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# STUDY OF VIMENTIN EXPRESSION IN TRIPLE NEGATIVE BREAST CANCER

**Histopathology**

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## ABSTRACT

**Background:** Breast carcinoma is one of the most common cancer among women globally. Increased vimentin expression has been reported in triple negative breast carcinoma (TNBC) cases. **Aims And Objective:** This study showed the expression of vimentin in TNBC cases and its correlation with various prognostic factors such as age, tumor size, histological grade and lymph node status. **Materials And Method:** This study was conducted at Department of Pathology, Gandhi Medical College, Bhopal from January 2021 to June 2022. Total 60 breast cancer specimens (mastectomy, lumpectomy, biopsy) were submitted for histopathological diagnosis and Estrogen receptor (ER), Progesteron receptor (PR) and HER2/neu expression. Out of 60 cases, 21 cases were showing triple negativity and selected for vimentin expression study. **Results:** In the present study out of 21 TNBC cases, 19 cases (90.5%) had shown positive vimentin expression. The mean age of cases was 52.6years and tumor size >2cm. Significant correlation was seen between vimentin expression and tumor size, as the tumor size increases vimentin expression also increases. Majority of cases (76.2%) were positive for nodal metastasis. In TNBC cases with positive vimentin expression, 47.6% cases belonged to grade III, 42.9% cases grade II, and 9.5% cases of grade I. As the grade of tumor increases, vimentin expression also increases. **Conclusion:** The vimentin expression in the present TNBC cases demonstrated significant vimentin expression which can be helpful in detection of metastasis and disease prognosis.

## KEYWORDS

Breast cancer, Triple negative breast cancer, Vimentin.

## INTRODUCTION

Breast cancer is the most common and deadly malignancy of women globally. With an estimated 1.68 million new cases per year, it is the most prevalent non-skin cancer in women. 23% of all malignancies in women are caused by it.[1] It ranks after cervical cancer as the second most common malignancy among women in our nation.[2]

Histologic type, histologic grade, tumour size, and lymph node status are significant prognostic indications in histopathology in addition to clinical factors such as age, illness presentation, and menopausal status.[3]

There are additional variables that not only predict outcomes but also direct therapy to specific biological targets. These variables include estrogen receptor (ER) and progesterone receptor (PR) status, HER2/neu status, basement membrane integrity and vimentin expression.

Compared to most other breast cancer types, triple negative breast cancers grow and spread more quickly. Hormone therapy is ineffective for treating certain tumours because the cancer cells lack hormone receptors. Drugs that target HER2 are useless because they don't have too much of it. Chemotherapy still has its uses.[4]

Vimentin, a major constituent of the intermediate filament family of proteins, is ubiquitously expressed in normal mesenchymal cells and known to maintain cellular integrity and provides resistance against stress. It's expression in breast cancers is one of the key prognostic factors. High grade tumours, accelerated tumour proliferation, low ER, low PR, increased basement membrane invasiveness, and treatment resistance are all linked to vimentin-positive cells.[5]

Therefore, in our investigation, we used immunohistochemistry to examine Vimentin expression in triple-negative breast cancer. Along with age of presentation, tumor size, histological grade, and lymph node metastases, we also looked at this marker expression in relation to these factors.

## MATERIALS AND METHODS

This study was conducted at Department of Pathology, Gandhi Medical College, Bhopal from January 2021 to June 2022. All the cases immunohistochemically diagnosed as triple negative breast carcinoma (ER -Ve, PR -Ve, HER2/neu -Ve) were included in the study. The clinical details like age, sex, laterality, duration of symptoms, size of the tumour, axillary lymph node status and imaging finding like USG/MRI were recorded in each case. Total 60 breast cancer specimens (mastectomy, lumpectomy and biopsy) were submitted and histopathological study of the specimens was done by Haematoxylin and Eosin staining as per standard protocol. IHC was done for ER, PR and HER2/neu expression and Vimentin expression study was done on triple negative breast carcinoma cases (ER -Ve, PR -Ve, HER2/neu -Ve).

### Vimentin Staining

A 5um thick paraffin tissue section was mounted on a poly-L-lysine-coated glass slide and fixed by baking on slide warmer table at 60°C for 1 hour. Anti-human vimentin antibody "mouse monoclonal" and the staining procedure were used for immune staining of the section for vimentin expression. One section from fibroadenoma breast was processed with the test batch as a positive control, and a section from skeletal muscle as negative control for vimentin expression. Fibrobalsts and vascular endothelial cells in surrounding connective tissue were used as internal positive control. Sections were stained, mounted with DPX, and allowed to dry. The tissue cells that expressed an antibody labelled for vimentin showed dark granular staining of the cytoplasm under a 400X light microscope.[6]

### Vimentin Scoring [6]

Vimentin scoring was done in each cases applying the criterion-

**1. Vimentin Cell Positivity Percentage(VPP):** The number of vimentin positive cells/100 tumor cells was recorded as Vimentin Percent Positivity (VPP) of the tumor.

**2. Vimentin Intensity Score (VIS) Recorded As-**

A) No staining score 0
B) Weak staining score 1
C) Moderate staining score 2
D) Strong staining score 3

**3. Vimentin Immune Expression Score (VIES)** was calculated by

multiplying the VPP score and VIS score observed for the tumor. The VIES score of more than thirty (>30) was considered as Positive Vimentin Expression.

**Vimentin Immune Expression Score (VIES)**
Very low: <30
Low: 31-50
Moderate: 51-100
High: >100
VIES <30- Not significant.
VIES >30- Significant.

## RESULTS

In the present study, in triple negative cases, more number of cases were in the age group of more than 45 years. The age of the patients was ranged from 27 to 80 years. Mean age of cases in our study was 52.6 years.

**Table 1: Distribution Of Study Cases According To Their Histopathological Grading In Triple Negative Breast Carcinoma Cases**

| S.N. | Histopathological Grading | No. of Cases (%) n=21 |
|---|---|---|
| 1. | Grade 1 | 02(9.5%) |
| 2. | Grade 2 | 09(42.9%) |
| 3. | Grade 3 | 10(47.6%) |

Grading was according to WHO 2019 guidelines (by using Elston's modification of the Bloom and Richardson method). Out of 21 cases 02 cases were Grade 1 carcinoma, 09 cases were grade 2 and 10 cases were grade 3. Hence majority of cases were grade 3 carcinoma.

**Table 2: Tumor Size Wise Distribution Of Cases In Triple Negative Breast Carcinoma Cases**

| | Tumor Size (In Cm) | No. Of Cases (N=21) | % |
|---|---|---|---|
| 1. | ≤2 | 00 | 00 |
| 2. | >2 TO ≤5 | 14 | 66.67 |
| 3. | >5 | 07 | 33.33 |

Invasive tumor size ranged from 2.5cm to 12cm with mean size of 5.5cm. Maximum cases, 14(66.7%) out of 21 ranged between tumor size 2cm to 5cm and 07 cases(33.3%) were of tumor size >5cm. In present study no TNBC cases had tumor size <2cm.

**Table 3: Lymph Node Metastasis In Triple Negative Breast Carcinoma Cases:**

| S.N. | Lymph Node Metastasis | No of Cases (%) N=21 |
|---|---|---|
| 1. | Present | 16(76%) |
| 2. | Absent | 05(24%) |

Nodal metastasis was found in 16 out of 21 cases. In our study, out of these 16 cases which showed nodal metastasis, 14 cases (87.5%) had shown positive vimentin expression. Tumors were considered positive when there was distinct brown cytoplasmic staining in cancer cells.

**Table 4: Vimentin Intensity Of Staining In Triple Negative Breast Carcinoma Cases:**

| S.N. | VIMENTIN Intensity of Staining in ER- PR- Her2- (Triple negative) | No of casesn=21 | | |
|---|---|---|---|---|
| | | Frequency | (%) | F value =12.85 P value= <.001 |
| 1. | Score 0 (No Stain) | 00 | 00 | |
| 2. | Score 1 (Weak stain) | 04 | 19 | |
| 3. | Score 2 (Moderate Stained) | 13 | 62 | |
| 4. | Score 3 (Intense Stain) | 04 | 19 | |

(F critical value = 3.29 at df is 3)

All 21 cases were taken for immunohistochemistry staining, analysis of vimentin expression and scoring was done to grade intensity of expression. Distinct brown granular cytoplasmic expression was considered as positive for vimentin expression.

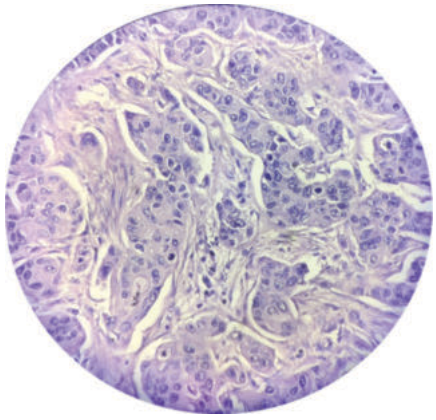
In our study intensity of staining for VIMENTIN in Triple negative cases of Breast Carcinoma, Majority of cases (62%) had score of 2 indicating moderate staining. 19 % cases had shown weak staining by obtaining a score of 1 while 19% cases had shown intense staining by obtaining a score of 3. Out of 21 cases, no case showed negative staining or score 0.

**Table 5: Vimentin Immune Expression Score (VIES) In Triple Negative Breast Carcinoma Cases**

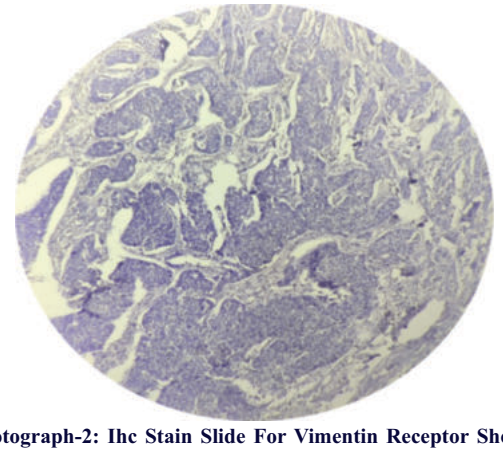
| S.N. | VIES in ER- PR- Her2- (Triple negative) cases | No of Cases n=21 | |
|---|---|---|---|
| | | Frequency (%) | Mean score ± SD |
| 1. | Very Low (<30) | 02(10) | 25 ± 7.07 |
| 2. | Low (31-50) | 03(14) | 40 ± 0.0 |
| 3. | Moderate (51-100) | 08(38) | 90 ± 15.12 |
| 4. | High (>100) | 08(38) | 120 ± 55.40 |
| | VIES score <30 – Not significant >30 - significant | F value =14.8 | P value = <0.001 |

(F critical value = 3.29 at df is 3)

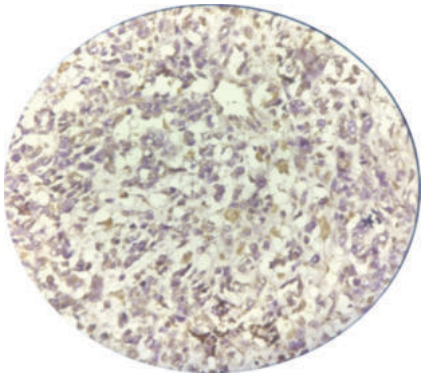
The Vimentin Immune Expression Score (VIES) for expression of VIMENTIN in Triple Negative Breast carcinoma cases were found to be very low (<30) which is not significant in 10% cases while 14% having score between 31- 50. Moderate VIES score between 51-100 were coming in 38% cases while High score >100 were present in 38% which are significant. The difference between the mean scores was found to be statistically significant ($p<0.05$) using ANOVA test.



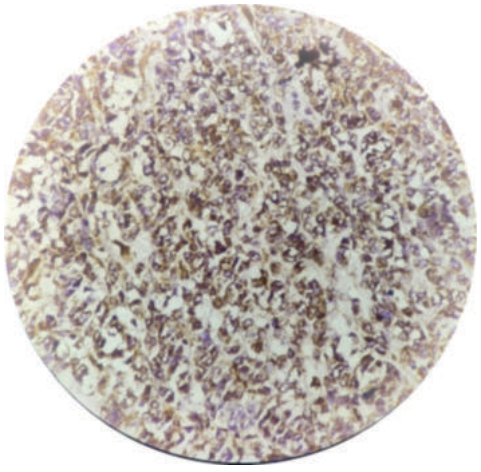
**Photograph-1: Infiltrating Ductal Carcinoma Breast, Modified Bloom Richardson Grade-2 (h & E Stain, 40x):**



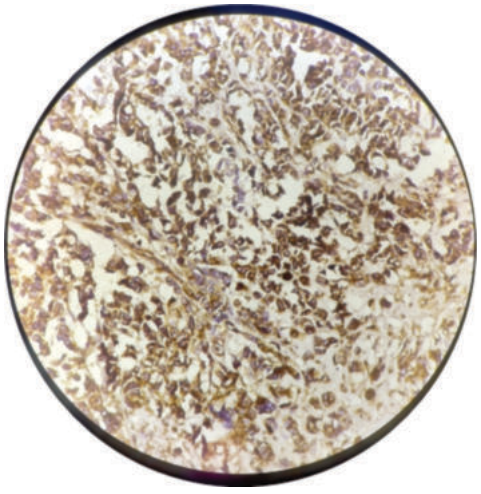
**Photograph-2: Ihc Stain Slide For Vimentin Receptor Showing Negative Stain (intensity Score 0) (40x)**



**Photograph-3: Ihc Stained Slide For Vimentin Receptor Showing Positive Stain (intensity Score 1+)**



**Photograph-4: Ihc Stain Slide For Vimentin Receptor Showing Positive Stain (intensity Score 2+)**



**Photograph-5: Ihc Stained Slide For Vimentin Receptor Showing Positive Stain (intensity Score 3+)**

## DISCUSSION

The clinical spectrum of breast cancer is broad. Despite having similar histologic presentations at the time of disease diagnosis, it is impossible to anticipate with precision how long it will take for the disease to proceed and spread.

Multiple molecular subtypes of breast cancer make up the heterogeneous illness. The existence of the hormone receptors ER and PR as well as the overexpression of the human EGFR-2 (HER-2) are crucial factors in determining the best course of treatment for breast cancer patients. These variables may influence the chance of a disease relapse in addition to predicting treatment response. As opposed to triple negative breast cancers, which typically have a bad prognosis due to their lack of ER, PR, and HER-2 expression as well as the unavailability of targeted therapy, hormone positive receptors have been thought to have a good outcome. Because of this, both scientists and physicians have developed an exponentially greater interest in this aggressive Triple Negative Breast Carcinoma.

Numerous theories have been put forward to define the role of vimentin in the pathogenesis of breast carcinoma. Vimentin expression is shown to be elevated in several aggressive breast cancer cell lines. Identifying vimentin positive cells may have a positive impact in prolonging the life of patients with breast cancer.

### Vimentin Immune Expression Score (VIES) And TNBC

In present study, the VIES score for expression of VIMENTIN for Triple Negative Breast carcinoma cases was found to be very low (<30) which is not significant in 2/21 cases (10%), while 3/21 cases (14%) had score between 31- 50. Moderate VIES score was found in 8 (38%) cases and 8 (38%) cases had high VIES score, which is significant for prognosis of breast carcinoma. Present study findings suggest that in majority of triple negative breast carcinoma cases vimentin immune expression score is high which is an indicator of high tumor grade and poor prognosis.

### Significance Of Age In TNBC And Its Correlation With Vimentin Expression

The incidence of TNBC in the present study was observed to be 21/60 (35%) of the total female breast carcinoma. Previous studies showed TNBC incidence of 10-30% of invasive breast cancers. In our study highest incidence of age in TNBC cases was observed to be within age group of 45- 65 years with mean age of 52.6years. Other studies reported that women with triple-negative breast cancers were under age of 40 years. Suresh et.al. reported median age for highest TNBC incidence in central India to be 49 years. Present study findings are in agreement with that of Suresh et.al.[7] Among vimentin positive tumors, age of younger patient was 27 years and that of older was 80 years with mean age of 52.6 years which is slightly more than WHO statistics who have described peak age of 45-50 years in Indian population.

### Vimentin Expression And Its Correlation With Tumor Size

**Table 6: Comparison Of Relationship Between Tumor Size And Vimentin Expression In TNBC Cases With Previous Studies:**

| Sr. No. | Study | No. of cases | Vimentin expression | Tumor size | |
|---|---|---|---|---|---|
| | | | | <2cm | >2cm |
| 1 | Heatley et al (1993) [8] | 67 | Vi-positive | 41.47% | 58.53% |
| | | | Vi-negative | 53.84% | 46.15% |
| 2 | Hemlatha et al (2014) [6] | 50 | Vi-positive | 12.5% | 19.04% |
| | | | Vi-negative | 87.5% | 80.96% |
| 3 | Current study | 21 | Vi-positive | - | 90.5% |
| | | | Vi-negative | - | 9.5% |

In the present study, in triple negative breast carcinoma cases the size of tumor varied between 2.5cm to 12cm with mean size of 5.5cm. Out of 21 cases, 14 cases (66.7%) varying in size between 2cm to 5cm. 12 out of these 14 cases (85.7%) showed good vimentin positivity and 2 cases (14.3%) did not show vimentin expression. Out of 21 cases, in 7 cases (33.3%) the size of tumor was >5cm and those 7/7 cases (100%) showed good vimentin expression. All the 21 TNBC cases had tumor size size >2cm. Out of these, 19 cases (90.5%) showed good vimentin expression and 2 cases (9.5%) did not show vimentin expression. Significant correlation was seen between vimentin expression and tumor size in the present study i.e., as the tumor size increases vimentin expression also increases. This positive correlation had been also described by Heatley et al[8].

In present study no TNBC cases had tumor size <2cm.

### Expression Of Vimentin And Its Correlation With Tumor Grade

**Table 7: Comparison Of Relationship Between Tumor Grade And Vimentin Expression In TNBC Cases With Previous Studies**

| Sr. No. | Study | Vimentin Expression in | | |
|---|---|---|---|---|
| | | Grade I | Grade II | Grade III |
| 1 | Domagala et al. (5) | 0/4 (0) | 0/34 (0) | 15/28(53.5%) |
| 2 | Sheshadri et al. (9) | 4/38(10.5%) | 10/105 (9.5%) | 26/86(30.2%) |
| 3 | Korsching et al.(10) | - | - | 19/21(90.5%) |
| 4 | Hemlatha et al. (6) | 0/22 (0) | 2/20 (10%) | 7/8 (87.5%) |
| 5 | Current study | 1/2 (50%) | 9/9 (100%) | 9/10 (90%) |

Cancer is evaluated according to the morphology and behaviour of the cells, which reflects how aggressive it is. In the present study, it was shown that Grade-2 and Grade-3 tumors predominated. Out of 21 cases there were 10 cases (47.6%) of grade-3, 9 cases (42.9%) of grade 2 and 2 cases (9.5%) of grade 1 TNBC.

Out of 10 cases of grade-3 TNBC, 9 cases (90%) showed vimentin positivity which was in concordance with study conducted by Hemalath et al[6] and Korsching et al[10]. Out of 9 cases of grade-2 TNBC all 9 cases (100%) showed vimentin expression and out of 2 cases of grade-1 TNBC, 1 case (50%) showed vimentin expression and 1 case did not show vimentin expression.

With increase in grade the cells undergo transition to mesenchymal cells with loss of adhesiveness, producing higher amount of vimentin and further leads to poorer prognosis in TNBC cases.

### Nodal Metastasis In TNBC And Its Correlation With Vimentin Expression

In the present study lymph node metastasis was found to be positive in 16/21 (76.2%) cases. Out of these 16 cases which showed nodal metastasis, 14 cases (87.5%) had shown positive vimentin expression (VIES >30 which is significant) and 2 cases (12.5%) did not show vimentin expression (VIES <30 which is not significant). This finding

is consistent with the findings reported by Heatley M. et al.[8] and not consistent with the findings reported be Domagla et al.[5] and Hemlatha et al.[6]

**Table 8: Comparison Of Relationship Between Lymph Node Metastasis And Vimentin Expression In TNBC Cases With Previous Studies**

| Sr. No. | Study | Vimentin Expression in Metastatic Lymph Node | |
|---|---|---|---|
| | | Present | Absent |
| 1. | Domagla et al.(5) (N=90) | 21.5% | 78.5% |
| 2. | Heatley M. et al.(8) (N=51) | 59.67% | 40.33% |
| 3. | Hemlatha et al.(6) (N=50) | 16.0% | 84.0% |
| 4. | Current study (N=16) | 87.5% | 12.5% |

## CONCLUSION

The age of the patient, tumor size, lymph node involvement, Scarf-Bloom Richardson grade and expression of vimentin are the important predictors for breast carcinoma.

Vimentin expression seems to be strongly associated with poor prognostic indicators in breast carcinoma. Strong significant correlation found between vimentin and young age, large tumors, high SBR grade with nodal metastasis.

In India, at the time of diagnosis, majority of new cases are in advanced stage. Delayed diagnosis with advanced carcinoma may be due to socio-economic factors, lack of awareness, and paucity of health and medical facilities.

For women at extremely high risk of getting breast cancer, such as those with a proven BRCA mutation, those who have already had breast cancer, and those with a significant family history of breast and ovarian cancer earlier, more frequent screening is advised.

Vimentin expression in breast carcinoma is linked with poor prognostic indicators and it also reflects the aggressive behaviour of breast carcinoma. For future significance of vimentin as biomarker for malignancy with clinical relevance, more research will be necessary to assess the major functions of vimentin in tumorigenesis. In view of available data, vimentin expression in cancer is likely to become an attractive and promising therapeutic target and has great potential for providing novel clinical prognostic and diagnostic tools. Further, the use of vimentin specific chemical inhibitors as well as novel therapeutic agents directed against vimentin in combination with other anticancer agents, must be encouraged.

## REFERENCES


1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012: Globocan 2012. International Journal of Cancer. 2015 Mar 1;136(5):E359–86.
2. Chopra R. The Indian Scene. J ClinOncol. 2001 Sep 15;19(suppl_1):106s–111.
3. Charles B, Dana KA, Timthy RB et al. Schwartz principle of surgery, 9th edition. United states: The McGraw-Hill companies, Inc.2010.
4. National Comprehensive Cancer Network (NCCN). Practise guidelines in oncology: Breast Cancer. Version 2.2017.
5. W Domagala J Losata J Bartkowiak K Weber M Osborn Vimentin is preferentially expressed in human breast carcinomas with low estrogen receptor and high ki67 growth fractionAm J Pathol199013621927.
6. Hemlatha A, Kumar MLH. Expression of vimentin in breast carcinoma, its correlation with Ki67 and other histopathological parameters. Indian Journal of Cancer.2013;50:1891-94.
7. Suresh P, UllasBatra, Doval DC. Epidemiological and clinical profile of triple negative breast cancer at a cancer hospital in North India, Indian J Med PaediatrOncol.2013; 34: 89-95.
8. Heatley M, Whiteside C, Maxwell P, Toner P. Vimentin expression in benign and malignant breast epithelium. J Clin Pathol 1993; 46: 441-445.
9. Sheshadri R, Raymond Wa, Leong AS, Horsfall DJ, Mccaul K. Vimentin expression is not associated with poor prognosis in breast cancer. Int J cancer 1996; 67: 353-356.
10. Korsching J, Packeisen C, Liedtke D, Hungermann P, Wülfing P, J Diest. The origin of vimentin expression in invasive breast cancer: epithelial–mesenchymal transition, myoepithelial histogenesis or histogenesis from progenitor cells with bilinear differentiation potential? J.Pathol20052064451710.1002/path.1797.