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JCIMCR OPEN ACCESS Journal of Clinical Images and Medical Case Reports

ISSN 2766-7820

Research Article

Open Access, Volume 3

HE4 elevated levels correlated with breast cancer progression

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Received: Jan 29, 2022

Accepted: Feb 24, 2022

Published: Mar 03, 2022

Archived: www.jcimcr.org

DOI: www.doi.org/10.52768/2766-7820/1707

Abstract

Background: Despite several, there were many Breast Cancer (BC)-related deaths around the world. Searching for new analytical tools for BC detection at early stages is widely demanded.

Aim: We aimed to evaluate Human Epididymal Protein 4 (HE4) clinical value in BC development and progression.

Methods: HE4 serum levels were determined by ELISA in females with BC (n=120), benign breast disease (n=40) and healthy women (n=40). Receiver operating characteristic curve was applied for evaluation HE4 diagnostic power.

Results: In BC patients, HE4 [5 (2-11.9) vs. 3.1 (1.8-5.4) and 1 (1-3.5); P=0.022] was significantly higher than that in benign and healthy females, respectively. Compared to CEA and CA-15.3, it had superior diagnostic ability (AUC=0.783; P<0.0001; 73.3% sensitivity; 63.7% specificity). This ability increase when comparing BC patients to only healthy controls (AUC=0.862; 73.3% sensitivity; 77.5% specificity). Elevated HE4 levels were associated disease progression including multiple lesions, late stages, high grades, large size, lymph node invasion and non-luminal molecular subtypes. Spearman correlation analysis revealed that HE4 high levels significantly (P<0.05) correlated with tumor stage, histological grade and tumor size.

Conclusions: HE4 is inexpensive, rapid, easy to perform and reliably BC biomarker. Moreover, its association with disease severity may support its potential role as prognostic BC marker.

Keywords: breast cancer; detection; HE4; serum; biomarker.

Introduction

Of all new tumor diagnoses in females, only colorectal, lung and breast (BC) cancers account for 51%, with BC alone accounting for almost one/third [1]. Usually, effective treatments are suitable for patients with early stages instead of metastatic patients [2]. Due to limited tools in triple-negative or metastatic BC treatment, overall BC patients morbidity is still increasing [3]. Thus, identifying easy and specific biomarkers associated with BC development and progression is vital for improving BC patients prognosis efficacy [2].

Human Epididymal Protein 4 (HE4) is secretory protein initially identified in human epididymis epithelial cells [4]. Its gene

is located on 20q13.12 chromosome long arm [5]. It is one of whey-acidic-proteins that have anti-microbial and anti-inflammatory activity against viruses and gram-negative bacteria [6], as well as a role in regulation of cell angiogenesis [7] and cell growth [8]. At mRNA and protein levels and compared to healthy controls, HE4 was reported to be elevated in ductal BC. Authors also reported that its relative expression may be correlated significantly with tumor progression [9].

As studies evaluating the association between HE4 protein and BC clinicopathological features are very limited, we aimed in this study to further evaluate its clinical value compared to established tumor markers (CEA and CA-15.3). Also, we aimed to evaluate its association with some tumor severity features including, number of lesions, stage, grade, size and lymph node invasion.

Material and methods

Patients

A retrospective study was carried out between Jan and May/2021 including women with BC (n=120), with breast benign diseases (n=40) and 40 age- and gender-matched healthy females. All patients were clinically, radiologically and pathologically screened at the Oncology Center, Mansoura University, Egypt. Patient with inflammatory tumors and metastases, other cancer types or undergone radio-/chemo- therapy were excluded. None of controls (healthy and benign) had any tumor history. BC was pathologically identified according to the tumor-nodes-metastasis Classification of Malignant Tumours of the Union for International Cancer Control [10]. Study design was approved by Mansoura University Hospitals Ethics and Scientific Committees. Written informed consent was obtained from all participants.

Laboratory assays

After BC diagnosis and according to the manufacturer's protocol, HE4 was determined by ELISA commercial kit (Sunred Biological Technology, Shanghai, China). Also, all participants were analysed for CEA and CA-15.3 using commercial ELISA kit (My Bio Source, San Diego, USA).

Statistical analysis

Data analyses were performed using Graph Pad Prism and SPSS programs. Based on normality distribution, variables levels were expressed as mean ± SD or median (inter quartile range), appropriately. Qualitative data was expressed as absolute numbers. For differences assessments, ANOVA, Chi-squared (X2) or Kruskal-Wall is tests were appropriately used follwed by LSD as post-hoc test. P value <0.05 is significant. HE4 Diagnostic power for BC was evaluated by Receiver Operating-Characteristic (ROC) curves and cut-off point was determined based on the point closest to the (0, 1) point (the minimal (1-sensitiity)2+(1-specifiity)2) [11]. Correlations were evaluated by Spearman's rank correlation analysis.

Results

HE4 levels and breast cancer

In comparison between patients and controls, there was no significant (P=0.563) difference in age. Most of participants were postmenopausal. Tumor features including stage, grade, size, lymph node status, hormonal status and HER-2 protein detection were shown in Table 1. Despite CEA and CA-15.3, it was reported that serum HE4 [5 (2-11.9) vs.3.1 (1.8-5.4) and 1 (1-3.5); P=0.022] in BC patients was more significantly higher than controls (Figure 1).

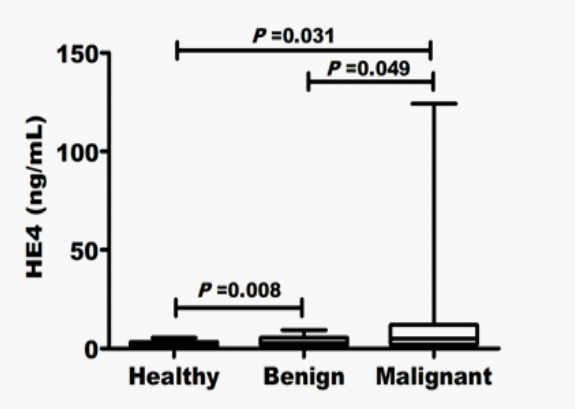

Figure 1: Distributions of HE4 between patients and controls. BC patients were significantly associated with elevated HE4 levels. HE4: Human Epididymal Protein 4.

Table 1: Age, menopause status and tumor characteristics of included females.

Parameter	Healthy	Benign	Breast cancer	*P* value
Number	40	40	120	-
Age (years)	48.0 ± 8.9	48.5 ± 8.9	49.5 ± 11.0	0.563[a]
Menopause (Pre-/post-menopausal)	18/22	16/24	43/77	0.455[b]
CEA (ng/mL)	2 (1-5)	3 (2-6)	4.6 (1-10.8)	0.101[c]
CA-15.3 (ng/mL)	12.5 (11-15)	20 (13-22)	20 (11-27)	0.181[c]
HE4 (ng/mL)	1 (1-3.5)	3.1 (1.8-5.4)	5 (2-11.9)	0.022[c]
Lesion (single/multiple)	-	-	96/24	-
Tumor size (<2 cm/>2 cm)	-	-	56/64	-
Tumor stage (T≤2/T>2)	-	-	78/42	-
Tumor grade (G1/G2-3)	-	-	36/84	-
Lymph node (negative/positive)	-	-	60/80	-
Estrogen receptor (negative/positive)	-	-	36/84	-
Progesterone receptor (negative/positive)	-	-	33/87	-
HER-2/neu (negative/positive)	-	-	42/78	-

Abbreviations: CEA: Carcinoembryonic Antigen; CA-15.3: Cancer Antigen 15.3; HE4: Human Epididymal Protein 4. Differences between groups were established by aANOVA,b Chi-squared (X2) or cKruskal-Wallis tests, appropriately.

HE4 clinical value in BC detection

According to ROC curve for BC detection, HE4 (AUC=0.783; P<0.0001) had superior diagnostic power compared to CEA and CA-15.3 (Figure 2 and Table 2). At cut-off >2 ng/mL, HE4 sensitivity and specificity were 73.3 and 63.7%, respectively.In early stages detection, this good diagnostic performance did not significantly affect (Table 2). This ability increase when comparing BC patients to only healthy controls (AUC=0.862; sensitivity=73.3%; specificity=77.5%; Table 2).

HE4 was correlated with disease progression

Elevated HE4 serum levels were associated disease progression (Figure 3). These increased levels were associated with multiple lesions (Figure 3A), late stages (Figure 3B), high grades (Figure 3C), large size (Figure 3D) and lymph node invasion (Figure E). Moreover, HE4 levels were higher in non-luminal compared to luminal tumor molecular subtypes (Figure 3F). Spearman correlation analysis revealed also that HE4 high levels significantly (P<0.05) correlated with tumor stage (r=0.29), grade (r=0.26) and tumor size (r=0.27) (Table 3).

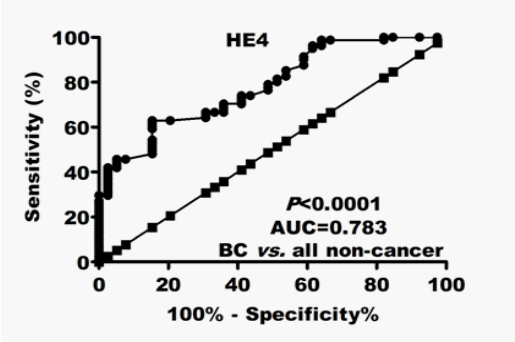

Figure 2: ROC curve for HE4 to discriminate BC patients from all non-cancer controls (benign and healthy). HE4: Human Epididymal Protein 4.

Table 2: Age, menopause status and tumor characteristics of included females.

Marker	AUC (95% CI)	*P* value	Cutoff	Sensitivity (%)	Specificity (%)
BC from all non-cancer individuals					
CEA (ng/mL)	0.570 (0.47-0.67)	0.229	2.6	61.7	50
CA-15.3 (ng/mL)	0.619 (0.52-0.72)	0.035	14.5	65	56.3
HE4 (ng/mL)	0.783 (0.69-0.86)	<0.0001	2.0	73.3	63.7
Patients with early stages (T>2) from all non-cancer individuals					
CEA (ng/mL)	0.547 (0.41-0.65)	0.434	2.6	56.4	50
CA-15.3 (ng/mL)	0.602 (0.50-0.70)	0.087	14.5	61.5	56.3
HE4 (ng/mL)	0.733 (0.65-0.85)	<0.0001	2.0	69.2	62.5
BC from healthy individuals					
CEA (ng/mL)	0.621 (0.50-0.74)	0.111	2.6	61.7	56.3
CA-15.3 (ng/mL)	0.690 (0.58-0.80)	0.011	14.5	65	72.5
HE4 (ng/mL)	0.862 (0.77-0.95)	<0.0001	2.0	73.3	77.5

Table 3: Correlation between HE4 and some tumor features.

Factor	Spearman correlation	
	r	*P* value
Lesions	0.17	0.128
Tumor T stage	0.19	0.116
Tumor grade	0.26	0.027
Lymph node	0.15	0.123
Clinical stage	0.14	0.691
Tumor size	0.27	0.016

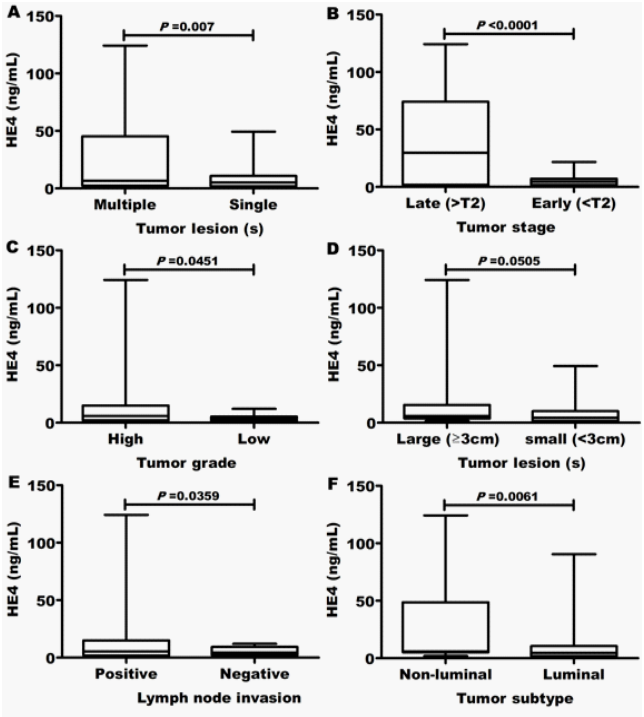

Figure 3: HE4 elevated levels were distributed according to tumor (A) number of lesions, (B) stage, (C) grades, (D) size, (E) lymph node invasion, and (F) molecular subtypes. HE4: Human Epididymal Protein 4.

Discussion

In clinical practice, BC is a heterogeneous disorder that is frequently diagnosed [12]. Despite several optional BC therapeutic strategies, there were many related deaths around the world [12]. Thus, BC analytical tools for its detection at disease early stages, as well as during its follow-up and management, are widely demanded [13].

After its first detection as a transcript expressed in epididymis of respiratory tract [14], HE4 was frequently used as epithelial ovarian tumor serum biomarker [15]. Subsequently, it was demonstrated to be expressed in ductal BC and limited studies reported its clinical value [14,16]. Here, we aimed to evaluate a blood HE4 easy test in BC detection using two controls group (patients with benign breast disorders and healthy females). Our observations indicate that serum HE4 [5 (2-11.9) vs.3.1 (1.8-5.4) and 1 (1-3.5); P=0.022] in malignant BC patients was

more significantly higher than benign and healthy controls, respectively. Also, it (AUC=0.783) had superior diagnostic ability for BC compared to CEA and CA-15.3. HE4 sensitivity (73.3%) and specificity (63.7%) were reasonable in distinguishing BC from benign and healthy females. This ability increase when comparing BC patients to only healthy controls (AUC=0.862; sensitivity=73.3%; specificity=77.5%).

These results demonstrate that HE4 may be used as a predictive BC marker. Galgano et al., reported HE4 mRNA and protein level of in normal and malignant BC tissues [14]. In addition using clear immunohistochemical staining, Kamei et al., found increased HE4 expression in BC cells and they reported its association with lymph node invasion [17]. Sai Baba et al. and Gündüz et al. also evaluated serum HE4 diagnostic potential of in BC detection and their results are consistent with our results [16,18]. In cancer cell migration, adhesion, growth and metastasis, it was demonstrated that HE4 can act as positive regulator through activating EGFR-MAPK and PI3K/AKT signalling pathways [19,20].

Elevated HE4 serum levels were associated disease progression including multiple lesions, late stages, high grades, large size and lymph node invasion. Also, HE4 levels were higher in non-luminal compared to luminal tumor molecular subtypes. Similar to these findings, Mirmohseni Namini et al. observed in BC patients that relative HE4 mRNA expression was significantly correlated with cancer cells differentiation grade, tumorstage, and tumor size. Also, they found that patients with lymph node involvement had significantly higher HE4 plasma levels than patients without involvement [9]. Moreover, Akoz et al. reported that HE4 expression rises in patients with BC subtypes that associated with poor prognosis [21].

Conclusion

In conclusion in this report, expression levels of HE4 were increased in BC patients compared to benign and healthy controls. It had superior diagnostic ability compared to other established biomarkers (CEA and CA-15.3) BC screening. This study may include some limitations like retrospective nature and single-center patient's cohort. Thus, future more multicentric comprehensive studies are required to examine HE4 prospective analysis.

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