 (G1), 8 cases were grade 2 (G2), and 2 cases were grade 3 (G3).

Multivariate stepwise linear regression for factors with significant differences between malignant and non malignant cases as age, parity, and duration of menopause are shown in table 4.

The level of HE4 in the malignant cases according to the tumor stage, grade and lymph node involvement is described in table 5.

**Discussion**

In this study, we focused on examining the role of HE4 in distinguishing malignant from non-malignant lesions of the thickened endometrium in women with PMB through histopathological examination of hysteroscopic directed endometrial curettage followed by hysterectomy, and to correlate HE4 level with the endometrial lesion.

The prevalence of EC in the present study was 13.3%. This is similar to that reported in previous studies [8].

HE4 is a new detection index. Being highly expressed in ovarian and endometrial carcinoma cells [7].

In this study, the preoperative level of HE4 was significantly higher in the endometrial carcinoma cases than its level in the non-malignant cases.

HE4 actually exists in normal tissues e.g. male vas deferens, mammary gland epithelium, female genital tract including the endometrium [9]. So its level is suspected to increase with increased endometrial thickness. As suspected its level is increased in cancers arising from these tissues [10].

The National comprehensive cancer network in 2012 signified the value of HE4 as a tumour marker for epithelial ovarian tumors and as both the uterus and the ovary share a common embryological origin so HE4 can be used as a marker for endometrial tumors [11].

In the present study, upon examining the diagnostic performance of HE4 in predicting the presence of EC among patients with PMB, using the cut-off value of 69.5 pmol/L for preoperative HE4 yielded a sensitivity of 75% and a specificity of 88.5%, and an AUC-ROC of 0.933, Having more serious consequences separating AEH and EC patients from SEH and atrophic endometrium cases ,HE4 was significantly higher in the former group 218.14 ± 273.46 versus 54.2 ± 22.45 , p <0.001 with a new cut off value calculated to help differentiation of AEH and EC cases, HE4 value of 62.5 pmol/L yielded a sensitivity of 85.9% and specificity of 62.9% with AUC of 0.832 .

Similar to our findings, previous study on 2015 reported the sensitivity and specificity of HE4 in distinguishing EC patients from healthy females were 62.2% and 95% respectively, with an AUC of 0.996 . Another one on the same year reported a sensitivity of 72.4% and a specificity of 75.4% for the cut-off 76.5 pmol/L [15]. Also, Capriglione et al in 2015 [16] reported sensitivity and specificity that are near to ours in detecting EC patients 83.3% and 96% respectively.

An earlier study on 2013 has reported that the sensitivity of HE4 in detecting malignant cases was 75% and the specificity was 65.5%, and that the sensitivity was improved after combining HE4 with other markers (CA-125, CEA, and serum amyloid –A) to be 84% [13].

Another publication in the same year revealed that the sensitivity of HE4 in detecting malignant endometrium was 59.4% with 100% specificity for the cut-off value of 70pmol/L. After adding CA-125, the sensitivity was elevated to be 60.4%. The authors concluded that HE4 at cutoff of 70 pmol/L yields the best sensitivity and specificity [12]. The lower sensitivity of the marker in their study compared to ours might be due to that they took into consideration other types of EC while all our cases were of the endometrioid type.

Previous study on 2016 have reported that HE4 was significantly higher in grade 3 (G3) carcinomas compared with grade 1 (G1) and 2 (G2), and that patients who needed lymphadenectomy had significantly higher HE4 level than those who had no indications for this procedure [14].

A recent study on 2017 stated that preoperative serum HE4 is significantly correlated with primary tumor diameter and depth of myometrial invasion, but not with tumor grade or cervical involvement and lymphovascular infiltration and that serum HE4 levels could be useful in identifying EC patients at high risk of lymphatic spread who would benefit from lymphadenectomy [17].

A meta-analysis done in 2014 reported that HE4 is the most accurate and sensitive EC marker identified to date. In particular, this new marker seems to have a good performance in diagnosis. The best cut-off of HE4 in diagnosis ranges between 50 and 70 pmol/L, resulting at least in 78.8% of sensitivity and 100% of specificity in all stages. Another important aspect to consider is HE4 capacity in predicting the stage of disease and myometrial involvement, which can help scheduling the appropriate timing of imaging and surgery in a more individualized fashion and as indicator of patient prognosis [18].

Our study confirmed the known fact that malignancy is suspected to be found in women with postmenopausal bleeding when they are older, lower parity, higher BMI and have longer interval between menopause and presentation.

ACOG confirmed these findings by stating that the clinically identified risks for carcinoma endometrium include age and high body fat [19].

The present study is strengthened by its prospective nature, and that it depended on hysterectomy specimen for diagnosis of different endometrial pathologies as well as malignancy, beside the analysis of positive results of lymphadenectomy.

The main limitation of the study is the small sample size included which resulted in a limited number of malignancy cases with the resultant limited variations in malignancy stages and pathological subtypes. Larger number of participants would have better detected the value of the studied marker (HE4) in diagnosis and prognosis of endometrial malignancies. Nevertheless, the study highlighted the presence of this new marker and pointed to its possible value in diagnosis of the disease and the prediction of its occurrence at certain cut-off value with the reported sensitivity and specificity.

Tables

Table (1): characteristics of the studied population

|  |  |  |  |
| --- | --- | --- | --- |
|  | Malignant group (n=12) | Non-malignant group (n= 78) | P value |
| Age (years) | 63.5 ± 6.86 | 55.97 ± 5.68 | <0.001 |
| Parity | 2.67 ± 1.49 | 4.71 ± 2.15 | 0.002 |
| BMI (Kg/m2) | 37.19 ± 5.58 | 32.95 ± 6.49 | 0.034 |
| Duration of menopause (years) | 11.67 ± 5.41 | 4.83 ± 4.26 | <0.001 |
| Endometrial thickness(mm) | 20.33 ± 7.4 | 12.68 ± 4.22 | <0.001 |
| Preoperative HE4 (pmol/ L) | 390.92 ± 351.72 | 61.25 ± 31.65 | <0.001 |

Data are presented as mean± SD

Table (2): Level of HE4 in different endometrial pathologies

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | EC (n= 12) | AEH (n= 16) | SEH (n= 51) | Atrophic endometrium (n= 11) | P value |
| **HE4 (**pmol/ **L)** | 390.92 ± 351.72 | 148.44 ± 67.34 | 51.26 ± 28.59 | 38.33 ± 27 | <0.001 |

Data are presented as mean± SD

Table (3): Tests of diagnostic accuracy of preoperative HE4 level in distinguishing malignant from non-malignant endometrium

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | Cut-off value | Sensitivity (%) | Specificity (%) | AUC-ROC | PPV | NPV | Accuracy |
| **HE4 level (pmol/L)** | Malignant versus non- malignant cases | 69.5 | 75 | 88.5 | 0.933 | 50 | 95.8 | 86.7 |

Table( 4) Multivariate stepwise linear regression for age, parity, and duration of menopause

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | Beta coefficient (adjusted | S.E. | Wald | p value | OR | 95% CI for OR | |
| Lower | Upper |
| Age | 0.259 | 0.294 | 0.779 | 0.377 | 1.296 | 0.729 | 2.304 |
| Parity | -1.064 | 0.492 | 4.676 | 0.031 | 0.345 | 0.132 | 0.905 |
| Duration of Menopause | 0.189 | 0.253 | 0.557 | 0.455 | 1.208 | 0.735 | 1.985 |

|  |  |  |  |
| --- | --- | --- | --- |
| Table (5): the level of HE4 in the malignant cases according to the tumor stage, grade and lymph node involvement. | | | |
|  | No. patients | HE4 level | P value |
| Figo stage  I  Ia  Ib  IIa | 10  5  5  2 | 322.56±182.66  262.32±319.51  435.4±293.59  501.72±423.74 | Ia vs Ib =0.012  Ib vs IIa=0.241  I vs II=0.001 |
| Grade  G1  G2  G3 | 2  8  2 | 82.16±55.23  308.89±275.85  920.54±166.17 | <0.001 |
| Lymph nodes  Positive  Negative | 3  9 | 635.42±426.88  167.84±112.43 | <0.001 |

**Figures**

Patients with PMB+ ET>5 mm (n=100)

Hysteroscopic guided FC + HE4 sample

Excluded (n=10)

Unfit for surgery (n=2)

Cervical carcinoma (n=2)

No follow up (n=6)

Atrophic endometrium (n=11)

Simple endometrial hyperplasia (n=51)

Atypical endometrial hyperplasia (n=16)

Endometrial carcinoma (n=12)

Total abdominal hysterectomy with bilateral salpingo-oophorectomy and pelvic lymphadenectomy (n=12)

Total abdominal hysterectomy with bilateral salpingo-oophorectomy (n=78)

Final analysis (n=90)

Figure 1: Study design.

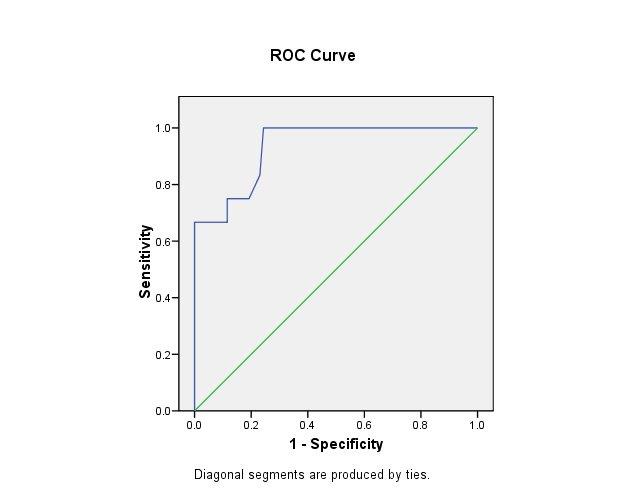


Figure 2:ROC curve

**Conclusion**

HE4 can predict endometrial pathology in women with PMB with a high specificity and a fair sensitivity.

**Conflict of interest**

The authors have no conflicts of interest.

**Source of funding**

Personal fund.

**Ethical committee approval**

Ethically approved by the department

.Clinical trial registry no. NCT03558321

**References**

1. Brennan DJ, Hackethal A, Metcalf AM. Serum HE4 as a prognostic marker in endometrial carcinoma: a population based study. Gynecologic Oncology 2014; 132(1): 159–165.
2. Bie Y, Zhang Z. Diagnostic value of serum HE4 in endometrial carcinoma: A meta-analysis. World J Surg Oncol. 2014; 12:169.
3. Bast RC, Klug TL, St John E, et al. A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian carcinoma. N Engl J Med 1983; 309: 883–887.
4. Liu X, Zhao F, Hu L, et al. Value of detection of serum human epididymis secretory protein 4 and carbohydrate antigen 125 in diagnosis of early endometrial carcinoma of different pathological subtypes. Onco Targets Ther. 2015 May 26; 8:1239-1243.
5. Kirchhoff C. Molecular characterization of epididymal proteins. Rev Reprod 1998, 3: 86–95.
6. Helistrom I, Raycraft J, Hayden-Ledbetter M. The HE4 (WFDC2) protein is a biomarker for ovarian carcinoma, Cancer Research 2003; 63 (13): 3695–3700.
7. Xia C, Ping Z, Xiaoyan L. Relationship between the serum human epididymis secretory protein 4 and clinical pathological features in patients with epithelial ovarian cancer. Labeled Immunoassays Clin Med. 2010; 17(6):365–367.
8. Damle RP, Dravid NV, Suryawanshi KH, et al. Clinicopathological Spectrum of Endometrial Changes in Peri-menopausal and Post-menopausal Abnormal Uterine Bleeding: A 2 Years Study. J Clin Diagn Res. 2013 Dec; 7(12):2774-2776.
9. Mehri Jafari-Shobeiri , Marzye Jangi , Ali Dastranj Tabrizi , Manizheh Sayyah-Melli , Parvin Mostafa-Gharabaghi , Elaheh Ouladsahebmadarek , Esmail Neginfar , Yasmin Pouraliakbar. Diagnostic Value of Novel Biomarker Human Epididymis Protein 4 (HE4) in Detecting Endometrial Cancer. International Journal of Women’s Health and Reproduction Sciences Vol. 4, No. 1, January 2016, 29–33
10. Simmons AR, Baggerly K, Bast RC Jr. The emerging role of HE4 in the evaluation of epithelial ovarian and endometrial carcinomas. Oncology (Williston Park) 2013;27:548-556.
11. Xiao Li, Yiping Gao, Mingzi Tan, et al., “Expression of HE4 in Endometrial Cancer and Its Clinical Significance,” BioMed Research International, vol. 2015, Article ID 437468, 8 pages, 2015. <https://doi.org/10.1155/2015/437468>
12. Angioli R, Plotti F, Capriglione S, et al. The role of novel biomarker HE4 in endometrial cancer: a case control prospective study. Tumour Biol. 2013; 34:571–576.
13. Omer B, Genc S, Takmaz O, et al. The diagnostic role of human epididymis protein 4 and serum amyloid-A in early-stage endometrial cancer patients. Tumour Biol. 2013 Oct; 34(5):2645-50.
14. Gąsiorowska E, Magnowska M, Iżycka N, et al. The role of HE4 in differentiating benign and malignant endometrial pathology. Ginekol Pol. 2016; 87(4):260-264.
15. Minář L, Klabenešová I, Jandáková E. The importance of HE4 in differential diagnosis of endometrial cancer .Ceska Gynekol. 2015 Aug; 80(4):256-63.
16. Capriglione S, Plotti F, Miranda A, et al. Utility of tumor marker HE4 as prognostic factor in endometrial cancer: a single-center controlled study. Tumour Biol. 2015 Jun; 36(6):4151-4156.
17. [Fanfani F](https://www.ncbi.nlm.nih.gov/pubmed/?term=Fanfani%20F%5BAuthor%5D&cauthor=true&cauthor_uid=28557834), [Restaino S](https://www.ncbi.nlm.nih.gov/pubmed/?term=Restaino%20S%5BAuthor%5D&cauthor=true&cauthor_uid=28557834), [Cicogna S](https://www.ncbi.nlm.nih.gov/pubmed/?term=Cicogna%20S%5BAuthor%5D&cauthor=true&cauthor_uid=28557834), et al. Preoperative Serum Human Epididymis Protein 4 Levels in Early Stage Endometrial Cancer: A Prospective Study. [Int J Gynecol Cancer.](https://www.ncbi.nlm.nih.gov/pubmed/28557834) 2017 Jul; 27(6):1200-1205.
18. Angioli R, Miranda A, Aloisi A, et al. A critical review on HE4 performance in endometrial cancer: where are we now? Tumour Biol. 2014 Feb; 35(2):881-887.
19. ACOG Committee Opinion No. 734 Summary: The Role of Transvaginal Ultrasonography in Evaluating the Endometrium of Women With Postmenopausal Bleeding. Obstet Gynecol. 2018 May;131(5):945-946. doi: 10.1097/AOG.0000000000002626.