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Research Article

Human Epididymis Protein-4 and Carbohydrate Antigen-125 for Diagnosis of Endometrial Carcinoma

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Abstract

Background: Endometrial carcinoma (EC) is a common genital tumor that frequently causes abnormal uterine bleeding (AUB). All gynecologists aimed to differentiate between malignant and benign causes of AUB using a noninvasive approach. Tumor markers like human epididymis protein-4 (HE4) and carbohydrate antigen-125 (CA125) were employed and assessed in this investigation. ***Objectives***: To quantify HE4 and CA125 levels in AUB patients and assess their significance in distinguishing between benign and malignant endometrial tumors. ***Methods***: This prospective observational case series analysis included 88 patients with AUB who were admitted for surgical surgery in Mosul hospitals between January 1st and September 30th, 2022. Preoperative blood samples were collected to assess HE4 and CA125 levels. After collecting histopathological results, the patients were separated into two groups: malignant (24) and benign (64) that were further divided into pre- and postmenopausal subgroups. The biomarker level was determined and statistically evaluated. ***Results***: All 24 malignant cases, reported among postmenopausal patients, showed statistically significant differences. The mean HE4 level was higher in the postmenopausal than premenopausal benign group. The mean CA125 level was substantially greater in the malignant than in the postmenopausal benign groups. CA125 showed greater validity markers than HE4. There was no significant relationship between HE4 and CA125 concentrations. ***Conclusions***: Measuring CA125 levels in postmenopausal patients with AUB may help differentiate EC in those with endometrial thickness greater than the threshold value.

Keywords: Abnormal uterine bleeding, Carbohydrate antigen-125, Endometrial carcinoma, Endometrial thickness, Human epididymis protein-4.

استخدام بروتين البربخ البشري-4 ومستضد الكربوهيدرات- 125 في تشخيص سرطان بطانة الرحم

الخلاصة

الخلفية: سرطان بطانة الرحم أحد أورام الأعضاء التناسلية الشائعة. نزيف الرحم غير الطبيعي عرضه الشائع و التمييز بين اسبابه الخبيثة عن الحميدة بطرق غير جراحية هو هدف الاطباء. تم استخدام علامات الورم (بروتين البربخ البشري 4 (HE4) ومستضد الكربوهيدرات-125 (CA125)) لهذا الغرض. **الهدف**: قياس مستوى HE4 و CA125 في المريضات اللواتي يعانين من نزيف الرحم غير الطبيعي وتقييم دورهم في التمييز بين الاسباب الحميدة والخبيثة لبطانة الرحم. **الطريقة**: دراسة سلسلة حالات مستقبلية تشمل 88 مريضةً من اللواتي يعانين من نزيف الرحم غير الطبيعي واللواتي خضعن للتداخل الجراحي في مستشفيات الموصل للفترة من 1 يناير إلى 30 سبتمبر 2022 وفقًا لمعايير الاشتمال في الدراسة. تم الحصول على عينات الدم قبل الجراحة لقياس مستويات HE4 وCA125. تم تقسيم المرضى بعد الحصول على نتائج التشريح المرضي إلى مجموعة خبيثة (24) ومجموعة حميدة (64) والتي قسمت إلى مجموعات فرعية قبل وبعد انقطاع الطمث. تم تحليل النتائج إحصائيا. **النتائج**: جميع الأورام الخبيثة سجلت بعد انقطاع الطمث مع وجود فرق إحصائي معنوي. كان متوسط مستوى HE4 أعلى بين المجموعة الحميدة بعد انقطاع الطمث عن قبل انقطاع الطمث. كان متوسط مستوى CA125 أعلى بكثير في المجموعة الخبيثة من المجموعة الحميدة بعد انقطاع الطمث. كانت علامات الصلاحية أعلى بالنسبة لـ CA125 مقارنة بـ HE4. لم يكن هناك ارتباط كبير بين مستويات HE4 وCA125. **الاستنتاج**: يمكن أن يكون لقياس مستوى CA125 دور في تمييز سرطان بطانة الرحم لدى المريضات المصابات بنزيف الرحم غير الطبيعي اللواتي لديهن سمك في بطانة الرحم أعلى من القيمة القطعية بعد انقطاع الطمث.

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INTRODUCTION

Endometrial carcinoma (EC) represents one of the common gynecological cancers. Risk factors have been identified, and most of them are related to long-term exposure to estrogen unopposed by progesterone [1]. The disease is associated with aging [2], and its incidence is rising due to various factors such as obesity, nulliparity, aging populations, early menarche, late menopause, and the use of unopposed estrogen [3]. Early detection of endometrial cancer is possible due to its common presentation of abnormal uterine bleeding (AUB) [4] and the easy access to the endometrium for endometrial biopsy (by curettage or hysteroscopy) with histopathology [5], which is considered the gold

standard method. However, this method is invasive and may not be well tolerated by patients [6]. However, there are cases of EC that occur in asymptomatic patients and are diagnosed late at an advanced stage [7]. Ultrasound is a non-invasive method measuring endometrial thickness, which can be used to decide further investigations. It depends on the cutoff value that is used. For example, in postmenopausal patients, a value of ≥ 4 mm had a sensitivity of 94.8% and a specificity of 46.7%. In premenopausal women, a value of 16.4 mm was reported to be of limited use [2]. In recent years, researchers have applied biomarkers, a non-invasive method with high patient tolerance, to distinguish benign from malignant endometrial conditions [6]. The role of tumor markers in endometrial cancer (EC) is a topic of debate, as there are currently no proven specific tumor markers for use in routine clinical practice for EC diagnosis, monitoring, or prognosis. Therefore, researchers persist in their quest to identify a tumor marker suitable for preoperative diagnosis and staging of EC [7]. Human epididymis protein 4 (HE4) is a whey acidic protein that was first identified in the epithelium of the distal epididymis [8]. Its biological function is unclear, although recent studies have shown that HE4 promoted EC proliferation, invasion, and growth [9]. Although it has been found to be overexpressed in patients with EC, meta-analyses reported that most of the studies of the role of HE4 as an EC biomarker remain controversial [6]. The diagnosis and monitoring of endometrial and ovarian cancer used the glycoprotein known as carbohydrate antigen-125 (CA125) [10]. Therefore, in this study, we measured the levels of HE4 and CA125 preoperatively in patients with AUB and compared them between benign and malignant conditions, thereby reducing the need for invasive methods to exclude malignant pathology.

METHODS

Study design and setting

This prospective observational case series study recruited 88 patients who were admitted to the gynecological department in Mosul hospitals, Iraq, from 1st January to 30th September 2022, with complaints originating from AUB.

Inclusion and exclusion criteria

The study included 88 patients with AUB (heavy menstrual bleeding, irregular menstrual cycles, and postcoital and postmenopausal bleeding) who needed surgical intervention (diagnostic curettage or hysterectomy). All patients were not lactating, had no history of acute infection, and had normal kidney function testing. We excluded patients with malignant disease, non-endometrial benign uterine pathology (uterine fibroid, adenomyosis), hormone therapy use, impaired renal function, liver cirrhosis, and pulmonary disease.

Data collection and outcome measurements

A detailed history, thorough examination, and ultrasound examination were done for all patients. A preoperative blood sample was obtained from all patients for measurement of HE4 and CA125 levels by using "Enzyme-Linked Immunosorbent Assays (ELISA)" and chemiluminescent immunoassay, respectively, using the Human Epididymal Protein 4 (HE4) ELISA Kit, Ylbinot, China, and MAGLUMI® CA 125 (CLIA), Snibe Diagnostic, China. For this study, standard cutoff values of HE4 (70 pmol/L) and CA-125 (35 u/ml), according to the manufacturer's indications and as suggested by other studies [4,7] were chosen. The cutoff value of ET in postmenopausal patients was ≥ 4 mm [11] and in premenopausal patients ≥ 16 mm [2]. After obtaining the histopathological results, the sample of patients was divided into benign and malignant (EC) groups, then subdivided into premenopausal and postmenopausal subgroups.

Ethical consideration

We verbally informed all patients about the study's aim and obtained their written consent to participate. Data were exclusively used for the purpose of this study. The Nineveh Health Directorate granted official approval for the study protocol.

Statistical analysis

Data coding and tabulation were performed via Microsoft Excel 2010 Descriptive and analytic statistics were performed using the Minitab version 18 software statistical program. The descriptive statistics include mean ± standard deviation (SD) for measurable variables and frequencies and percentages for categorical variables. Pearson`s correlation coefficient (r) was estimated between HE4 and CA125. An independent t-test of the two means was used for comparison between mean tumor markers. Furthermore, the Chi-square test was performed for comparison between categorical variables. *p*-values < 0.05 were considered statistically significant throughout the data analysis.

RESULTS

This study included eighty-eight patients who were admitted for surgical intervention due to complaints from AUB. The age of the study-selected patients

ranged from (41–81) years. Moreover, the mean age of patients with malignancy was 62.1±6.13 years, with no significant statistical difference from those with benign conditions (58.1±10.23) years. The mean body mass index (BMI) didn't show significant statistical differences between the two groups. Nulliparous patients constituted 4.6% of cases among both groups, with no statistically significant difference from parous patients. All cases with EC were postmenopause, with a significant statistical difference (p= 0.001) as shown in Table 1.

Table 1: Maternal characteristics of the patients studied

Characteristics	Benign group (n = 64)	Malignant group (n = 24)	All (n = 88)	*p*-value*
Age (years)	58.1±10.23	62.1±6.13	59.2±9.43	0.077
BMI (kg/m2)	32.1±5.65	33.0±6.09	32.3±5.76	0.499
Parity				
Nullipara	2(3.1)	2(8.3)	4(4.6)	0.296
≥ 1	61(95.3)	22(91.7)	84(95.4)	
Menopausal status				
Premenopause	22(34.4)	0(0.0)	22(25.0)	0.001
Postmenopause	42(65.6)	24(100.0)	66(75.0)	

Values are expressed as frequency, percentage and mean±SD. *Independent t-test of two means was used for quantitative variables and Chi-square test for categorical variables.

The malignant group had a higher number of patients with a higher level of CA125, showing a significant statistical difference (p=0.003). However, the HE4 cutoff value between the two groups did not reveal this difference. Table 2 shows that all cases in the malignant group had ET levels higher than the cutoff value, with a significant statistical difference (p=0.005).

Table 2: Validity of tumor markers in the diagnosis of endometrial histopathology in the patients studied

Tumor markers	Cutoff value	Evidence of malignancy by histopathology		*p*-value*
		Absent (Benign)	Present (Malignant]	
HE4 (pmol/l)	≥ 70	26(40.6)	8(33.3)	0.532
	< 70	38(59.4)	16(66.7)	
CA125 (U/ml)	≥ 35	10(15.6)	11(45.8)	0.003
	< 35	54(84.4)	13(54.2)	
ET (mm)	Pre ≥ 16, Post ≥ 4	47(73.4)	24(100)	0.005
	Pre < 16, Post < 4	17(26.6)	0(0.0)	
Total		64(100)	24 (100)	

Values are expressed as frequency and percentage. * Chi-square test was used.

Comparing the mean levels of the studied variables between the malignant group (all post-menopausal) and the benign (pre- and post-menopausal) group revealed a higher HE4 level among the benign post-menopausal subgroup, with a significant statistical difference from benign premenopausal patients (p=0.01). There was a significant statistical difference in CA125 levels between malignant post-menopausal patients and benign post-menopausal patients, with the former having a higher level (p=0.006). Table 3 shows that the malignant group reported higher ET than the others, but it did not reach a significant statistical difference.

Table 3: Comparison of the variables studied among the patients' groups

Tumor markers	Benign group		Malignant group (n. = 24)	*p*-value*
	Premenopause (n = 22)	Postmenopause (n = 42)		
HE4 (pmol/l)	41.2±53.4^{b}	102.0±98.5^{a}	56.2±52.1^{ab}	0.01
CA125 (U/ml)	30.2±25.87^{ab}	20.9±18.45^{b}	43.94±38.83^{a}	0.006
ET (mm)	13.0±6.42^{a}	15.3±9.14^{a}	18.2±6.88 ^{a}	0.089

* One-way ANOVA-test with Tukey's *post hoc* test was applied. Values with different superscripts (a,b) are significantly different (p<0.05).

As shown in Table 4, CA125 reported higher statistical validity (sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV)) than HE4. Table 4 also reported high sensitivity and NPV (100%) but low specificity and PPV with a cutoff value of ET.

Table 4: Validity of tumor markers in the diagnosis of endometrial carcinoma in the patients studied and their standard cutoffs

Tumor markers	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
HE4 (pmol/l)	33.3	59.4	26.0	67.5
CA125 (U/ml)	45.8	84.4	55.7	78.4
ET (mm)	100.0	26.6	37.0	100.0

PPV: Positive Predictive value, NPV: Negative Predictive value.

Table 5 shows that the CA125 did not show a correlation with age, but it did show an indirect, weak, and not significant correlation with HE4.

DISCUSSION

Because of the high number of patients who refuse endometrial sampling in the study area, and the fact that many AUB patients have other health issues that put them at risk during the procedure to obtain an endometrial biopsy, all gynecologists are interested in using tumor markers as a quick and painless way to distinguish between benign and malignant pathology. Researchers are now using HE4 to diagnose ovarian cancer and exploring its use in various other malignant tumors. It is overexpressed in about 90 percent of EC. CA125, another well-established biomarker in the detection of ovarian cancer, has increased its level in EC [8]. As there was growing interest in studying HE4 and CA125 in the diagnosis of EC around the world, this study was conducted to assess their involvement in the diagnosis of EC. The study population ranged in

age from 41 to 81 years, whereas other investigations found ranges of 22 to 78 years [6].

Table 5: Correlation matrix between the studied variables in the patients studied

Parameters	Correlation coefficient*	Age	HE4	CA125
HE4	*r*	0.228	---	---
	p	0.033	---	---
CA125	*r*	0.057	- 0.03	---
	p	0.597	0.779	---
ET (mm)	*r*	- 0.044	0.109	0.171
	p	0.682	0.313	0.112

* Pearson correlation.

Endometrial carcinoma is more common in older patients, so the mean age in this study for malignant tumors was higher than the mean age in the benign group, but there was no significant statistical difference. Patients' mean BMI and parity did not differ significantly, as observed in previous research [12]. Like the results of earlier studies [12,13], all cases of EC in this investigation were among postmenopausal patients; however, this contrasted from the study [4], in which EC was also observed in premenopausal patients. One-third of the patients in the malignant group in this study had a level above the HE4 cutoff value. This was a lower percentage compared to previous studies [7,14], which reported 59.4% and 83.33%, respectively. This could be because the sample sizes were different. Researchers found a level of CA125 above the cutoff value in 45.8% of the malignant group. This was higher than other studies [7,14] that used the same cutoff value and found that 19.8% and 36.67% of the EC group, respectively, had a level of CA125 above the threshold. This could be due to differences in the sample size and study design. In line with prior research [11], all patients in the malignant group had ET levels that were above the threshold value. The study found no significant difference in HE4 levels between benign and malignant endometrial situations. Four meta-analyses [5,8,15,16] reported many differences in this regard. This was the study's flaw when it came to evaluating HE4 in EC diagnosis, and they said that people should wait for the results of the multicenter trial [17]. This study's findings differed from those of previous studies [6,7,10,12,14] in that they saw HE4 as a promising marker for EC diagnosis, which could be related to differences in study sample and methodology. All cases in our study complained of AUB, whereas prior studies [7,10] included healthy patients. This study included both pre- and postmenopausal women, in contrast to the previous study [7], which only included postmenopausal women, and this influenced the amount of HE4, which tends to rise with age [18]. Postmenopausal women's hormones change, which can affect the levels of tumor markers [4]. The benign group in this study also showed that postmenopausal women had higher levels of HE4 than premenopausal women. This investigation excluded additional benign non-endometrial gynecological disorders, whereas previous studies [6] included prolapse, fibroid, and ovarian cyst. Some studies [19,20] separated cases of endometrial hyperplasia from other benign disorders and found higher levels of HE4 in their serum. However, this study included cases of endometrial hyperplasia with other benign endometrial pathologies. Different stages of EC were reported in different studies and HE4 level was higher among advanced stage [7,13,21] and grades [21]. This study found a difference in cutoff values [6], while others found two different cutoff values for pre- and postmenopausal patients [12]. The postmenopausal benign category had a higher level of HE4, showing a significant statistical difference from the premenopausal benign subgroup. This difference is consistent with the findings of the Bolstad *et al*. study [18], which attributed this increase to age rather than tumor. This study reported a greater mean level of CA125 in patients with EC compared to the postmenopausal benign group, a significant statistical difference consistent with other studies' findings [4,7,13] and explained by enhanced synthesis by tumor [4]. Other research [1,14] did not report this difference, which can be attributed to differences in participant numbers and study designs. Although ET was more common in malignant groups than benign subgroups, there was no meaningful statistical difference, like the Abdel-Rahman *et al*. study [11] findings. A large proportion of patients with endometrial hyperplasia in the benign group could explain the small difference. Studies vary in the validity markers (sensitivity, specificity, PPV, and NPV) of the examined biomarkers, potentially due to variations in sample size, measurement methods, cutoff values, study design, and tumor type, stage, and grade [7]. For one or more of the aforementioned factors, this study found lower sensitivity (33.3%) and specificity (59.4%) of HE4 than previous investigations [6], which reported sensitivity (57.35%) and specificity (76.38%). CA125 has a higher sensitivity and specificity (45.8%, 84.4%) for detecting endometrial cancer than HE4. Angioli *et al*. [14] reported a lower number (19.8% and 62.1%), although another study [7] recorded 43.3% and 100%, respectively. HE4 had a higher PPV (26.0%) and NPV (67.5%) than prior studies (72% and 62.58%, respectively). [6]. CA 125 had a PPV (55.7%) and NPV (78.4%) that differed from earlier studies (33.9% and 44.1%) [10], as well as (85.2% and 73.7%) [14]. In this investigation, ET had sensitivity (100%) and specificity (26.6%), in contrast to the Jones *et al*. study [2], which revealed sensitivity of 94.8% and specificity (46.7%). The PPV for ET was 37%, while the NPV was 100%. This is consistent with another study [22] conducted in America, which revealed an NPV of over 99% for excluding EC at a cutoff value of ≥4 mm. Compared to the finding of Antonsen *et al*. [23], there is a significant weak direct link between HE4 and patient age in our study but no significant correlation

between CA125 and HE4. There was no significant association between CA125 and age, which is consistent with prior findings [18,24]. This study's strength was its prospective study of symptomatic cases, which excluded non-endometrial gynecological benign diseases.

Study limitations

The main study's limitation was the designated timeframe for research completion, which restricted the selection of patients, particularly those with malignant cases, and included premenopausal patients.

Conclusion

It is advised to measure CA125 levels in postmenopausal patients with abnormal uterine bleeding and endometrial thickness beyond the threshold value, particularly for those who are unable to have surgery or decline it, as well as in resource-constrained clinical environments.

Conflict of interests

No conflict of interest was declared by the authors.

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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