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Evaluation of some biomarkers in breast cancer patients

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Abstract

Background. Osteopontin (OPN) is an extracellularmatrix protein that is overexpressed in various cancers and promotes onco-genic features including cell proliferation, survival, migration, and angiogenesis. Here, we analyzed the correlation between the expression pattern of (osteopontin and CA15.3) and clinic-pathological status of breast cancer and other standard prognostic factors.

Patients and methods. A case control study with 60 female breast cancer patients and 20 controls. All patients were subjected to complete medical history uptake , clinical examination and laboratory investigations include histopathological type of cancer, hormone receptor status ,CA 15-3 serum concentratios and osteopontin plasma level by ELISA.

Results. Our study showed that osteopontin plasma level was significantly different among breast cancers and controls ($p<0.0001$) but no association between osteopontin plasma level and histological type , stage or grade. Also we found significant association between CA 15-3 and cancer stage ($p<0.000$). Interestingly CA15.3 serum concentrations increased in hormone receptor positive cancer.

Conclusion. OPN and CA15.3 are overexpressed in breast cancer. OPN overexpression is associated with poor prognosis. Moreover CA15.3 has minimal prognostic value in non metastatic breast cancer.

Introduction

Breast cancer is the most common cause of cancer-related mortality in women in less developed regions, and the second most common (after lung cancer) in developed regions [1][2]. It emerges through a multi-step process starting from hyperplasia to premalignant change, in situ carcinoma, and invasive breast cancer [3]. Up to 2013, breast cancer reportedly accounted for 29% of all new cancer cases and 14% of all cancer-related deaths among women worldwide . Breast cancer-related mortality is closely associated with the development of metastatic potential of the primary tumor. Despite the significant efforts to reduce breast cancer metastasis and mortality, its prognosis remains poor [4].

Improvements in chemotherapy, surgery, lymph node evaluation and hormone receptor blocking therapy have successfully doubled the survival of breast cancer patients however it is still the leading cause of cancer-related mortality in women [5][6]. It is a heterogeneous disease whose component includes many molecular subtypes [1]. Different molecular subtypes of breast cancer have various prognoses and responses to therapy[5]. Due to the heterogeneous clinical nature of breast cancer, it is necessary to identify new biomarkers that are associated with tumor growth, angiogenesis and metastasis[7].

Serum tumor markers have been widely used as non-invasive tools for measuring treatment response, early diagnosis of recurrence and predicting prognosis. Breast cancer biomarkers

include tissue markers such as estrogen and progesterone receptors and the human epidermal growth factor receptor 2 (HER2), and circulating markers such as carcinoembryonic antigen (CEA) and cancer antigen 15-3 (CA15-3) [8].

CA 15-3 is transmembrane glycoprotein encoded by the MUC1 gene. The protein is normally found in the luminal secretion of glandular cells whose normal function is cell protection and lubrication. It is typically seen to be overexpressed on breast tumor cells, reaching measurable concentrations in the circulation due to the lack of an intact basal membrane. CA15.3 interacts with cell adhesion, cell-cell aggregation and cell extracellular matrix adhesion. Consequently it can affect formation of metastasis. Elevated levels of CA 15-3 are found in the majority of breast cancer patients with distant metastasis; about 60–75% of women with metastasized cancer present elevated levels of CA 15-3. However CA 15-3 levels are only elevated in 10% of patients with stage 1 breast cancer so it has little value in early detection [2].

Osteopontin (OPN), a secreted, non-collagenous, extracellular matrix protein that belongs to the small integrin-binding ligand N-linked glycoprotein (SIBLING) family. It's encoded by secreted phosphoprotein 1 (SPP1) gene which is mapped to chromosome 4q24-q25, Several polymorphisms in the SPP1 gene affect OPN expression[9]. OPN plays a significant role in determining the oncogenic potential of various cancers and is recognized as a key marker in the processes of tumorigenicity and metastasis[10].High OPN levels in breast cancer are associated with a poor prognosis and disease progression[7].

However up to date , there are no data with regard to OPN and CA15.3 overexpression and their correlation with clinic-pathological status of breast cancer and other standard prognostic factors. The present study was designed to analyze the correlation between the expression pattern and clinic-pathological status of breast cancer and other standard prognostic factors.

Aim of the work: this work aims to Provide new insights about the possible role of osteopontin and CA 15-3 in development , progression and metastasis of breast cancer. And to assess the relation of osteopontin expression and CA15-3 serum concentrations with the clinico-pathological status of breast cancer and other standard prognostic factors.

Patients and methods: The study was approved by the ethical committee of Faculty of Medicine Sohag university and an informed consent was obtained from each patient. 60 female patients with breast cancer recruited from The Department of General Surgery Sohag university hospital. In addition 20 healthy controls with no evidence of acute or chronic medical disorders. Each patient was subjected to complete medical history uptake and clinical examination. The demographic data of patients include: age, parity, menopausal status, stage, ER status, PR status, grade, histopathological

type of cancer and CA 15-3 serum concentrations which were retrieved from their medical records. Osteopontin plasma level was measured by ELISA.

Exclusion criteria: Patients were excluded if with chronic medical diseases (cardiac, hepatic or renal) and diabetes or those currently pregnant or breast feeding. Also patients with distant metastasis and those who were received chemotherapy or radiation therapy or have done surgery or with malignancies elsewhere in the body.

Statistical analysis: Data was analyzed using STATA intercooled version 14.2 using student t-test to compare means of two groups. When the data was not normally distributed Mann-Whitney test was used to compare two groups **.**

Results

The current study was carried out on newly diagnosed 60 female breast cancer patients and 20 healthy controls the demographic data of patients and controls was summarized in Table 1. The clinical Characteristics of breast cancer , osteopontin plasma level and CA15.3 serum concentrations were summarized in Table 2.

Our results showed higher osteopontin plasma level among patients than controls. But no difference in its level according to histological type of cancer , cancer stage or grade. Osteopontin plasma level was higher in ER-ve and PR-ve tumors than ER+ve and PR+ve tumors. Also, CA15.3 serum concentrations were higher in stage 2 and stage 3 than in stage 1. But showed no difference according to tumor grade. Interestingly CA15.3 serum concentrations were higher in ER+ve and PR+ve tumors than ER-ve and PR-ve tumors.

Table 1 .Age, parity and menopausal status of studied population

Variable	Cases N=60	Controls N=20	P value
Age/years			
Mean ± SE	**57.42±1.26**	**55.05±1.80**	**0.33**
Median (range)	**57 (39-75)**	**55 (40-70)**	
Parity			
Mean ± SE	**4.32±0.23**	**4.1±0.19**	**0.42**
Median (range)	**4 (0-8)**	**4 (3-6)**	
Menopausal status			
Pre-menopause	**23 (38.33%)**	**7 (35.00%)**	**0.79**
Post-menopause	**37 (61.67%)**	**13 (65.00%)**	
Osteopontin level µg/L±SE	**194.84±5.57**	**80.55±7.75**	**<0.0001**

Table 2. Characteristics of breast cancer

Variable	Number (%)	Osteopontin plasma level µg/L±SE	P value	CA15.3 serum concentrations U/mL±SE	P value
Histological type					
Ductal carcinoma	39 (65.00%)	199.40±6.27			
Lobular carcinoma	21 (35.00%)	186.38±10.82	0.27		
Stage					
Stage 1	15 (25.00%)	198.83±13.92		10± 0.72	
Stage 2	22 (36.67%)	195.64±6.22	0.87	22.2±1.4	0.000
Stage 3	23 (38.33%)	191.48±9.99		23.7±1.79	
Grade					
Grade 1	10 (16.67%)	174.65±8.74		23.8±1.3	
Grade 2	23 (38.33%)	195.5±9.58	0.24	20.5±1.4	0.05
Grade 3	27 (45.00%)	201.76±8.52		16.5±1.3	
Estrogen receptors					
Negative	30 (50.00%)	216.87±3.34	0.0001	15.8±0.97	0.000
Positive	30 (50.00%)	172.82±9.03		24.7±1.6	
Progesterone receptors					
Negative	39 (65.00%)	205.38±6.17	0.009	16.9±1.2	0.005
Positive	21 (35.00%)	175.26±9.86		23.8±1.95	

Discussion

OPN is a secreted glycolphosphoprotein that may physiologically serve as a cytokine and an extracellular matrix molecule. The gene encoding OPN known as secreted phosphoprotein 1 (SPP1) is mapped to chromosome 4q24-q25. OPN is implicated in physiological as well as pathological processes ,It is expressed and secreted by various cells, and plays a role in bone remodeling, reconfiguration of tissue integrity during inflammatory processes, coronary stenosis, and cancer metastasis [4]. It has been demonstrated that OPN is associated with more than 30 cancers so far and a marker for breast, cervical, colorectal, head and neck, liver, lung, ovarian and prostate cancers, as well as for sarcoma.

Abundant production of osteopontin is correlated with aggressiveness here we tested this hypothesis. We found that osteopontin plasma level was significantly higher in breast cancers than controls (p<0.0001) . Consistent with ***,Liu et al*** [9],who found that OPN expression was significantly higher in human cancers tissues than in matched normal tissues . But there was no significant difference between patients and controls as regard to age , parity and menopausal status.

Also We found that there were no association between osteopontin plasma level and age ,parity or menopausal status. This finding is concordant with ***Xu et al.***[4]**,** in their meta-analysis study found that there was no correlation between OPN expression and age (P=0.572) and between OPN expression and menopausal status (p=0.688). Moreover osteopontin plasma level showed no correlation with histological subtype of cancer (p=0.27).This result is in concordance with, ***Thorat et al.,*** [7] who found that OPN expression didn't correlate with histological subtype of cancer. In our study OPN plasma level didn't differ significantly by T stage(p=0.87). Similar to ***Thorat et al.,*** [7] who found that OPN expression did not correlate with tumor stage.

According to osteopontin plasma level and tumor grade there was no significant differences (p=0.24). Similar to , ***Bramwell et al ;*** [10] who found that OPN plasma level didn't differ significantly by tumor grade (1 vs 2 vs 3). Moreover osteopontin plasma level was higher in ER -ve and PR -ve than the patients with ER+ve and PR+ve tumors. Inconsistent with ***,Bramwell et al.,*** [10] who found that OPN plasma level didn't differ significantly by hormone receptor status (negative vs positive).

According to the possible prognostic role of CA 15-3 in non metastatic breast cancer patients we found that CA 15-3 serum concentrations were higher in stage 2 and stage 3 than in stage 1 with statistically significant difference (P<0.000) but no statistical difference p=0.05 as regard to tumor grade. These results are in concordance with , ***Brouckaert et al.,***[11] who found Significant different values for preoperative CA15.3 in function of tumor size, nodal stage, phenotype and detection mode.

Interestingly CA15.3 serum concentrations showed significant difference among ER+ve, PR+ve tumors and ER-ve , PR-ve tumors. Similar to, ***Li et al.,*** [12] who found higher CA 15-3 serum concentrations with the positive hormone receptor status.

Conclusion OPN and CA15.3 are overexpressed in breast cancer. OPN overexpression is associated with poor prognosis as detected by higher grade and hormone receptors negative status**.** Although CA15.3 is the only adopted serum tumor marker, it has little value in early detection of breast cancer.

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