HE4 concentration in women with endometrial cancer

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SUMMARY

Introduction. Endometrial cancer is one of the most common female genital cancers, but it still does not have a valid and reliable diagnostic marker. Doubts concerning the genuine behavior of HE4 in endometrial cancer, which are mentioned in the literature, are the basis for attempts made for their resolution.

Aim of the study. The aim of the study was to evaluate HE4 levels in patients with endometrial cancer and compare the results with those of healthy age-matching women.

Material and methods. The study involved 50 patients with endometrial cancer and 50 healthy controls treated in the Clinical Unit of Gynecology, Obstetrics and Gynecologic Oncology in Bytom, Silesian University of Medicine.

Results. The mean HE4 concentration in healthy women was lower in a statistically significant way compared with women with endometrial cancer. Moreover, HE4 levels in endometrial cancer patients increase with the depth of endometrial infiltration specified after a histopathological examination. The mean concentration of CA125 in healthy women is lower in a statistically significant way compared with women with endometrial cancer.

Conclusions. Endometrial cancer is a potent stimulus inducing HE4 and CA125 synthesis. A marked increase in HE4 expression in women with this disease suggests that this protein can be the searched endometrial cancer marker that strongly correlates with the depth of endometrial infiltration. An HE4 increase in patients with endometrial cancer may be mediated by higher erythrocyte, INR and potassium values. This suggests that HE4 is more valuable as a tumor marker in endometrial cancer patients with no comorbidities.

Key words: HE4; endometrial cancer; tumor marker

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INTRODUCTION

Endometrial cancer is one of the most common malignancies of the female reproductive system in developed countries. Its incidence has been increasing for over 30 years. According to the data of the Ministry of Health, the prevalence per 100,000 women in Poland is 19.3, which places this disease on the fourth place, after breast, lung and ovarian cancers. Endometrial cancer develops mainly in the sixth decade of life. An increase in its incidence is associated with exogenous estrogen use and lifestyle diseases, such as arterial hypertension, diabetes and obesity.

HE4 protein (*human epididymis protein 4*) is a glycoprotein that takes part in numerous physiological and pathological processes in the human organism. Apart from healthy tissues (e.g. kidneys, lungs or thyroid), it is expressed in various pathological cell lines, e.g. ovarian, endometrial, lung and colorectal cancers. However, all authors concur that its greatest expression is observed in epithelial ovarian carcinoma [1]. The recent years have brought reports suggesting that HE4 plays a role in endometrial cancer prediction. Published data indicate a statistically significant increase in HE4 levels with an increase in FIGO grades of this cancer, depending on tumor grade, level of endometrial infiltration and presence of lymph node metastases. Moreover, a positive correlation between pre-operative HE4 levels and FIGO grades has been suggested [2]. HE4 is promising as a pre-operative indicator enabling identification of patients with myometrial infiltration exceeding 50% of its thickness, i.e. patients in whom pelvic lymphadenectomy should be considered [3]. However, the clinical usefulness of HE4 in women with endometrial cancer requires further research. Doubts concerning the actual behavior of HE4 protein in endometrial cancer underlie the attempts made for their resolution. It is still a challenge of medicine to develop a reliable diagnostic tool enabling rapid, non-invasive and certain identification of this cancer and prompt initiation

of therapy. Despite huge progress in the knowledge on this issue, there are still lots of questions without answers, which empowers research in this field.

MAIN AIM

The main aim of the study was to evaluate HE4 levels in patients with endometrial cancer and compare the results with those of healthy age-matching women.

MATERIAL AND METHODS

In total, 100 women were enrolled. The investigated group included 50 patients with endometrial cancer, and there were 50 controls (healthy age-matching women who report to the Clinic of Gynecology and Obstetrics for regular check-ups). All the cancer patients were hospitalized and treated surgically in the Clinical Unit of Gynecology, Obstetrics and Gynecologic Oncology in Bytom, Silesian University of Medicine. The medical history and course of the disease from its diagnosis to the end of treatment in the clinic were analyzed based on medical records for all women from the investigated group. The study excluded women with any chronic diseases beyond the reproductive system (particularly of the kidneys and liver) as well as those who smoked and used alcohol. Also, women reporting irregular and abnormal menstruation in the reproductive period were excluded. Another exclusion criterion was BMI below 18.5 kg^2 or above 30 kg/m^2. The study was approved by the Ethics Committee of the Medical University of Silesia in Katowice (Resolution KNW/0022/KB1/115/13 of 19 November 2013). All patients were exhaustively informed about the aim of the study and all expressed consent to participate in writing. The study was a part of statutory research work carried out in the Department and Clinical Unit of Gynecology, Obstetrics and Gynecologic Oncology in Bytom of the Silesian University of Medicine in Katowice, Poland.

Blood samples of 5 mL were collected from the patients before surgery into a clot tube. In the control group, blood was drawn once only during a routine check-up in the Clinic of Gynecology and Obstetrics. Directly after obtaining a sample, the blood was centrifuged, and the obtained serum was stored in -70°C until testing. Assays were made using an immunoenzymatic method with commercial kits for quantitative HE4 assay in human serum. All tests were performed in the Department of Pharmacology of the School of Medicine with the Division of Dentistry in Zabrze, Silesian University of Medicine, Katowice.

Statistical calculations were made in the Statistica 10.0 (StatSoft Inc., 2011). Results for variables in the nominal scale were presented in frequency tables. As for quantitative variables, basic descriptive statistics were calculated, i.e. mean, median, quartile and standard deviation. The normality of distribution was tested with the Shapiro-Wilk test. Since distributions (including that for the key HE4 parameter) deviated from the normal distribution considerably, hypotheses were verified with nonparametric methods. For intergroup comparisons, the Mann-Whitney U test was used for two comparison groups, the Kruskala-Wallis ANOVA with post-hoc Dunn test for more than two groups or the chi-squared test for qualitative variables. The Wilcoxon matched-pairs test was used for comparisons between parameters measured before and after surgery. The correlation analysis was performed using the Spearman's correlation coefficient. The significance level was á=0.05. The results fulfilling the inequality of $p<0.05$ were considered statistically significant.

RESULTS

Preliminary results are presented in Table 1. and Figures 1–5. The mean age of patients in the group with endometrial cancer was 63.7 ± 9.0 years, and in the control group: 55.3 ± 7.1 years. There were no statistically significant differences between age and subject groups. The

Tab. 1. HE4 [pmol/L] and CA125 [U/mL] levels (median values, mean ± standard deviation) in the patients before surgery and in controls

	Patients N=50	Controls N=50
HE4 [pmol/l]	55,5 [a]	22,5
	75,9 ± 50,2	23,1 ± 5,1
CA125 [U/ml]	42,7 [b]	17,2
	42,7 ± 19,8	17,2 ± 10,5

a) p<0.000001 compared with controls
b) p<0.00009 compared with controls

mean concentration of HE4 protein in healthy women was lower in a statistically significant way compared with women with endometrial cancer (p=0.000000) (Tab. 1.). Moreover, HE4 levels in endometrial cancer patients increase with the depth of endometrial infiltration, specified in a histological examination. Also, CA125 was determined in both patients and controls. The mean concentration of CA125 protein in healthy women (17.2 U/mL) was lower in a statistically significant way compared with women with endometrial cancer (42.7 U/mL; p=0.00009) (Tab. 1.).

Furthermore, factors that could have an impact on HE4 concentration in the studied groups were analyzed. In the group of patients with endometrial cancer, several linear correlations were found between HE4 levels and biochemical parameters, both before and after surgery. These relationships are illustrated in Figures 1–5.
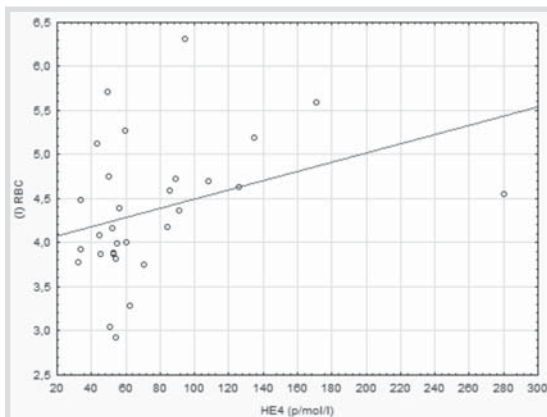

Fig. 1 Relationship between HE4 [pmol/L] in women with endometrial cancer and pre-operative red blood cell count [mg/dL]
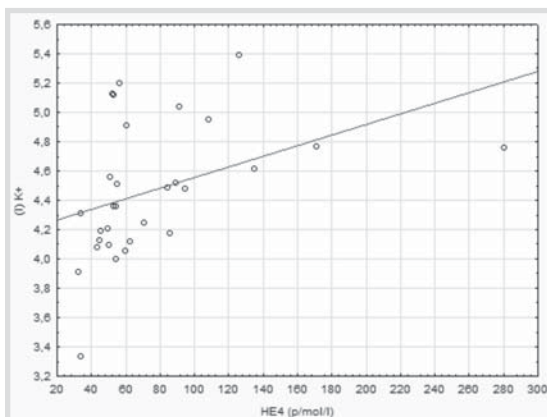

Fig. 2 Relationship between HE4 [pmol/L] in women with endometrial cancer and pre-operative potassium level [mmol/L]

DISCUSSION

Several reports on the usefulness of HE4 in women with endometrial cancer have appeared in the recent years. Despite the fact that this cancer produces symptoms relatively early, which means that it is detected at an early stage, to date no tumor marker specific for endometrial cancer has been introduced to the clinical practice [4]. The literature contains reports on the usefulness of various serum markers for endometrial cancer, such as: CEA, CA72.4, CA19.9, CA15.3, M-CSF as well as CA125, which is also sometimes used for this purpose. Nevertheless, these markers are overexpressed in merely 20–30% patients [5]. Finding of such a marker is of particular significance for patients with a considerable risk of endometrial cancer and in those with additional risk factors, such as extreme obesity, Lynch syndrome or history of tamoxifen therapy for breast cancer. In the remaining patients, a sensitive marker would enable one to monitor supportive treatment and predict recurrences early. In the recent years, it has been proven that HE4 plays a role in endometrial cancer prediction [6,7]. Zanotti et al. [2] published data that indicate a statistically significant increase in HE4 levels with an increase in FIGO grades, depending on tumor grade, level of endometrial infiltration and presence of lymph node metastases, which is also confirmed by other authors [4,5,8–11]. Moreover, Zanotti et al. [2] found a positive correlation between pre-operative HE4 levels and FIGO grades. According to Bignotti et al. [4], assessment of HE4 values in patients with endometrial cancer can be useful as it is a simple, early and highly effective tool to select high-risk
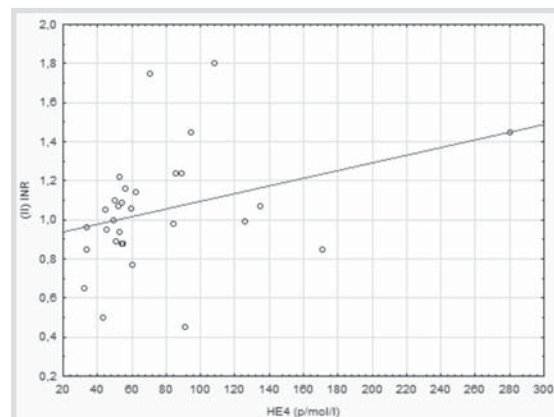

Fig. 3 Relationship between HE4 [pmol/L] in women with endometrial cancer and the difference in post- and pre-operative INR values [mmol/L]

patients in whom conventional treatment can be modified by adding lymphadenectomy or by using an additional therapy, such as radiotherapy field extension or systemic chemotherapy. Our observations are in line with those described above: HE4 is indeed a useful marker in the diagnosis and prediction of endometrial cancer in women. This opinion has been confirmed by Capriglione et al. [3] who see HE4 as a promising pre-operative indicator enabling identification of patients with myometrial infiltration exceeding 50% of its thickness, i.e. patients in whom pelvic lymphadenectomy should be considered. This would help refer patients to appropriate surgical oncology units [3]. Angioli et al. [12], to the fashion of ROMA, proposed the introduction of a REM (risk of endometrial malignancy) algorithm to the clinical practice in patients with endometrial cancer. It would encompass serum assays, ultrasonography and analysis of clinical symptoms. The aim of this new tool would be to identify patients with a high risk of endometrial cancer and refer them to centers that might offer adequate and optimal treatment, thereby improving treatment outcome and reducing therapy cost. The above considerations indicate that HE4 assay in women with endometrial cancer may be useful in preoperative selection of appropriate surgical method even in early stages of the disease [2,3,5,13] and that HE4 is the most appropriate and sensitive endometrial cancer marker identified thus far [8,13,14]. However, the clinical usefulness of HE4 in women with endometrial cancer does require further research even if only to determine the upper limit of normal for this marker in this disease entity [3].

The author's own study has revealed an impact of certain biochemical blood parameters on HE4 levels. A linear positive correlation was found between erythrocyte count and HE4 concentration before surgery (Fig. 1). An analogous relationship was proven for potassium (Fig. 2). It is difficult to interpret these findings since such correlations have not been fully described in the literature thus far. An increase in potassium levels that shows a positive correlation with an increase in HE4 in women with endometrial cancer can be explained by weaker renal filtration in older women [15]. HE4 is a protein with a low molecular mass and is therefore easily excreted by the kidneys. Nagy et al. [16] observed that HE4 levels increase in a statistically significant way and proportionally to a decrease in glomerular filtration rate (GFR) in various stages of chronic kidney disease. Moreover, the impact of renal dysfunction on HE4 levels bas been confirmed by Escudero et al. [17], who strongly suggest that renal and hepatic diseases change its concentrations. Also, Bolstad et al. [18] report that renal dysfunction and associated serum creatinine concentrations in women affect HE4 levels: they increase proportionally to serum creatinine concentrations. Assuming a creatinine level of 50 μmol/L as normal, its increase to 70 μmol/L entails a 12% increase in HE4, and creatinine at the level of 100 μmol/L is associated with an HE4 increase by 27% [18]. Renal failure is currently believed to be one of the main factors causing HE4 increase in women without cancer [17]. This disease affects CA125 to a lesser degree [14], but can be a source of false positive results of other markers, such as squamous cell carcinoma (SCC) antigen, S-100 or ProGRP
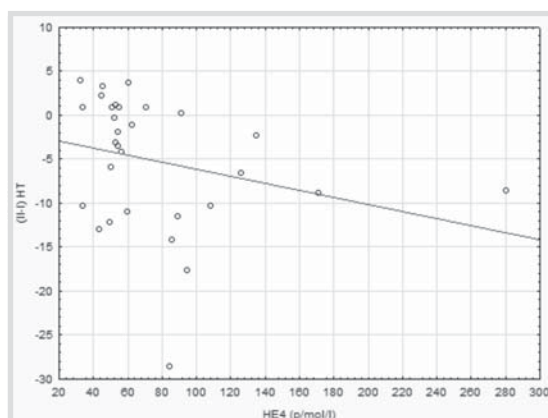

Fig. 4 Relationship between HE4 [pmol/L] in women with endometrial cancer and the difference in post- and pre-operative hematocrit values [mmol/L]
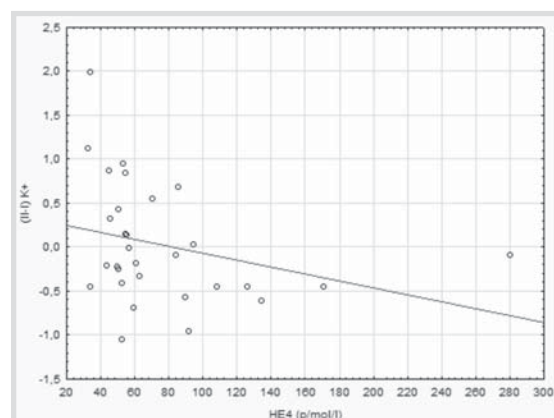

Fig. 5 Relationship between HE4 [pmol/L] in women with endometrial cancer and the difference in post- and pre-operative potassium values [mmol/L]

[17]. The analyzed group did not include patients with renal failure, which was one of the exclusion criteria. It seems, however, that renal filtration can be weaker in postmenopausal patients due to their age, but this observation requires further studies. In women with renal failure, levels of HE4 marker, which is used in the clinical practice in these cases, should be interpreted cautiously, especially when creatinine concentration is abnormal (higher than 115 μmol/L or 1.3 mg/dL) [17]. This is particularly significant when monitoring cancer treatment effects since adjuvant therapy may cause permanent or transient renal dysfunction [19,20].

CONCLUSIONS

1. Endometrial cancer is a potent stimulus inducing HE4 and CA125 synthesis. A marked increase in HE4 expression in women with this disease suggests that this protein can be the searched endometrial cancer marker that is strongly associated with the depth of endometrial infiltration.
2. An HE4 increase in patients with endometrial cancer may be mediated by higher erythrocyte, INR and potassium values. This suggests that HE4 is more valuable as a tumor marker in endometrial cancer patients with no comorbidities.

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