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RESEARCH ARTICLE

Soluble CD44 is a promising biomarker with a prognostic value in breast cancer patients

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ABSTRACT

Background:: Cancer is a heterogeneous disease encompassing various subtypes. Exploration of novel biomarkers is important to tailor therapy. **Aim:** The main aim of this study was to explore the prognostic value of soluble CD44 in the sera of breast cancer patients (BCPs), and to correlate its levels with hyaluronic acid (HA), VEGF and p53 levels. **Subjects and Methods:** Fifty female BCPs were enrolled in the study as a patient group and 50 healthy females as a control group. Cancerous and neighboring normal breast tissues were collected from BCPs. Blood samples were collected from BCPs at diagnosis and control group. CD44 tissue expression was evaluated by immunohistochemistry (IHC). Serum levels of sCD44, HA, VEGF, and p53 levels were measured by ELISA. **Results**: CD44 showed high expression in cancerous breast tissues as compared to control tissues. Circulating sCD44 was highly increased in BCPs and was associated significantly with the expression levels of CD44 in tissues. Serum HA and VEGF levels were highly elevated in BCPs along with the increase in CD44 positivity. In contrast, the decrease in p53 level was associated with higher CD44 expression in BCPs. According to Kaplan-Meier curves, BCPs who expressed high levels of sCD44 or tissue CD44 showed shorter DFS time than CD44 negative. **Conclusion:** Tissue and soluble CD44 are significantly elevated in BCPs and associated with increases in HA and VEGF. Increased sCD44 is related to bad patients' survival. As such, CD44 can be used as a prognostic marker in breast cancer patients.

Keywords: Breast cancer, CD44, hyaluronic acid, VEGF, p53

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INTRODUCTION

Breast tumors are well known to be composed of phenotypically different groups of cells. However, it is unclear which of these cells contribute to tumor development. Unlike the theory that all cell populaces can become tumorigenic by accumulating mutations, anoth er speculation limits this capacity to a group of cells that share the classic characteristics of stem cells, commonly known as cancer stem cells (CSCs) or cancer-initiating cells (Gupta et al., 2019). CSCs are recognized by their capacity to self-renew, differentiate into various lineages, and reconstitute the tumors cell hierarchy from which they are derived in serial xenotransplant assays. These cells are greatly resistant to anti-cancer therapy and are liable for tumor recurrence after therapy (Chen et al, 2018; Zhang et al., 2019). CD44 is recognized as one of the foremost reliable markers of CSCs in numerous malignancies, including leukemia, breast, colon, ovarian, prostate and pancreatic cancers (Chen et al, 2018; Zhang et al., 2019). $CD44^+$ subpopulation of BC cells express higher levels of proinvasive genes and accordingly they have greatly invasive properties (Kang et al, 2016; Bourguignon et al., 2014).

CD44 is a ubiquitous cell surface glycoprotein consolidated in adhesive cell-cell and cell-matrix interactions, cell movement and cell homing.

CD44 protein undergoe extensive alternative splicing, where all CD44 isoforms contain the link module hyaluronic acid (HA)-binding location. The interaction of CD44 to HA affects cell attachment to extracellular matrix components and is involved in the stimulation of aggregation, expansion, migration, and angiogenesis (Mishra et al., 2019; Chen and Bourguignon, 2014).

The main target of this work was to explore the prognostic value of soluble CD44 in the serum of BC patients as well as its correlation with HA, VEGF and apoptosis stimulating protein p53 was also considered.

Patients and Methods

This study included 50 female patients newly diagnosed with breast cancer as the patient's group and 50 healthy females with normal mammography findings and no previous history of any kind of cancer as a control group, matched for age and menopausal status with the patient's group. The Clinico-pathological characteristics of BCPs are represented in Table 1.

Patients were selected from those admitted to the Department of Cancer Management and Research, Medical Research Institute, Alexandria University. Written consent for participating in the study was taken according to the Declaration of Helsinki and approved by the Ethical Committee of the Medical Research Institute. Metastatic patients at diagnosis and patients receiving chemotherapy or radiotherapy before surgery were excluded from this study.

Patients were subjected to preoperative evaluation that included history taking, clinical examination to detect the tumor site and the presence of enlarged axillary lymph nodes. Radiological investigations included mammogram, abdominal ultrasound and chest x-ray. Preoperative investigations also included fine needle aspiration cytology (FNAC) to diagnose the presence of malignancy. Patients were subjected to surgery (modified radical mastectomy or conservative surgery). Postoperative pathological evaluation of the tumor included type, grade, size of the tumor, numbers of axillary lymph nodes involved, and the presence or absence of vascular invasion. Assessments of estrogen, progesterone receptors (ER, PR) and Her2/neu expression were also confirmed.

Tissue samples collection

Tumor tissues and adjacent normal breast tissues were collected from patients with breast cancer at the time of surgery and fixed in 10% formalin solution for immunohistochemical staining.

Blood samples collection

A total of 5 ml fasting venous blood sample was drawn from each control subject and within a week before surgery for breast cancer patients. Blood samples were allowed to clot for 30 minutes before centrifugation, centrifuged at 3000 rpm for 10 minutes to isolate sera. Serum was stored at -80°C until used for quantification of sCD44, hyaluronic acid, VEGF, and p53 levels by enzyme-linked Immunosorbent assay (ELISA) according to the manufacturer's instructions (eBioscience, USA).

Immunohistochemical assessment of CD44 standard form

Breast tissue specimens were fixed in 10% formalin solution at room temperature and embedded in paraffin. Thin sections (5 micrometer) were obtained by microtome from representative paraffin blocks and transferred into gelatin-coated slides. The immunolocation of CD44 was carried out on deparaffinized five micrometer sections using streptavidin-biotin-peroxidase complex method. Five-micrometre paraffin sections were deparaffinized in xylene bath overnight, hydrated gradually through a series of graded alcohols and then in distilled water. The slides were then rinsed briefly in phosphate-buffered saline (PBS). To facilitate the access of the antibody to the antigen, the slides were treated with heat prior to immunohistochemical staining procedure. Target retrieval was done by immersion of tissue sections in a pre-heated target retrieval solution (Dako, Denmark) and maintaining heat at (95-99^{0}C) for 40 minutes in a thermoresistant container. The buffer was added periodically in between when evaporation occurred to prevent drying during the incubation process. The primary monoclonal antibody (monoclonal

mouse anti-human CD44 antibody, ready-to-use Dako, Denmark, N1587, clone 124), was applied to the samples and incubated in a humidity chamber overnight at room temperature. Biotinylated rabbit anti-mouse, immunoglobulin (IgG) was used as a secondary antibody against the monoclonal antibody and is incubated with slides for 10 minutes. CD44 was detected with standard streptavidin-biotin-peroxidase techniques using diaminobenzidine as the chromogen. Afterwards, the slides were briefly counterstained with hematoxylin, dehydrated and mounted. (Jang, et al, 2016).

Immunostained samples were evaluated by the pathologist. Epithelial reactivity was semi-quantified taking into consideration the percentage of positive epithelial cells and the intensity of the reaction. Scoring of CD44 was ranged from negative (0%) if no staining was seen in the stained cells with concurrent positive staining in the control tissues, weak (< 25% positive cells), moderate (25% to 50% positive cells), or strong (> 50% positive cells). Immunoreactivity (nuclear or cytoplasmic) was quantified as the percentage of positive cells in relation to the total number of cells encountered in ten representative high power fields covering the whole section and averaging the results.

Determination of serum p53 levels

Sandwich ELISA was used to measure p53 levels in serum samples. An anti-human p53 coating antibody was adsorbed onto microwells. Human p53 present in the sample or standard binds to antibodies was adsorbed to the microwells. A biotin-conjugated anti-human p53 antibody was added and binds to human p53 captured by the first antibody. Following incubation unbound biotin-conjugated anti-human p53 antibody was removed during a wash step. Streptavidin-HRP was added and binds to the biotin-conjugated anti-human p53 antibody. Following incubation unbound Streptavidin-HRP was removed during a wash step, and substrate solution reactive with HRP was added to the wells. A coloured product is formed in proportion to the number of human p53 present in the sample or standard. The reaction was terminated by addition of acid and absorbance was measured at 450 nm. A standard curve is prepared from 7 human p53 standard dilutions and human p53 sample concentration was determined.

Determination of soluble CD44 levels

Soluble CD44 in serum samples was measured by ELISA technique according to the manufactures instructions (eBioscience, USA). This technique is a double antibody sandwich enzyme-linked immunosorbent assay for in vitro determination of CD44 in serum samples. An anti-human CD44 coating antibody was adsorbed onto microwells. Human CD44 present in the sample or standard binds to antibodies was adsorbed to the microwells. Following incubation unbound biological components were removed during a wash step. A biotin-conjugated anti-human CD44 antibody was added and binds to human CD44 captured by the first antibody. Following incubation unbound biotin-conjugated anti-human CD44 antibody was removed during a wash step. Streptavidin-HRP was added and binds to the biotin-conjugated anti-human CD44 antibody. Following incubation unbound Streptavidin-HRP was removed during a wash step, and substrate solution reactive with HRP was added to the wells. A coloured product was formed in proportion to the amount of human CD44 present in the sample or standard. The reaction was terminated by addition of acid and absorbance was measured at 450 nm. A standard curve is prepared from 7 human CD44 standard dilutions and human CD44 sample concentration determined.

Determination of serum HA levels

A double-antibody sandwich enzyme-linked immunosorbent assay was used to measure the level of HA in samples. HA was added to monoclonal antibody Enzyme well which is pre-coated with HA monoclonal antibody, incubation; then, HA antibodies labeled with biotin, and combined with Streptavidin-HRP was added to form an immune complex; then incubation and washing again to remove the uncombined enzyme was carried out. Then Chromogen Solution A, B were added, the color of the liquid changes into the blue, and at the effect of acid, the color finally becomes yellow. The chroma of color and the concentration of

the Human HA of the sample were positively correlated.

Determination of serum VEGF levels

Serum VEGF was measured by ELISA technique according to the manufactures instructions (eBioscience, USA). The present method uses sandwich ELISA to measure VEGF levels in serum samples. An anti-human VEGF coating antibody was adsorbed onto microwells. Human VEGF present in the sample or standard binds to antibodies was adsorbed to the microwells. A biotin-conjugated anti-human VEGF antibody was added and binds to human VEGF captured by the first antibody. Following incubation unbound biotin-conjugated anti-human VEGF antibody was removed during a wash step. Streptavidin-HRP was added and binds to the biotin-conjugated anti-human VEGF antibody. Following incubation unbound Streptavidin-HRP was removed during a wash step, and substrate solution reactive with HRP was added to the wells. A coloured product is formed in proportion to the amount of human VEGF present in the sample or standard. The reaction was terminated by addition of acid and absorbance was measured at 450 nm. A standard curve is prepared from 7 human VEGF standard dilutions and human VEGF sample concentration was determined.

Statistical analyses:

Data were supplied to the computer and analyzed using IBM SPSS software package version 20. **(**Armonk, New York: IBM Corp**).** The two-sided chi-square test determined the differences between groups. Paerson correlation coefficient was used to assess the correlations between quantitative variables. Using log-rank test, Kaplan-Meier curves were used to calculate disease-free survival (DFS). We identified DFS as the duration between the date of diagnosis and the date of adverse outcome including local recurrence, distant metastasis or contralateral breast cancer. The significance of the obtained results was assessed at the 5% level.

RESULTS

CD44 expression in BC tissues

CD44 protein expression in BC tissues was positive in (70%) and negative in (30%) of patients while it was not recognized in normal breast tissues. The number of cases with positive CD44 receptor expression was significantly higher than those with negative CD44 receptor expression (P <0.001*). Figure 1 illustrated intra-ductal breast carcinoma tissue samples showing strong positive staining for CD44 by IHC.

Serum levels of soluble CD44

Serum sCD44 Range and Mean ± SD in healthy controls and BCPs at diagnosis were illustrated in Table 2. As presented in Table 2, serum sCD44 levels in BCPs were significantly higher than those in normal controls (P<0.001).

Serum levels of p53

Serum p53 levels in BCPs were significantly lower than those in normal control group (P<0.001) (Table 2).

Serum levels of VEGF

Serum VEGF levels were significantly elevated in BCPs than in the normal control group (P<0.001) (Table 2).

Serum levels of HA

Hyaluronic acid levels were significantly elevated in the serum of BCPs than in the normal control group (P<0.001) (Table 2).

Correlations between the biochemical parameters and the clinicopathological characteristic of the tumor

According to Table 3, p53 was significantly negatively correlated with histological grade (r_s=-0.462, P=0.003*). Serum VEGF was significantly positively correlated with histological grade (r_s=0.438, P=0.005*) and clinical stage (r_s=0.342, P=0.008*). Serum HA was significantly positively correlated with histological grade (r_s=0.361, P=0.022*) vascular invasion (r_s= 0.580, P<0.001*), significantly negatively correlated with ER expression (rs=-0.392, P=0.012*) and PR expression (r_s=-0.392, P=0.012*). Tissue CD44 is correlated significantly with histological grade (r_s=0.480, P=0.002*), vascular invasion (r_s=0.403, P=0.010*), clinical stage (r_s=0.420, P=0.010*) and Her-2/neu expression (r_s=0.405, P=0.014*) while significantly negatively correlated with ER expression (rs=-0.394, P=0.012*) and PR expression (r_s=-0.394, P=0.012*).

Table 1. Patients' demographics and tumor characteristics.

	Breast Cancer patients (n =50)
Age	
Mean ± SD	48.83 ± 7.26
Range	38.0 – 65.0
Menopausal status	
Pre	15 (30%)
Post	35 (70%)
Histological grade	
II	33 (65%)
III	17 (34%)
Clinical Stage	
I	10 (20%)
II	35 (70%)
III	5 (10%)
ER status	
Positive	31 (62%)
Negative	19 (38%)
PR status	
Positive	32 (64%)
Negative	18 (36%)
Her-2/neu expression	
Positive	22 (44%)
Negative	28 (56%)
Vascular invasion	
Yes	32 (64%)
No	18 (36%)
Tumor size (cm)	
<2	4 (8%)
2 – 5	44 (88%)
>5	2 (4%)
Axillary lymph node involvement	
Positive	37 (74%)
Negative	13(26%)

ER: estrogen receptor, PR: progesterone receptor, Her-2/neu: human epidermal growth factor receptor-2.

Table 2. Studied biomarkers in serum of breast cancer patients and in normal control subjects.

	Control group (n=50)	Patients (n=50)
sCD44 (ng/ml)		
Range	124.90–295.20	163 – 1931.10
Mean ± SD.	175.61± 62.72	610.04±322.86
p	<0.001*	
p53 (ng/mL)		
Range	71.0–195.0	21.0 – 91.0
Mean ± SD.	113.68± 44.36	58.68±27.0
p	<0.001*	
VEGF (ng/ml)		
Range	75.0 – 162.0	50.0 – 772.0
Mean ± SD.	98.23±24.70	191.80±120.12
P	<0.001*	
HA (ng/ml)		
Range	12.0 – 52.0	29.0 – 221.0
Mean ± SD.	22.97±6.76	81.13±44.08
p	<0.001*	

p: p-value for comparing between control group and patients group. *:Statistically significant at two-sided P value<0.05.

Regarding soluble sCD44, it was positively significantly correlated with clinical stage (r_s=-0.374, P=0.017*) and Her-2 expression (r_s=0.316, P=0.047*) while there was insignificant correlation between this parameter and the other clinicopathological characters.

Correlations among the studied biomarkers in breast cancer patients

It was noticed that tissue CD44 is significantly positively correlated with VEGF (r_s=0.429, P= 0.006*), HA (r_s=0.340, P= 0.032*) and sCD44 (r_s=0.395, P= 0.003*). Moreover, tissue CD44 is significantly negatively correlated with serum p53 (r_s=-0.400, P= 0.011*). Regarding soluble CD44s, it was significantly positively correlated with VEGF (r_s=0.415, P= 0.008*) and HA (r_s=0.352, P= 0.037*) while it was significantly negatively correlated with serum p53 (r_s=-0.385, P= 0.021*).

Prognostic value of tissue CD44, serum sCD44, HA, P53 and VEGF in breast cancer patients

To study the prognostic role of these biomarkers, the Kaplan-Meier disease-free survival (DFS) curves were constructed. As shown in Table 4 and figures (2-6), DFS curves showed that BCPs with positive tissue CD44 protein expression had shorter mean DFS time than patients with negative CD44 expression (0.028*). Regarding serum sCD44, elevated levels of this biomarker were associated with bad patients disease-free survival (P = 0.009*). Moreover, patients with elevated levels of serum VEGF and HA than its corresponding cut-off points had shorter DSF time than patients with lower levels of serum VEGF and HA (P= 0.030, P= 0.021* respectively). However, BCPs with lower levels of serum P53 had shorter DFS time than patients with higher serum P53 levels (P=0.004*) according to log-rank test.

DISCUSSION

Studies proposed that BC originates from CSC, which strongly express adhesion molecules CD44 (Masciale et al., 2020; Oliveira et al., 2010). In our study, the prognostic significance of CD44 in the serum of BCPs was studied. Also, its correlation with HA, VEGF and p53 serum levels was considered. In the current study, the

Table 3. Correlations between studied biochemical parameters and clinicopathological characteristic of the tumor.

	p53		VEGF		HA		Tissue CD44		sCD44	
	r_s	p	r_s	p	r_s	p	r_s	p	r_s	p
Histological grade	-0.462	0.003*	0.438	0.005*	0.361	0.022*	0.480	0.002*	-0.150	0.356
Tumor Size (cm)	0.205	0.206	-0.050	0.761	-0.125	0.444	-0.164	0.313	0.018	0.911
Lymph node (LN)	0.206	0.202	0.024	0.881	-0.127	0.435	-0.112	0.493	-0.227	0.160
Vascular invasion (VI)	-0.284	0.075	0.236	0.142	0.580	<0.001*	0.403	0.010*	0.090	0.582
Clinical Stage	-0.293	0.078	0.432*	0.008*	0.220	0.191	0.420	0.010*	0.374*	0.017*
ER expression	0.164	0.313	-0.177	0.274	-0.392	0.012*	-0.394	0.012*	0.136	0.401
PR expression	0.164	0.313	-0.177	0.274	-0.392	0.012*	-0.394	0.012*	0.136	0.401
Her-2/neu expression	0.219	0.174	-0.159	0.328	-0.238	0.139	0.405	0.014*	0.316*	0.047*

rs: Person coefficient, *: Statistically significant at two sided P value<0.05.

Table 4. Test of significance of disease free survival for tissue CD44 protein expression, sCD44, serum HA, P53 and VEGF in breast cancer patients.

	Mean Survival (months)	Log rank	
		χ^2	p
p53			
≤89	8.0	8.139*	0.004*
>89	20.6		
VEGF			
≤148	22.9	4.730*	0.030*
>148	18.3		
HA			
≤50	21.1	5.321*	0.021*
>50	17.7		
tissue CD44			
Negative	22.5	4.841*	0.028*
Positive	19.1		
sCD44			
≤291.8	23.5	6.844*	0.009*
>291.8	14.96		

*: Statistically significant at two-sided P value<0.05.

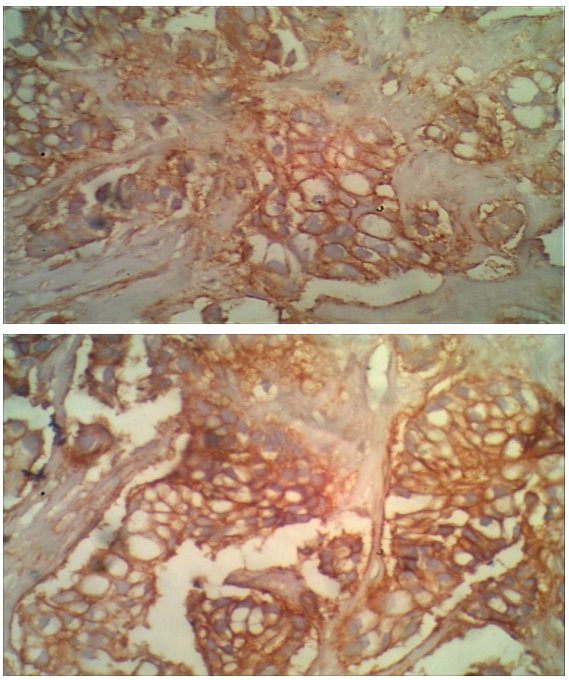

Figure 1. Intra-ductal breast carcinoma tissue samples showing strong positive staining for CD44 by IHC.

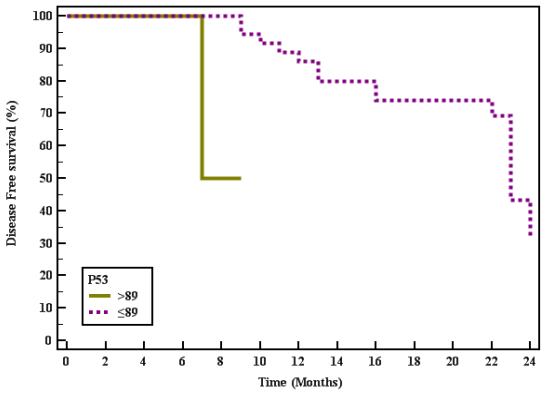

Figure 2. Kaplan-Meier DFS curve according to serum P53.

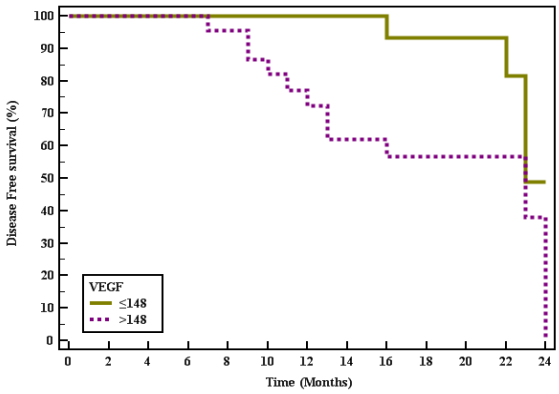

Figure 3. Kaplan-Meier DFS curve according to serum VEGF.

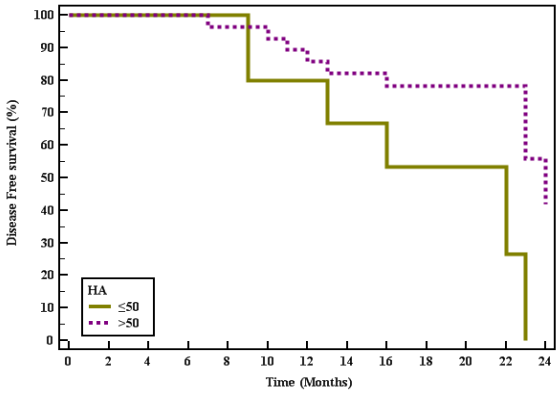

Figure 4. Kaplan-Meier DFS curve according to serum HA.

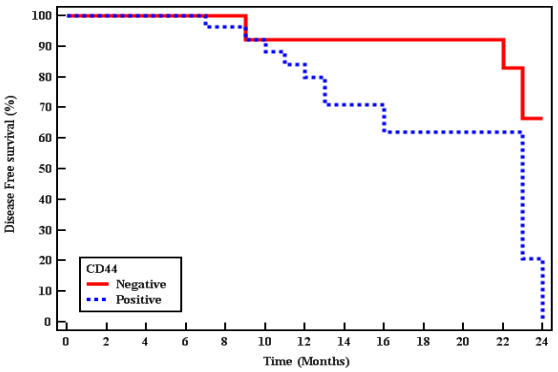

Figure 5. Kaplan-Meier DFS curve according to tissue CD44 expression.

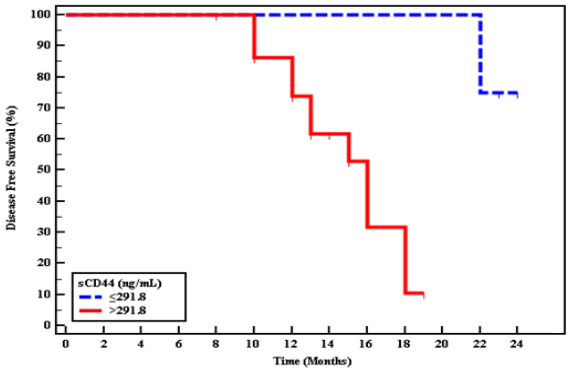

Figure 6. Kaplan-Meier DFS curve according to serum sCD44.

expression of tissue CD44 protein and serum sCD44 were examined. In line with previous studies, our result also offered evidence that CD44 protein was remarkably upregulated in BC tissues and significantly elevated in the serum of BC patients in comparison to normal counterparts indicating that CD44 might participate in the tumorigenesis of breast cancer (Xu et al., 2016; Khamis et al., 2017; Wu et al., 2015).

CD44 was originally characterized as HA and lymphocyte homing receptor. CD44 has an imperative role in the prognosis of malignancies including BC (Perez et al., 2013). CD44 exists as both the standard form (CD44s) and alternative splicing of different exons (CD44v) generated several isoforms (Anand and Kumar, 2014). The standard form, CD44s, is related to the expansion, invasion, and metastasis of BC (Afify et al., 2009).

The current study proposes a positive significant correlation between tumor grade and tissue CD44 protein expression. This demonstrated that patients with increased CD44 protein might have a bad prognosis, because high-grade tumor tends to be more aggressive and prefers early recurrence. This correlation is in agreement with previous research of 448 primary breast tumors by McFarlane (2015) showing that breast tumors with high CD44 protein abundance tended to be high grade. In addition elevated CD44 protein in both BC tissues and serum tend to be positively significantly correlated with clinical stage indicating that this molecule may be considered as a biological tumor marker for estimating the occurrence and progression of BC regardless of treatment. This correlation was proven in a previous study carried out by Wu (2015). Moreover, the current study demonstrated that both tissue CD44 and circulating sCD44 were positively correlated with HER-2, a well-known bad prognostic marker. Therefore CD44 could play a significant role in tumor aggressiveness and invasion. This result is coincided with that reported by Auvinen (2013) who demonstrated that CD44 might acquire an oncogenic signaling pathway as it was discovered to interfere with the expression of well-known oncogenic marker such as HER-2.

After a median follow up of 24 months survival analysis showed that patients with elevated serum sCD44 levels or whose tumor tissues overexpress CD44 by IHC were prone to have shorter disease-free survival time than patients with lower serum sCD44 or CD44 negative tumors. Our data confirmed the previous finding and revealed that the prevalence of $CD44^+$ tumor cells is correlated significantly with bad prognostic factors and favors a poor patient survival (Wang et al., 2019; Lv et al., 2016; Liu et al., 2013).

Furthermore, the current study showed that both tissue and serum CD44 were significantly positively correlated suggesting that soluble sCD44 detection in peripheral blood using ELISA is recommended to be a promising method may have a prognostic value in the management of BC patients especially after the tumor has been removed surgically. Soluble CD44 present within the circulation of patients with cancer most likely came from tumor cells instead of normal cells. Elevated serum-CD44 levels in cancer patients may be because of active shedding of CD44 molecules by tumor cells.

CD44 on tumor cells may be released into circulation by some proteolytic enzymatic cleavage (Wang et al., 2018). Proteolytic ectodermal cleavage of CD44 results in the discharge of soluble extracellular CD44 and release of CD44 intracellular domain which is considered as an important event in the activation of CD44-signalling pathway and enhances the invasiveness of cancer cells (Cho et al, 2015).

The present study revealed that serum HA levels in BCPs were significantly elevated in BCPs in comparison to controls. This finding suggesting that, tumor cells synthesize HA. Moreover, our finding showed that this parameter is significantly correlated with CD44 positivity and poor prognosis in patients with BC. HA is regarded as the major ligand for CD44 and capable of binding CD44v isoforms that are ubiquitously expressed. Interaction of CD44, with HA activates matrix metalloproteinases (MMPs) which involved in tumor progression (Bourguignon et al., 2014). The mechanism of how HA affects cell invasion was described by the previous report (Senbanjo and Chellaiah, 2017). Bourguignon (2014) demonstrated that binding of HA to CD44 plays a major role in the metastasis of BC.

We found higher levels of VEGF in blood samples of BC patients when compared to controls. This result is not in contradiction with the hypothesis that circulating VEGF levels may reflect the extent of active angiogenesis in patients with cancers. BC cells with $CD44^{+}$ express higher levels of proinvasive genes and have extremely invasive characteristics (Chen et al, 2018). This association was proven in the present research since patients with tissue positive CD44 and those with higher circulating sCD44 had elevated VEGF in serum. Thus, we conclude that CD44 positivity served as an upregulator of VEGF to function in the expansion of BC.

Our study revealed that p53 levels in serum were significantly lower in BCPs than in the control group. Moreover, decreased p53 serum levels are correlated with higher CD44 expression in both tissue and serum of BCPs. These findings lead us to suggest that abnormal CD44 protein expression is advantageous for the growth and dissemination of tumor cells (Thapa and Wilson., 2016). In response to strong cellular stresses, such as DNA damage or oncogenic signals, the wild type form of p53 regulates expression of a large cohort of genes that affect a cell-cycle arrest, senescence and apoptosis (Chen J, 2016). CD44 is known to act predominantly in a growth-promoting and anti-apoptotic fashion (Chanmee et al., 2015). Godar (2008) observed that CD44 mRNAs were upregulated 4-to-5-fold and CD44 protein 4-fold in BPEC-T cells in where p53 function was mainly abolished. Hence, the expression of CD44 was mainly suppressed by p53 by impacts on CD44 mRNA levels.

Detecting CD44 is vital for identifying effective anticancer drugs. Especially, when HA binds with CD44, this binding mediated signals trigger cytological activities such as up-regulation of VEGF and p53 downregulation to function in the progression of BC (Misra et al., 2011; Lee et al., 2013; Cortes-Dericks et al., 2017).

CONCLUSION

Tissue and soluble CD44 are significantly elevated in BCPs and related to bad patients' survival. Serum sCD44 detection in peripheral blood has a prognostic role in the management of BCPs. Serum HA levels are highly elevated in BCPs with significant correlation with CD44 positivity which points to the role of CD44 in up-regulating HA. CD44 abundance may be important for driving angiogenesis by secreting VEGF. Decreased p53 levels are correlated with higher CD44 expression in BCPs, suggesting that aberrant CD44 protein expression is advantageous for the growth, survival and metastasis of tumor cells.

RECOMMENDATIONS

- We recommend using ELISA technique for monitoring soluble levels of serum sCD44 in BCPs after the cancerous tissue is removed surgically and/or after chemotherapy as an indicator of the effectiveness of the treatment.
- Further studies targeting CD44 to prevent aggressiveness of breast cancer are needed. Future studies with longer follow-up and a larger number of patients are important to confirm our results.

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CONFLICTS OF INTEREST

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