Gynecologic Oncology 143 (2016) 128–134

Contents lists available at ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

Diagnostic performance and establishment of reference limits of HE4 in Korean healthy women

Shinae Yu [a], Jin Kyung Lee [b], Jea-Hoon Kim [c], Hyosoon Park [a], Mi Yeon Lee [d], Seungho Ryu [e,f,g], Min-Jung Kwon [a,e,*], Hee-Yeon Woo [a,*]

[a] Department of Laboratory Medicine, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea
[b] KIRAMS Radiation Biobank, Korea Institute of Radiological and Medical Sciences, Seoul, Republic of Korea
[c] Department of Obstetrics and Gynecology, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Republic of Korea
[d] Department of Biostatistics, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea
[e] Center for Cohort Studies, Total Healthcare Center, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea
[f] Department of Occupational and Environmental Medicine, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea
[g] Department of Health Sciences and Technology, Samsung Advanced Institute for Health Sciences and Technology, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea

HIGHLIGHTS

- HE4 levels increase over 50 year-old and influence by age, not by menopausal status.
- Reference limit of HE4 is different by racial or regional difference.
- HE4 is less affected by benign conditions than CA125 in patients with ovary mass.

ARTICLE INFO

Article history:
Received 5 January 2016
Received in revised form 27 June 2016
Accepted 10 July 2016
Available online 15 July 2016

Keywords:
Human epididymis protein 4
CA125
Risk of ovarian malignancy algorithm
Ovarian cancer
Benign ovarian tumor

ABSTRACT

Objective. We aimed to establish distribution and reference limits of HE4 and risk of ovarian malignancy algorithm (ROMA) in healthy Korean women and investigated the factors influencing HE4 levels. We also investigated the diagnostic performances of HE4 and ROMA score, compared with CA125.

Methods. We collected specimens from 1809 healthy Korean women, 140 specimens from patients with ovarian cancers (OCs) and 123 specimens from patients with benign ovarian tumor. Serum HE4 and CA125 concentrations were measured using an electrochemiluminescence immunoassay. The receiver operator characteristic (ROC) curve analysis was done for ROMA, HE4, CA125 and combining of HE4 and CA125.

Results. HE4 level was influenced by age, not by menopausal status. The 97.5th percentile upper reference limit of HE4 of subjects <50 years and ≥50 year-old was 63.87 pmol/L and 88.28 pmol/L, respectively. The 97.5th percentile upper reference limits of ROMA score were 13.66 in premenopausal and 19.30 in postmenopausal women. The serum HE4 level was even lower in the patients with benign tumor compared to those in healthy controls. HE4 had significantly higher concentrations in OCs than benign ovarian tumor ($P < 0.001$). ROMA and HE4 combined with CA125 or not performed better diagnostically than CA125 alone for distinguishing OCs, with AUCs of 0.844 for ROMA, 0.827 for combining of HE4 and CA125, 0.825 for HE4, and 0.795 for CA125.

Conclusions. The reference limit of HE4 was different from those reported by other studies, suggesting racial or regional difference. HE4 and ROMA were better than CA125 for differentiation normal and benign ovarian tumor from OCs. (Word count: 253)

1. Introduction

Ovarian cancer (OC) is one of the most lethal malignant diseases. More than 200,000 female patients undergo exploratory laparotomy for a differential diagnosis of pelvic mass in the United States [1,2]. A total number of 21,980 new OC patients and 14,270 OC deaths, ranking fifth in causes of cancer-related death in women in the United States,

* Corresponding authors at: Department of Laboratory Medicine, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, 29, Saemunan-ro, Jongno-gu, Seoul 110-746, Republic of Korea.
E-mail addresses: mjkkmd@gmail.com (M.-J. Kwon), drwhy@hanmail.net (H.-Y. Woo).

http://dx.doi.org/10.1016/j.ygyno.2016.07.100
0090-8258/

occurred in 2014 [3]. Maximal cytoreduction with staging work up should be performed in case of OC, while benign ovarian tumor is simply removed by a general gynecologist or surgeon [4]. The majority (about 66%) of OCs is recognized in advanced stages, since symptoms are not initially apparent, or symptoms are nonspecific and similar to those of gynecological benign diseases at early stages [5,6]. The discrepancy between survival rates of 80%–90% in early stage and 15%–20% in advanced stages has reinforced the need for biomarkers with higher diagnostic accuracy to set up screening and to distinguish malignancy from benign pelvic mass early [7,8].

Serum carbohydrate antigen 125 (CA125) is the most widely used biomarker for detecting and therapy response monitoring for OCs [9]. However, CA125 is associated with a high false-positive rate among premenopausal women with benign gynecological conditions such as ovarian cysts, endometriosis and pregnancy, is not increased in up to 20% of OCs and shows wide range of sensitivity from 27% to 66% for identifying early stage OCs [10].

There have been attempts to find other biomarkers that complement or replace CA125 to detect early stage OCs. Human epididymis protein 4 (HE4) has been identified as a potential serum biomarker in the diagnosis of a pelvic mass [11]. HE4 is frequently overexpressed in OCs, especially in OCs with serous and endometrioid histologic types [12,13]. Recent guidelines based on a meta-analysis have suggested HE4 to be used as an aid in OCs diagnosis [14]. However, the information about serum HE4 levels in healthy population is still very limited [15–17]. In addition, there are several multivariate index algorithms developed in combination with CA125, such as risk of malignancy index (RMI); ultrasound and menopausal status, OVA1; apolipoprotein A1, β2-microglobulin transferrin and transthyretin [18], and risk of ovarian malignancy algorithm (ROMA); HE4 and menopausal status [4,19]. The ROMA was developed and validated by Moore et al. in 383 women diagnosed with benign disease and 89 women with malignancy, and provided 94% sensitivity and 75% specificity overall [20]. This result showed that ROMA was more sensitive than RMI [21–23] and more specific than OVA1 [24,25]. ROMA showed higher ROC-AUC values than RMI (0.741 vs. 0.688) in 50 patients, including 16 OCs [26], and had greater specificity than OVA1 (83% vs. 55%) in 146 patients with a total of 31 OCs [27].

In this study, we aimed to establish our own reference limits of HE4 by age and of the ROMA by menopausal status, and to investigate the factors which could influence serum HE4 levels in healthy Korean women. We compared our own reference limits with those of other studies. In addition, we investigated the cut-offs of HE4 and ROMA for distinguishing between OCs and benign ovarian tumor, and compared their clinical performances.

2. Materials and methods

2.1. Reference subjects

A total of 1809 healthy women, aged 18 to 83 years (mean, 49.6 years), who underwent a comprehensive examination at our institute from January through March 2013 were enrolled. After antecubital venipuncture for 10 mL of whole blood using a serum separator tube (SST), serum was separated within 30 min at room temperature after arrival at laboratory of Kangbuk Samsung Hospital Total Health Care Center and CA125 were measured and then stored at −70 °C until assay of HE4.

The subjects were classified into five groups according to age; <30 years (n = 126), 30–39 years (n = 238), 40–49 years (n = 513), 50–59 years (n = 575) and ≥60 years (n = 357). The upper reference limits of 95th, 97.5th and 99th percentile of HE4 were analyzed according to the Clinical and Laboratory Standards Statistical analysis Institute (CLSI) document C28-A2 [28]. Based on retrospective review of a questionnaire sent to the subjects two weeks before blood was drawn, we examined menopausal status, female hormonal therapy, smoking and body mass index (BMI). Number of reference subjects according to the investigating factors is described in Supplemental Table S1. This study was approved by the institutional review board of Kangbuk Samsung Hospital (KBC14058).

2.2. Patients

A total of 272 serum samples from patients diagnosed with OC or benign ovarian tumors and histologically confirmed by two or more expert pathologists were examined. The 140 samples from OC patients and 123 samples from the patients with benign ovarian tumor were provided by the Korea Gynecologic Cancer Bank through Bio & Medical Technology Development Program of the Ministry of Education, Science and Technology, Korea. Nine samples from OC patients were distributed by the Korea Institute of Radiological and Medical Sciences (KIRAMS) Radiation Biobank (KRB). Venous blood was collected from January 2004 to December 2013 by using sterile 10 mL SST before beginning tumor therapy. Samples were centrifuged at 3000 rpm for 10 min at room temperature within 30 min of collection; serum was collected and immediately stored at (−70 °C). In 272 patients, serum levels of HE4 and CA125 were measured concurrently in our laboratory. We collected information of menopausal status of the patients except one patient aged 50 with serous EOC. Number of patients and their histological types of OCs and benign ovarian tumors are described in Table 1.

Table 1
Histological types of ovarian tumor in patients.

Benign ovarian tumor (n = 123)	Number (%)	Ovarian cancer (n = 149)	Number (%)
Mature teratoma	29 (23.6)	Epithelial ovarian cancer (EOC)	
Endometrioma	26 (21.1)	Serous	74 (49.7)
Mucinous cystadenoma	26 (21.1)	Mucinous	12 (8.1)
Hemorrhagic corpus luteal cyst/corpus luteal cyst	13 (10.6)	Clear cell	11 (7.4)
Serous cystadenoma	10 (8.1)	Endometrioid	8 (5.4)
Follicular cyst/simple cyst	4 (3.3)	Transitional cell	3 (2.0)
Struma ovarii	4 (3.3)	Brenner	2 (1.3)
Endometrioid cystadenoma	3 (2.4)	Mixed (serous + mucinous)	1 (0.7)
Fibrothecoma	3 (2.4)	Borderline tumor	
Fibrothecoma + endometriosis	1 (0.8)	Mucinous	19 (12.8)
Benign inclusion cyst	1 (0.8)	Serous	7 (4.7)
Brenner tumor, benign	1 (0.8)	Brenner	1 (0.7)
Mature teratoma + hemorrhagic corpus luteal cyst	1 (0.8)	Non-EOC	
Luteinized thecoma	1 (0.8)	Yolk sac tumor	3 (2.0)
		Immature teratoma	3 (2.0)
		Sertoli-Leydig cell tumor	1 (0.7)
		Granulosa cell tumor	1 (0.7)
		Malignant mixed mesodermal tumor	1 (0.7)
		Metastatic adenocarcinoma	2 (1.3)