ORIGINAL ARTICLE

HE4 AS A BIOMARKER FOR DIAGNOSIS OF MALIGNANT OVARIAN MASSES IN BANGLADESHI POPULATION

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ABSTRACT

Ovarian cancer is one of the commonest cancers in gynecology with very high fatality to case ratio. In approximately 70% of all cases of ovarian cancers, the disease is not diagnosed before reaching an advanced stage. Thus early diagnosis of ovarian malignant tumor becomes a key factor in improving the survival rate of patients. This study was aimed to evaluate HE4 (Human Epididymis 4) biomarker for the diagnosis of ovarian malignancy in admitted patients with pelvic mass of ovarian origin scheduled for surgery from Obstetrics and Gynecology department of different medical colleges of Dhaka city and Bangabandhu Sheikh Mujib Medical University (BSMMU). It was a cross-sectional analytical study carried out by non-probability sampling. For this study, 110 admitted patients with ovarian tumor scheduled for surgery were selected.Purpose and procedure of the study was explained in details and informed written consent was taken from all the study subjects before collection of blood sample. Clinical information was taken from the patients' hospital notes. Before surgery serum HE4 was measured and after surgery, histopathology reports were collected for each patient.Depending on histopathology reports, patients were categorized as benign and malignant. Diagnostic efficacy of HE4 was evaluated with respect to sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and likelihood ratios considering histopathology report as gold standard. To evaluate the performance of HE4 based on optimal cut-off (34.3 pmol/L), we found better sensitivity, NPV and satisfactory likelihood ratios but low specificity and PPV compared to suggested cut-off. The findings of this study shows that HE4 is not a useful biomarker to help in the assessment and management of patients with suspected ovarian cancer.

Key words: HE4, Ovarian tumor, Biomarker, Screening tool

Introduction

Ovarian cancer includes cancers of the ovary, fallopian tubes, and peritoneum due to their origination from similar tissue types and similar clinical management and treatment[1]. Ovarian cancer is the fifth most common cause of cancer death in women[2] and mortality is strongly related to disease stage[3]. It is due to less effective screening for this preventable and curable cancer. Early diagnosis is very important for decreasing mortality, performing satisfying surgery, increasing the patients' quality of life, and minimizing treatment costs in ovarian cancer[4]. Given the poor prognosis for patients with advanced stage disease, effective

screening modalities are needed to identify patients with early stage disease[5]. This has led to efforts over the past two decades to develop early detection strategies using serum CA125 and ultrasound[6].

At present, no screening techniques are recommended for early detection of ovarian cancer in the general population. HE4 (Human Epididymis 4) is a precursor of the protein It is encoded by a gene located in chromosome 20q. HE4 is frequently overexpressed in ovarian cancers[3]. Though CA125 is the present "gold standard" for diagnosis of ovarian carcinoma using serum samples, it is elevated in several nonmalignant conditions, which can lead to false-positive results whereas the specificity and sensitivity of HE4 shows promise as a serum marker for ovarian cancer in the early detection process[7].

Currently, FDA has approved the use of HE4 as a tumor marker for monitoring relapse or progression of epithelial ovarian carcinoma[8]. Human epididymis protein 4 (HE4) has received much attention recently due to its diagnostic and prognostic abilities for epithelial ovarian cancer. Since its inclusion in the Risk of Ovarian Malignancy Algorithm (ROMA), studies have focused on its functional effects in ovarian cancer[9]. Therefore, this study was designed and expected to help in early differential diagnosis of patients with pelvic mass of ovarian origin to help in better management of these patients and thereby to reduce the morbidity and mortality of ovarian malignancy.

Materials and Methods

After getting ethical clearance from Institutional Ethical Clearance Committee, this cross-sectional analytical study was performed from March 2018 to February 2019. Women with a pelvic mass of suspected ovarian origin documented by USG and scheduled for surgical intervention were included and women with a previous bilateral oophorectomy and pregnancy were excluded. Finally, one hundred and ten admitted patients with ovarian tumors scheduled for surgery were selected by non-probability purposive sampling technique from Department of Obstetrics and Gynecology of BSMMU and different medical colleges of Dhaka city. Purpose and procedure of the study were explained in details and informed written consent was taken from all the study subjects before collection of blood sample. Clinical information was taken from the patients' hospital notes. Before surgery, serum HE4 was measured and after surgery, histopathology reports were collected from each patient. Depending on histopathology reports, patients were categorized as having benign and malignant ovarian mass. Serum HE4 assays were done in the department of Biochemistry and Molecular Biology, BSMMU, Dhaka by a two-step immunoassay using the Architect i2000 SR Immunoassay Analyzer (Abbott Laboratories, Illinois, US), which uses chemiluminescence microparticle immunoassay technology. All manufacturer recommendation for maintenance, calibration, and internal quality assessment were followed for the assay.

Results were expressed as median for quantitative data and as absolute or relative frequencies for qualitative data and p-value ≤ 0.05 was considered as statistically significant. Evaluation of HE4 for diagnosis of ovarian malignancy was done with respect to sensitivity, specificity, positive predictive value, negative predictive value and likelihood ratios, considering histopathology report as gold standard.

Results

In this study, benign ovarian tumors and malignant ovarian tumors were 67 (61.0%) and 43 (39.0%) respectively. HE4 concentration was found significantly higher in malignant ovarian

tumor cases compared to benign ovarian tumor cases (Table I). Figure 1 reveals that AUC of HE4 for diagnosing ovarian cancer was 0.821 and optimal cut-off was 34.3 pmol/L determined by Youden Index. Using suggested cut-off (>140pmol/L), HE4 showed higher frequency for benign tumors than malignant tumors with high interval (Table II) than using optimal cut-off determined by Youden Index (>34.3pmol/L) (TableIII). Based on that optimal cut-off (34.3pml/L), we found better sensitivity, NPV and satisfactory likelihood ratios but low specificity and PPV compared to suggested cut-off (Table IV).

Table I: Distribution of study subjects depending on histopathology report (N = 110)

Variables	Benign ovarian tumor	Malignant ovarian tumor	P value
Number (%)	67 (61.0%)	43 (39.0%)	
Serum HE4 (pmol/L)[a]	24.7	55.2	0.00[b]

Comparison[b] was done by Mann-Whitney U test

TableII: Distribution of subjects according to HE4 biomarker using suggested cut-off point and histopathology report

HE4 (pmol/L)	Histopathology (Gold standard)		Total(%)
	Malignant	Benign	
Malignant (>140)	17	2	19(18)
Benign (<140)	24	67	91 (82)
Total	41	69	110

Table III: Distribution of ovarian malignancy according to HE4 biomarker using optimal cut-off point (determined by Youden Index) and histopathology report

HE4 (pmol/L)	Histopathology (Gold standard)		Total (%)
	Malignant	Benign	
Malignant (>34.3)	32	19	51 (46)
Benign (<34.3)	11	48	59 (54)
Total	43	67	110

Table IV: Performance of HE4 in suggested and optimal cut-off points for diagnosis of ovarian cancer

Biomarker	Cut-off	Performance					
		Sensitivity	Specificity	PPV	NPV	LR +	LR -
HE4 (pmol/L)	Suggested >140	41.5	97.1	89.5	73.6	14.3	0.6
	Optimal >34.3	74.4	71.6	62.7	81.3	2.6	0.36

Diagnostic efficacy in terms of sensitivity, specificity, PPV, NPV and likelihood ratios

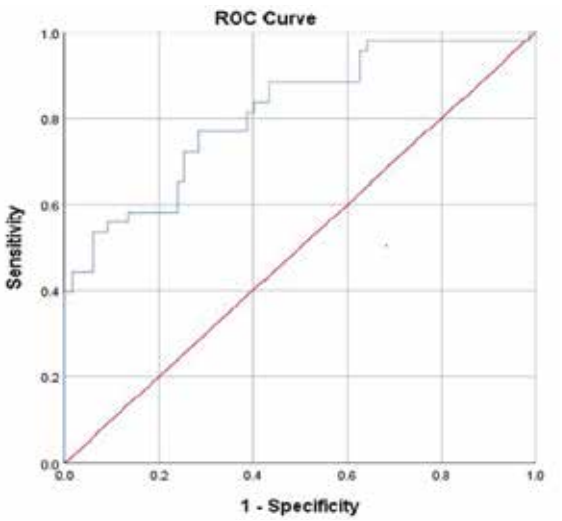

Fig 1. *ROC curve of HE4 as a biomarker for ovarian cancer*
Area under curve (AUC) = 0.812; p value =0.00
Optimal cut-off Point = 34.3
(Determined by Youdan Index)

Discussion

This cross-sectional analytical study was aimed to evaluate the efficacy of Human Epididymis 4 (HE4) protein in diagnosing malignant ovarian masses.We recruited 112 patients with pelvic mass of suspected ovarian origin documented by USG and scheduled for surgical intervention in our study. Two patients of them died before surgery, so finally we enrolled 110 patients. Among them benign ovarian tumor and malignant ovarian tumor were 67(61.0%) and 43(39.0%) respectively and HE4 concentration was found significantly higher in malignant ovarian tumor group compared to benign ovarian tumor group (Table I).Abdel-Azeez et al.also found that HE4 was significantly increased in malignant compared to benign cases and healthy subjects, and in benign cases compared to healthy subjects ($p < 0.001$)[10].

A diagnostic test with high sensitivity and high NPV is useful for screening and exclude disease, whereas a diagnostic test with high specificity and high PPV is useful to confirm diagnosis[11]. Different types of tumor markers have certain specificity and sensitivity and they are important for tumor diagnosis, assessment, prognosis, and recurrence and metastasis prediction[12].

Therefore, we evaluated the performance of HE4 biomarker for diagnosis of ovarian masses in our patients.To evaluate the performance of HE4 as an early detection biomarker, we calculated optimal cut-off point of HE4 on the basis of Youden Index and found 34.3 pmol/L as the optimal cut-off for HE4 (Figure 1) which was lower than the suggested cut-off value of 140 pmol/L. Based on that optimal cut-off (34.3pmol/L); we found better sensitivity, NPV and satisfactory likelihood ratios but low specificity and PPV compared to suggested cutoff (Table IV). From the view point of sensitivity and NPV; using optimal cut-off for HE4 found to be satisfactory to minimize the false negative results and could be used as an effective screening tool for ovarian cancer. This finding was in close agreement with Dolgun et al at 2017 where they proposed that by lowering the cut-off point at 25 pmol/L for serum HE4 level improves sensitivity, NPV and positive likelihood ratio to perform as a diagnostic test for confirming ovarian cancer[4]. Hellstrom and Hellstromat 2008 found the specificity and sensitivity of HE4 promising as a serum marker for ovarian cancer in the early detection process[7]. In contrast with that, Yanaranop et al at 2017 reported a specificity of 86% for HE4 at suggested cutoff point but sensitivity was low compared to that[13].

So, at the optimal cut-off point, HE4 biomarker found to perform better as a screening tool for diagnosis of ovarian cancer. Huy et al 2018 proposed HE4 as a novel and effective marker for diagnosis of ovarian cancer at a reduced cut-off point (55.4 pmol/L) compared to standard cut-off[14].

Limitation of our study is that we had limited sample of patients with ovarian mass which is not truly representative.

The findings of this study shows that HE4 is not a useful biomarker to help in the assessment and management of patients with suspected ovarian cancer. We like to suggest further comprehensive studies in this regards.

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