Original Article

Expression of Vimentin in Breast Carcinoma & its Correlation with Histopathological Parameters

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ABSTRACT

Objective: To explore vimentin expression in different forms of breast cancer 2) to establish whether vimentin expression has a relationship to prognostic markers such as tumor size, grades, and status of the lymph node.

Study Design: Observational study

Place and Duration of Study: This study was conducted at the Department of Pathology Hayatabad Medical Complex Peshawar from March 2019 to March 2020.

Materials and Methods: 50 Specimens of radical modified mastectomy have been received by the Department of Pathology Hayatabad Medical Complex Peshawar. After history has been collected, specs of hematoxylin and eosin and immunehistochemically stained sections were checked and fixed to 10 percent forms of formalin.

Results: In this research, the number of patients was 40-60 years of age. (It's about 51 years old on average.) In 23/50 (46%) of cases, Vimentin expression has been identified. It has demonstrated that its expression is highly linked to progressive malignancies (P-value 0,05) and that tumor cells transition from the epithelial to the mesenchymal. The bulk (48 percent) were classified as Invasive Carcinoma NST in Grade 2 and histopathologically identified. No link was found between the expression and status of the lymph node and the tumor size (P-value 0.05).

Conclusion: Vimentin is more typically seen in advanced cancers. Its expression is unrelated to tumor size or nodal metastasis, implying that it may assist patients to obtain early treatment and live longer lives regardless of other prognostic variables.

Key Words: Vimentin, Breast Carcinoma, Correlation, Histopathological Parameters

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INTRODUCTION

Cancer is a disorder group in which the cells of the body uncontrolled change and spread. The incidence of cancer and mortality are growing alarmingly on a global scale. The mammalian gland is a highly dynamic organ that is submitted to branching, morphogenesis in adolescence, alterations during the menstrual cycle, and goes in the course of pregnancy, lactation, and involution.

Breast neoplasm is the most frequent cancer in women all over the world.[1-2]

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Cervical cancer has previously been the most common disease in Indian women, but breast cancer has now outweighed cervical cancer as one of the principal reasons for mortality, however cervical cancer remains the most common cancer in rural India.[3]

Breast cancer has become more common as a result of changes in risk factors. According to GLOBOCAN 2018, 2 million new cancer cases were diagnosed in women, accounting for 25% of all new cancer cases. Breast cancer is a complex disease in which a variety of factors have a role in its development. Many investigations and experiments have shown that carcinomatous cells take on the characteristics of mesenchymal cells and express mesenchymal markers. That is, high-grade epithelial tumors lose their epithelial form and develop mesenchymal features, a process known as epithelial-mesenchymal transition (EMT), which is linked to tumor invasiveness and metastatic potential. EMT causes epithelial markers like vimentin and fibronectin to be downregulated while mesenchymal markers like vimentin and fibronectin are upregulated.[4-5]

In normal mesenchymal cells, vimentin, a class 3 intermediate filament, is widely expressed. It is well known for maintaining cellular integrity and providing stress tolerance. Vimentin is either laterally or

terminally connected to the nucleus, endoplasmic reticulum, and mitochondria. Vimentin is a multifunctional protein that can interact with a wide range of proteins, making it a possible controller of a variety of physiological activities. Expression of Vimentin is higher in a variety of epithelial malignancies, including prostate cancer.[6]

According to much research, the expression of Vimentin in breast cancer is a major prognostic predictor. High-grade tumors, increased tumor proliferation6, low-PR, low-AR, invasive membrane basement, and therapeutic resistances are connected to Vimentin positive cells.[7]

As a result, we used immunohistochemistry to examine the expression of Vimentin in breast cancer in our study. We also looked at how this marker's expression correlated with grade related to histopathologically, size of the tumor, & metastasis of lymph node.

MATERIALS AND METHODS

Lumpectomy specimens delivered to the pathology laboratoryof Hayatabad Medical Complex provided the data for this investigation. Age, sex, presentation style (side of a lump or nipple discharge), technique, gross tumor size, and axillary nodal status are all things to think about. Our college's ethics committee permitted us to do just that.

The sections of Haematoxylin& Eosin (H&E) for the histological type, grade, and status of lymph node were obtained and analyzed. The grades were calculated with the grading system of Nottingham. All portions for IHC have been selected after testing for representative tumor paraffin blocks (Immunohistochemistry).

The slide was covered and incubated overnight at 58 degrees Celsius, 4 micron neoplastic tissue blocks were sliced. Sections of xylene, purified alcohol, 90 percent, and 70 percent were de-affining and dehydration. The antigen recovery resulted in the microwave and subsequent coverage of the slides with three-sodium citrate buffer solutions by a peroxide block to suppress endogenous peroxidase. The areas were stained for an hour with main antimicrobials. After secondary stains with peroxidase anti-peroxidase, the antigen body complex was colored with DAB.

RESULTS

Brown color development was regarded as positive, as well as the method outlined below was employed to score it. Immunohistochemical staining was evaluated.

For scoring immunostaining patterns, the most typical tumor regions were chosen. Light microscopy was used to score the samples. Vimentin staining of the cytoplasmic granules was regarded as a good result. Positive was defined as the absence of positivity in 10% of tumor cells. (Table 1).

Table No.1: Score with vimentin expression

Score	Vimentin expression
0	Negative with on staining of tumor cells
1+	Weak staining of more than 10% of tumor cells
2+	Moderate staining of more than 10% of tumor cells
3+	Strong staining of more than 10% of tumor cells

In this study, the majority of instances were in the under 40-year-old age group, while the least number of instances were in the over 70-year-old age group. The patients ranged in age from 28 to 90 years old. In our investigation, the average age of the cases was 51.0 years. The average age of patients with vimentin-positive tumors was 29 years old for the younger patients and 75 years old for the older patients.

Breast cancer that has spread across the body NST was found in practically every instance of breast carcinoma in the study, except two cases of carcinoma with medullary features such as mucinous carcinoma and only one case of either invasive papillary carcinoma, lobular carcinoma, as well as mixed type invasive & lobular carcinoma. The WHO's 2012 recommendations were used to grade the students. (By adapting Bloom and Richardson's method to suit Elston's needs). There were 18 cases of Grade 1/well-differentiated carcinoma, 24 cases of Grade 2/ moderately differentiated carcinoma, and 8 cases of Grade 3/ badly differentiated carcinoma in the 50 cases studied. As a result, grade 2 carcinomas with considerable differentiation accounted for the vast majority of cases. The invasive tumors ranged in size from 2cm - 9cm in diameter, with a mean of 5cm. Only one case had a tumor size of 4, 14 cases had metastatic deposits in 1-3 lymph nodes, & 10 cases had none. The bulk of the patients, 36 (72%) out of 50, had tumor sizes ranging from 2 to 5 cm, with 13 instances (26%) having tumor sizes more than 5 cm.

Vimentin expression was found to be significant in 23/50 (46%) of the patients in our investigation. When cancer cells had a pronounced brown cytoplasmic staining, the tumor was declared positive. Fibroblasts, endothelial cells, lymphocytes, and macrophages were labeled positively, while non-neoplastic tubule epithelial cells were stained negatively. All 50 cases were subjected to immunohistochemical staining, vimentin expression analysis, and scoring to determine the intensity of expression. When noticeable brown granular cytoplasmic expression was detected, vimentin expression was considered to be positive. There were 27 cases with a negative/score 0 expression, 1 case with a score 1+ expression, 21 cases with a score of 2+ expression, and 1 case with a score of 3+ expression out of 50. (Table 2)

Table No.2: Vimentin score with number of cases

Sr.No.	Vimentin Score	No. of cases (total 50)	% of cases
1	Score 0 / Negative	27	54
2	Score 1+	1	2
3	Score 2+	21	42
4	Score 3+	1	2

Vimentin is preferably expressed in invasive NST breast carcinomas (49%) and medullary-like carcinomas (100%) but not in invasive papillary carcinomas, lobular carcinomas, invasive and lobster-like mixed carcinomas.

Vimentin positivity was found in 6/8 cases of grade 3/poorly differentiated tumors, 15/24 cases of grade 2/moderately differentiated tumors, and only 2/18 cases of grade 2/moderately differentiated tumors. The expression of vimentin was correlated with the tumor grade. Score 0 expression was prominent in grade 1 carcinoma. Grade 2 and 3 carcinomas, on the other hand, revealed a lot of score 2+ expression. With a P-value of 0.05, there was a significant link between carcinoma's grade & expression of and vimentin in breast carcinoma.

With a P-value of 0.05, there was no statistically significant relationship between vimentin expression as well as the size of the carcinoma. The total number of lymph nodes affected was correlated with vimentin expression. In the current investigation, there was no statistically significant link between expression of vimentin & involvement of lymph node. (P-value > 0.05).

DISCUSSION

Breast cancer manifests itself in a variety of ways. Clinical behaviors including the time to tumor growth and metastasis cannot be predicted with accuracy, despite histologic similarity at the time of sickness diagnosis. Breast cancer is a multi-faceted molecular disorder. Breast cancer. Hormone receptor ER and PR as well as human EGFR-2 over-expression (HER-2) is crucial in the treatment judgment process for patients with breast cancer. These characteristics may determine the chance of illness relapse in addition to predicting response to therapy. Negative triple breast cancer has a poor prognosis due to lack of ER, PR, and HER-2 expression and lack of targeted therapy. Hormone-positive receptors have good outcomes, while negative triple breast cancer has a poor prognosis due to lack of ER, PR, and HER-2 expression and lack of targeted therapy. As a result, doctors and researchers are increasingly paying attention to this aggressive TNBC. Consequently, identification of aggressive phenotypes, such as the presence of EMT (epithelial-mesenchymal tumor) cells, is important for predicting cancer cell behavior. Interactions between multiple secretory soluble molecules, growth factors, their effects, and many extracellular signals, including multiple transcription factors such as PDGF, Notch, and NF-KB, trigger EMT.[8] Since the Trans positive value of this EMT score is unknown, in this study we investigated the EMT marker vimentin in breast cancer patients.

Wendy A Raymond et al. were the first to describe vimentin expression in breast cancer in 1989.[8] In the development of breast cancer in the young, the patient's age is a crucial predictor. The patients were between the ages of 28 and 90. The mean age of the cases in our research was 51.0 years, which is marginally greater than WHO estimates of the Indian population's peak age of 45-50 years. The average age of the 6 patients with well moderately, & poorly differentiated tumors was 55.2, 49.2, and 45.5 years. Expression of Vimentin was shown to be significant in 23 of 50 instances (46 percent). Our results were lower than those of Thomas et al[9], who detected vimentin expression in 25/53 patients (47.1%) and observed a strong link between vimentin expression and high histological grade 3 tumors, as well as ki67 and EGFR expression. Vimentin expression in tumor cells has been found to vary between 18 and 22.7 percent, 17.4 percent, and 18 percent in other investigations. A cutoff of positive in 10% tumor cells was regarded as substantial positivity in the majority of the studies mentioned above. In our research, we used a cut off value of positive in more than 10% of tumor cells.

Vimentin expression was also discovered in invasive carcinoma breast NST and mucinous carcinoma by Rakshith et al., whereas vimentin negative was identified in lobular carcinoma breast. The shape and behavior of cells are used to grade cancer and determine its aggressiveness. In the current study, grade 1 and grade 2 tumors were found to be the most common, indicating that the patients had low-grade carcinoma. According to a study conducted, vimentin expression was substantial with grade 3 malignancies, by Hemalath et al, Rakshith et al., Korsching et al.[9]

The findings backed up the EMT theory, which claims that because E-cadherin and other proteins are present in low-grade cancer cells, they preserve their adhesion properties. Cells transition to mesenchymal cells as they progress in grade, losing their adhesiveness & producing more vimentin.[10] An elevation in vimentin expression is linked to epithelial keratin loss, which is a sign of breast cancer progression. Furthermore Vora et al[11] in their study that in In terms of illness state, those who experienced recurrence or metastatic outgrowth gained more vimentin as compared to those who did not.

The size of invasive tumors in our samples consisted of 3 to 9 centimeters, with a mean of 5 centimeters. Vimentin positive was observed in one case with a tumor that was less than 2 cm in size, however, the majority of vimentin positivity was found in tumors that

were 2-5 cm in size. The expression of vimentin and the size of the tumor had no obvious relationship. Domagala and colleagues. This unfavorable relationship has also been described by Hemalat and Rakshith V et al. Vimentin expression was found to be unaffected by the destructive size of a tumor, which would be linked to patient survival.

The existence of axillary lymph nodes is by far the most commonly utilized prognostic indication in breast carcinomas. While a positive lymph node does not rule out severe illness or distant metastases, a negative lymph node does not rule out serious illnesses or distant metastases.[12] In our investigation,[13] (34%) of the 23 vimentin-positive cases in the initial tumor had lymph node metastases, and except for a few studies by Vora et al., who showed that patients with lymph node-positive status had greater vimentin expression, we found no statistically significant association with nodal metastasis, which was consistent with many previous investigations.

Clinical Significance of EMT: Although EMT-prone cells can spread, they may only account for a small portion of the overall population of tumor cells. The presence of a single cancer cell or a tiny cluster of cancer cells at the invasive front of tumor tissues is known as tumor budding. Cancer cells in tumor buds have already been demonstrated to downregulate E cadherin expression & enhance vimentin expression, as well as having cancer stem cell characteristics.[14,15] It has been established that cells undergoing EMT have evolved the potential to infiltrate and gain resistance to most anticancer treatments as a result of numerous stressors such as radiation and hypoxia.[16] Targeted therapies are being developed and used to treat a wide range of malignancies, with improved survival rates & clinical outcomes.[17,18] EMT, on the other hand, was said to provide resistance to certain specific compounds. As a result, it has been demonstrated that EMT causes medication resistance and allows tumor development to be rapid. Clarifying the relationship between EMT and drug resistance may aid clinicians in selecting the best anticancer medication treatment as well as the mode of invasion for high-risk situations.

CONCLUSION

When taken as a whole, the idea of EMT provides a useful framework for understanding the morphologic and molecular alterations that occur during tumor cell invasion and metastasis. In malignancy, there is a well-established link between EMT-like cellular phenotype as seen by changes in marker protein expression and tumor aggressiveness. As a result, high-grade tumors expressed vimentin preferentially in our investigation, with no link to tumor size or nodal metastasis. Further research is necessary to investigate major cancer activities in vimentin to detect the future utility of vimentin as a biomarker for clinically relevant malignancy. Vimentin expression in cancer is expected based on existing evidence to become a popular and promising therapeutic objective with great potential to develop new predictions and diagnostics. Furthermore, the usage in conjunction with existing anti-cancer therapies of vimentin-specific pharmacologic inhibitors and new therapeutic drugs should be supported.

Author's Contribution:

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Conflict of Interest: The study has no conflict of interest to declare by any author.

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