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ORIGINAL ARTICLE

Comparison of Serum Human Epididymis Protein 4 (HE4) Levels in Breast Cancer Patients and Healthy Individuals

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Abstract

Purpose Human Epididymis Protein 4 (HE4) is one of serum biomarkers that recently reported in gynecological cancers, including breast cancer. This study aimed to compare serum HE4 levels in breast cancer patients and healthy individuals.

Methods This cross-sectional study examined serum HE4 levels in 42 patients and 36 healthy women in 2019. Samples were easily selected from women referring to a teaching hospital. Healthy women were selected from the normal population who did not have a risk factor for breast cancer. Demographic information were recorded in a checklist. Then 10 cc of venous blood was taken from each patient. After centrifugation, serum and plasma were kept at minus 80 °C. The HE4 level was measured with Immunoassay Chemi Luminescence Method and using HE4 kits made by German company Cobas.

Results The mean age of the patients and healthy individuals was 48.76 ± 14.97 and 39.88 ± 14.05 years, respectively. The average size of tumor in patients was 4.70 ± 2.48 cm. The average serum HE4 level in patients and healthy individuals was 68.01 ± 63.39 and 50.06 ± 14.97, respectively ($P = 0.46$). There was a significant relationship between serum HE4 levels and age of patients ($P = 0.01$).

Conclusion This study showed an increase in HE4 levels in breast cancer patients compared to healthy individuals. Preliminary results suggest that HE4 may serve as a new biomarker for breast cancer. However, more large-scale clinical studies are needed to further determine the predictive value of this biomarker as well as its molecular mechanisms in carcinogenesis and tumor progression, especially in breast cancer.

Keywords Human epididymis protein 4 (HE4) · Patients · Breast neoplasms · Healthy people

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Introduction

Breast cancer is the most common cancer among women and the third most common cause of cancer death in women [1]. Although some prevention methods or early screening programs may reduce the risk of breast cancer, most cases cannot be eliminated, especially in developing countries where breast cancer is diagnosed in the late stages. Early diagnosis is therefore critical to improving outcomes and survival and patient management. Tumor markers are widely used to evaluate treatment responses, early detection of recurrence, and prognosis [2].

In breast cancer, the most common tumor marker is usually the cancer antigen (CA 15-3), while its sensitivity and specificity are not appropriate [3, 4]. Several serum markers have been identified with a potential prognostic role in breast cancer, but none has been used for routine clinical practice [5].

Among the serum biomarkers recently reported in gynecological cancers, one of the most promising proteins is the human epididymis protein 4 (HE4 or WFDC2), which is a member of the four whey acidic protein (WAP), the nucleus disulfide of the cluster gene, which has a protective role in several protease inhibitors [6]. HE4 was first described by Kirchhoff et al. [7]. HE4 is a secreted protein that was first observed in human epididymis epithelial cells and subsequently in many other normal tissues, especially in the reproductive system and respiratory tract of the central respiratory system, as well as in female malignancies [8–13].

Increased HE4 expression has been shown in a wide range of malignant neoplasms, especially those in women such as ovarian cancer and those of pulmonary and gastrointestinal origin [14–18]. As recently reported, HE4 is sometimes expressed in cancers of the breast tissue [14]. However, the level of serum expression and its diagnostic and prognostic potential in breast cancer have not yet been determined. Therefore, the aim of this study was to compare serum HE4 levels in breast cancer patients and healthy individuals.

Materials and Methods

The present descriptive cross-sectional study examined serum HE4 levels in 42 women with breast cancer and 36 healthy women in 2019. Samples were easily selected from women referring to the clinic or department of obstetrics and gynecology, Afzalipour Hospital in Kerman. Non-breast cancer women were selected from the normal population who did not have a risk factor for breast cancer.

Inclusion criteria in the breast cancer group were the following:

No early menarche or late menopause, no history of taking hormonal drugs, no family history of breast cancer in grade 1 and 2, no history of precancerous breast lesions, failure to perform chest radiotherapy, no alchohol drinking, and normal examination of both breasts in the examination performed by a gynecologist.

Diagnosis of any other malignancies in patients, having a history of chemotherapy or radiotherapy, and the presence of any chronic liver and kidney disease led to the exclusion of individuals.

After obtaining informed consent from the patients, first the demographic information of each patient including age, tumor stage and underlying disease was recorded in the checklist. Then 10 cc of venous blood was taken from each patient. After centrifugation, serum and plasma were kept at minus 80° C until HE4 analysis.

The samples were then sent to Besat Clinic to determine the serum level of HE4 and the HE4 level was measured by Immunoassay Chemi Luminescence Method using a HE4 kit made by the German company Cobas. According to that company description, the HE4 level between 0.5 and 150 pmol/lit was regarded as normal and higher level was regarded as abnormal.

Descriptive and analytical statistical methods and SPSS software version 20 were used to analyze the data.

This study was approved by the ethics committee of Kerman University of Medical Sciences (Ethics Code: IR.KMU.AH.REC.1396.1684).

Results

In this study, 78 people were examined. The mean age of the persons was 44.66 ± 15.13 (13 to 87 years old). More than three-quarters of the persons were in the active phase of menstruation (61 individuals, 78.2%) and the rest were in the menopausal phase. On average, 9.29 ± 5.58 years had passed since menopause (2 to 20 years).

Forty-two of the subjects had breast cancer and 36 were healthy. The mean age of breast cancer patients and healthy individuals was 48.76 ± 14.97 and 39.88 ± 14.05 years, respectively ($P = 0.001$). Out of 61 people who were in the active phase of menstruation, 30 had breast cancer (71.4%) and 31 were healthy (86.1%). During the menopausal phase, 12 patients had breast cancer (28.6%) and 5 patients were healthy (13.9%). The mean tumor size in patients with breast cancer was 4.70 ± 2.48 cm (1 to 10 cm). In more than half of the patients, the tumor was grade II (64.28%).

Measurement of serum HE4 levels could not be measured for 5 persons due to sample drying. In other persons,

the mean level of HE4 was 50.04 ± 46.62 pmol per deciliter (21 to 312.70 pmol per deciliter). The mean serum level of HE4 in patients with breast cancer was 68.01 ± 63.39 and in healthy individuals was 50.06 ± 14.97 ($P = 0.46$). There was a significant relationship between serum HE4 levels and age of people with breast cancer ($P = 0.01$). With age, the mean HE4 also increased. There was no significant relationship between serum HE4 levels and tumor size in breast cancer patients ($P = 0.64$).

In each of the study groups, the mean serum level of HE4 was higher in women who were in the active phase of menstruation than in postmenopausal women. There was no significant relationship between serum HE4 levels and menopausal status in patients and healthy individuals (Table 1). The mean serum level of HE4 in patients with breast cancer was not significantly different based on pathological findings (Table 2).

Table 2 Comparison of serum HE4 levels in breast cancer patients based on pathological findings

Pathological findings	*N* (%)	Mean ± SD	*P* value
Degree of histology			
I	7 (16.66)	64.16 ± 38.66	0.73
II	27 (64.28)	74.14 ± 75.47	
III	8 (19.04)	51.17 ± 28.74	
Vascular invasion			
Negative	11 (26.19)	57.42 ± 20.14	0.64
Positive	31 (73.80)	71.54 ± 72.34	
Infiltration			
Negative	15 (35.71)	68.15 ± 73.60	0.43
Positive	27 (64.28)	67.93 ± 58.64	
Microscopic			
Negative	18 (42.85)	74.41 ± 66.56	0.37
Positive	24 (57.14)	62.89 ± 62	
Estrogen receptor			
Negative	19 (45.23)	82.03 ± 88.11	0.97
Positive	23 (54.76)	55.47 ± 23.70	
Progesterone receptor			
Negative	19 (45.23)	68.17 ± 69.05	0.56
Positive	23 (54.76)	67.87 ± 59.80	
Human epidermal growth factor receptor			
Negative	21 (50)	72.52 ± 65.18	0.09
Positive	21 (50)	60.50 ± 63.50	
Ki 76 antigen			
Negative	13 (30.95)	49.42 ± 20.48	0.34
Positive	29 (69.04)	76.17 ± 73.89	
Tumor histology type			
Invasive ductal carcinoma	38 (90.5)	70.41 ± 66.74	0.61
Invasive lubolar carsinom	4 (9.5)	48.80 ± 17.48	

Discussion

Breast cancer is a heterogeneous group of diseases that differ in terms of pathological characteristics and clinical manifestations. The risk of recurrence and prognosis of this disease is affected by the stage of diagnosis and the biological characteristics of the tumor. The main problem with biomarkers for breast cancer diagnosis is the improved accuracy in diagnosing malignancies in the early stages. Numerous serum biomarkers have been studied, including BR 27.29 (CA 27.29), mucin-like carcinoma-associated antigen, CA 549, and CEA, but none have achieved the sensitivity and specificity required for standard clinical practice [5].

The present study examined the level of HE4 biomarker in breast cancer patients and healthy individuals. According to the results, the mean serum level of HE4 in breast cancer patients was higher than healthy individuals, but this difference was not significant; while in a study by Gündüz et al., there was a significant difference between serum HE4 levels in breast cancer patients, ovarian cancer patients and healthy individuals, so that serum HE4 levels were significantly higher in patients with breast and ovarian cancer than in healthy individuals [2].

Galgano et al. reported the expression of mRNA and HE4 protein in normal and malignant tissues. HE4 positive immunoreactivity was present in ovarian cancer as well as other cancers including lung, endometrial, breast and gastrointestinal cancers, and mesothelioma. The highest expression was observed in serous ovarian cancer and significant positive staining was detected in breast cancer tissues. However, malignant breast tissue showed variable

Table 1 Comparison of serum HE4 levels in breast cancer patients and healthy individuals by menopausal status

Group	Variable	Mean ± SD	*P* value
Patients with breast cancer	Active phase of menstruation	70.06 ± 76.04	0.22
	Menopausal phase	63.91 ± 25.90	
Healthy individuals	Active phase of menstruation	50.88 ± 15.77	0.62
	Menopausal phase	45 ± 7.58	

expression and an average increase in HE4 expression of ductal carcinoma was observed [14].

Kamei et al. also showed that HE4 expression in ductal carcinoma increased moderately and increased HE4 expression in breast cancer tissues was associated with lymph node invasion. They believe that increased expression of HE4 is a predictor of breast cancer recurrence. Five-year disease-free survival in the HE4-positive group (58.6%) was significantly worse than the negative group (85.6%). These findings indicate that HE4 is significantly associated with breast cancer [19].

The results of other similar studies also show that HE4 is associated with breast cancer and some other serious diseases, despite being associated with ovarian, cervical and lung cancers [15, 20]. In Galgano and Plebani study, the mammary epithelium showed the expression of HE4 variable with higher staining in ducts compared to lobules [14, 21].

In each of the groups studied in the present study, the mean serum level of HE4 in people who were in the active phase of menstruation was higher than menopausal people. There was no significant relationship between serum HE4 levels and menopausal status in patients and healthy individuals. In Gündüz et al. study, no significant relationship was observed between serum HE4 levels and menopausal status of breast cancer patients [2].

In our study, the mean serum level of HE4 in breast cancer patients was not significantly different based on pathological findings. In Gündüz et al. study, multivariate analysis showed no positive and significant correlation between serum HE4 level with histological grade, lymph node involvement and clinical stage in breast cancer patients. But findings on the sensitivity and specificity of HE4 for the diagnosis of breast cancer patients suggest that HE4 may be used as a biomarker predicting breast cancer [2].

In the present study, the serum level of HE4 had a significant relationship with the age of people with breast cancer, so that with age, the mean of HE4 also increased.

Conclusion

The present study showed an increase in serum HE4 levels in breast cancer patients compared to healthy individuals. Preliminary results suggest that HE4 may serve as a new biomarker for breast cancer. However, more large-scale clinical studies are needed to further determine the predictive value of this biomarker as well as its molecular mechanisms in carcinogenesis and tumor progression, especially in breast cancer.

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Declarations

Conflict of interest The authors have no funding or conflicts of interest to disclose.

Ethics approval This study was approved by the ethics committee of Kerman University of Medical Sciences in Iran (Ethical Code: IR.KMU.AH.REC.1396.1684).

Consent to participate Informed consent was taken from all patients.

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