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The role of HE4 in differentiating benign and malignant endometrial pathology

Rola HE4 w różnicowaniu złośliwych i niezłośliwych patologii endometrium

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Abstract

Objectives: *The incidence of endometrial cancer is constantly growing. More aggressive types of endometrial cancer as well as the incidence in younger women is being observed. More than 80% of cases is diagnosed in early stages due to early symptoms like abnormal bleeding. The remaining 20% of asymptomatic cases of endometrial cancer as well as the cases of false negative histopathological diagnoses are mostly the incidences of serous endometrial cancer and are a true diagnostic and therapeutic challenge. This was the reason of our study in which we proposed investigation of HE4 levels as a complementary diagnostic method in management and diagnosing of EC.*

Material and methods: *Serum HE4 level was measured in 92 patients with abnormal vaginal bleeding. Based on histhology after curretage the study group was divided into the benign and malignant endometrial pathology groups. Statistical analysis was performed using Mann-Whitney test*

Results: *The difference of serum HE4 level between benign endometrial pathology and cancer was significant (p=0.000) and the cut-off for identification of patients with endometrial cancer was 58.08 pmol/l. There was a significant difference between G2 and G3 endometrial cancer, and G1 and G3. (p=0,4 and p=0,008 respectively) Patients who needed lymphadenectomy had significantly higher HE4 level than those who had no indications for this procedure (p=0,001).*

Conclusions: *HE4 is a useful biomarker in diagnosing endometrial cancer. HE4 is associated with high grade endometrial cancer. It can also serve as an useful preoperative counseling tool to identify patients, who may require pelvic and paraaortic lymphadenectomy.*

Key words: **endometrial cancer** / **endometrial hypertrophy** / **HE4** /

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Streszczenie

Obecnie obserwuje się wzrost zapadalności na raka endometrium, w szczególności bardziej agresywnych typów tej choroby oraz jej występowanie u coraz młodszych pacjentek. Wprawdzie więcej niż 80% przypadków jest diagnozowanych we wczesnych stadiach zaawansowania, dzięki wczesnych objawom, takim jak nieprawidłowe krwawienia. Jednak pacjentki bezobjawowe, jak również przypadki fałszywie negatywnych wyników histopatologicznych są wyzwaniem diagnostycznym i terapeutycznym. W niniejszej pracy dokonano analizy wartości białka HE4 jako metody uzupełniającej diagnostykę raka endometrium.

Materiał i metody: *Stężenie markera w surowicy krwi zostało oznaczone u 92 pacjentek z nieprawidłowymi krwawieniami. Opierając się na wyniku badania histopatologicznego zostały podzielone na grupę z niezłośliwymi i złośliwymi zmianami endometrium. Analiza statystyczna została wykonana za pomocą testu Mann-Withney.*

Wyniki: *Różnica stężenia HE4 między zmianami niezłośliwymi endometrium a rakiem była istotna statystycznie (p=0,000) i wartość odcięcia wyniosła 58,08 pmol/l. Różnica stężenia HE4 w stopniu złośliwości raka endometrium G2 i G3 była istotna statystycznie jak również między G1 a G3. (p=0,4 i p=0,008 odpowiednio) Pacjentki, które wymagały limfadenektomii miały istotnie wyższe stężenia HE4 od tych, które nie wymagały takiego postępowania. (p=0,001)*

Wnioski: *HE4 jest użytecznym biomarkerem raka endometrium. Jest związany z niskim stopniem zróżnicowania nowotworu. HE4 może być użyteczne w identyfikacji pacjentek wymagających okołoaortalnej i miednicznej limfadenektomii.*

Słowa kluczowe: **rak endometrium** / **przerost endometrium** / **HE4** /

Introduction

Endometrial cancer (EC) is one of the most common female genital cancer in Poland and the incidence is constantly rising. EC mostly occurs after menopause in the sixth or seventh decade of life, but in about 25% it can appear also before menopause. Up to 10% of premenopausal women with EC diagnosis are younger than 40. It is then more malignant form of adenocarcinoma, with worse prognosis [1].

The need for any biomarker for the process of diagnostics, treatment or follow up seems to be doubtful. However in some cases such biomarker could be useful, especially for screening women with high risk of endometrial cancer. It can be helpful to make decision about interventional diagnostic in young, still menstruating women with ambiguous symptoms. Another group of patients, who may benefit from the additional differentiating marker are asymptomatic women with pathological endometrial thickness.

Human epididymis protein 4 (HE4) is a novel tumor marker of growing importance in recent years. According to the available studies its significance begins to be perceived not only in ovarian cancer but also in other malignancies [2, 3].

The aim of the study was to evaluate the significance of HE4 in differential diagnosis of endometrial cancer and benign endometrial pathology.

Materials and methods

Data analysis was performed prospectively, 92 patients were admitted to Department of Obstetrics and Gynecology (Poznań University of Medical Sciences, Poland) due to pathology of endometrium. Serum HE4 was measured before curettage. The study was approved by the Institutional Review Board. The cases were not stratified for any known prognostic factors. The patients distribution in each clinicopathological feature in connection with HE4 level is summarized in table I.

Statistical analysis

For statistical analyses, GraphPad Prism 6 for Windows from GraphPad Software and Microsoft Excel were used. The Mann-Whitney test was used for comparing the studied subgroups. The outcome was recognized as statistically significant when $p<0.05$. All p-values are given for two-sided tests.

Results

The difference of serum HE4 level between benign endometrial pathology and cancer was significant (p=0.000). Based on ROC curve, the HE4 cut-off for identification of patients with endometrial cancer was 58.08 pmol/l, with the sensitivity of 91% and the specificity of 75% (NPV81,8% and PPV87,2%).

Patients with serous histological type had higher HE4 level than those with endometrioid type (p=0.05) and the cut-off value of HE4 was 111,08 pmol/l. (Figure 3). Comparing HE4 in respect of staging was not statistically important. The HE4 difference between G1 and G2 endometrial cancer was not statistically relevant. (p=0.11) But there was a significant difference between G2 and G3, and G1 and G3. (p=0,04 and p=0,008 respectively).

Patients who needed lymphadenectomy had significantly higher HE4 level than those who had no indications for this procedure (FIGO Ia, G1,G2).(p=0,001) Comparing patients who would benefit from lymphadenectomy and those who would not require this procedure, we estimated the cut-off for lymphadenectomy 76,89 pmol/l (sensitivity 76% and specificity 75%). (Figure 5).

Discussion

Currently, HE4 is used in diagnosing of ovarian cancer, due to its significant tissue overexpression and high serum concentrations in ovarian cancer patients [2, 4].

Blood levels of this protein are also elevated in other malignancies like breast or lung cancer, but its expression is low in normal, nonmalignant tissues [3].

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Table I. Patients characteristics.

Characteristics	No of patients	HE4 Mean ± mean error	HE4 min. – max.
Benign endometrial pathology: - polyp - hypertrophy	46 4 42	52.2±3.2	27 – 138.7
Endometrial cancer: - type I - type II	46 36 10	161.9±32.4 144.7±35.5 271.7±91.8	40.15 – 1130 42.5 – 1130 40.15 – 700
Histopathology - Endometrioides - Serosum - Other	 39 3 4	 144.7±35.5 353.1±179.8	 42.5 – 1130 97.6 – 700
Endometrial cancer: - G1 - G2 - G3 - Gx	 17 20 4 5	 90.3±11.7 197.3±60.8 389.2±205.5	 42.5 – 217 53 – 1130 130 – 1000
Endometrial cancer: - FIGO Ia - FIGO Ib - FIGO II - FIGO IIIa - FIGO IV - undetermined FIGO	 15 12 4 6 1 7	 130.7±58.4 105.6±12.6 216±122.2 328.3±162.5 659.2	 40.15 – 1000 61.3 – 217 66 – 700 97.6 - 1130
Limfadenectomy not indicated Limfadenectomy indicated	12 29	72.5±8.2 222.9±52.0	42.5 – 131.6 61.3 – 1130

Although serum levels of HE 4 in endometrial cancer are not as high as in ovarian cancer there is a statistically significant difference between malignant and benign endometrial diseases. Our study revealed the cut-off level of HE4 is 58,08 pmol/l.

In 2008 Moore et al. demonstrated that HE4 diagnoses endometrial type of EC with a 45,5% sensitivity and 95% specifity. He has also suggested the superiority of this marker to CA125 which is useless in distinguishing patients with type I and II of EC, although it correlates with the stage and grade of the disease, depth of myometrial invasion and infiltration of the lymph nodes [5]. Angioli in 2013 took under consideration not only the endometrial type but also other types of EC in his study group. He set a threshold of HE4 at the level of 70 pmol/l, with a 59,4% sensitivity and 100% specificity. In addition, he has shown a growing marker level correlated with the stage of the disease [6]. The study of Liu et al. demonstrated a threshold value of HE4 useful in diagnosing EC, which was 141,5 pmol/l. He has also proved that HE4 is more sensitive in detecting the serous rather than endometrial type of cancer [7]. The cut-off value that could help to distinguish serous and endometrioid type of EC was 111,8 pmol/l presented in our study, and patients with serous type had higher HE4 levels although the differences were not statistically significant.

In 2012 Mutz-Dehbalaie has reported that HE4 protein is an independent prognostic factor for endometrial cancer . The study has shown a correlation between serum HE 4 level and the depth of myometrial invasion and the age of the patient. Although no correspondence with histological subtype, grade or the lymph node infiltration was found, an increase in HE4 serum level in endometrial type of EC was proven to be a prognostic factor of overall survival [8]. Indeed, our study revealed that the HE4 level correlates closely with the tumor grade (G1 vs G3, G2 vs G3.) These results showed a significant correlation of higher serum concentrations of HE4 with a more aggressive type of EC and a poor prognosis.

Moreover, in 2011 Bignotti proved that HE4 is an independent prognostic factor in endometrial cancer, which could aid in adequate planning of the surgical procedure [9]. The marker could help in selecting a group of patients, who will distinctively benefit from performing paraaortic and pelvic lymphadenectomy. In such patients the procedure could improve the prognosis, despite increased risk of post-surgical complications [10].

Lymphadenectomy in endometrial cancer still remains controversial. Clinical trials did not settle the value of this procedure in endometrial cancer in terms of progression free survival and overall survival [11, 12, 13, 14].

The procedure is not recommended in patients with low-risk endometrial cancer. On the other hand, the infiltration of lymphatic space is an absolute indication for postoperative radiotherapy. It is an independent prognostic factor of overall survival of EC patients.

Cancerous invasion of 1/3 of the myometrium is associated with 5% risk of nodal metastases, whereas when the myometrial invasion is more than 1/3-2/3 of myometrium, the risk of metastases to the lymphatic space reaches 23%.

In type II endometrial cancer, paraaortic lymphadenectomy is always recommended and performing a radical surgery leads to a 20% higher survival rate [15, 16].

The best way of selecting patients who would benefit from surgical lymphadenectomy, despite potential complications of this procedure, would bean objective and easy method such as detecting the marker serum level.

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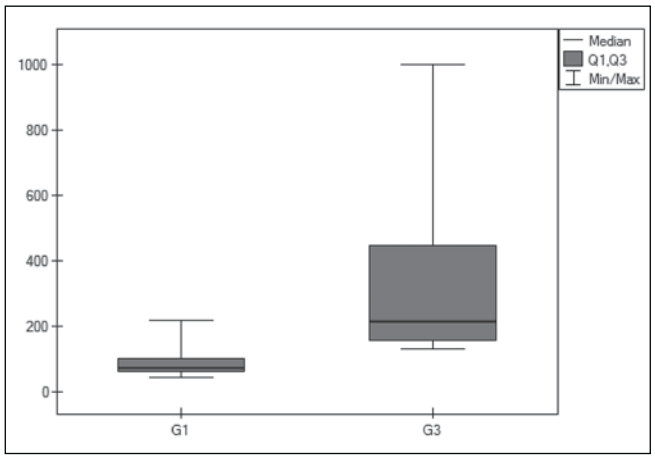

Figure 1. The difference between HE4 in G1 and G3 endometrial cancer was statistically relevant, p=0.008.

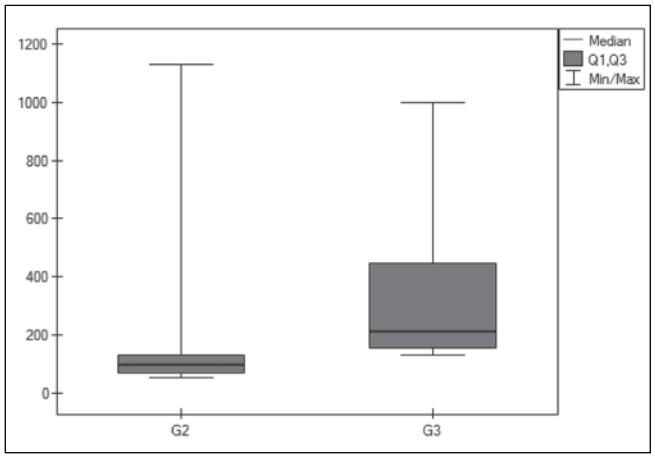

Figure 2. The difference between HE4 in G2 and G3 endometrial cancer was statistically relevant, p=0.04.

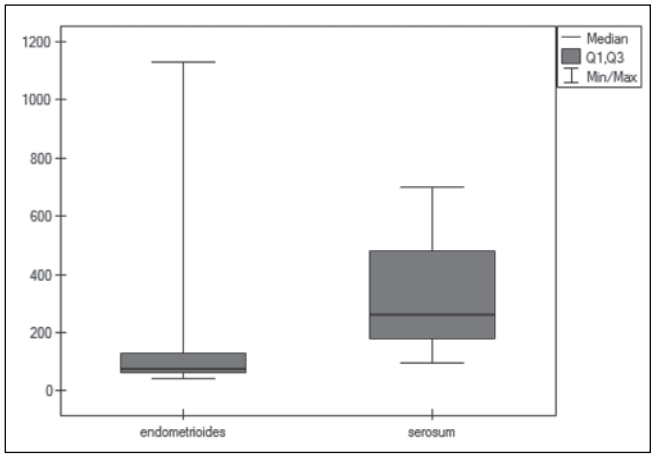

Figure 3. HE4 comparison depending on the histology, p=0.05. (HE4 cut-off value was 111,08 pmol/l).

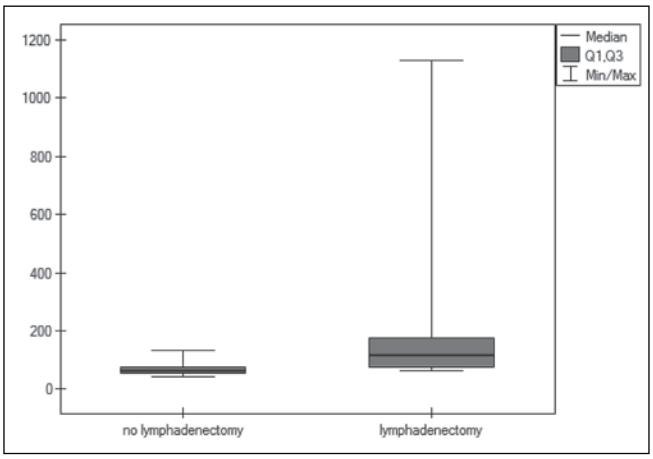

Figure 4. HE4 level and the indications for lymphadenectomy, p=0.001.

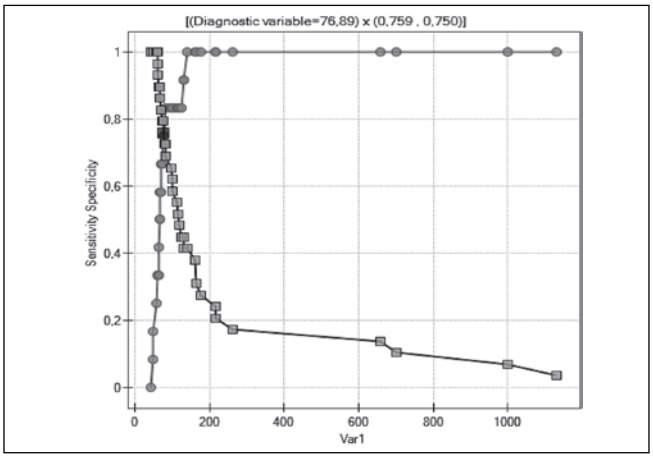

Figure 5. ROC curves for HE4 cut-off for lymphadenectomy.

We set a HE4 serum level for stages of EC in which the paraaortic and pelvic lymphadenectomy should be performed. In our study it was 76,89 pmol/l and it was similar to the level set by Dobrzycka and colleagues- 78 pmol/l [17].

Threshold showed in our study is only a guide value set on a small group of patients. It could serve as a useful preoperative counselling tool in patients without histopathological grading after the curettage to identify women, who may require lymphadenectomy.

The limitation to our study is that there's no analyses of positive results of lymphadenectomy.

The reason for increase of HE4 serum level in malignant diseases (such as ovarian, endometrial, breast, lung, pancreatic cancer or others) remains unknown. Single studies on cell lines revealed the positive influence of this protein on proliferation of cancer cells. It suggests an important role of HE 4 in cancerogenesis. Other studies showed that physiologically, this protein is located in cells of the epithelium and is a part of immune response in inflammatory diseases. Perhaps its contribution in cancerogenesis is different than suspected and

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HE4 overexpression observed in malignant diseases could be a consequence of immune reaction to the presence of cancer cells. It can also be released by the tumor cells to defend it from host's immunology system. Further studies on HE4 in pathological and physiological mechanisms are needed to investigate the role of the protein in carcinogenesis.

Previous studies showed that HE4 is a sensitive marker of malignant process and setting of a reference values for healthy patients could be helpful in detecting pathologies in early stages as well as ongoing malignant diseases.

Conclusions

1. Our findings indicate that HE4 is a useful biomarker in diagnosing endometrial cancer.
2. HE4 is associated with more aggressive G3 endometrial cancer.
3. It can also serve as an useful preoperative counseling tool to identify patients, who may require pelvic and paraaortic lymphadenectomy. The limitation to our study is a relatively small sample size.

Authors' Contribution:

1. Emilia Gąsiorowska – concept, analysis and interpretation of data, collection of the material, article draft, corresponding author.
2. Magdalena Magnowska – collection of the material, statystical analysis, article draft.
3. Natalia Iżycka – translation of the manuscript, collection of the material.
4. Wojciech Warchoł – statistical analysis.
5. Ewa Nowak-Markwitz – obtaining the funds necessary to perform the study, concept, storgae of patient's files, final version and acceptance of the manuscript.

Authors' statement

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