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Caveolin-1 Gene, Hyaluronan and CA 15-3 as Potential Biomarkers for Diagnosis and Prognosis of Primary Breast Cancer

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Authors' contributions

This work was carried out in collaboration between all authors. Authors WAH and NTEM designed the study. Authors HMEB, RIED, EHA and BMA performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. All authors managed the analyses of the study, managed the literature searches, read and approved the final manuscript.

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ABSTRACT

Aims: Breast cancer (BC) is the greatest female malignancy and the leading causes of cancer death in the less developed countries. The determination of markers that help in diagnosis, prognosis, discovery of recurrence and metastasis is a valuable tool for management in BC patients. The present study aimed to provide insights about the role of Caveolin-1 (Cav-1) gene, hyaluronan (HA) and cancer antigen (CA) 15-3 in development and progression of BC, evaluate the possible correlations between these biomarkers and the clinico-pathological status of BC and compare between validity of these biomarkers with CA 15-3.
Methodology: This case-control and cohort study included 49 female patients from the South Egypt Cancer Institute, Surgery Department, Assiut University, from December, 2013 to March, 2015 and

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10 healthy controls with matched age and sex. The patients were divided into 4 groups, group I: Included 39 female patients with breast cancer before operation, group II: Included 17 women from group I followed for 6 months after operation, group III: Included 9 women from group I followed for 12 months after operation, group IV: Included 10 female patients with benign breast diseases. Cav-1 gene analysis was performed by thermal cycler PCR method. Estimation of serum HA and CA 15-3 by ELISA and related clinico-pathological features were assessed.

Results: positive Cav-1 gene in 26 (66.7%) of 39 breast cancer patients was found. Benign and control groups were negative for the presence of the gene. Cav-1 gene was associated with larger tumor size ($p<0.05$), grade ($p<0.05$), advanced stage ($p<0.01$) and lymphovascular invasion ($p<0.05$).The mean serum levels of HA and CA 15-3 were significantly higher in BC women before operation when compared to benign and control groups. Patients after 6 and 12 months follow up showed a decrement of CA 15-3 levels. Also, HA levels were changed towards normalization, 6 months after treatment.

Conclusion: presence of Cav-1 gene and high circulating HA, CA were significantly associated with breast carcinogenesis and metastasis. Accordingly, estimation of these biomarkers may expect the breast disease behavior and its prognosis.

Keywords: Biomarker; breast cancer; CA 15-3; cancer diagnosis; Cav-1 gene; hyaluronan.

1. INTRODUCTION

Breast cancer is the most common malignancy worldwide [1]. One in eight women in the United States will develop the illness in their lifetimes [2]. In Egypt, 37.7% of total cancer cases among women is breast cancer [3]. The addition of a blood-based tumor marker test may rise patient compliance as blood analysis is more acceptable [4].

Caveolin-1 is the principal structural constituent of caveolae micro domains, elaborates in vesicular trafficking and signal transduction, its gene maps to 7q31.1 and encodes a 21 to 24 kDa integral membrane protein [5]. Caveolin-1 overexpression is associated with tumor malignant progression and plays an important role in carcinogenesis because it interacts with many factors involved in mitogenic signaling, angiogenesis, and senescence processes [6].

Hyaluronan (also hyaluronic acid or hyaluronate) is one of the most important polymers of extracellular matrix and could be affected with the physiological alterations in cell cycle during carcinogenesis. Hyaluronan binds mainly to CD44 receptor and promotes tumor growth, survival as well as cancer cell invasion [7].

Cancer antigen 15-3 is an epitope of a large transmembrane glycoprotein named mucin (MUC) that is resulting from the MUC1 gene. This protein is often overexpressed and aberrantly glycosylated on its extracellular region in breast cancer [8]. High tumor marker CA 15-3 before operation was significantly related with tumor size, axillary node involvement and advanced stage [9]. The CA 15-3 tumor marker test is specific, but not sensitive enough. The addition of other markers to the CA 15-3 may increase the sensitivity during the post-surgical follow up of breast cancer patients [10], so the present study clarify the possibility of using Cav-1 gene, HA, CA 15-3 as biomarkers for diagnosis and prognosis of breast cancer and correlate them with the clinico-pathological status of BC.

2. MATERIALS AND METHODS

2.1 Material

This case-control and cohort study included 49 female patients who were selected from the South Egypt Cancer Institute, Surgery Department, Assiut University, from December, 2013 to March, 2015. Their ages ranged between 30-70 years, with a mean ± SD (50.7 ± 10.3 years). In addition to 10 age and sex matched healthy controls. All participants signed a written informed consent for participation in the study. The study was approved by the Faculty of Medicine, Assiut University ethical committee in accordance with Helsinki declaration (1975).

Patients were divided into 4 groups as follows: Group I: Included 39 female patients with breast cancer before operation. Group II: Included 17 women from group I followed for 6 months after the surgical removal of their breast cancers. Group III: Included 9 women from group I followed for 12 months after the surgical removal of their breast cancers. Group IV: Included 10 female patients with benign breast diseases.

Three pathological types of benign breast diseases were selected: 7 cases had fibroadenoma (70%), 2 cases had fibrocystic diseases (20%) and 1 case had phylloid tumor (10%).

Group I women received treatment after surgery in the form of chemotherapy which included fluorouracil 500 mg/ m^2, epirubicin 100 mg/ m^2 or Adriamycin 50 mg/ m^2 and cyclophosphamide 500 mg/ m^2 (FEC or FAC) every 3 weeks for 6 cycles, then radiotherapy for 1.5 months followed by hormonal therapy (tamoxifen 20 mg/day) for patients whose tumors were positive for estrogen and/or progesterone receptors and to be continued for 3-5 years.

The following was done for all participants including demographic characteristics, family history of breast cancer, smoking habits, therapeutic history and personal obstetric history. Imaging studies including mammography and ultrasound were done. Body mass index was calculated for each female.

Exclusion criteria included: the presence of any previous tumors, getting neoadjuvant chemotherapy or undergoing surgical operations for tumor resection, Patients with chronic medical diseases and patients who are unfit for surgery.

2.2 Methods

Six ml venous blood was drawn by venipuncture, and divided into 2 parts. Two ml were collected on EDTA for DNA extraction, and the other 4 ml were left in room temperature for serum separation which was stored at -70°C till the assay of biochemical markers.

The level of serum hyaluronan was measured using an ELISA kit, supplied by WKEA Med Supplies (Chenguang Gardon, China) according to the methods of [11]. Serum Cancer antigen 15-3 was measured using an ELISA kit, supplied by BioTina GmbH (Freiburg, Germany) according to [12].

Genomic DNA was isolated from peripheral whole blood collected on EDTA using QIAamp DNA mini kit (Qiagen) according to the manufacturer's instructions. Primers were purchased from InvitrogenTM by life technologies, UK. The following primers were used to amplify 100 bp fragment of DNA.

Forward primer
5' AACGTTCTCACTCGCTCTCTGCTCGCTGCG 3',

Reverse primer
5' GTACACTTGCTTCTCGCTCAGCAC 3'

PCR was carried out using 25 µl of Dream Taq PCR master mix (2x) (Sigma), 1 µg of template DNA, 12.5 µl of nuclease-free water and 10 µm of each forward and reverse primers. The amplification was conducted on ARKTIK 96 Thermal Cycler (thermoscientific) as follows: initial 5-min denaturation at 94°C, followed by 45 cycles denaturation at 94°C for 30s , annealing at 55°C for 30s and extension at 72°C for 60s, then a final extension step of 7 min at 72°C. PCR products were electrophoresed on ethidium bromide stained (0.5 µg/ml) 1% agarose gel containing 1X TEA buffer and visualized by TRZol UV transilluminator (USA).

2.3 Statistical Analysis

Data collected were analyzed by computer program SPSS" ver. 20" Chicago. USA. Data expressed as mean, standard deviation and percentage. Whereas the cut-off point, sensitivity and specificity were made using MedCalc program. Prior to analysis the variables were tested for normal distribution using the Shapiro-Wilk W test. Student t-test was used for normally distributed data and Mann-Whitney was used for skewed data for the purpose of identifying differences between the tested groups. ANOVA test was used for comparison between different groups. Differences were considered significant at $p \leq 0.05$. Chi Square ($\chi 2$) was used to determine significance for categorical variables. Spearman correlation was used for correlations between groups.

3. RESULTS

The demographic and clinical data of patients and controls of the current study were clarified in Table (1). The age of breast cancer disease group was significantly higher than benign breast diseases. The age at menarche and age at menopause were not relevant to either benign breast diseases or breast cancer diseases groups. The mean levels of BMI were significantly higher in benign and breast cancer groups in comparison to those of controls. Family history of breast cancer and history of contraception in patients and controls were shown in Table 1. Table 2 showed the clinicopathological characteristics of the breast cancer patients.

Table 1. The demographic and the clinical data of patients and controls

Variables	Controls (n=10)	Benign breast diseases group (n=10)	Breast cancer (group I) (n=39)
Age (years) mean ± SD	44.20±10.81	36.30±9.14 p1 NS	50.67±10.32 p1 NS p2 < 0.01
Age at menarche (years) mean ± SD	13.10±1.28	13.30 ±1.25 p1 NS	13.46±1.62 p1 NS p2 NS
Age at menopause (years) mean ± SD	52.25±3.20	48.50±2.12 p1 NS	48.74±3.4 p1 NS p2 NS
Body mass index (kg /m2) mean ± SD	29.20±2.66	32.97±6.93 p1 < 0.05	32.84± 5.20 p1 < 0.05 p2 NS
Family history of breast cancer N (%)			
-Yes	1 (10.0%)	1 (10.0%)	4 (10.3%)
-No	9 (90.0%)	9 (90.0%)	35 (89.7%)
History of using contraceptives N (%)			
-Yes	6 (60.0%)	4 (40.0%)	21 (53.8%)
-No	4 (40.0%)	6 (60.0%)	18 (46.2%)

Group I: Breast cancer patients at diagnosis.
p1: Compared to controls, p2: Compared to benign breast diseases group, NS: non-significant

There were 26 (66.7%) of 39 BC patients had positive Caveolin 1gene as shown in Fig. (1). Fig. (2) showed positive and negative samples for the presence of caveolin1 gene by agarose gel electrophoresis. The statistical relationship between clinicopathological features and other molecular markers with caveolin-1 gene analysis in breast cancer patients were shown in Table 3.Caveolin-1 gene was associated with larger tumor size ($p<0.05$), grade ($p<0.05$), advanced stage ($p<0.01$) and lymphovascular invasion ($p<0.05$).

Table 4. showed the levels of studied parameters in patients and controls. The current study showed a high significant difference ($p<0.001$) in levels of hyaluronan, CA 15-3 between breast cancer patients before operation and control groups and between breast cancer patients before operation and benign breast diseases groups. The mean±SD levels of serum hyaluronan in breast cancer patients showed a decrement towards normalization of levels 6 month after the surgical removal of their tumors and after they received medical treatment. After 12 months, hyaluronan levels showed no significant difference from pretherapy levels. Also, CA 15-3 levels were changed towards normalization, 12 months after treatment.

The sensitivity and specificity based on the cut-off levels for studied variables in breast cancer patients were demonstrated in Table 5.

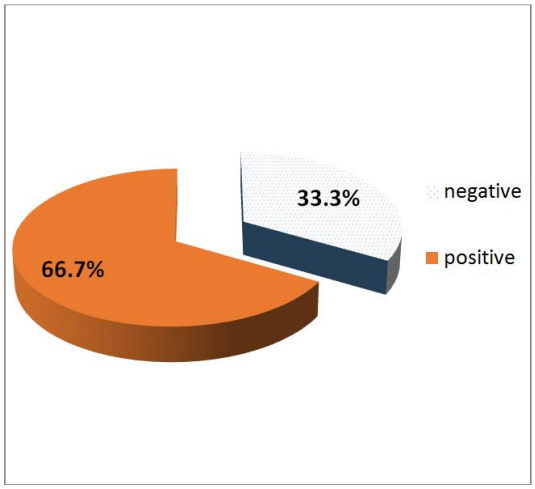

Fig. 1. Percentage of caveolin1 gene in breast cancer group before operation

The result of the current study showed significant relation between hyaluronan and clinicopathological characteristic of breast cancer patients before operation as regarding axillary lymph node involvement and distant metastasis (Table 6). Interestingly, only CA 15-3's correlation with lymphovascular invasion was statistically significant.

Table 2. The clinicopathological characteristics of the breast cancer patients

	Number of patients	Percentage
Grade:		
- Grade I	2	5.1%
- Grade II	24	61.5%
- Grade III	13	33.3%
Tumor size (T)		
- T1	5	12.8%
- T2	24	61.5%
- T3	9	23.1%
- T4	1	2.6%
Regional L.N.		
- N0	10	25.6%
- N1	15	38.5%
- N2	8	20.5%
- N3	6	15.4%
Distant metastasis (M)		
- M0	33	84.6%
- M1	6	15.4%
Stages		
- I	3	7.7%
- II	21	53.8%
- III	10	25.6%
- IV	5	12.8%
Estrogen receptor (ER)		
-Positive	20	51.3%
-Negative	18	46.2%
-Not available	1	2.5%
Progesterone receptor (PR)		
- Positive	14	35.9%
- Negative	24	61.5%
- Not available	1	2.6%
HER2\ neu		
- Positive	8	20.5%
- Negative	14	35.9%
- Not available	17	43.6%
Distribution of breast cancers		
- Right-sided tumor	18	46.2%
- Left-sided tumor	21	53.8%
Lymphovascular invasion		
- Yes	25	64.1%
- No	14	35.9%
Type of surgery		
- Modified radical mastectomy (MRM)	24	61.6%
- Conservative breast surgery (CBS)	10	25.6%
- Palliative mastectomy	5	12.8%
Menopausal state		
- Pre-menopausal	16	41%
- Post-menopausal	23	59%

The relation between the Caveolin-1 gene analysis and biochemical parameters among breast cancer patients were presented in Table 7. Caveolin1 gene showed significant associations with serum CA 15-3 levels in breast cancer patients.

4. DISCUSSION

Breast cancer detection relies commonly on mammography, which associated with diminished breast cancer mortality [13]. However, mammography screening has made controversy due to the hazards of false-positive outcomes and over finding of indolent disease [14]. There is thus a critical requisite to recognize the biochemical bases of carcinogenesis, invasion and metastasis. The adding of a blood-based tumor indicator test may raise patient agreement as blood testing is more suitable and would also avoid the difficulties related with imaging high-density breast tissue [1].

In spite of the discovery of a number of breast cancer susceptibility genes in the last decades (e.g. TP53, PTEN, CDH1, STK11, CHEK2, ATM, BRIP1, BRCA1 and BRCA2), there are many genetic variants and triggers required to be recognized [15]. The present study found caveolin-1 gene in 26 (66.7%) of 39 cases of breast cancer group. On the other hand, caveolin-1 gene could not be detected in 33.3% of malignant cases. This may be due to decreased Cav-1 gene expression in non-metastatic primary tumors [16]. No Caveolin-1 gene could be found in benign breast disease group (n=10) and control group (n=10). These results agreed with Thompson et al. [17] and Chung et al. [18].

Lamaze and Torrino [19] found a strong association among Cav-1 expression and a basal like phenotype of breast cancer, as 52% of cancers that expressed Cav-1 had this phenotype, linked with only 9% of Cav1 negative carcinomas. In addition, 90% of metaplastic breast cancers revealed Cav1 expression.

Caveolin-1 can prevent apoptosis through numerous signal pathways and develops breast cancer cell survival. It is associated with cell transformation, cancer growth, cell migration, invasion and angiogenesis. In addition, Cav-1 shields cancer cells from death by speeding aerobic glycolysis [20]. The gene can facilitate multidrug resistance (MDR) by positively regulating the action of the ATP-binding cassette

transporter breast cancer resistance protein in breast cancer [21]. Caveolin-1 knockdown could significantly decrease the tumorigenicity of breast cancer stem cells by downregulating the β-catenin/ ATP-binding cassette subfamily G member 2 signaling pathway [22]. Moreover, breast cancer violence is associated with Cav-1 CGI shore methylation levels, a newly described areas that flank CpG islands with less CG-density [23].

Table 3. Statistical relationship between caveolin 1 gene analysis and the studied clinicopathological characteristics of the breast cancer patients

Variables	Total N (39)	Caveolin 1 gene positive cases (n=26) {N (%)}	Caveolin1 gene negative cases (n=13) {N (%)}	p value
Grade:				
-Grade I	2	0 (0.0%)	2 (100%)	p<0.05
-Grade II	24	19 (79.2%)	5 (20.8%)	
-Grade III	13	7 (53.8%)	6 (46.2%)	
Tumor size				
-T1	5	1(20%)	4 (80%)	p<0.05
-T2	24	16 (66.7%)	8 (33.3%)	
-T3	9	8 (88.9%)	1(11.1%)	
-T4	1	1(100%)	0 (0.0%)	
Regional L.N.				
-N0	10	5 (50%)	5 (50%)	NS
-N1	15	8 (53.3%)	7 (46.7%)	
-N2	8	8 (100%)	0 (0.0%)	
-N3	6	5 (83.3%)	1 (16.7%)	
Distant metastasis (M)				
-M0	33	20 (60.6%)	13 (39.4%)	NS
-M1	6	6 (100%)	0 (0.0%)	
Stages				p<0.01
-I	3	0 (0.0%)	3 (100%)	
-II	21	12 (57.1%)	9(42.9%)	
-III	10	9 (90%)	1 (10%)	
-IV	5	5 (100%)	0 (0.0%)	
Estrogen receptor				
-Positive	20	12(60%)	8 (40.9%)	NS
-Negative	18	14 (77.8%)	4 (22.2%)	
-Not available	1	0 (0.0%)	1 (100%)	
Progesterone receptor				
-Positive	14	9 (64.3%)	5 (35.7%)	NS
-Negative	24	17 (70.8%)	7 (29.2%)	
-Not available	1	0 (0.0%)	1 (100%)	
HER2\ neu				
-Positive	8	3 (37.5%)	5 (62.5%)	NS
-Negative	14	12 (85.7%)	2 (14.3%)	
-Not available	17	11 (64.7%)	6 (35.3%)	
Triple negative				
-Yes	11	9 (81.8%)	2 (18.2%)	NS
-No	28	17 (60.7%)	11(39.3%)	
Age				
- ≤50 years	19	11 (57.9%)	8 (42.1%)	NS
- >50 years	20	15 (75%)	5 (25%)	
Pathological tumor type				
-Carcinoma insitu	1	0 (0.0%)	1 (100%)	NS
-Invasive ductal	37	26 (70.3%)	11 (29.7%)	
-Atypical medullary carcinoma	1	0 (0.0%)	1 (100%)	
Lymphovascular invasion				
- Yes	25	20 (80%)	5 (20%)	p<0.05
- No	14	6 (42.9%)	8 (57.1%)	
Body mass index (BMI)				
- <30	11	7 (63.6%)	4 (36.4%)	
- ≥30	28	19 (67.9%)	9 (32.1%)	

Table 4. The levels of studied parameters in patients and controls

Variables	Controls (n=10)	Benign breast diseases group (n=10)	Group I (n=39)	Group II (n=17)	Group III (n=9)	ANOVA p-value
Serum hyaluronan (ng/l)						
range	125-146	113-165	106-368	110-205	117.8-239.9	< 0.001
mean ± SD	135.5±7.4	128.54±15.04 *ac***	***ab	*bc	**ab	
Serum cancer antigen15-3 (U/ml)						
range	14-40	16-42	22.34-100	10.57-62	14-50	< 0.001
mean ± SD	25.2± 7.5	24.20 ±8.91 ***c	***ab	**c	**c	

*Group I, II, III correspond to breast cancer patients at diagnosis, 6 months after treatment, 12 months after treatment respectively, a: Compared to controls, b: Compared to benign breast diseases group, c: Compared to breast cancer group at diagnosis, *p<0.05, **p<0.01, ***p<0.001*

Table 5. Cut-off points, sensitivity, specificity and area under ROC curve of studied parameters in breast cancer patients

Variables	Cut-off (discriminant analysis)	Sensitivity%	Specificity%	Area under ROC curve
Serum hyaluronan (ng/l)	144. 6	79	90	0.877
CA 15-3 (U/ml)	30.2	87.18	80	0.926

A scaffolding amino acid sequence identified in Cav-1 permits this protein to interact with signaling molecules, such as epidermal growth factor receptor (EGFR), G-proteins, Src family tyrosine kinases, Rho-GTPases, protein kinase C, eNOS, and integrin. In several types of cancer cells, Cav-1 is linked or co-localized with EGFR and appears to modify EGFR signaling [24]. Cav-1 can adjust these signaling molecules, thus playing a dynamic role in cancer advancement. The cooperation between cav-1 and Rho-GTPases helps tumor metastasis, which mostly depend on the raised expression of α5-integrin and the higher activation of Src, Ras and Erk. Besides, an amplified expression of Cav-1 can stimulate the activation of AKT1, leading to the increased phosphorylation of Rho- GTPase. As aconsequence, the invasion capability of breast cancer cells is significantly raised [21]. Also, cav-1 contributes in the remodelling of the extracellular matrix by promoting communications with matrix metalloproteinases [25].

In contrast, Chiu et al. [26] revealed that Cav-1 overexpression can decrease the primary breast cancer growth and brain metastasis via the oncoprotein signal transducer and activator of transcription 3 (Stat3) inhibition. Furthermore, Thomas et al. [27] showed Cav-1 downregulation (compared with normal tissue) in breast cancer cells and demonstrated that loss of Cav-1 expression was linked with tamoxifen resistance.

Six months after treatment, caveolin-1gene was detected in 5 of 17 cases (2 of them from stage II, 3 were from stage III) due to difference in response of patients to treatment. Twelve months after treatment, no caveolin-1gene was observed in the followed up breast cancer women (n=9). Caveolin-1 gene modifies breast cancer response to chemotherapy and radiation therapy [20]. On the other hand, Chatterjee et al. [22] reported that radiation and chemotherapy up-regulate Cav-1 expression, while Cav-1 reduction induces both chemosensitization and radiosensitization through changed apoptotic and DNA repair signaling.

In the current study, caveolin-1 gene has been linked to increased tumor diameter ($p<0.05$), poor histopathologic grade ($p<0.05$) in BC patients at diagnosis as were found in previous studies (Chatterjee et al. [22] and Zhao et al. [28]). Also, Cav-1 level was observed to have

association with advanced stage ($p<0.01$) and lymphovascular invasion ($p<0.05$) as shown in Qian et al. [29] and Mao et al. [30] studies. These data designate its involvement in BC as a possible molecular goal and its ability to expect the patient's response to treatment. Cav-1 has been documented as a janus-faced tumor regulator in different types of cancers [30]. A microarray analysis also reinforced Cav-1 as a metastasis-related gene by matching gene expression profiles between poorly and highly invasive breast cancer cells [31].

Caveolin-1gene found in ER negative (14 of 18) patients more than ER positive (12 of 20) patients. This in line with Rao et al. [23] that reported that cav-1 level correlated with ERα-negative BC patients. Also, Liedtke et al. [32] found Caveolin-1 positivity significantly linked with lack of ER, PR, and HER2 expression.

The tumor promotion by HA may be mediated in several ways. The first way is accumulation of HA creates highly hydrated and gel-filled spaces in extra cellular matrices. The formation of the less dense matrix separates collagen layers and enhances tumor cell migration and penetration through physical barriers in the matrices [33]. The second way is the interaction of HA with its cell surface receptors CD44 and RHAMM influenced cellular processes such as differentiated cell growth, migration, and invasion which support carcinogenesis [7]. Elevated synthesis of HA is associated with enhanced cell proliferation and angiogenesis, and subsequently tangled in carcinogenesis [34].

In the current study, the mean serum hyaluronan levels were significantly higher in breast cancer women as compared to that of controls and benign groups (Table 4). These results are in accordance with the finding of Yahya et al. [35] and Wu et al. [36]. Hyaluronic acid may be formed by tumor cells or as a consequence of interactions between cancer and the adjacent connective tissue.

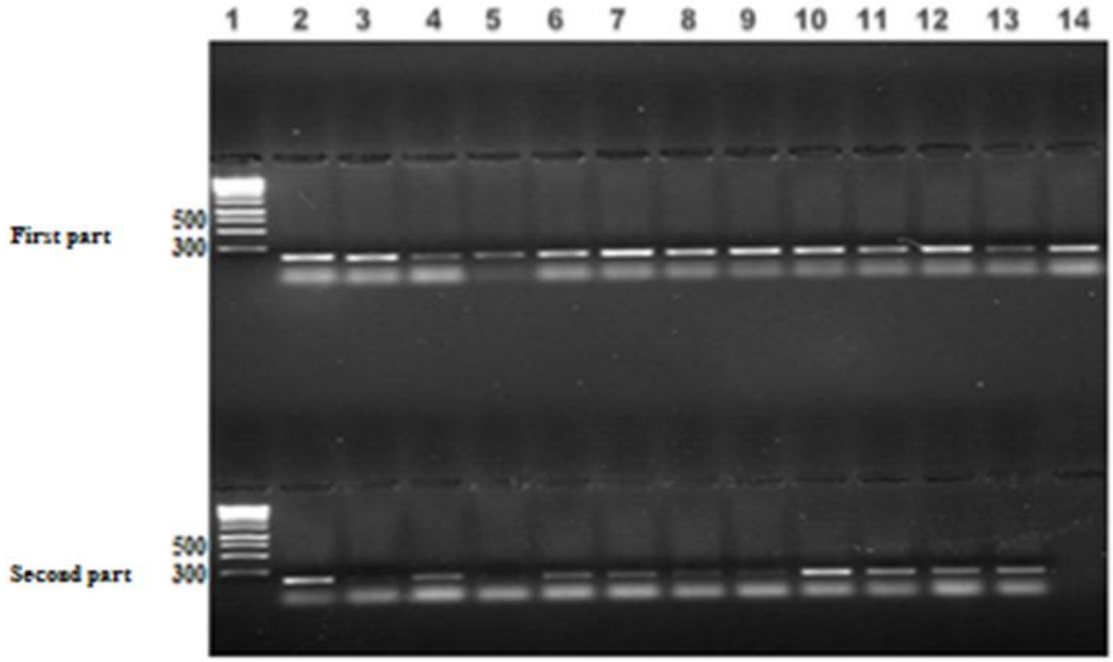

Fig. 2. Agarose gel electrophoresis (1.5%) for PCR products of caveolin 1 gene. Lane 1 represented DNA marker. The first part: Lanes 2-14 represented positive samples for the presence of caveolin1 gene (100 bp PCR products). The second part: Lanes 3, 5, 8, 9 represented negative samples for caveolin1 gene, other lanes were positive for the gene. The faint band indicated decrease gene expression which correlated with early stage

Table 6. Statistical relation between the studied biochemical parameters and clinicopathological characteristics of the breast cancer patients before operation

Variables	Number of patients	Hyaluronan	CA 15-3
Grade			
- G1 + G2	26	196.5±64.8	56±23.5
- G3	13	205.6±74.7	59.4±27.6
TNM stage			
-I + II	24	175.4±45 [b]	51.5±23.7
-III + VI	15	238.2±79.9	66 ±24.2
Tumor size			
-T1+T2	29	194.9±62	54.5±22.7
-T3+T4	10	213±82	64.6±29.7
Axillary lymph node involvement			
-Negative	10	163±33.5 [a]	50±24
-Positive	29	212±71.9	59±24
Pathological tumor type			
- Ductal invasive	37	200±68.9	57.7±24.9
- Other tumor type	2	189±36	47±22
Distant metastasis			
- Yes	6	276±66 [b]	68±31.4
- No	33	185.6±58	55±23.3
Age			
- ≤50 years	19	195.5±71	52±20.3
- >50 years	20	203.4±65	61.8±27.9
Lymphovascular invasion			
- Yes	25	214.5±77	64.9±23.9 b
- No	14	172.9±32	43.3±20.2
Menopausal status			
-Premenopausal	23	202±71	51.9±21
-Postmenopausal	16	197±66	61.2±26.9
Estrogen receptor			
-Positive	20	201±78	56±24.5
-Negative	18	197±56	58±25.5
-Not available	1		
Progesterone receptor			
-Positive	14	203±88	58±27
-Negative	24	196.6±55	57.6±23.7
-Not available	1		
HER2\ neu			
-Positive	8	189.8±50	51.7±22.8
-Negative	14	216.6±70	60.8±25
-Not available	17		56.6±26

a: p< 0.001, b: p< 0.01, c: p< 0.05

Table 7. Statistical relationship between the studied biochemical parameters and caveolin1 gene analysis of the breast cancer patients

Variables	Caveolin1 gene positive cases (n=26) Mean ± SD	Caveolin1 gene negative cases (n=13) Mean ± SD	p value
Serum hyaluronan (ng/l)	210.6±76.5	177.5±37.8	NS
Serum CA 15-3 (U/ml)	62.53±24.7	46.3±21.8	p<0.05

The present study also showed that the mean serum hyaluronan levels were significantly decreased (p<0.05) in BC women after 6 months of surgical removal of tumors besides medical treatment. These results are in accordance with the finding of Yahya et al. [35] and Peng et al. [37] which established the blocking of HA roles by FAC chemotherapy have therapeutic significance in breast malignancy.

However, there was non-significant difference between levels of serum hyaluronan of

pretreated BC women and patients 12 months after treatment. This may be due to non-evident distant metastasis and/or non-specific rise of HA. High serum HA has been reported with liver disease and various inflammatory conditions, including rheumatoid arthritis, psoriasis, scleroderma, and osteoarthritis. Although, this biomarker is not specific to BC diagnosis, serum HA levels may be useful to recognize potentially high-risk sets for additional diagnostic work-ups or nearer follow-ups [38].

There is a significant correlation between serum hyaluronan and TNM stage ($p<0.01$) as also found by Corte et al. [39] and Wu et al. [36]. Serum hyaluronan was correlated with lymph node positivity ($p<0.05$) and distant metastasis ($p<0.01$) as shown by Auvinen et al. [40]. These results suggest that HA achieves a number of roles in developing tumors and especially contributes to invasion in initial and advanced stage breast cancer [41]. In the contrary, Wu et al. [36] found no relationship between the serum HA levels and BC metastasis.

High preoperative CA 15-3 level is directly linked to tumor burden. CA15-3 is possibly the best identified, non-invasive marker of breast cancer, even though its suggested medical use is limited to checking of patients with metastatic disease during treatment [42]. This indicator plays the biological roles such as cell connection, immunity and it is responsible for metastasis [43]. So it can be used in combination with investigative imaging and history, physical examination in assessing return of disease and response to treatment [44].

Concerning CA 15-3, the present study found that its preoperative levels in breast cancer patients were significantly higher than control group and benign group (Table 4) with no significant difference between control and benign groups. Similar observations were reported by previous studies (Gautam et al. [44] and Nieder et al. [9]). CA 15-3 levels decreased significantly after 6, 12 months of operation and treatment to be non-significant from control and benign group levels. These findings are in line with Gautam et al. [44] and Khan et al. [42]. The only clinicopathological feature related to elevated CA 15-3 levels was lymphovascular invasion. In addition, CA 15-3 levels was found to be increased across different stages (stage II 49±17 U/ml, stage III 73.5±26 U/ml, stage IV 99.2 U/ml) but did not reach a statistically significant level. Lee et al. [45] demonstrated that raised CA 15-3 before surgery was significantly associated with tumor size, axillary node involvement and advanced stage. On the other hand, Geng et al. [46] found a correlation with metastatic sites. The result was in agreement with previous studies (Incoronato et al. [47] and Śliwowska et al. [48]).

This study revealed significant correlation between cav-1 gene and serum CA 15-3 ($p<0.05$) levels in breast cancer patients.This association may indicate their associated role in breast cancer formation, invasion and metastasis.

5. CONCLUSIONS

The current study revealed that presence of caveolin-1gene, high circulating hyaluronan and CA 15-3 are significantly associated with breast carcinogenesis and act together as a battery for tumor progression and metastasis. Accordingly, estimation of these biomarkers may expect the breast disease behavior and its prognosis. Also, the study throws the light for the future use of these biomarkers as ideal chemopreventive or therapeutic agents for breast cancer.

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COMPETING INTERESTS

Authors have reported that no competing interests exist.

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