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Correlations of CD44 and CD133 with the grade and subtypes of invasive ductal carcinoma

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Abstract

Breast cancer (BC) is the most commonly diagnosed cancer and the main cause of mortality among women worldwide. Cancer stem cells are subpopulations of cancer cells characterized by self-renewal, tumorigenesis, maintenance of cancer heterogeneity, and metastasis. The current study was designed to identify the presence of cancer stem cells (CSCs) within tumor tissue and their role in metastasis and to determine the relationships between the expression of these markers and the grade and subtype of invasive ductal carcinoma (IDC). Sixty paraffin blocks from patients with IDC, where 49 blocks were from patients without metastasis (M- group), and 11 blocks from patients with distant metastasis (M+ group), were used. All cases were subjected to histopathological and immunohistochemistry investigations to determine the tumor grade and BC subtypes, as well as to identify the presence of CSCs on the basis of the expression of CD44 and CD133. IHC revealed that the subtype (luminal A/B) had the highest percentage (90.9%), triple-negative subtype (9.1%) and no HER2-enriched subtype (0%) in the M+ group compared with the M- group, and this difference was significant. Furthermore, IHC revealed the presence of CSCs within the tumors of both groups, but the odds ratios (ORs) of CD44 and CD133 revealed that these markers are 3.1 and 1.29 times more likely to be expressed in metastatic cases than in other cases without metastasis. There was no significant correlation between the expression of stem cell (CD) markers and tumor grade, but the expression of CD44 and CD133 was significantly lower in the triple-negative IDC subtype than in the luminal IDC subtype (r=-0.3424, P=0.007; and r=-0.2787, P=0.031, respectively). Female patients with the luminal A subtype and positive expression of CD44 and CD133 are more likely to experience metastasis. Therefore, along with IHC investigations of ER, PR, and HER2, the expression of these markers, especially CD44, is recommended as a predictor marker for metastasis.

Keywords: *invasive ductal carcinoma, cancer stem cell, breast cancer subtypes.*

Introduction

Breast cancer is a common disease that causes death among women and is considered the fifth leading cause of cancer death among women globally, with an estimated 2.3 million new cases in 2020, approximately 11.7% of all cases, followed by lung cancer (approximately 11.4%), and the number of deaths reached approximately 684,996 (1,2). In Iraq, breast cancer is one of the most common causes of death among women, as the number of new cases of breast cancer increased from 52.00/100,000 in 2000 to 91.66/100,000 in 2019 (3). The incidence of breast cancer in females is approximately 100 times higher than that in males. It is the first leading cause of mortality among women after cardiovascular diseases (4). Although BC is a highly heterogeneous malignant tumor, estrogen, progesterone, and epidermal growth factor-2 receptors (ER, PR, and HER2, respectively) remain the main receptors relevant for breast cancer classification (5,6). On the other hand, the diverse stem and progenitor cell populations in the mammary glands could cause a qualitative shift in the current understanding of its heterogeneity. Despite significant advances in treatment, many breast cancer patients experience drug resistance and tumor recurrence, which is believed to be attributed to the small population of cells inside breast cancer. Therefore, eliminating breast cancer stem cells represents a promising therapeutic approach for preventing drug resistance and tumor recurrence (7). Cancer stem cells (CSCs) are a subgroup of cells with highly tumorigenic characteristics that exhibit properties similar to those of normal stem cells, including self-renewal, proliferation, autophagy, invasion, multiple differentiation, metastasis, endocrine disruption, and

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chemoresistance (8). These cancer stem cells express many specific surface cell markers, including CD44 (9) and CD133 (10). Accurate biomarkers of breast cancer stem cells can help in cancer identification, diagnosis, prognosis evaluation, and therapy monitoring (11). Therefore, the current study was designed to investigate the role of patient age and menopausal status, tumor stage, tumor grade, tumor subtype, and cancer stem cells (CD44, CD133) in tumor metastasis and to determine the relationship of the expression of cancer stem cell markers (CD44, CD133) with the grade and subtype of invasive ductal carcinoma (IDC).

Materials and methods

This retrospective study was conducted on sixty paraffin-embedded tumor tissue blocks from patients with invasive ductal carcinoma (IDC), 11 from patients with distant metastasis, and 49 from patients without metastasis. These paraffin-embedded blocks were obtained from the National Center for Teaching Laboratories/Medical City. The ethics committee of the College of Science/Mustansiriyah University approved this work (Ref. No: BCSMU/0822/00018Z), and consent from the patient was not required because of the retrospective nature of this study. Age, menopausal status, and tumor stage were recorded from medical profiles of patients at the first diagnosis of their cancer.

Determination of tumor grade

The tumor blocks of all the patients were subjected to histopathological investigation to determine the tumor grade by a pathologist on the basis of the Nottingham Bloom–Richardson system (NGS) (12). All the paraffin-embedded tumor samples were prepared and stained via the conventional laboratory method described by Bancroft and Steven (13) to determine the tumor grade.

Determination of tumor subtypes

Paraffin-embedded tissue blocks were subjected to immunohistochemistry (IHC) to identify the different subtypes of IDC on the basis of the expression of ER, PR, and HER 2 (14). The tumor subtypes of BC were determined by using monoclonal rabbit anti-human estrogen receptor α (ER), monoclonal mouse anti-human progesterone receptor (PR), and polyclonal rabbit anti-human c-erbB-2 oncoprotein (HER2) from Dako, Denmark. The procedure of this IHC method can be summarized as follows:

The paraffin-embedded tissue for each sample was cut into three sections of approximately 4 µm thickness and mounted on positively charged slides. Then, the slides were placed vertically in a hot air oven at 65°C overnight. For deparaffinization and rehydration of the tissue sections, each slide was submerged in serial jars containing the following solutions: (xylenes (twice/fifteen min), absolute ethyl alcohol (twice/5 min), 95%, 90%, 80%, 70% ethyl alcohol/5 min, and distilled water/5 min). For antigen retrieval, the slides were immersed in a jar containing antigen retrieval solution and placed in an autoclave. The slides were kept at a temperature below 121°C for 15 minutes before the autoclave was turned off. Once the solution had cooled, the slides were removed and rinsed in distilled water for 5 min. A sufficient amount of dihydrogen dioxide drops was added to the slides, which were then incubated at 37°C for 10 min in a humidity chamber; thereafter, the slides were soaked in the buffer twice every 5 min. A sufficient number of protein block drops were applied to the slides, which were then incubated at 37°C for 10 min), after which they were washed twice in the buffer for five min, drained, and gently blotted. The diluted primary Ab (specific for ER, PR, or HER2) was applied to every slide and incubated in a humidified chamber overnight at 37°C. Early the next day, the slides were cleaned four times in buffer for 5 min each and then drained and gently blotted. Sufficient yellow drops of secondary Ab reagent (link Ab) were added to every slide and placed in a humidified chamber at 37°C for 20 min. The slides were washed in buffer four times for 5 min each, then eventually drained and gently blotted. Sufficient red drops of streptavidin-horse radish peroxidase (HRP) Ab were applied to the tissue, which was subsequently incubated at 37°C for 20 min. The slides were then washed in buffer four times for 5 min each, after which they were subsequently drained and gently blotted. In a dark room, diluted (DAB) substrate was applied to the tissue and incubated in the humidified chamber at 37°C for 10 min. Then, the slides were washed carefully in tap water for 5 min. The slides were submerged in a hematoxylin counterstain bath for 1–2 min and then rinsed for 10 min in tap water. The slides were dehydrated by submerging them into serial jars containing the following solutions: 70%, 80%, 90%, 95% ethyl alcohol/1 min, ethyl alcohol absolute twice each/1 min, xylenes/1 min, and fresh xylenes/1 min. After dehydration and clearing, drops (one or two) of DPX were added to the sections moistened with xylene, coverslipped, and left to dry throughout the night. The slides were subsequently examined at 100X and 400X with a light microscope. The results of the immunohistochemical staining and scoring system were confirmed by the presence of immune staining (light to dark brown color) in the positive control slides and its absence in the negative control slides (positive controls included one tissue block from a normal human liver for CD44 and one tissue block from human lung cancer tissue for CD133). The cytological staining pattern of ER and PR is nuclear, whereas the staining patterns of HER2 are located around the plasma membrane according to the Allred scoring system (15).

According to the results of the IHC staining of these three receptors, the subtypes of IDC are commonly grouped into four categories: the luminal (A) subtypes are positive for ER or PR and negative for HER2. Luminal (B) subtypes are positive for ER and can be negative for PR. HER2 positivity is identified by elevated expression of HER2 and negativity for ER and PR. However, triple-negative breast cancer (TNBC) is characterized by a lack of expression of any of the above receptors (16).

Identification of cancer stem cells (CSCs)

IHC is used to detect the presence of cancer stem cells (CSCs) within tumor tissue on the basis of the expression of certain clusters of differentiation, including CD44 and CD133. The same procedure of the IHC method was used to determine the tumor subtypes. Cancer stem cells in the tumor sections of all patients with BC were identified by their ability to express one or more CD markers on their plasma membrane, including CD44 and CD133. The expression of these CD markers was investigated by using polyclonal rabbit anti-human CD 44 and polyclonal rabbit anti-human CD 133 antibodies from

Elabscience, USA. Dako, Denmark.
Tumor cells with brown staining of their plasma membrane and/or cytoplasm were positive for cancer stem cell markers. The number of stained cells in ten felids was counted via a light microscope at high power (40X). The immunostaining score was calculated according to the number of stained cancer cells (17) as follows: ((-) negative = 0–10%, (+) slightly positive = 11–25%, (++) moderately positive = 26–50%, (+++) strongly positive = 51–100%).

Statistical analysis:

The Vassarstats website for statistical computation was used to analyze the statistical findings (18). The present study data are presented as the means ± standard deviations (M ± SDs), and differences between the two dependent groups were statistically analyzed via t tests. On the other hand, category-specific data were reported as percentile values, and a comparison of these data between different groups was carried out via the chi-square test. Pearson's correlation coefficient was used to examine the relationship between two variables, whereas the sensitivity, specificity, and odds ratio were used to identify the ability of CD markers to identify metastatic tumor cells. Any difference at a P value of less than 0.05 was regarded as significant. Pearson's correlation coefficient was used to examine the relationship between two variables, whereas the sensitivity, specificity, and odds ratio were used to identify the ability of CD markers to identify metastatic tumor cells. Any difference at a P value of less than 0.05 was regarded as significant.

Results:

The present study was conducted on 60 paraffin blocks containing tumor biopsies from women who had invasive ductal carcinoma. According to the status of the tumor, two groups were recognized among those patients: 49 patients (81.7%) presented without metastasis (the M- group), and the remaining 11 patients (18.3%) presented with distant metastasis (the M+ group), as shown in Figures. 3-1. The average age of patients in the M+ group (53.4 ± 9.5 years) was significantly higher than that in the M- group (48.8 ± 9 years).

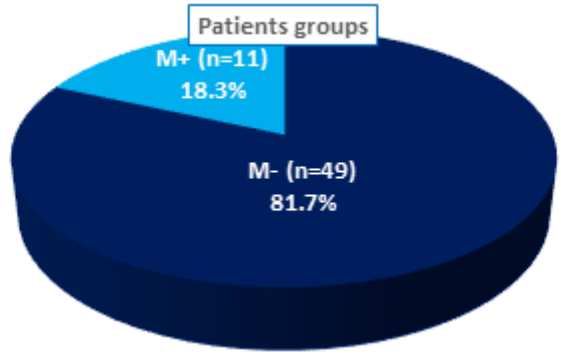

Figure 3-1: Grouping of patients according to the presence or absence of metastasis

Grades of tumors

Figure. 3-2 shows no significant difference in the frequency of tumor grades I, II, and III between patients in the M- group (2%, 49%, and 49%, respectively) and those in the M+ group (9.1%, 72.7%, and 18.2%, respectively).

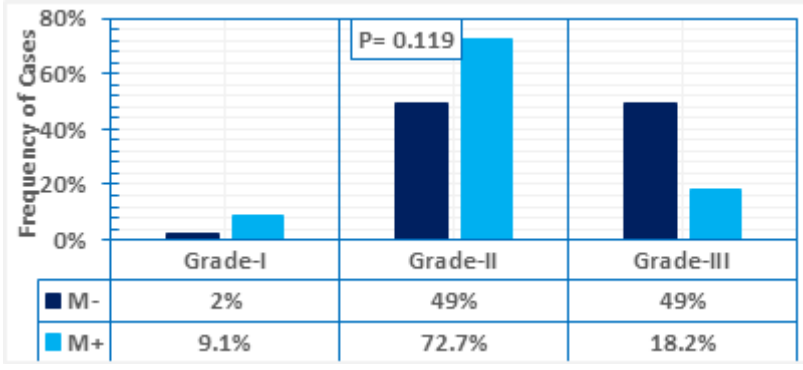

Figure 3-2: Frequency of cases in the two groups stratified by tumor grade

Identification of molecular IDC subtypes

By using immunohistochemistry, subtypes of IDC were determined on the basis of their expression of ER, PR), and HER2. The cellular staining pattern for ER and PR is nuclear; thus, a positive result is defined as nuclear staining in ≥ 1% of tumor cells. In the Allred scoring system, a score of 0 5 is given to the cells depending on the proportion of stained cells (PS), and a score of 0 3 is given depending on the intensity of staining (IS). Then, the final Allred score can be calculated by adding the PS and IS. Zero PS and zero IS are interpreted as negative expressions of ER and PR (Figure. 3-3A), whereas any PS and IS rather than zero can be interpreted as positive expressions of ER and PR (Figure. 3-3 B, C, D).

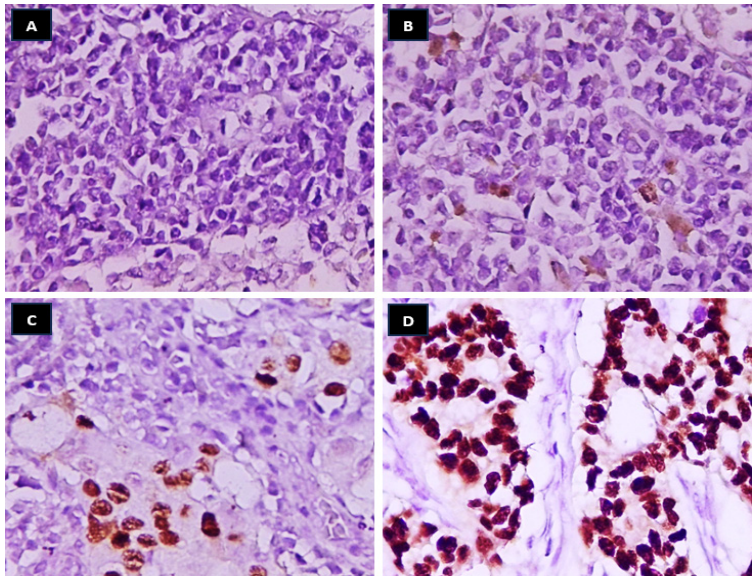

Figure 3-3 Histologic section of IDC showing the expression of ER and PR (IHC staining, 40X) [negative expression (A), positive expression with low PS & IS (B), moderate PS &IS (C), high PS & IS (D)]

On the other hand, the scoring method for HER2 expression is based on the cell membrane staining pattern. Uniform intense membrane staining of more than 30% of the tumor cells was interpreted as positive for HER2 expression (Figures. 3-4).

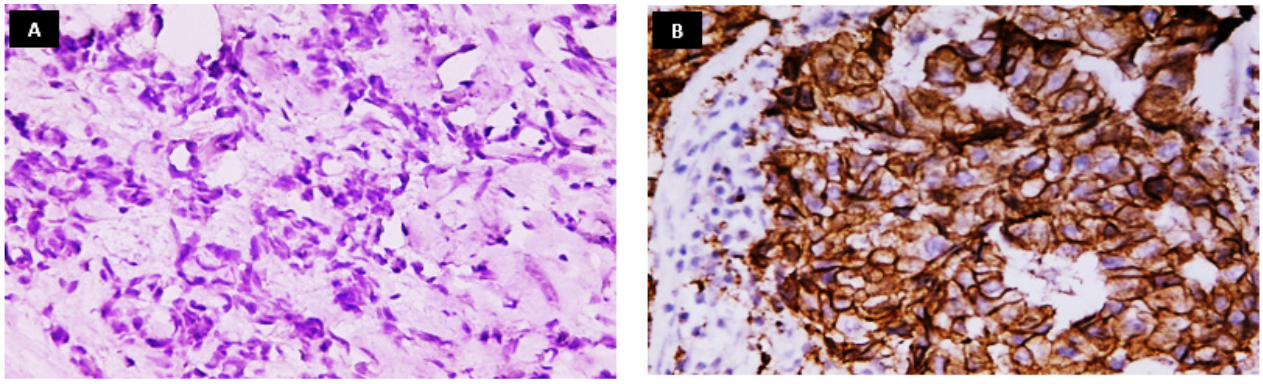

Figure 3-4: Histologic section of IDC showing the expression of HER-2 [A: negative expression, B: positive expression] (IHC staining, 40X)

Three subtypes of IDC were identified on the basis of the results of ER, PR, and HER-2 expression. Table 3-1 shows that 90.9% of the patients in the M+ group had luminal subtypes A/B, and the remaining patients had the triple-negative subtype (9.1%), but no patients had the HER2-enriched subtype (0%). These results are significantly different from those in patients in the M- group, which constitute approximately 42.8%, 18.4%, and 38.8%, respectively.

Table 3-1: Frequency of patients with different IDC subtypes on the basis of their ER, PR, and HER-2 expression

IDC subtype (n, %)	Receptor expression	M- (n=49)	M+ (n=11)	P value
Luminal A/B	ER+, PR+, HER2-/HER2+	(42.8%) 21	(90.9%) 10	0.003
HER2-enriched	ER-, PR-, HER2+	(18.4%) 9	(0%) 0	
Basal-like (triple negative)	ER-, PR-, HER2-	(38.8%) 19	(9.1%) 1	

Identification of cancer stem cells (CSCs)

By using immunohistochemistry, cancer stem cells (CSCs), a subpopulation of tumor cells, have been identified on the basis of their expression of four CD markers, namely, CD44 and CD133, as follows:

Expression of the CD44 marker

By using the IHC technique, Figure. 3-5 shows negative and positive expression of CD44 on the surface of tumor cells.

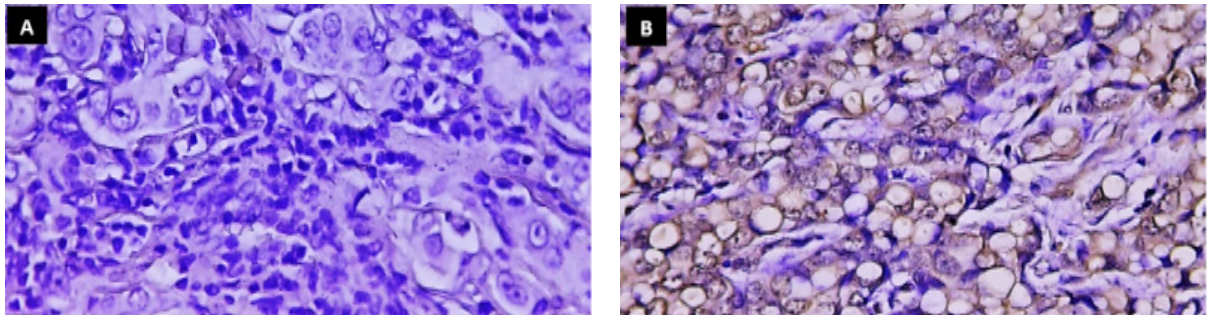

Figure 3-5: Histologic section of IDC showing negative expression of CD44 (A) and positive expression (B) (IHC staining, 40X).

Table 3-2 shows that the sensitivity and specificity of the use of the CD44 marker for identifying tumor cells in these patients are 81.3% and 40.8%, respectively, which results in an odds ratio of approximately 3.1 (i.e., the CD44 marker is 3.1 times more likely to be expressed in metastatic patients than in nonmetastatic patients).

Table 3-2: Evaluation of CD44 as a marker for identifying metastatic cases in patients with IDC

CD44 marker expression		M- (n=49)	M+ (n=11)
Number of Cases	Positive	29	9
	Negative	20	2
Sensitivity			81.8 %
Specificity			40.8 %
Odd Ratio (OR)			3.1

To evaluate the correlation of CD44 expression versus tumor grade and subtype, Table 3-3 shows no significant correlation between CD44 expression and tumor grade, but its expression was significantly lower in triple-negative IDC patients (r= - 0.3424, P= 0.007) and nearly greater in luminal IDC patients (r= 0.2330, P= 0.073).

Table 3-3: Correlation of CD44 expression with tumor grade and subtype

Tumor status		Correlation of CD44 expression versus	
		R	P
Grade	I	0.1413	0.281
	II	0.0508	0.699
	III	-0.1024	0.436
Subtype	Luminal	0.2330	0.073
	HER2-enriched	0.1259	0.337
	Triple negative	-0.3424	0.007

Expression of the CD133 marker

By using the IHC technique, Figure. 3-6 shows negative and positive expression of CD133, which is detected in the cell membrane as well as in the cytoplasm of cancer cells.

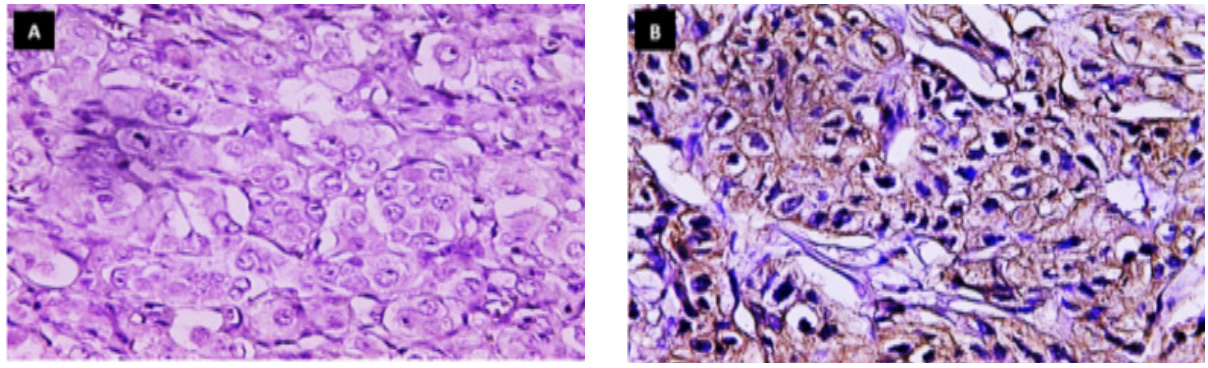

Figure 3-6: Histologic section of IDC showing negative expression of CD133 (A) and positive expression (B) (IHC staining, 40X).

Tables 3-4 show that the sensitivity, specificity, and odds ratio of using CD133 as a marker for identifying tumor cells in metastatic patients are 72.7%, 32.6%, and 1.29, respectively (i.e., the CD133 marker is more likely to be expressed in metastatic cases 1.29 times than non-metastatic).

Table 3-4: Evaluation of CD133 as a marker for identifying metastatic cases in patients with IDC

CD133 expression	M- (n=49)	M+ (n=11)
Positive	33	8
Negative	16	3
Sensitivity	72.7 %	
Specificity	32.6 %	
Odd Ratio	1.29	

To evaluate the correlation of CD133 expression with tumor grade and subtype, Table 3-5 shows no significant correlation between CD133 expression and tumor grade, but CD133 expression is significantly decreased in triple-negative IDC patients (r= - 0.2787, P= 0.031).

Table 3-5: Correlation of CD133 expression with tumor grade and subtype

Tumor status		Correlation of CD133 expression versus	
		R	P
Grade	I	0.1264	0.335
	II	-0.1341	0.307
	III	0.0892	0.497
Subtype	Luminal	0.1303	0.321
	HER2-enriched	0.1856	0.155
	Triple negative	-0.2787	0.031

Discussion

As shown in the present results, the mean age of the M+ patients (53.4 ± 9.5 years) was significantly greater than that of the M- patients (48.8 ± 9 years).

In terms of tumor grade, Grade II tumors were more prevalent in patients in both groups. In general, the percentage of patients with other grades was greater in the present study. In agreement with these results, several Iraqi studies have shown that Grade II is the most common grade among Iraqi women (4,19,20). Additionally, other studies have shown that grades II and III BC are the most common tumor cells (21,22).

Histologic grade has prognostic importance for overall survival in patients with breast carcinoma (23,24,25). A strong link between tumor grade and metastasis to axillary lymph nodes has been shown; thus, tumors of Grade II and Grade III had a greater number of positive axillary nodes, whereas tumors of Grade I had a lower rate of axillary metastases (26,27). Therefore, patients in the M- group in this study who were Grade II or III may be at high risk of metastasis later.

As shown in Table (3-1), the luminal A/B subtype appears to be more prone to metastasis than the other subtypes are, so a woman diagnosed with the luminal subtype is at risk of metastasis to other organs. Therefore, knowing which subtypes are most likely to spread is important for monitoring patient survival and treatment, as well as for physician and patient expectations.

Consistent with these results, a previous Iraqi study reported that the luminal subtype constituted approximately 80.6% of IDC cases among Iraqi patients with BC, 9.7% of triple-negative IDC cases, and 9.7% of HER2+-enriched IDC cases (28). Additionally, a recent Iraqi study revealed that 46.67% of all BC cases belong to the luminal subtype, followed by 28.89% for triple-negative BC and 8.89% for HER2+ BC (20). Several previous studies reported that subtypes of BC are linked with different patterns of metastasis and have various prognostic influences. These subtypes can identify patients at high risk for developing specific metastases (29,30) and can also predict preferred sites for distant spread (31). For example, Tabor et al. (32) reported that metastasis is greater in the BC luminal A/B subtype than in the other subtypes, mainly in the long term, because in the luminal A/B subtype, several factors play great importance, such as hormone cross-talk and cancer cell dormancy, remodeling of the extracellular matrix, the involvement of immune cells and stroma in dissemination and survival, and that cancer cells are constantly developing and gaining new properties, such as estrogen resistance in the ER+ subtype. Furthermore, hormonal receptor+/HER2+ patients had bone metastases, and hormonal receptor-/HER2+ patients had a significantly increased number of liver metastases, whereas lung and brain metastases were more common in hormonal receptor-/HER2- women (33).

In contrast to previous results, Helmi et al. (34) reported that BC women with metastasis constitute approximately 61.5% of those with luminal B BC, 21.5% of those with HER+ BC, 14.6% of those with triple-negative BC, and 2.3% of those with luminal BC, and they reported that triple-negative BC had a greater risk of metastasis (OR, 7.74), followed by B luminal BC (OR, 3.76). Moreover, Guo et al. (6) revealed that HER2+ BC patients had a higher metastasis rate than HER2+ BC patients did, regardless of hormonal receptor status, and reported that HER2+ and triple-negative BC patients had high metastasis rates and worse overall survival, whereas hormonal receptor-positive BC patients had lower metastasis rates and good overall survival. However, another study examined the median survival duration concerning distant area metastasis and reported that A luminal patients had (2.2) years, B luminal patients had (1.6) years, Luminal-HER2+ patients had (0.7) years, and triple-negative patients had (0.5) years (35).

Identification of cancer stem cells (CSCs)

Expression of the CD44 marker

Some studies have revealed that the expression of CD44 is common in breast cancer tissue (36,17). Other studies have examined CD44 expression in lymph node metastases and have shown high expression of CD44 in metastatic lymph nodes, which indicates that CD44 expression is significantly associated with metastasis to the lymph nodes (37,38).

With respect to the relationship between CD44 expression and BC grade, several studies have shown that there is no significant relationship between CD44 expression and BC grade (4,17,39). However, Xu et al. (41) reported that CD44 protein expression was significantly elevated in

Grade III BC tissues.

With respect to the level of CD44 expression in the BC subtypes, the statistical analysis in the present study revealed that the highest expression of CD44 was in the luminal A/B subtypes compared with the triple-negative subtype. Many previous studies have shown compatibility with the present results. For example, the expression of standard CD44 (CD44st) is closely related to the luminal subtype (42). Additionally, Olsson et al. (43) reported that the luminal A subtype has higher total expression of CD44 than triple-negative, luminal B, and HER2-enriched subtypes do. In contrast, many previous studies have shown that CD44 expression is significantly elevated in the triple-negative BC subtype (37,44,45,46), and a high expression level of CD44 is associated with low estrogen and progesterone receptor status in the A luminal subtype, while its expression is also low in the HER2 subtype (41).

To explain the role of CD44 in the aggressiveness of BC, CD44 is one of several markers that have been identified on the cell surface of cancer stem cells and is responsible for adhesion and communication between neighboring cells and between the extracellular matrix and cells to regulate and preserve their integrity. Therefore, misregulation or disruption of the adhesive relationship leads to loss of tissue architecture and is characteristic of the transformation of neoplasms (47,48).

In addition to its function in cell adhesion, CD44 may contribute to many cancers, including breast cancer, by directing intracellular signals related to motility and growth (49). It regulates the metastatic process by interacting with suitable matrix ligands and is favorable for the processes of invasion and migration involved in metastasis (50). The formation of the CD44-hyaluronic acid complex activates the exchange of Na+/H+ activity, which promotes intracellular acidification and creates the environment of an acidic extracellular matrix. This results in hyaluronic acid catabolism and modification and the activation of thiol proteases, resulting in the invasion of breast cancer cells (51). Additionally, the formation of the CD44-hyaluronic acid complex leads to the activation of the OCT3/4 and Nanog transcription factors in embryonic stem cells, which in turn results in the activation of REX1, SOX2, and the multidrug resistance protein MDRRP1 (52). Recently, it has been reported that the formation of this complex regulates downstream pathways of the cytoskeleton involved in cell survival or cell death that lead to cell invasion and proliferation and, ultimately, metastasis (53).

For a tumor to grow to distant areas, metastatic cancer cells need to leak through the endothelial barriers through the interaction of these cells with the endothelial cells that line the blood vessels, a process that occurs through integrin adhesion (54). These findings suggest that CD44 may play a role in distant spread (metastasis) through its ability to act as an adhesion receptor. It facilitates the escape of cancer cells from the circulation. Thus, high expression of CD44 can initiate cell adhesion to distant monolayers of the endothelium (55). Moreover, the microenvironment is a crucial regulator of cancer stem cell-derived cancer metastases. For metastatic lesion establishment, it is important to have a permissive microenvironment in distant areas, such as at the site of the primary cancer. Therefore, when cancer stem cells are in metastatic areas, their proliferation, survival, and differentiation may be activated through deregulated specialized signals from the new microenvironment (56).

With respect to the tissue expression of the CD133 marker, in agreement with the results shown in Figures. 3-5, many studies have shown that CD133 is expressed in the membrane and cytoplasm of cancer cells (57, 58). Compared with the results in Tables 3-4, several studies have shown that the expression of CD133 in a high number of cancer cells is associated with greater tumor aggressiveness and poor prognosis in BC patients and enables them not only to self-renew and proliferate but also to invade and be involved in drug resistance and metastasis (59,60). Furthermore, Utnal et al. (61) reported that the expression level of CD133 in BC patients was approximately 77.08%, and its expression is essential for the spread of tumors along lymph channels through the epithelial–mesenchymal transition process; thus, increased CD133 expression helps cells enter the lymphatic system and then spread throughout lymph channels. Similarly, Ahmed and Mohammed (58) reported positive CD133 expression in 68% of IDC patients and reported a significant association between the CD133 expression level and lymph node metastasis and lymphovascular invasion. In contrast to these findings, one study demonstrated that CD133 was significantly decreased in BC patients with metastatic tumors, which was not consistent with the findings of the current study (62).

To explain the role of CD133 in the aggressiveness of cancer cells, Boumahdi et al. (63) reported that the expression of CD133 is a well-known marker for cancer stem cells in various cancer types and that its expression is accompanied by the expression of essential transcription factors of stemness, such as OCT4, NANOG, c-MYC, and SOX2, which are suggested to play important roles in tumor growth, recurrence, and cancer metastasis. Recently, Moreno-Londoño and Robles-Flores (64) reported that CD133 partially regulates signal transduction pathways, some of which are frequently deregulated in cancer, such as the Wnt/β-catenin and PI3K/Akt signaling pathways. Additionally, they reported that in addition to intrinsic cellular mechanisms that control the expression of CD133 in any cell type, extrinsic factors surrounding the microenvironment can affect CD33 levels. Moreover, previous studies revealed a positive correlation between CD44 and CD133 expression in BC patients, which are associated with a poor prognosis (44,57).

As shown in Tables 3-5, the present study did not find a significant correlation between CD133 expression and tumor grade, but its expression was significantly decreased in triple-negative IDC patients (r= - 0.2787, P= 0.031). The correlation between CD133 expression and tumor grade has been controversial among previous studies; some authors reported that there was no statistical correlation between the expression of CD133 and BC grade (61), whereas other studies have shown a significant correlation between CD133 expression and BC grade (57,58).

Similarly, the relationship between CD133 expression

and IDC subtypes has been controversial among previous studies. Martin and Jiang (62) reported that the expression of CD133 is increased in ER+ tumors, but this increase is nonsignificant, which is consistent with the present study. Additionally, Li et al. (65) demonstrated a significant correlation between the overexpression of CD133 and ER, PR, and HER2 status but did not determine the type of this correlation. In contrast, other studies reported that there was a positive significant association between the overexpression of CD133 and triple-negative IDC subtypes and negative hormonal receptors (53, 58).

Conclusions:

The luminal A subtype of IDC appears to be more prone to metastasis than the other subtypes are, so a woman diagnosed with the luminal subtype is at risk of metastasis to other organs. Therefore, knowing which subtypes are most likely to spread is important for monitoring patient survival and treatment, as well as for physician and patient expectations. The increased expression of markers (CD44 and CD133) confirmed the presence of subpopulations of cancer stem cells, which in turn contributed to increasing the proliferation of tumor cells and their tendency to metastasize. Although there was no significant correlation between the expression of CD44 or CD133 and tumor grade, the expression of these markers was significantly lower in the triple-negative subtype of IDC than in the other subtypes.

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Author contributions: Eqbal Awadh Gatea collected data for the research project and interpreted and discussed the results, whereas Dr. Khalid Mahdi Salih conceived and designed the study, analyzed it statistically, and generally supervised the research project.

Ethical approval: The ethics committee of the College of Science/Mustansiriyah University approved this work (Ref. No: BCSMU/0822/00018Z), and consent from the patient was not required because of the retrospective nature of this study.

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