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RESEARCH ARTICLE

EXPRESSION LEVELS OF *E-CADHERIN* AND *VIMENTIN* IN BREAST CANCER

***,[1]Rakshith, V., [2]Harendra Kumar, M. L., [3]Bhaskaran, A. and [4]Suresh, T. N.**

[1]Department of Pathology, Sri Devaraj Medical College, Sri Devaraj Academy of Higher Education & Research, Kolar 563101, Karnataka, India
[2]Professor, Department of Pathology, Sri Devaraj Medical College, Sri Devaraj Academy of Higher Education & Research, Kolar 563101, Karnataka, India
[3]Professor, Department of Surgery, Sri Devaraj Medical College, Sri Devaraj Academy of Higher Education & Research, Kolar 563101, Karnataka, India
[4]Professor, Department of Pathology, Sri Devaraj Medical College, Sri Devaraj Academy of Higher Education & Research, Kolar 563101

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ABSTRACT

Background: Despite various treatment and diagnostic options, breast cancer remains one of the most prevalent forms of cancer, globally. Such scenario is due to studies, which are still lagging behind in preventing the recurrence of breast cancer and its progression. The present study focuses on evaluating the expression of *E-cadherin* and *vimentin* in breast cancer cases.

Methods: The study comprised of 58 breast cancer patients admitted at the Department of Surgery between January 2014 and July 2015. Representative sections from formalin-fixed paraffin embedded tissue blocks of the cancer patients were stained with hematoxylin and eosin staining to evaluate tumor grade, size, and type by tumor, lymph node, and metastasis classification of malignant tumor staging system and Nottingham Prognostic Index along with skin involvement and vascular invasion analysis. Expression of proteins—*vimentin* and *E-cadherin*—was evaluated by immunohistochemical staining. Statistical analysis was performed using chi-square and Pearson's correlation test; $p<0.05$ was considered statistically significant.

Results: Majority of the patients were in the age-group of 41–50 years (31.03%). *Vimentin* and *E-cadherin* expressions were found to be significantly associated with tumor grade and stages ($p<0.05$), indicating epithelial to mesenchymal transition of tumor cells. Majority of the tumors were of grade I (37.93%) and II (41.37%) and diagnosed histopathologically as in filterating ductal carcinoma type (79.31%). Association of protein expression with other prognostic parameters was non-significant ($p>0.05$). Inverse correlation was observed between *vimentin* and E cadherin expression ($r = -0.2196$; $P = 0.09$).

Conclusion: *E-cadherin* and *vimentin* could be used as a prognostic marker in the diagnosis of the cancer stage and grade, which could in turn aid in early treatment and prolonged survival rate among the patients independent of other prognostic parameters.

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INTRODUCTION

Breast cancer is one of the most prevalent form of cancer globally (Parkin *et al.,* 2001). In India, it ranks second after cervical cancer with peak occurrence at ≥40 years of age. Breast cancer has been considered as the leading cause of death, especially among women in developing countries (Kamath *et al.,* 2013).

****Corresponding author: Rakshith, V.,***
Department of Pathology, Sri Devaraj Urs Medical College, Sri Devaraj Urs Academy of Higher Education & Research, Kolar 563101, Karnataka, India

High mortality rate due to breast cancer was observed in 2015with approximately 0.57 million deaths worldwide when compared to 0.37million mortality cases in 2001(Organization 2006; Parkin *et al.,* 2001). Education and awareness about menarche after 13 years of age, late marriage, first pregnancy after 30 years of age, induced abortion and non-vegetarian diet play a major role in reducing the risks of breast cancer in women (Kamath *et al.,* 2013). Diagnosis at the initial stage or early screening of the individuals (mammography screening in the case of breast cancer) further improves the treatment and survival rate, aiding in recovery of the cancer patients with low chances of recurrence. Prognostic factors of breast cancer such

as size of tumor, histopathological grade and type, status of axillary lymph node and vascular invasion, hormone receptor, human epidermal growth factor receptor 2 (HER2), mutation in p53 gene, and angiogenesis markers are evaluated to monitor the treatment of cancer patients. These factors are also helpful to estimate the chances of progression or recurrence of breast cancer (Li *et al.,* 2002; McCarthy *et al.,* 2002). However, a large number of cancer cases progresses towards the end stage despite breakthroughs in treatment modalities. This may be due to the increased aggressiveness and transition of cancerous cells from epithelial cells to mesenchymal cells, known as epithelial–mesenchymal transition (EMT) (Tomaskovic-Crook *et al.,* 2009). The transition is characterized by the altered expression of proteins such as *E-cadherin* (Singhai *et al.,*, 2011), alpha-smooth muscle actin (Yamashita *et al.,*, 2012), and *vimentin* (Hemalatha *et al.,*, 2013), which play a major role in the diagnosis of breast cancer. *E-cadherin* proteins are localized on the epithelial cell surfaces and are involved in cell–cell adhesion. Therefore, its dysfunction leads to transformation of normal cells to malignant forms leading to progression of tumor (Pećina-Šlaus 2003). On the other hand, *vimentin* is limited to the mesenchymal cells and helps in maintaining the integrity of the cells and protecting them against stress. It is reported to be over expressed in various types of cancer, as it aids in cell proliferation and growth. As a result, *vimentin* has been recognized as a well-known marker for the prognosis of EMT(Satelli and Li 2011). Irrespective of available modern therapies, there is an increased morbidity and mortality due to breast cancer. Prognosis of breast cancer may be improved by focusing on EMT and metastasis, which lead to advanced stage of breast cancer. Therefore, the present study evaluated the expression of proteins—*E-cadherin* and *vimentin* in breast cancer cases and correlated their expression with histological parameters such as grade, type and stage of tumor.

MATERIALS AND METHODS

Study design

The study comprised of 58 breast cancer patients admitted at the Department of pathology and Department of surgery were included in the study during January 2014 to July 2015.Age group in between 23 and 85 years was considered for the study, with the mean age of 52.3 years. Out of 58 cases of mastectomy, 51 cases were of modified radical mastectomy. Ethical clearance was obtained from the Institutional Ethics Committee. Informed consent was obtained from all the participants in the study before collecting the sample.

Evaluation of prognostic parameters

Staining with hematoxyl in and eosin (H&E) stain was performed for recognizing cancer tissue types and the morphologic changes. The grading of all the tissue samples were carried out using Bloom–Richardson grading system(Bloom and Richardson 1957). Lymph node status, tumor size, and stages were classified on the basis of tumor, lymph node, and metastasis (TNM) classification of malignant tumor staging system (Staging *et al.,* 1978). Nottingham Prognostic Index (NPI) (Albergaria *et al.,* 2011). Was calculated for 51 cases to perform the prognosis of post-mastectomy cancer cases. Skin involvement for 58 tissue samples was considered positive only when the tumor was detected within the dermis. Similarly, presence of tumor cell nests within the vascular lumina, outside of the tumor region was considered positive for vascular invasion (Nassar *et al.,* 2001).

Immunohistochemistry

Formalin-soaked paraffin-embedded tissue blocks were cut into 4-µm thin sections and fixed on 1% organosilane-coated slides for immunohistochemical staining. The tissue sections were dewaxed and dehydrated in organic solvents, (xylene, and absolute 70% and 90% ethyl alcohol), prior to the staining procedure (Andriani *et al.,* 2015). Tissue samples were washed using trisodium citrate buffer (pH 6) and subsequently treated with 3% hydrogen peroxidase for 15 min at room temperature to quench the endogenous peroxidase activity. Before immunohistochemical processing, antigen retrieval was performed in microwave at95˚C for 6 min in tris EDTA buffer (pH 9) for 2 cycles (Shi *et al.,* 1997). Staining procedure consisted of primary and secondary staining. Primary staining was performed by incubating the slides with pre-diluted antibodies (Biogenix, USA) against *vimentin* and *E-cadherin* for 45 min and 1 h, respectively. Fibroblasts and endothelial cells were used as internal controls for *vimentin*, whereas normal skin tissues were used as control for *E-cadherin*. The peroxidase–antiperoxidase method of Sternberger(Sternberger *et al.,* 1970)was followed for secondary staining of the tissue samples.

Image analysis

The stained tissue sections were first examined at 40X and 100X using Olympus CX21i microscope (Olympus Corporation, Tokyo, Japan) to identify and select the areas of high positivity. Individual cells present in the selected areas were counted under high magnification (200X). Calculated average number of cells were taken for further evaluation.

Evaluation of immunoscore

Vimentin expression was evaluated by counting the cells with positive cytoplasmic staining in the region of hotspots. Similarly, *E-cadherin* 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 the cases were assigned a scores such as unstained (0), weak (1+), moderate (2+), and strong (3+) stained cells. Immunoscore was calculated with the help of percentage of cells and staining intensity in each of the cases.(Galon *et al.,* 2012) A score of >30 was considered as significant in the case of *vimentin* expression, whereas score >2 was considered significant in *E-cadherin* expression in the present study (0 =0, 1–5 = 1, 6–8 = 2, 9–12 = 3).

Statistical analysis

Statistical analysis was performed using SPSS 11 software package. Data were represented as frequencies. Chi-square test were employed to see the significance level for categorical data and Pearson's correlation method was used to evaluate the correlation between the protein expressions in the studied population. $p<0.05$ was considered as statistically significant.

RESULTS

Majority of the patients were in the age group of 41-50 years (31.03%).

Table 1. Expression of *vimentin* and *E-cadherin* along with other prognostic parameters

Study parameters		*Vimentin*		*P* value	*E-cadherin*		*P* value
		Negative, n (%)	Positive, n (%)		Negative, n (%)	Positive, n (%)	
Final grade	I	21 (36.21)	1 (1.72)	0.001	8 (13.79)	14 (24.14)	0.051
	II	15 (25.86)	9 (15.52)		6 (10.34)	18 (31.03)	
	III	4 (6.90)	8 (13.79)		8 (13.79)	4 (6.90)	
Nottingham prognostic index	< 3.4	9 (17.65)	3 (5.88)	0.537	3 (5.88)	11 (21.57)	0.579
	3.4–5.4	16 (31.37)	7 (13.73)		9 (17.65)	12 (23.53)	
	>5.4	9 (17.65)	7 (13.73)		7 (13.73)	9 (17.65)	
Lymph node status	0	12 (23.53)	9 (17.65)	0.415	8 (15.69)	13 (25.49)	0.562
	1–3	14 (27.45)	6 (11.76)		6 (11.76)	14 (27.45)	
	>4	8 (15.69)	2 (3.92)		5 (9.80)	5 (9.80)	
Vascular invasion	No	29 (50)	11 (18.97)	0.386	14 (24.14)	26 (44.83)	0.493
	Yes	11 (18.97)	7 (12.07)		8 (13.79)	10 (17.24)	
Skin involvement	No	37 (63.79)	12 (20.69)	0.012	17 (29.31)	32 (55.17)	0.236
	Yes	3 (5.17)	6 (10.34)		5 (8.62)	4 (6.90)	
Histopathological type	IDC	31 (53.45)	15 (25.86)	0.152	10 (17.24)	36 (62.07)	<0.0001
	ILC	6 (10.34)	0 (0)		6 (10.34)	0 (0)	
	MC	3 (5.17)	3 (5.17)		6 (10.34)	0 (0)	
Tumor size	<2	0 (0)	2 (3.45)	0.080	1 (1.72)	1 (1.72)	0.926
	2–5	31 (53.45)	11 (18.97)		16 (27.59)	26 (44.83)	
	> 5	9 (15.52)	5 (8.62)		5 (8.62)	9 (15.52)	
Tumor stage	I	0 (0)	1 (1.72)	0.011	1 (1.72)	0 (0)	0.488
	II A	13 (22.41)	7 (12.07)		7 (12.07)	13 (22.41)	
	II B	13 (22.41)	4 (6.90)		5 (8.62)	12 (20.69)	
	III	11 (18.97)	0 (0)		4 (6.90)	7 (12.07)	
	III B	3 (5.17)	6 (10.34)		5 ((8.62)	4 (6.90)	

IDC-Infiltrating ductal carcinoma, ILC-Invasive lobular carcinoma, MC-Medullary carcinoma, $p<0.05$ is considered statistically significant,

The data obtained for parameters such as tumor size, stage and grade, NPI, lymph node status, vascular invasion, skin involvement and type of histology with respect to expression pattern of *vimentin* and E cadherin has been represented in Table 1. Analysis of correlation between *vimentin* and E cadherin expression revealed inverse correlation with r value of -0.2196 and *P*value of 0.097.

DISCUSSION

Detection of protein expressionin cancer is an intensive area of research, as it is hypothesized to diagnose the stages of cancer rapidly and effectively. Alterations in protein expression may also improve the monitoring of patients and could also prevent the chances of progression or recurrence. The present study focused on detection of expression of *vimentin* and *E-cadherin* and correlating it with the various prognostic parameters of breast cancer.

Association of stages and grades with expression of *vimentin* and *E-cadherin*

The knowledge on stage and grade of cancer in a patient can help in evaluating for the better prognosis of the disease. It also facilitates the selection of appropriate treatment regimen for patients (Buhmeida *et al.,* 2006). Grading of a cancer reveals its aggressiveness and is evaluated on the basis of morphology and behavior of the cells (Cancer 2002).Grade I and II were observed to be predominant in the present study, indicating low-grade and intermediate-grade breast carcinoma among the patients. In addition, the expression of *vimentin* was significant with the final grade tumor which was in accordance with a study conducted by Husian *et al* (Husain *et al.,* 2016). Similar results were obtained in the cases of *E-cadherin* expression from the present study, in accordance with the observation made by Mohammadizadeh *et al.,* in which the alteration in *E-cadherin* expression led to significant changes in lymph node metastasis and grading of tumor(Mohammadizadeh *et al.,* 2009).

In the present study, expression of *E-cadherin* reduced withthe increases in grade and stage of breast cancer. In contrast, the expression of *vimentin* was observed to increase with the grade and stage of breast cancer, indicating the transition of cells from epithelial to mesenchymal type. The results werein accordance with the theory of EMT where low-grade cancer cells are known to retain their adhesion properties due to the presence of *E-cadherin* and alike proteins. However, with the increase in grade the cells undergo transition to mesenchymal cells, thereby producing higher amount of *vimentin* and reduced expression of *E-cadherin* leading to loss of adhesiveness (Tomaskovic-Crook *et al.,* 2009). In addition, increase in the expression of *vimentin* in higher stage and grade may aid in detecting progressive cancer (Satelli and Li 2011).

Association of expression of *vimentin* and *E-cadherin* with histopathological typing

The immunohistopathological typing of the cancer tissues demonstrated predominance of IDC cases, especially in cases of *vimentin* expression. Studies conducted by Hemalatha *et al.,*(Hemalatha *et al.,* 2013) (18%), Niveditha *et al.,* (Niveditha and Bajaj 2003) (18%), Domagala *et al.,* (Domagala *et al.,* 1990) (16%) on IDC type with *vimentin* expression observed similar results as that of the present study. However, difference in expression of *vimentin* among the studies can be due to the type of fixative used during the processing of the tissue samples (Niveditha and Bajaj 2003; Yagasaki *et al.,* 1996). In addition, most of the E cadherin expressing cases were also of IDC type in the present study, with highly significant difference among the histological types ($P< 0.0001$). Similar findings of positivity was observed by many other studies (Howard *et al.,* 2004; Kowalski *et al.,* 2003; Moll *et al.,* 1993; Younis *et al.,* 2007). In a study conducted by Qureshi *et al.,* (Qureshi *et al.,*, 2006), 99% of cells expressed E-cadher in in IDC type. However, few studies have reported the loss of *E-cadherin* expression in lobular carcinoma, indicating an association of lobular carcinoma with diffuse invasive carcinoma (Gamallo *et al.,* 1993; Moll *et al.,* 1993; Younis *et*

al., 2007). The present study revealed anassociation between immunohistopathological typing and *E-cadherin* expression levels. Similar findings were reported by Quershi *et al.,*(Qureshi *et al.,* 2006) in which a correlation was observed between *E-cadherin* expression and immunohistopathological typing of breast cancer. Loss of *E-cadherin* expression in ILC was also observed in various other studies in which similar significance between *E-cadherin* expression and type of tumor was reported concluding that expression of the proteins in breast cancer may have minimal prognostic value; however, play a significant role as phenotypic marker (Acs *et al.,* 2001; de Leeuw *et al.,* 1997; Gamallo *et al.,* 1993; Parker *et al.,* 2001; Wahed *et al.,* 2002).

Association of *vimentin* and *E-cadherin* with prognostic parameters

An increase in the expression of viment in with the loss of epithelial keratin is an indicator of progressive breast cancer (Vora *et al.,* 2009). In the present study, association was observed between *vimentin* expression and the skin involvement, in contrast to the results of skin involvement and *E-cadherin* expression. In addition, no significant association was observed between the *vimentin* and *E-cadherin* expressions with the prognostic parameters such as vascular invasion, NPI, tumor size, and lymph node status. Similar results with respect to *vimentin* expression and parameters such as lymph node status, NPI, and tumor size was reported by Hemalatha *et al.,* (Hemalatha *et al.,* 2013). However, Liesheng *et al.,*(Liesheng *et al.,* 2014) reported an association between the prognostic parameters and E cadherin expression, in contrast to the present study.

Correlation of expression of *vimentin*and *E-cadherin* in the study population

Studies reported that EMT transition is characterized by the altered expression of *E-cadherin* (Singhai *et al.,* 2011) and *vimentin* (Hemalatha *et al.,* 2013). Differential expression of *E-cadherin* has beenreported and involved in tumor progression (Pećina-Šlaus 2003). In contrast, *vimentin* expression is essential in maintaining cell integrity, and studies have identified the increased expression of *vimentin* in various types of cancer (Satelli and Li 2011). In accordance with others findings in breast and oral cancers, (Myong 2012; Vandewalle *et al.,* 1994)our study found similar observations in which correlation between *E-cadherin* and *vimentin* was inverse in nature. Thus, it can be inferred that cells might have undergone EMT transition. Loss of *E-cadherin* expression and increase in *vimentin* expression is associated with the increase in aggressiveness of tumor cells, possibly due to transition of cancerous cells to EMT cells(Tomaskovic-Crook *et al.,* 2009). *Vimentin* expression was associated with higher grade and stage of cancer and thus could be used as a prognostic marker in identifying aggressive tumors independent of lymph node status and tumor grades. Anti-*vimentin* drugs such as with aferin-A could be used for the treatment as *vimentin* has become a therapeutic target for the treatment of cancer, especially those arising from EMT. Therefore, identification of tumor cells, stages and/or grades with the help of *vimentin* and *E-cadherin* expression levels can help in early detection of cancer, which can prolong the survival periods.

Conflict of Interest: Authors have no conflict of interest to disclose.

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