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Original Research Article

Evaluation of E-Cadherin and Vimentin Expression as Prognostic Markers for Epithelial-Mesenchymal Transition and Tumor Aggressiveness in Breast Cancer – Sudan

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Abstract

This is a prospective laboratory-based study, conducted at Rahma medical centre, Khartoum, during the period from March to July 2018. The study aims to evaluate the expression of E-cadherin and Vimentin as prognostic markers for epithelial- mesenchymal transition and tumour aggressiveness in breast cancer. Fifty six paraffin blocks are collected from archive for women previously diagnosed as breast cancer. Tissue microarrays are prepared, and then stained by immunohistochemistry method. The data obtained was analyzed using SPSS program version 22.0. The age of patients ranged between 30 to 80 years with a mean of 51.1. The histopathological diagnosis reveals that the invasive ductal carcinoma is 71.4%, while medullary carcinoma and mucinous carcinoma are 25% and 3.6% respectively. The result of histological grade shows, grade I is 10.7%, grade II is 35.7% and 53.6% for grade III. The study shows E-cadherin expression is negative in 12.5%, weakly expressed in 32.1%, moderately expressed in 48.2% and strongly expressed in 7.1%. The study reveals a significant correlation between E- cadherin expression with ages and histological grades (*p*.value= 0.028 – 0.027) respectively. Vimentin expression is negative in 1.8%, weakly expressed in 51.8%, moderately expressed in 46.4%. This results show a significant correlation between Vimentin expression with ages and histological types (*p*.value= 0.016 – 0.004) respectively. The study reveals an inverse correlation between E-cadherin and Vimentin (***r*** = -0.389) with a significant correlation (*p*.value= 0.002). The study concludes that, decreased expression of E-cadherin and increased expression of Vimentin are associated with epithelial- mesenchymal transition and breast carcinoma aggressiveness.

Keywords: Breast Carcinoma, E-cadherin, Vimentin, Shendi University.

INTRODUCTION

Breast cancer was the most frequently diagnosed cancer and a leading cause of cancer death among females worldwide, with an estimated 1.7 million cases and 521,900 deaths in 2012 [1]. International variation in breast cancer incidence rates reflected differences in the availability of early detection as well as risk factors [1]. Annual mortality rates among breast cancer patients were significantly greater in low middle-income countries, compared to high-income countrie s[2]. More than 90% of breast cancer-related mortalities are not caused by the primary tumour, but by its metastases at distant sites [3]. In Sudanese population cases comprised 1255 women from central Sudan diagnosed with breast cancer and referred to and treated at Institute of Nuclear Medicine, Molecular Biology, and Oncology, from January 1999 to December 2006 [4]. Studies on breast cancer in Sudan had been limited. The reasons for that included the lack of population-based cancer registry [5].

Breast cancer was broadly categorized into in situ carcinoma and invasive carcinoma [6]. The major invasive tumor types included invasive lobular (ILC), invasive ductal (IDC) carcinomas and ILC comprised up to 15% of all cases [7]. IDC was the most common subtype accounting for 70–80% of all invasive lesions [8]. The grading system for breast cancer evaluates tubule formation, nuclear grade, and mitotic rate. Well-differentiated carcinomas are associated with a significantly better prognosis than poorly differentiated carcinomas. Moderately differentiated carcinomas

initially have a good prognosis, but survival rate of 20 years approaches for poorly differentiated type [9]. Epithelial- mesenchymal transition (EMT) is a physiological phenotypic shift in which epithelial cells break down (cell to cell and cell to extracellular matrix connections) and then migrate to other locations in the body [10]. EMT may also be involved in metastasis of epithelial cell malignancies [11]. In cancer cell when E-cadherin (E-C) protein activity decreased can increase the rate of EMT and therefore disruption of cell to cell junctions and loss of cell polarity. At the same time, cancer cells that undergo EMT demonstrate increased expression of mesenchymal cell proteins such as Vimentin [12]. E-C is a calcium-regulated adhesion molecule expressed in most normal epithelial tissue [13]. E-C knockout has been associated with non-viability and abnormal epithelial morphogenesis. Selective loss of E-C can cause dedifferentiation and invasiveness in human carcinomas. In various cell lines, a reciprocal relationship has been shown between levels of E-C expression and invasiveness [14].

Vimentin is an intermediate filament used as a marker of mesenchymal cells to distinguish them from epithelial cells [15]. Vimentin is expressed at sites of cellular elongation, and associated with a migratory phenotype. Increased Vimentin expression is frequently used as an EMT marker in cancer [16, 17]. There is a positive correlation of Vimentin expression and the rate of invasiveness and metastasis [18].

E-C and Vimentin are now regarded as major and conventional canonical markers of EMT [19]. The reports of previous studies showed that the elevated expression of Vimentin contributed to the aggressiveness of invasive breast cancer. However, the role of E-C in breast cancer biology might be unclear and more complex. The aim of this study is to reveal the prognostic importance of the expression of E-C and Vimentin in breast cancer.

MATERIALS AND METHODS

Study Design

This is an analytical descriptive laboratory-based study which aims to detect the expression of E-C and Vimentin among Sudanese breast cancer patients using immunohistochemistry.

Study Sample

Fifty six (56) paraffin blocks previously diagnosed as breast cancer are selected from El-rahama medical centre. Patient's identification information (age, histological type and cancer grade) is obtained from patient records.

Study Area

This study was conducted at El-rahama medical centre in Khartoum State during the period from March to July 2018.

Sample Processing

Tissue microarray (TMA) samples were selected, Fifty six formalin-fixed-paraffin-embedded tissue blocks, processed, cut at thickness of (3µm), and mounted on positively charged glass slides (Thermo) and baked at 60 C^o for 30 minutes.

Immunohistochemical Staining

The immunohistochemical procedure was done as follows: Following deparaffinization in xylene, TMA slides were rehydrated through a graded series of alcohol and were placed in distilled water. TMAs were steamed for antigen retrieval for E-C and Vimentin using high PH (9) by water bath at 95 C^o for 40 min. After washing with phosphate buffer solution (PBS) for 3 min Endogenous peroxides activity were blocked with 3% hydrogen peroxide and methanol for 10 min, and after washing with PBS for 3 min, then each TMA slide were treated separately with (100 µ L) of (mouse monoclonal antibody against E-cadherin, Dako), and (100 µ L) of (mouse monoclonal antibody against Vimentin, Dako) for 30 min at room temperature in a moisture chamber. After washing with PBS for 3 min, binding of antibodies will be detected by incubating for 20 min with dextran labelled polymer (Dako). Finally, the sections washed in three changes of PBS, followed by adding 3,3 diaminobenzidinetetrahydrochloride (DAB) as a chromogen to produce the characteristic brown stain for the visualization of the antibody/enzyme complex for up to 5 min. After washing with distal water for 3 min slides were counterstained with haematoxylin (Mayer's) for one minute and washed in running tap water for several minutes 7-10 (bluing), then dehydrated, cleaned, and mounted in DBX.

Data Analysis

Analysis was done using SPSS 22.0 computer program. Frequencies, mean and Chi-square test values were calculated. Pearson's correlation method was used to evaluate the correlation between the protein expressions in the studied cases. P.value <0.05 was considered as statistically significant.

Result Interpretation

Vimentin expression was evaluated by counting the cells with positive cytoplasmic staining in the region of hot spots. Similarly, E-C expression was evaluated by enumeration of cells exhibiting positive reaction in the membrane and cytoplasmic region as the protein is present in major concentration on the membrane. Staining intensity in both cases were assigned a scores such as unstained (0), weak (1+), moderate (2+), and strong (3+) stained cells. Immuno-score was calculated with the percentage of cells and staining intensity in each of the cases [20].

Ethical Consideration

The samples were collected after permission according to the laboratory guidelines and regulations.

RESULTS

The study included fifty six samples, previously diagnosed as breast cancer. The age of patients ranged between 30 to 80 years with mean age of 50.1. Majority of the patients were in the age group of (50-59).The histopathological diagnosis of the study cases included invasive ductal carcinoma (IDC), medullary carcinoma (MC) and mucinous carcinoma (colloid) (CC). The histological grade of the study cases included grade I, II and III. The reminder of the result is explained in the following tables.

Table-1: Distribution of E-cadherin expression among the study cases

E-cadherin expression	Frequency	Percentage
Negative	7	12.5
Weak	18	32.1
Moderate	27	48.2
Strong	4	7.1
Total	**56**	**100**

Table-2: Distribution of Vimentin expression among the study cases

Vimentin expression	No	Percentage
Negative	1	1.8
Weak	29	51.8
Moderate	26	46.4
Strong	0	0
Total	**56**	**100**

Table-3: The correlation between age group and expression of E-cadherin

E-cadherin Expression	Age group					Total
	30-39	40-49	50-59	60-69	70-79	
Negative	3	3	1	0	0	**7**
Weak	4	4	7	3	0	**18**
Moderate	1	7	11	6	2	**27**
Strong	1	2	0	1	0	**4**
Total	**9**	**16**	**19**	**10**	**2**	**56**

P.value= 0.028

Table-4: The correlation between age group and expression of Vimentin

Vimentin Expression	Age group					Total
	30-39	40-49	50-59	60-69	70-79	
Negative	0	0	0	1	0	**1**
Weak	1	10	10	7	1	**29**
Moderate	8	6	9	2	1	**26**
Strong	0	0	0	0	0	**0**
Total	**9**	**16**	**19**	**10**	**2**	**56**

Table-5: The correlation between histological type and expression of E-cadherin

E-cadherin Expression	Histological type			Total
	IDC	MC	CC	
Negative	5	1	1	**7**
Weak	10	7	1	**18**
Moderate	22	5	0	**27**
Strong	3	1	0	**4**
Total	**40**	**14**	**2**	**56**

Table-6: The correlation between histological type and expression of Vimentin

Vimentin Expression	Histological type			Total
	IDC	M C	CC	
Negative	1	0	0	**1**
Weak expression	14	14	1	**29**
Moderate expression	25	0	1	**26**
Strong expression	0	0	0	**0**
Total	**40**	**14**	**2**	**56**

Table-7: The correlation between histological grade and expression of E-cadherin

E-cadherin Expression	Histological grade			Total
	Grade I	Grade II	Grade III	
Negative	2	1	4	**7**
Weak	3	10	5	**18**
Moderate	1	8	18	**27**
Strong	0	1	3	**4**
Total	**6**	**20**	**30**	**56**

Table-8: The correlation between histological grade and expression of Vimentin

Vimentin Expression	Cancer grade			Total
	Grade I	Grade II	Grade III	
Negative	0	0	1	**1**
Weak	5	13	11	**29**
Moderate	1	7	18	**26**
Strong	0	0	0	**0**
Total	**6**	**20**	**30**	**56**

Table-9: The correlation between E-cadherin and Vimentin expression:

Marker		E-cadherin	Vimentin
E-cadherin	**Pearson Correlation**	1	-.398*
	Sig. (2-tailed)		.002
	No	**56**	**56**
Vimentin	**Pearson Correlation**	-.398*	1
	Sig. (2-tailed)	.002	
	No	**56**	**56**

*There is an inverse correlation between E-cadherin and Vimentin expression, (r = -398). (*P.value= 0.002*).

DISCUSSION

The study is focused on detection of expression of E-C and Vimentin, and correlating their expression with various diagnostic parameters of breast cancer. It involved 56 cases of women with breast cancer stained by immunohistochemistry for EMT markers (E-C & Vimentin).

The age group of the study population reveals that majority of patients are more than 40 years 80% (45/56), indicated that older women were more susceptible to breast cancer than younger women. This result agreed with Hemalatha *et al.,* who reported that age is an important factor in occurrence of carcinoma, but breast carcinoma rarely occurred in young [21]. The result is also compatible with Bakhet *et al.,* who reported that the risk of developing breast carcinoma increased with age [22]. The study shows (40/56) cases are invasive ductal carcinoma, while (14/56) and (2/56) are medullary and mucinous carcinomas respectively. This finding agreed with Domagala *et al.,* who their study reported (214/262) cases of breast cancinoma is invasive ductal carcinoma [23], also compatible with Bakhet *et al.,* who reported most frequent type is invasive ductal carcinoma [22].

The study reveals the most frequent histologlcal grade is grade III, indicating that delay in diagnosis lead to delay in treatment. The result is compatible with Bakhet *et al.,* who reported that grade III was more frequent malignant carcinoma grade, and this is associated with poor prognosis [22]. But it's not compatible with Hemalatha *et al.,* who reported that (22/50) cases were of grade I [21].

The study reveals that the expression of E-C is negative in (7/56) cases, weakly expressed in (18/56) cases, moderately expressed in (27/56) cases and strongly expressed in (4/56) cases indicating that down-regulation of E-C acted as a major role in EMT and tumour cells metastasis (Table-1). This result agrees with Shiozaki *et al*., who reported that reduced expression of E-C may be a characteristic acquired during malignant transformation [24]. Also agrees with Gamallo, C *et al.,* who reported a correlation had been suggested between a loss of E-C and increased invasiveness of neoplastic cells [25]. The Vimentin expression reveals negativity in (1/56) cases, weakly expressed in (29/56), moderately expressed in (26/56) and strongly expressed in (0/56) cases (Table-2). That indicated Vimentin expression works as a prognostic marker for EMT and plays a major role in prognosis of breast cancer. This result agrees with Heatley *et al.,* who reported that Vimentin expression in breast tumours is an indicator of prognosis [26], and also agrees with Hemalatha *et al.,* who reported that Vimentin-positive cells associated with increased tumour proliferation [21].

The correlation between age and expression of E-C and Vimentin in the study is significant (*p*.value= 0.028 / 0.016) respectively (Table 3 & 4), indicated an

association between age and the EMT markers under investigation. There is no significant correlation between the histological type of the study cases and E-C expression (*p*.value= 0.126) (Table-5). This result may be due to the limited number and types of the study cases, so we suggest to use all types of breast cancer with reasonably sample size which may reveal a significant correlation. The study disagrees with Gamallo *et al.,* and Qureshi et al., who reported that E-cadherin expression correlates with histological type in breast carcinomas [14, 25]. On the other hand, there is a significant correlation between the histological type of the study cases and Vimentin expression (p.value= 0.004) (Table-6). The result agrees with Domagala *et al.,* who reported that Vimentin expression was unevenly distributed among the various histologic types of breast cancers and seems to be associated with ductal carcinomas [23].

There is a significant correlation between the hitological grading of the study cases and E-C expression (*p*.value= 0.027) (Table-7). This result agrees with Gamallo *et al.,* and Shiozaki *et al*., who reported that an association between E-cadherin expression and histological grade in breast cancer [24, 25]. There is no significant correlation between the histological grade and Vimentin expression (*p*.value= 0.051) (Table-8), and this result disagrees with Domagala *et al.,* who reported that there was a correlation between V imentin expression and histological grade of ductal breast carcinoma [23]. Also disagrees with Hemalatha *et al.,* who reported that a significant correlation was present between Vimentin expression and tumour grade [21].

The study finds that there is an inverse correlation between E-C and Vimentin expression (*r* = -0.389; *p*.value= 0.002) (Table-9), indicating that loss of E-C expression and increased in Vimentin expression is associated with the increase in aggressiveness of tumour cells. The detection of E-C and Vimentin as prognostic markers will help in evaluation of EMT, and thus help in prognosis and treatment of breast cancer.

CONCLUSION

Decreased expression of E-C and increased expression of Vimentin are associated with epithelial-mesenchymal transition (EMT) and tumour aggressiveness. The study recommends to use E-C and Vimentin in tumour markers panel for breast carcinoma prognosis assessment and to choose the suitable treatment in combination with tumour grading and staging systems.

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