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OCT3/4, ALDH-1 and CD44 Expression Levels in Iraqi Women with Stage II-III Breast Cancer

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ABSTRACT

Cancer stem cell (CSC) markers OCT3/4, ALDH-1 and CD44 play important roles in metastasis and resistance to conventional cytotoxic agents. In this study, we investigated the expression levels of CSC markers OCT3/4, ALDH-1 and CD44 in Iraqi women with stage II-III breast cancer or with benign breast tumor. We also investigated the association between the expression levels of these markers and some clinicopathological features. The expression of OCT 3/4, ALDH-1 and CD44 in breast tissues was determined using real-time PCR and immunohistochemistry in 64 patients with breast cancer stage II or III samples as well as in 21 corresponding benign tumors. OCT3/4, ALDH-1 and CD44 mRNA levels were highly expressed in stage III (p= 0.004, 0.015 and 0.008, respectively) and in stage II (p= 0.043, 0.045 and 0.028, respectively) as compared to those in benign tumors. There was no significant variation in the expression of these markers between stages II and III (p= 0.18, 0.30 and 0.49). In addition, immunohistochemistry showed that OCT3/4, ALDH-1 and CD44 expression levels were increased in 82.2%, 68.8% and 53.1%, respectively in stage II-III breast cancer as compared to 14%, 28%, and 33% in benign tumor. The expression levels of OCT3/4, ALDH-1 and CD44 were correlated to tumor grades, types and lymph node metastasis. These results indicate that the expression levels of CSC markers OCT3/4, ALDH-1 and CD44 were increased in stage II-III breast cancer but not in benign breast tumor in Iraqi women.

Key Words: Benign tumor, breast cancer stem marker, cancer stem cells.

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INTRODUCTION

Breast cancer is one of the most frequent malignant tumors among women, with an estimate of more than 1.4 million new breast cancer cases diagnosed worldwide each year and accounting for 23% of all new neoplasm cases (Jemal et al., 2011). Approximately half a million women die from breast cancer (DeSantis et al., 2011). In Iraq, breast cancer ranks the first among cancers diagnosed in women, its incidence rate in Iraqi women has increased from 26.6 per 100,000 in 2000 to 31.5 per 100,000 women in 2009 (Al-Hashimi and Wang, 2014).

Tumorgenesity and metastasis in human cancers may result from a small population of cells called tumor initiation cells or cancer stem cells (CSCs). These cells have the capacity to form new tumors in immune-deficient mice since they have the ability of self-renewal and differentiation. The expression of potential biomarkers of cancer stem cells was studied extensively to understand the behavior of these cells in cancer progression and resistance to conventional therapy (Voranta et al., 2013). Cancer stem cells have the ability of self-renewal and differentiation. They may be generated from disturbances in normal stem cells or differentiated cells. These cancer stem cells are involved in the initiation of malignant growth and metastasis and they have been found in many human cancers (Raya et al., 2001; Li et al., 2009).

Cytotoxic agents that are used in cancer treatments target the bulk tumor by reducing the volume of the tumor but they may not exert this effect on CSCs, therefore patients suffer from the relapse of breast cancer. Cancer stem cells can be identified and separated from different solid tumors by using putative CSC markers (Wakamatsu et al., 2012).

CD44 is a transmembrane glycoprotein that plays important roles in several cellular properties including differentiation, growth, motility and survival. It plays a key role in the metastasis of cancer cells by acting as an intermediate link between cell-to-cell and cell-to-environment interactions through its ability to bind with hyaluronic acid, a transmembrane glycosaminoglycan as well as to other ligands (Nagano et al., 2004). In cancer, CD44 plays important roles in tumorigenesis by allowing colony formation through increased adhesion to its multiple ligands in the surrounding environment, induction of cellular growth factors, degradation of the surrounding extracellular matrix allowing cellular migration and tumor expansion (Herrera and Jothy, 2009). In breast cancer, CD44 enhances tumor growth and cancer cell migration in response to environment signaling (Al-Hajj et al., 2003).

Aldehyde dehydrogenase-1 (ALDH-1) is a detoxifying enzyme belonging to a family of 19 NADP-dependent enzymes that play key roles in the oxidization of intracellular and extracellular aldehydes to their corresponding carboxylic acids. It is important in protecting cellular homeostasis, by scavenging reactive aldehydes resulting from lipid peroxidation (Vasiliou et al., 2000). High ALDH-1 expression level is associated with chemoresistance by interfering with cytotoxic drugs used in the treatment of cancer (Thomas and Matthew, 2013).

OCT3/4 or POU5f1 is a transcriptional factor that belongs to homobox Pit-Oct-Unc (POU) family and plays an important role in self-renewal and pluripotency of embryonic stem cells. It is expressed in unfertilized oocyte cells, in the inner cell mass of the blastocyst, embryonic stem cells and germ cells. The stemness of stem cells disappears after knockout of the OCT3/4 gene from mouse embryos. Two-fold increase in OCT3/4 expression induced conversion of embryonic stem cells towards mesoderm and endoderm while a 50% decrease in OCT3/4 expression induced differentiation of embryonic stem cells to trophectoderm. On the other hand, up-regulation of OCT3/4 expression was associated with tumorgenesity, metastasis and resistance to conventional cancer therapy (Pardo et al., 2010; Du et al., 2011).

In this study, the expression levels of CSC markers OCT3/4, ALDH1 and CD44 were determined in Iraqi women with stage II-III breast cancer and the association between the expression levels of these markers and tumor stages, grades, types and lymph node metastasis were examined.

MATERIALS AND METHODS

Patients and tumor characteristics

Sixty-four malignant breast cancers and twenty one corresponding benign tumors were obtained from women with breast tumors admitted to the Alkarama Teaching Hospital in Baghdad, Iraq, during the period from June 2013 to April 2014.

The Institutional Review Board at Beirut Arab University (BAU), Lebanon, approved this study. The patients with breast cancer enrolled in this study were diagnosed as stage II (60.9%) and stage III (39.1%) (Table 1). The mean age of patients was 64 years (range 36 to 77 years).

Mammogram and fine needle aspiration (FNA) technique as well as histopathological examination were used for the diagnosis of all cases. None of the patients recruited in this study had received chemotherapy or radiation therapy.

RNA extraction and cDNA preparation

Fresh breast tissues samples were stored in liquid

Table 1. Clincopathological features of patients with stage II-III breast cancer.

Parameter	Patients (n)	Percentage (%)
Age of patients		
≤50 years	17	26.6 (%)
≥50 years	47	73.4 (%)
Tumor Stages		
Stage II	39	60.9 (%)
Stage III	25	39.1 (%)
Tumor Grades		
Grade I	8	12.5 (%)
Grade II	36	56.3 (%)
Grade III	20	31.2 (%)
Tumor Types		
IDC	48	75 (%)
ILC	10	15.6 (%)
Other	6	9.4 (%)
Lymph Node Metastasis		
Positive	29	45.3 (%)
Negative	35	54.7 (%)
ER Status		
Positive	47	73.4 (%)
Negative	17	26.6 (%)
PR Status		
Positive	44	68.8 (%)
Negative	20	31.3 (%)
HER2/new Receptor Status		
Positive	21	32.8 (%)
Negative	43	67.2 (%)

IDC: Invasive Ductal Carcinoma, **ILC**: Invasive lobular carcinoma, **ER**: Estrogen Receptor, **PR**: Progesterone Receptor, **HER2/New**: Epidermal growth Factor Receptor.

nitrogen for RNA extraction. Total RNA was extracted from these frozen tissues using an RNA miniprep kit (Agilent biotechnology Inc., USA) according to the manufacture instructions. Pure RNA was used for the synthesis of cDNA by using a Revert Aid First Strand cDNA synthesis kit (Thermo Fisher Scientific Inc., USA). A volume of 2 µl of total RNA (400 µg/µl) was added to 1 µl oligo $(dt)_{18}$ primer (100 µM) and nuclease-free water was added to a final volume of 12 µl. Then, 4 µl of 5X reaction buffer, 1 µl ribolock RNase inhibiter (20 U/µl), 2 µl dNTP (200 µM) and 1 µl of Revert Aid M-Mulv Reverse transcriptase (200 U/µl) were added. The reaction mixture was incubated for 60 Min. at 42° C for cDNA synthesis followed by incubation for 5 Min. at 70° C for termination of the activity of the reverse transcriptase enzyme.

Real-time PCR

The mRNA levels of OCT3/4, ALDH-1, CD 44, and β-actin in breast tissues from breast cancers and benign tumors were determined by real-time PCR. The accession numbers for mRNA sequences were obtained from NCBI database (Table 2) and were used for designing specific primers for these mRNA molecules using NCBI/Primer-BLAST. The sequences of the primer pairs used are shown in Table 2.

The PCR amplification reaction was carried out by AccuPower GreenStar qPCR Premix (Bioneer, Korea) using Stratagene Mx3005PqPCR system (Agilent Technologies, CA, USA). The thermal profile reaction used was 5 Min. at 95° C for 1 cycle, followed by 40 cycles at 94° C for 15 Sec., 58° C for 30 Sec. and 72° C

Table 2. The sequences of specific primers used for determination of ALDH-1, CD44, OCT3/4 and β-actin by real-time PCR

	Primer sequence (5' to 3')	PCR product (bp)	NCBI reference sequence
ALDH-1	F: GCAACTGAGGAGGAGCTCTG R: AGCATCCATAGTACGCCACG	117	NM-000689.4
CD44	F: TTACAGCCTCAGCAGAGCAC R: TGACCTAAGACGGAGGGAGG	145	NM-001202557.1
OCT3/4	F: ATGTGGTCCGAGTGTGGTTC R: ACAGTGCAGTGAAGTGAGGG	186	NM-001285987.1
β-actin	F: CCGCAAATGCTTCTAGGCG R: TGTTTTCTGCGCAAGTTAGGT	78	NM-001101.3

for 30 Sec. The calculation of relative amount of gene expression was performed by using the equation $=2^{-\Delta\Delta ct}$. The Mann-Whitney U test was used to compare the median expression levels.

Immunohistochemistry staining

Fresh breast tissue samples were fixed in 10% formalin and embedded in paraffin for immunohistochemistry staining and pathological examination for determination of tumor type, grade, lymph node metastasis and estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2/neu) status. Tumor staging was evaluated according to a modified Bloom-Richardson grading system. The expression of OCT3/4, ALDH-1, and CD44 was determined by using immunohistochemistry. Paraffin embedded sections were cut and mounted on positively charged glass slides and deparaffinized in xylene.

The sections were treated with heat induced retrieval antigen (HIRA) in citrate buffer at 121°C and washed twice. Slides were incubated with bovine serum albumin (BSA) and the slides were incubated with anti-ALDH-1 antibody (A1334-33W US-bio, USA), anti-CD44 antibody (HCAM (F-4): Sc-9960 Santa Cruz biotechnology, Inc, USA) or anti-OCT3/4 antibody (Sc-5279 Santa Cruz biotechnology, Inc.). Secondary antibody (DAKO biotechnology) was added followed by incubation with avidin-biotin for 30 Min. at room temperature. Slides were treated with diaminobenzidine (DAB), counter-stained with hematoxiline, dehydrated with ethanol, mounted with water-free mounting medium (DPX) and analyzed by light microscope. Placental tissue was used as a positive control. The negative control was treated with all the above steps except the incubation with primary antibody.

Staining evaluation

Normal cells were scored as zero (no positive staining), score 1: (1-10% staining), score 2: (11-50%) and score 3 (51-100 %). The positive intensity was considered as zero (none), weak positive, moderate positive and strong positive. Both zero and score 1 were considered as low expression and both scores 2 and 3 were recorded as high expression.

Statistical analysis

The comparison between breast carcinoma stage II-III and benign tumors was performed by using prism pad graph version 6 (Graphic pad Software Inc., San Diego CA, USA). Comparison of expression values was performed by Mann-Whitney U test. A chi-square (χ^2) statistic was used to investigate whether expression values differed between breast cancer tissues stage II-III and benign tumors. A $P<0.05$ value was considered as statistically significant.

RESULTS

This study examined the expression levels of CSC markers OCT3/4, ALDH-1, and CD44 in 64 patients with stage II-III breast cancer and 21 benign tumors using quantitative RT-PCR and immunohistochemistry.

Clinical and pathological data

Table 1 represents the clinical data and pathological features of patients with stage II-III breast carcinoma enrolled in this study. The diagnosis of the stages of breast cancer was based on the modified Bloom-Richardson grading system. Histopathological tumor

grades showed that the patients with well differentiated (grade 1) represented 12.5% of all patients, while the moderate (grade 2) and poorly differentiated (grade 3) were 56.3% and 31.2%, respectively. Tumor histopathological examination showed that the majority of tumors were ductal carcinomas, where 75% of the cases were pure invasive ductal carcinomas, 15.6% were invasive lobular carcinomas and 9.4% others (Invasive medullary carcinomas and mixed infiltrative ductal and lobular carcinomas). Twenty-nine patients (45.4%) had lymph node metastasis. The hormone receptor status for ER, PR and HER2/neu were 73.4%, 68.8% and 32.8%, respectively.

mRNA levels of OCT3/4, ALDH-1 and CD44

Figure 1A-1C shows the steady-state mRNA levels of OCT3/4, ALDH-1 and CD44 in breast tissues from stage II-III breast carcinoma and benign tumors as determined by real-time RT-PCR. The mRNA level of OCT3/4 was increased in breast cancer stage III (13.49±2.07, p= 0.0004) and in stage II (9.43±2.57, p=0.043) as compared to that in benign tumors (3.96±0.76) (Figure 1A). The mRNA level of ALDH-1 showed an increased expression in malignant tumor stages III and II of breast carcinoma (16.41±5.41, p=0.015 and 10.34±3.24, p= 0.045, respectively) as compared to that of benign tumors (2.47±0.35) (Figure 1B). The mRNA level of CD44 in breast cancer tissues was increased in stages III and II of breast cancer tissues (5.57 ±0.91, p=0.008 and 4.95±0.42, p= 0.028, respectively) as compared to that in benign tumors (2.49±0.47) (Figure 1C).

Protein expression of OCT3/4, ALDH-1 and CD44

Immunohistochemistry data showed that OCT3/4, ALDH-1 and CD44 levels were higher in stage II-III breast cancer than those in benign tumors (Figures 2-4). The expression of OCT3/4 was mainly located in the cell nuclei while ALDH1 was mainly expressed in the cytoplasm. OCT3/4, ALDH-1 and CD44 markers were highly expressed in 82.2%, 68.8% and 53.1% of cancerous tissues as compared to 14.3%, 28.6% and 33.3% of benign tumors, respectively (Table 3). Human placental tissue was used as positive control for the expression levels of CSC markers.

Relationship between OCT3/4, ALDH-1 and CD44 expression and clinicopathological data

Expression levels of OCT3/4, ALDH-1 and CD44 were significantly increased with tumor grade (p=0.003, 0.004 and 0.01, respectively), and with tumor type (p=0.001, 0.003, and 0.001) (Table 4). OCT3/4 ALDH-1 and CD44 expression levels were higher in patients with lymph node metastasis when compared to the negative metastatic group (p=0.005, 0.05, and 0.002). There was no significant variation between the expression levels of OCT3/4, ALDH-1 or CD44 with age, stages, and hormone receptor status (Table 4).

DISCUSSION

Cancer stem cells represent a small population of cancer cells and are responsible for initiation, progression, recurrence and tumor metastasis (Wicha et al., 2006). They play a role in chemotherapy resistance due to their ability to survive by self-renewal and differentiation (Raya et al., 2001). Different putative cancer stem cell markers were used to identify and isolate these cells (Yata et al., 2012).

OCT3/4 belongs to POU5F1 transcriptional factors family. It plays a key role in maintaining self-renewal in embryonic stem and germ cells. It has been shown that OCT3/4 is reduced or even disappeared in differentiated tissues (Rao et al., 2012). OCT3/4 can be considered as a diagnostic marker for different tumor types and the positive immunostaining of OCT3/4 indicates the presence of human testicular germ cell tumors (TGCTs). The present study investigated the expression levels of OCT3/4, ALDH-1 and CD44 in Iraqi women with stage II-III breast cancer using real-time PCR and immunohistochemistry. We found that OCT3/4 was highly expressed in breast tissues of 82.2 % of cases with stage II-III breast cancer as compared to 14.3 % in benign tumors. In addition, the high expression of OCT3/4 was associated with the tumor grade and metastasis in patients with stage II-III breast cancer that reflects the role of OCT3/4 in progression and aggressiveness of breast cancer in Iraqi women.

Our results were in line with another study concerning the relation between the OCT3/4 expression level and tumor grade, progression and tumor metastasis. Up-regulation of OCT3/4 may enhance the malignant potential of breast cancer and tumor cell invasion, and metastasis may be suppressed by down-regulation of OCT3/4 expression levels (Wang et al., 2009, Beltran *et al.,* 2011). Immunostaining studies demonstrated that OCT3/4 was highly expressed in the nuclei of cells in stage II-III breast cancer, and this

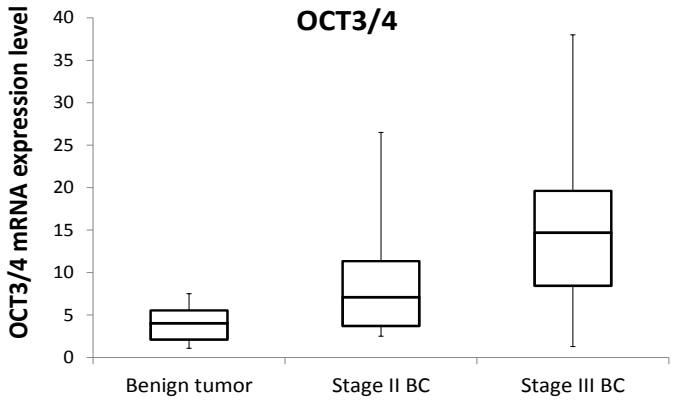

Figure 1A. Boxplot analysis of mRNA levels of OCT3/4 in benign breast tumors (n=17) and stage II (n=32) and stage III (n=18) breast cancer in Iraqi women as determined by qPCR. The expression level of each mRNA was normalized to the corresponding expression of β-actin. The horizontal line within the box represents the median value, and the lines extending from the box indicate the maximum and minimum expression levels.

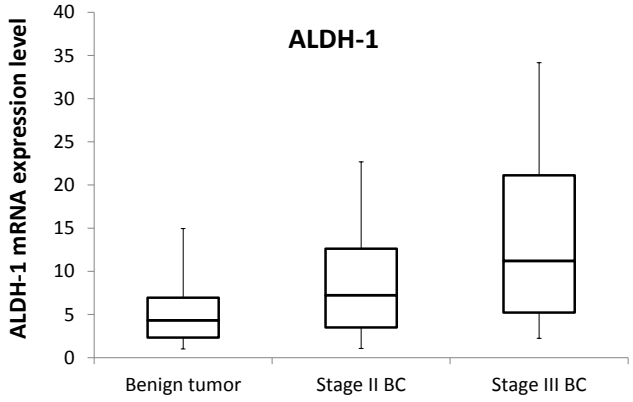

Figure 1B. Boxplot analysis of mRNA levels of ALDH-1 in benign breast tumors (n=17) and stage II (n=32) and stage III (n=18) breast cancer in Iraqi women as determined by qPCR. The expression level of each mRNA was normalized to the corresponding expression of β-actin. The horizontal line within the box represents the median value, and the lines extending from the box indicate the maximum and minimum expression levels.

observation suggested that a sub-population of cells with stem cell characteristics may be present in breast cancer. OCT3/4 in stem cells acts as a master key during differentiation by regulating the pluripotent potential (Ark et al., 2008).

During the past few decades, the Iraqi population was exposed to wars, particularly people in the middle and south regions of the country. Around eight hundred tonnes of depleted uranium were dropped on the middle and southern area of Iraq during the first Gulf war in 1991 and a similar amount during the second Gulf war, in 2003 (Bertell, 2006). Some of these toxic substances enter into the body by ingestion, inhalation or through the skin and remain within the body for years. The toxic

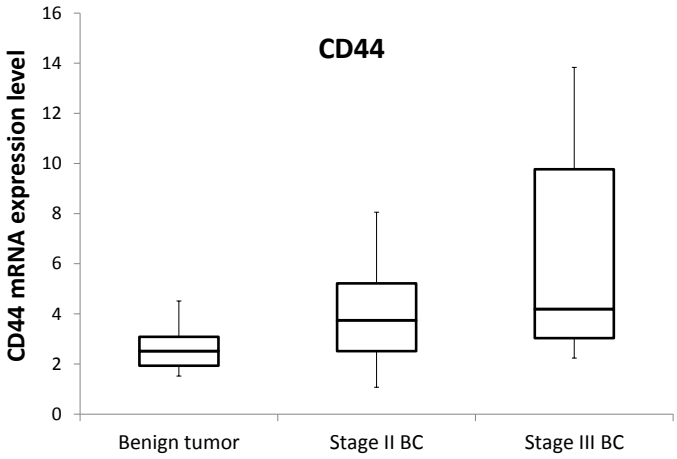

Figure 1C. Boxplot analysis of mRNA levels of CD44 in benign breast tumors (n=17) and stage II (n=32) and stage III (n=18) breast cancer in Iraqi women as determined by qPCR. The expression level of each mRNA was normalized to the corresponding expression of β-actin. The horizontal line within the box represents the median value, and the lines extending from the box indicate the maximum and minimum expression levels.

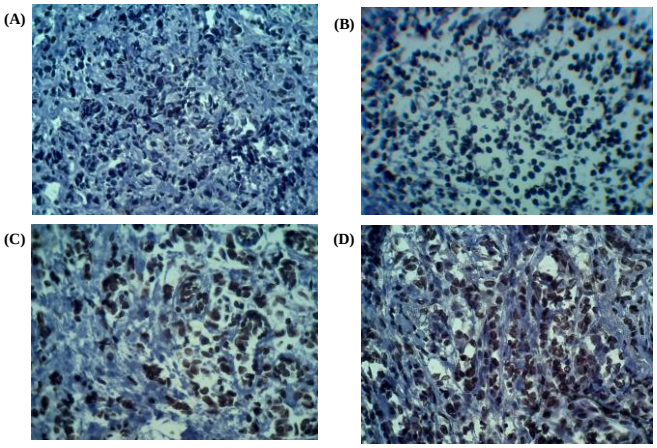

Figure 2. Immunohistochemical staining (X400) of OCT3/4 in benign breast tumors, stage II and stage III breast cancers in Iraqi women. **(A)** Negative control showing no expression of OCT3/4. **(B)** Benign breast tumor. **(C)** Stage II breast carcinoma with positive expression of OCT3/4. **(D)** Stage III breast carcinoma with high OCT3/4 expression.

effects of these substances may cause damage to lymphatics and kidneys, lung fibrosis or increased risk of cancer. There is an increased prevalence of breast cancer in Iraqi women and increased progression and aggressiveness of breast cancer. Cancer stem cells may develop from disturbances in self-renewal processes that

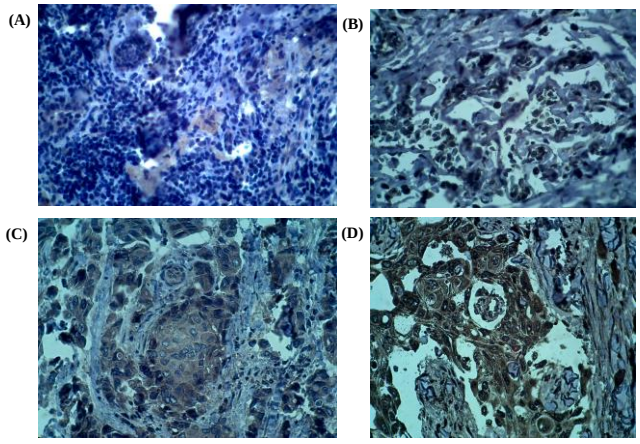

Figure 3. Immunohistochemical staining (X400) of ALDH-1 in benign breast tumor, stage II and stage III breast cancer in Iraqi women. **(A)** Negative control. **(B)** Benign breast tumor with relatively slight positive expression. **(C)** Stage II breast carcinoma showing positively stained tumor mass. **(D)** Stage III breast carcinoma with strong positive invasive tumor nests.

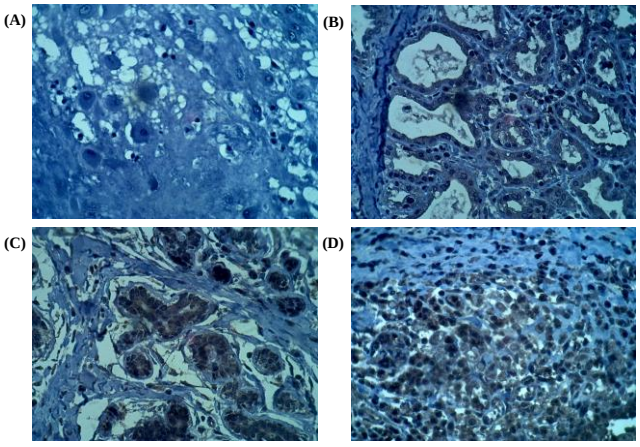

Figure 4. Immunohistochemical staining (X400) of CD44 in benign breast tumor, stage II and stage III breast cancer in Iraqi women. **(A)** Negative control. **(B)** Benign tumor **(C)** Stage II breast carcinoma showing the nests of tumor. **(D)** Stage III breast carcinoma with highly CD44 protein expression.

may result from exposure to these toxic substances.

The results of the current study showed that there is no statistically significant association between the expression level of OCT3/4 and age, stages of the cancer, ER, PR or HER2/neu hormone receptors in breast cancer patients at stage II-III.ALDH-1 is an enzyme that plays important roles in the detoxification of intracellular and extracellular aldehyde molecules, such as malondialdehyde resulting from lipid peroxidation. It may play a role in cancer resistance to conventional chemotherapy especially those utilizing alkylating agents as intermediate molecules (Dylla et al., 2008). Breast

Table 3. OCT3/4, ALDH and CD44 expression levels in breast carcinoma tissues (n=64) and benign tumors (n=21).

	High expression n(%)	Low expression n(%)	P value
OCT3/4			
Cancer Tissues	53(82.2 %)	11(17.8%)	0.001
Benign Tumors	3(14.3%)	18(85.7%)	
ALDH-1			
Cancer Tissues	44(68.8%)	20(31.2%)	0.001
Benign Tumors	6(28.6%)	15(71.4%)	
CD44			
Cancer Tissues	34(53.1%)	30(46.9%)	0.01
Benign Tumors	7(33.3%)	14(66.7%)	

cancer cells that express high levels of ALDH-1 could be protected from oxidative stress resulted from radiation or chemotherapy used for the treatment of cancer (Croker and Allan, 2012). ALDH-1 is highly expressed in solid tumors such as bladder cancer and head-and-neck small cell carcinoma as well as breast cancer (Su, 2010; Chen et al., 2009). The eexpression level of ALDH-1 was increased in 30% of the breast cancer population and thus ALDH-1 may be used as a prognostic factor for breast cancer (Ginesteir et al., 2007). The current study revealed that ALDH-1 is highly expressed in 68.8% cases of breast cancer in Iraqi women. This variation in the expression levels of ALDH-1 may result from the differences in genetic or environmental factors such as toxic substances that affect ALDH-1 expression. Moreover, this study showed that a high expression level of ALDH-1 was associated with tumor histopathological grade, type and lymph node metastasis. It has also been shown that there is a correlation between ALDH-1 expression level and tumor grade and metastasis (Ying et al., 2014). ALDH-1 over-expression was associated with invasive ductal carcinoma (IDC) in breast cancer tissues compared with low expression in ductal carcinoma in situ (DCIS) (Ping et al., 2013). Therefore, there is a link between ALDH-1 expression levels and tumor grade and metastasis in breast cancer.

CD44 plays important roles in normal tissues in different cellular activities such as adhesion, aggregation and migration (Sneath and Mangham, 1989). In cancerous tissues, CD44 plays a pivotal role in tumorigenisity through several mechanisms including induction of growth factors, degradation of extracellular matrix resulting in cells migration and metastasis (Culty et al., 1992). A previous study has shown the role of CD44 in breast CSCs initiation, metastasis and survival (Jaggupilli and Elkord, 2012). This study demonstrated that CD44 may play important roles in cancer progression and metastasis. In the present study, there was no significant association between the expression level of CD44 and age, stages of cancer or hormone receptors status. A previous study also indicated a negative correlation between the expression of CD44 and patients clinical characteristics such as age, stages of cancer and hormone receptor status. CD44 expression level was inversely correlated with ER and PR, with no correlation to Her2 in breast cancer patients (Bernardi et al., 2012). In the current study, there was high association between the expression level of CD44 and tumor type, grade and lymph node metastasis. Up-regulation of CD44 was found to be linked to transcription factors such as OCT3/4, B cell-specific Moloney murine leukemia virus integration site 1 (Bmi-1), and β-catenin in colorectal cancer (Lei et al., 2008). We also found that higher expression of CD44 was associated with highly expressed OCT3/4 in patients with stage II-III breast cancer as compared to that in benign tumors.

The evaluation of CSC makers OCT3/4, ALDH-1 and CD44 in breast tissues of Iraqi women with stage II-III breast cancer may clarify the breast cancer metastasis and chemotherapy resistance. This will pave the way for efficient treatment of breast cancer in women.

In conclusion, these results indicate that the expression levels of OCT3/4, ALDH-1 and CD44 were increased in Iraqi women with stage II-III breast cancer. The high expression levels of OCT3/4, ALDH-1 and CD44 were associated with tumor grades, types and lymph node metastasis and may play important roles in cancer progression and metastasis. The mechanisms by which

Table 4. Relationship between the OCT3/4, ALDH and CD44 expression levels and pathological data of patients.

		OCT3/4			ALDH-1			CD44		
Pathological data	**Patients number**	**High expression**	**Low expression**	**P value**	**High expression**	**Low expression**	**P value**	**High expression**	**Low expression**	**P value**
Age of patients										
≤ 50 Years	17	9(53%)	8(47%)		7(41%)	10(59%)		6(35%)	11(65%)	
≥ 50 Years	47	28(59.8%)	19(40.2%)	0.51	25(53%)	22(47%)	0.18	29(61.7%)	18(38.3%)	0.053
Tumor Stages										
Stage II	39	28(72(%)	11(28%)		26(67%)	13(33%)		24(62%)	15(38%)	
Stage III	25	21(84%)	4(16%)	0.37	20(80%)	5(20%)	0.32	18(72%)	7(28%)	0.43
Tumor Grades										
Grade I	8	3(38%)	5(62%)		2(25%)	6(75%)		3(37%)	5(63%)	
Grade II	36	23(61%)	13(39%)		20(56%)	16(44%)		24(66%)	12(34%)	
Grade III	20	17(85%)	3(15%)	0.003†	15(75%)	5(25%)	0.004†	16(80%)	4(20%)	0.01**
Tumor Types										
IDC	48	36(75%)	12(25%)		34(71%)	14(29%)		39(81.2%)	9(18.8%)	
ILC	10	3(30%)	7(70%)		4(40%)	6(60%)		3(30%)	7(70%)	
Other	6	1(17%)	5(83%)	0.001†	2(33%)	4(67%)	0.003†	2(33%)	4(67%)	0.001†
ER status										
Positive	49	23(49%)	26(51%)		26(53%)	23(49%)		22(45%)	27(55%)	
Negative	15	8(53%)	7(47%)	0.91	10(67%)	5(33%)	0.63	6(40%)	9(60%)	0.2
PR status										
Positive	47	23(49%)	24(51%)		27(57%)	20(43%)		24(51%)	23(49%)	
Negative	17	11(64%)	6(36%)	0.158	10(59%)	7(41%)	0.85	9(52%)	8(47%)	0.62
Her2/new Stats										
Positive	24	14(58%)	10(42%)		16(67%)	8(33%)		11(46%)	13(54%)	
Negative	40	26(65%)	14(35%)	0.98	22(55%)	18(45%)	0.2	23(57%)	17(43%)	0.42
Lymph node metastasis										
Positive	27	22(81%)	5(19%)		20(69%)	7(31%)		19(70.3%)	8(29.7%)	
Negative	37	18(49%)	19(51%)	0.005†	17(46%)	20(54%)	0.05*	12(32.4%)	25(67.6%)	0.001†

IDC: Invasive Ductal Carcinoma, **ILC**: Invasive lobular carcinoma, **ER**: Estrogen Receptor, **PR**: Progesterone Receptor, **Her2/New**: Epidermal growth Factor Receptor.*P≥0.05, **P≥0.01 and †P≥0.001.

these markers are involved in the progression and metastasis of breast cancer in Iraqi women require further investigation.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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