RESEARCH ARTICLE

Comparison of Diagnostic Accuracies of Serum HE-4 Levels and 3D Power Doppler Angiography Parameters between Benign Endometrial Pathologies and Endometrial Cancer

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Abstract

Purpose: To study the diagnostic accuracies of serum human epididymis protein 4 (HE-4) levels, virtual organ computer-aided analysis (VOCAL) parameters and endometrial volume in endometrial cancer cases. Materials and Methods: One hundred and seven patients (37 with endometrial cancer and 70 with benign endometrial pathology) were included in this study. VOCAL parameters and serum HE-4 levels were compared between the groups. Results: Area under the curve (AUC) values were 0.702, 0.658, 0.706 for vascularization index (VI), the flow index (FI) and the vascularization flow index (VFI), respectively. A cut off value of 0.568 for VI demonstrated 70% sensitivity, 72% specificity, 56% positive predictive value (PPV) and a81% negative predictive value (NPV). A cut off value of 25.8 for showed a senitivith of 70% and a specificity of 58% with aPPV of 46% and NPV of 78%, and with a cut off value of 0.12 for VFI 70%, 69%, 54% and 81%, respectively. The area under the curve for HE-4 was 0.814. A cut off value of 458 pmol/L was predictive of malignancy with 86% sensitivity and 63% specificity. Conclusions: VOCAL parameters and serum HE-4 levels were statistically significantly higher in the endometrial cancer patients. Serum HE-4 levels provided a greater sensitivity compared to power doppler angiography for predicting malignancy or benign endometrial pathology.

Keywords: Endometrial cancer - colour doppler - HE-4 protein - three-dimensional - ultrasonography

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Introduction

Endometrial carcinoma (EC) is the most common gynecologic malignant tumor in the developed countries (Amant et al., 2005). Fortunately, 70% of the cases are diagnosed in the early stage and they have good prognosis (Creutzberg et al., 2000; Smith et al., 2004; Papanikolaou et al., 2006). Most patients are diagnosed at an early stage due to postmenopausal bleeding and approximately 75% of women with endometrial cancer are postmenopausal. Endometrial cancers have been divided into two types; estrogen dependent type-1 and estrogen nondependent type 2 endometrial cancer. Type 2 endometrial cancers are not hormone dependent and they are associated with early spread and worse prognosis. FIGO stage, myometrial invasion, histological type and grade are most important prognostic factors in EC (Amant et al., 2005).

Althoughseveral tumor markers have been studied for EC, there are no markers routinely used in the diagnosis of EC. Among these markers the most extensively researched is Cancer Antigen 125 or Carbohydrate Antigen 125 (CA-125) and Sood et al. (1997) found elevated serum CA125 levels to be a strong predictor of extrauterine disease and mortality (Sood et al., 1997). Atguden et al. (2016) showed predictive value of CA-125 in depth of myometrial invasion (Atguden et al., 2016). However CA-125 values are not useful in the diagnosis of early stage EC. Recent studies focused on human epididymis protein 4 (HE-4) which is known as a novel marker for the diagnosis of ovarian cancer. HE-4 has a better performance than CA-125 in the diagnosis of premenopausal ovarian cancer (Ortiz et al., 2014). A recent systematic review showed that the diagnostic accuracy of HE-4 + CA-125 in differentiating ovarian cancer from other benign gynecologic diseases is better than HE-4 alone (Lin et al., 2012). Furthermore, HE-4 also showed good performance in EC diagnosis even at early stages (Angioli et al., 2014).

In recent years, three-dimensional (3D) ultrasonography and power Doppler angiography (PDA) have become a significant instrument for diagnosis of endometrial pathologies. Endometrial volume (EV) and evaluation of the vascularity of the entire endometrium can be obtained with this technology. Rotational measurement of volume has become possible through the introduction of virtual organ computer-aided analysis (VOCAL™), which is an extension of 3D-VIEW™ software (Kretz Technik, Zipf, Austria) (Raine et al., 2002). Using VOCAL, three vascularity indices can be calculated automatically: the vascularization index (VI), the flow index (FI) and the vascularization flow index (VFI) (Raine-Fenning

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et al., 2002). Objective assessment of vasculature and endometrial volume can be performed with this method even in patients with irregular-shaped endometrium. According to the study of Merce et al. (2007), 3D power Doppler indices (VI, FI, and VFI) were significantly increased in endometrial carcinoma (Mercé et al., 2007). EV and its vascularity evaluated by 3D power Doppler angiography were found superior to endometrial thickness in the diagnosis of EC (Mercé et al., 2007).

There is study comparing VOCAL parameters and serum HE-4 in the prediction of EC. Therefore, we aimed to study the diagnostic accuracies of serum HE-4 levels, VOCAL parameters and endometrial volume in endometrial cancer.

Materials and Methods

One hundred and seven patients (37 endometrial cancer and 70 benign endometrial pathology) with endometrial carcinoma and benign endometrial pathology treated at the Department of Obstetrics and Gynecology, Istanbul University Cerrahpasa Medical Faculty, between 2010 and 2012 were included in this study. The study was approved by the Ethics Committee of Istanbul University Cerrahpasa Medicine Faculty (07.09.2010- No: 32723).

Inclusion criteria were the following for dilation and curettage (D&C) procedure; abnormal uterine bleeding after 40 years of age, postmenopausal endometrial thickness (double layer) greater than 8 mm on transvaginal ultrasound and new onset postmenopausal endometrial bleeding. Patients with other gynecological malignancies or breast cancer were excluded. Blood samples were collected and all patients underwent ultrasonographic examination prior to endometrial curettage or operation. Blood samples were centrifuged at 5000 rpm for 10 minutes and kept at - 80 degree until ELISA measurement. HE-4 levels were calculated in pmol/l using an Quantikine® ELISA Human HE-4/WFDC2 Immunassay kit provided by R&D Systems.

All ultrasonographic examinations were performed by one of the authors (H. E.) with transvaginal probe (5-7.5-MHz) in lithotomy position after emptying the bladder. General Electric Voluson 730 ultrasound machine (GE Healthcare, Milwaukee, WI, USA) was used to measure maximum endometrial thickness (double-layer) with B- mode in longitudinal plane. Power Doppler settings were set to achieve maximum sensitivity to detect low velocity flow (frequency, 5 MHz; power Doppler gain, 0.8; dynamic range, 20-40 dB; edge, 1; persistence, 2; color map, 5; gate, 2; filter, L1; pulse repetition frequency, 0.6 kHz).Vascularized areas within the endometrium with lowest vascular resistance were detected with colour doppler. The 3D volume was activated to obtain a 3D box from the uterus. The volume acquisition interval was 10-15 second. Endometrial volume and 3D- power doppler angiography (3D-PDA) parameters (VI, FI, VFI) were measured by virtual organ computer-aided analysis (VOCAL) software automatically. A rotation step of 15° was choosen and endometrial volume measurement was performed by rotating in the longitudinal plane. We recorded measurements to harddisk driver of ultrasound device. The VOCAL program automatically calculates the EV and 3D power Doppler indices: VI, FI and VFI.

The patients were divided into two groups based on endometrial pathology. Patients were grouped as 70 cases of benign endometrial pathology (group 1) and 37 cases of endometrial cancer (group 2).

Endometrial cancer patients were operated in the division of gynecologic oncology. Total abdominal hysterectomy, bilateral salpingo-oophorectomy and pelvic lymphadenectomy were performed routinely. Para-aortic lymphadenectomy was not performed for patients with histologic grade 1 according to the FIGO 2009 criteria and body mass index greater than 40 kg/m^2. All group 1 patients underwent D&C under general anesthesia. All pathology specimens were evaluated in the Pathology Department of Cerrahpasa Medical Faculty.

Statistical analysis was performed using SPSS 17 software (SPSS, Chicago, IL). Serum CA-125, HE-4 levels and 3D-PDA parameters were compared between group 1 and group 2 using Student's t test. Mann-Whitney U test was used for non-normaly distributed data. Receiver operator curves (ROC) were used to compare the ability of HE-4 with 3D-PDA parameters to identify patients with endometrial carcinoma. Positive and negative predictive value were calculated for HE-4, VI, FI and VFI. A P value of <0.05 was considered to be statistically significant.

Results

During the study period, 70 women who underwent D&C (group 1) and 37 women with diagnosis of endometrial carcinoma (group 2) were evaluated at our center. The mean age was 53 years (range: 40-82 years) for group 1 and 64 years (range: 45-85 years) for group 2. Demographic characteristics and pathology results of group 1 are shown in Table 1. Demographic characteristics and histologic diagnosis of endometrial carcinoma patients are given in Table 2. Mean BMI was 30 kg/m^2 in group 1 and 34.5kg/m^2 in group 2 (p<0.001).

Endometrial thickness (ET), endometrial volume (EV) and resistance index (RI) were compared between benign and malign endometrial pathologies. Even though endometrial thickness was significantly higher in the cancer patients (p<0.05), there was no such difference

Table 1. Demographic Characteristics and Histologic Diagnosis of Patients who Underwent D&C

	(n)	%
Age	53 (40-82)	
BMI	30 kg/m^2	
Symptom		
Menometrorrhagia	(34)	49
Postmenopausalbleeding	(20)	28
İncreased ET	(16)	23
Histologic Diagnosis		
Polyp	(52)	74.2
Atrophy	(4)	5.7
Simple non atypical hyperplasia	(3)	4.3
Chronic non specific endometritis	(1)	1.4
Other*	(10)	14.4

*irregular proliferative endometrium, irregular secretory endometrium

between the groups for endometrial volume; 12.76 Cm^3 versus 15.42 CM^3 p:0.524. RI was lower in cancer patients but the difference was not statistically significant.

Mean VI, FI, VFI levels of benign endometrial pathology and endometrial carcinoma patients are shown in Table 3. Vascularization index (VI), flow index (FI), and vascularization-FI (VFI) were significantly higher in endometrial carcinoma patients.

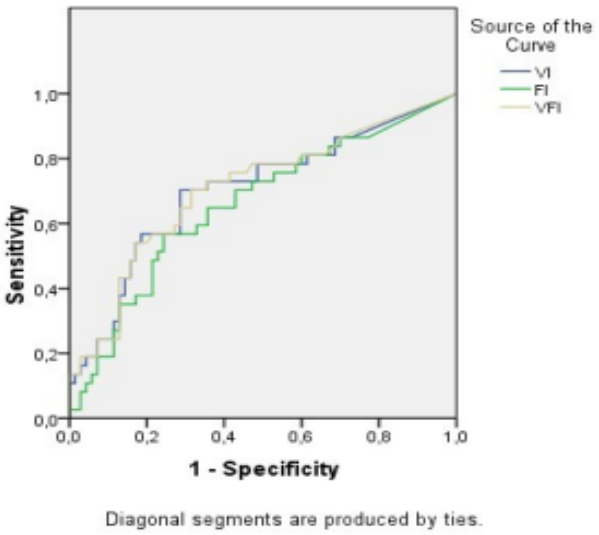

Figure 1. ROC Analysis for VI, FI, and VFI

Table 2. Demographic Characteristics and Histologic Diagnosis of Endometrial Carcinoma Patients

	(n)	%
Age	64(45-85)	
BMI	34,5 kg/m2	
Histologic type		
Endometrioid	(26)	70
Other*	(11)	30
Histologic grade**		
Grade 1	(10)	30
Grade 2	(13)	40
Grade 3	(10)	30
Myometrial invasion		
None	(4)	10
<50%	(17)	46
>50%	(16)	44
Lymph node metastasis‡		
Negative	(28)	85
Positive	(5)	15
Tumor stage†		
1A	(18)	48
1B	(8)	22
2	(3)	8
3	(7)	19
4	(1)	3

*non-endometrioid or malign mixed mullerian tumour; **4 cases with sarcomatoid components were not graded; ‡lymphadenectomy was not performed in four cases with morbid obesity; †FIGO 2009 criteria were considered in staging.

ROC analyses for VI, FI and VFI are shown in Figure 1. Area under the curve (AUC) was 0.702, 0.658, 0.706 for VI, FI and VFI respectively. A cut off value of 0.568 for VI revealed 70% sensitivity, 72% specificity, 56% PPV and 81% NPV. A cut off value of 25,80 for FI showed a senditivity of 70% and a specificity of 58% with a PPV of 46% and NPV of 78%. A cut off value of 0.12 for VFI show sensitivity, specificity. PPV, and NPV of 70%, 69%, 54%, and 81%. RI was lower than 0.4 only in six patients with endometrial cancer. Mean RI value was 0.29.

CA-125 measurement was not performed for benign cases but mean CA-125 value was 46 mIU/ml in the endometrium cancer group. Mean serum HE-4 levels were compared between the groups. Mean serum HE-4 levels were significantly higher in endometrium cancer group; 892 pmol/L versus 467 pmol/L (p<0.001).

A receiver operating characteristic (ROC) curve analysis to determine the serum HE-4 level with the maximal prognostic power in predicting endometrial carcinoma was performed. Area under the curve for HE-4 was 0.814 (Figure 2). The operating characteristic curve revealed that using serum HE-4 levels rather than power doppler angiography provided a greater sensitivity for predicting malignancy or benign endometrial pathology. A cut off value of 458 pmol/L was predictive of malignancy with 86% sensitivity and 63% specificity.

Discussion

Transvaginal ultrasonography is a common diagnostic

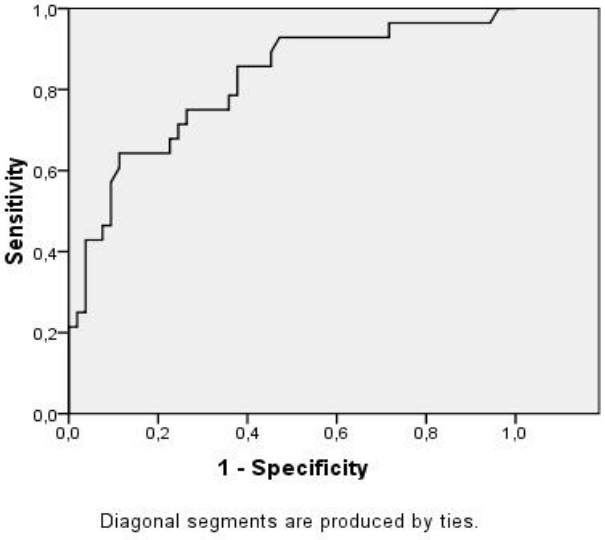

Figure 2. ROC Analysis for HE-4

Table 3. Comparison of VI, FI, VFI Parameters Between Groups

Parameter	VI			FI		VFI	
	(n)	VI(%)	p	FI	p	VFI	p
			0.001**		0.031*		<0.001**
Benign	(70)	0.84		20.47		0.26	
Malign	(30)	3.51		25.85		1.24	

* Student's T test, ** Mann-Whitney U test

tool for patients with premenopousal abnormal uterine bleeding and postmenopousal bleeding. However, gold standard is biopsy and histopathologic evaluation, with the progress in ultrasound technology recent studies aimed to compare the 2D and 3D ultrasonography. We investigated the benefits of 3D ultrasonography and serum HE-4 levels in the diagnosis of endometrial cancer.

In our study, we compared VI, FI, VFI parameters of patients with benign endometrial pathology and endometrial carcinoma. VI, FI and VFI values were found to be statistically significantly higher in the endometrial carcinoma patients. In the study of Alcazar and Galvan, 99 patients with postmenopausal bleeding were evaluated with ultrasonography and VI, VFI values were higher in cancer patients (Alcazar et al., 2009). Mean VI value was 18.97 in the endometrial cancer patients; however, in our study mean VI value was 3.57. In the same study, sensitivity and specificity measurements were not performed; however, diagnostic accuracy of VI was found to be superior to other parameters for endometrial carcinoma. Area under the curve was 0.90 for VI (Alcazar et al., 2009). Opolskiene et al. (2010) evaluated 75 patients with postmenopausal bleeding and they found no advantage of volume and VOCAL parameters over endometrial thickness in the diagnosis of endometrial carcinoma but VOCAL parameters was statistically significantly different between malign and benign lesions (Opolskiene et al., 2010). Area under the curve was 0.82 for VI. Using the cut off value of 5%, the overall sensitivity and specificity for endometrial carcinoma of VI was 69% and 84% respectively (Opolskiene et al., 2010). Makled et al. (2013) reported that VI have better diagnostic performance for endometrial carcinoma compared to endometrial thickness and endometrial volume in patients with postmenopausal bleeding (Makled et al., 2013). Odeh et al. (2007) compared VOCAL parameters between 90 patients with benign endometrial pathology, 18 patients hyperplasia and 11 patients with carcinoma. Mean VI value was found 2.95 for hyperlasia and carcinoma together (Odeh et al., 2007). In our study, mean VI value was 3.51 for carcinoma and the difference can be related with less vascularity of hyperplasia than carcinoma. Odeh et al. (2007) showed in their study that, area under the curve for VI was 0.62 and using the cut-off value of 0.055, the sensitivity and specificity of VI for endometrial carcinoma was 89% and 33% respectively (Odeh et al., 2007). In our study, area under the curve for VI was found to be 0.70 and when a cut-off value of 0.568 was taken, sensitivity and specificity were found to be 70% and 72% respectively. In a recent study, 38 patients with benign endometrial pathology were compared with 10 endometrial carcinoma patients. Mean VI was 13.7 for endometrial carcinoma and area under the curve was 0.78. Cut-off value of 6.8 provided 77% sensitivity and 82% specificity for endometrial carcinoma (Rossi et al., 2012). There are very different results of mean values among these different studies. Measurement techniques, ultrasonography device and examination after biopsy can affect the results. Further studies are needed to prove that 3D parameters are superior to conventional B mode ultrasonography in the diagnosis of malign endometrial pathology.

Moreover, we also measured serum HE-4 levels in the same groups and compared diagnostic accuracy of HE-4 with VOCAL parameters. Serum HE-4 levels were significantly higher in endometrial carcinoma patients. Moore et al. (2012) showed better specificity than CA-125 for HE-4 (Moore et al., 2012). In another study of Moore et al. (2008), 171 endometrial carcinoma patients were compared with control group and area under the curve for HE-4 was 0.787 at specificity of 95%, serum HE-4 levels had a sensitivity of 45.5% whereas ca-125 had sensitivity of 24.6 (Moore et al., 2008). In our study, HE-4 showed a 43% sensitivity at a specificity of 95%. Area under the curve for HE-4 was 0.814. Our results demonstrated concordancy with previous study. Moore et al. observed an increase in sensitivity by combining CA-125 and HE-4 (Moore et al., 2008). Area under the curve was 0.831 for HE-4 levels in predicting endometrial carcinoma in a recent meta-analysis (Bie et al., 2014). According to Minar et al. (2015), serum HE-4 level is a better predictor in distinguishing the high-risk patients (absence of at least one of the following factors; myometrial invasion <50%, tumor diameter <2 cm, no cervical stromal involvement, tumor grade 1 or 2, and the absence of extrauterine disease) than CA125 (Minar et al., 2015). In another study, serum HE-4 levels were significantly higher in recurrent endometrial carcinoma. A HE-4 level of 70 pmol/L was associated with a sensitivity of 84%, a specificity of 74% and a negative predictive value of 93% in detecting recurrent endometrial carcinoma (Brennan et al., 2015). HE-4 was superior to CA-125 levels in the detecting recurrent endometrial carcinoma (Brennan et al., 2015). Therefore, the role of HE-4 levels in endometrial carcinoma follow-up and diagnose of high risk group of endometrial carcinoma patients should be investigated in further studies.

To date there are no studies comparing three dimensional power doppler angiography parameters and serum HE-4 in the diagnose of endometrial cancer. We used both important diagnostic tools in endometrial carcinoma cases and compared them with the benign endometrial pathologies. In our study serum HE-4 levels showed better distinction than VOCAL parameters between benign and malign cases, however future studies are needed to confirm the diagnostic role of serum HE-4 levels in detecting endometrial carcinoma.

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