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VIMENTIN EXPRESSION IN CIRCULATING TUMOR CELLS AS A PROGNOSTIC BIOMARKER FOR BREAST CANCER PROGRESSION

Oncology

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ABSTRACT

Circulating tumor cells (CTCs) play a critical role in cancer metastasis. Vimentin, an intermediate filament protein and a hallmark of epithelial-to-mesenchymal transition (EMT), is implicated in cancer progression and dissemination. This study aimed to evaluate vimentin gene expression in CTCs as a potential prognostic biomarker for breast cancer. Peripheral blood samples were collected from 30 patients with histologically confirmed breast cancer and 20 age-matched healthy controls. CTCs were isolated using Ficoll-Paque density gradient centrifugation. Following initial separate on, a negative selection approach was employed using CD45-conjugated magnetic beads to deplete leukocytes, thereby enriching the CTC population. Vimentin mRNA expression was quantified by reverse transcription quantitative PCR (RT-qPCR). Vimentin expression was significantly upregulated in CTCs from breast cancer patients compared to healthy controls ($p < 0.01$). Elevated expression levels were positively associated with advanced tumor stage (stage III/IV) and adverse clinical outcomes. Vimentin gene expression in CTCs is a potential non-invasive biomarker for predicting breast cancer progression. Its prognostic value may assist in tailoring personalized treatment strategies. Future studies should validate these findings in larger cohorts and explore the mechanistic role of vimentin in breast cancer metastasis

KEYWORDS

Circulating Tumor Cells, Vimentin, Breast Cancer, Biomarker

INTRODUCTION

Breast cancer is the most commonly diagnosed cancer and the leading cause of cancer-related deaths among women globally. In India, breast cancer accounted for 28.2% of all female cancers in 2022 (Sathishkumar et al., 2024). Despite advances in detection and treatment, metastatic breast cancer (MBC) remains a significant challenge, responsible for approximately 90% of breast cancer-related deaths(J. Li et al., 2023).

Metastasis involves cancer cells spreading from the primary tumor to distant organs, a process facilitated by epithelial-to-mesenchymal transition (EMT), which enables cancer cells to acquire migratory and invasive properties(Noubissi Nzeteu et al., 2022). Circulating tumor cells (CTCs) are key biomarkers in this process and offer insights into tumor progression and metastatic potential(Noubissi Nzeteu et al., 2022). Vimentin, an intermediate filament protein upregulated during EMT, plays a critical role in tumor invasiveness and metastasis.

Recent studies suggest that vimentin expression in CTCs can predict metastatic spread and poor prognosis in breast cancer(Xie et al., 2021). Liquid biopsy analysis of CTCs provides a minimally invasive alternative to traditional tissue biopsies, offering real-time insights into tumor dynamics. This study aims to assess the correlation between vimentin expression in CTCs and clinical outcomes in breast cancer patients.

MATERIALS AND METHODS

Study Design And Participant Enrolment

This prospective cohort study included 30 breast cancer patients and 20 healthy controls. Patients were classified into early-stage (I/II) and advanced-stage (III/IV) groups based on the AJCC staging system, with the latter including cases with distant metastasis or locally advanced disease. Healthy controls had no history of cancer. Informed consent was obtained from all participants under SCMH-Institutional Ethics Committee approval. Clinical data, demographics, and treatment details were collected from medical records.

Isolation Of Circulating Tumor Cell From Blood Sample

Peripheral blood samples (10 mL) were collected in heparinized tubes at diagnosis. CTCs were isolated using density gradient centrifugation by layering blood over 10 mL Ficoll-Paque and centrifuging at 1200 rpm for 30 minutes at 4°C. The buffy coat containing PBMCs(Ho et al., 2024) and CTCs was collected. To enrich CTCs, leukocytes were depleted using CD45-conjugated magnetic beads. After incubation with 20 µL beads per 1×10^7 cells at 4°C for 15 minutes, the sample was passed through a magnetic column(Hu et al., 2021). CD45-positive cells were retained, and the CD45-negative CTC-enriched fraction was collected, washed with PBS, and resuspended in 500 µL TRIzol™ for RNA extraction and further analysis.

RNA Isolation And cDNA Synthesis From Circulating Tumor Cells (CTCs)

Total RNA was isolated from CTCs using TRIzol™ reagent (Thermo Fisher Scientific) (Zhao et al., 2019). Cells were lysed, followed by chloroform-induced phase separation and centrifugation. RNA was precipitated with isopropanol, washed with 75% ethanol, and resuspended in DEPC-treated water. RNA concentration and purity were assessed using a Nanodrop spectrophotometer, and samples were stored at -80°C. For cDNA synthesis, 2 µg of total RNA was reverse transcribed using the Verso cDNA Synthesis Kit with oligo(dT) primers. The reaction was incubated at 42°C for 30 minutes and inactivated at 95°C for 2 minutes(Kaller, Forné, Imhof, & Hermeking, 2024). The synthesized cDNA was diluted and stored at -20°C for subsequent qPCR analysis.

Quantitative Real-Time PCR (qPCR) Analysis

Vimentin gene expression was analyzed using PowerUp™ SYBR™ Green Master Mix (Thermo Fisher Scientific) in a 20 µL reaction containing 10 µL master mix, 1 µL each of forward and reverse primers, 2 µL cDNA, and 6 µL nuclease-free water(Gupta, Sen, Priyadarshi, & Ta, 2023). GAPDH was used as the internal control. The qPCR conditions included an initial denaturation at 95°C for 5 minutes, followed by 40 cycles of 95°C for 45 seconds, 57°C for 1 minute, and 72°C for 45 seconds, with a final extension at 72°C for 8 minutes. Melting curve analysis confirmed product specificity. Relative gene expression was calculated using the ΔΔCt method. Amplified products were verified by 1.5% agarose gel electrophoresis and visualized using the Bio-Rad ChemiDoc MP Imaging System.

Statistical Analysis

Statistical analysis of gene expression data was performed using SPSS (Version 26.0). Vimentin expression was normalized to GAPDH using the ΔΔCt method. Differences in expression between conditions were assessed using t-tests for continuous variables and chi-square tests for

categorical variables. Associations between vimentin expression and clinical variables (e.g., tumor stage, lymph node involvement) were analyzed with Spearman's rank correlation. A p-value <0.05 was considered statistically significant.

RESULTS

Patient Characteristics

The study included 30 breast cancer patients (median age 58, range 34–76) and 20 healthy controls. Patients were grouped into early-stage (n=16) and advanced-stage (n=14) based on AJCC criteria. Table 1 summarizes baseline characteristics.

Hormone receptor-positive tumors were more common in early-stage patients (81%) than in advanced-stage (64%), while HER2-positive (57%) and triple-negative tumors (29%) were more frequent in advanced stages. Invasive ductal carcinoma (IDC) was the predominant histological type across both groups. Early-stage tumors were more often low-grade and smaller in size (<2 cm in 44% vs. 14% in advanced-stage).

Lymph node involvement (79%) and high Ki-67 proliferation index were more prevalent in advanced-stage patients, indicating more aggressive disease.

Table 1: Baseline Characteristics Of Breast Cancer Patients

Characteristic	Early-Stage (Stage I/II) (n=16)	Advanced-Stage (Stage III/IV) (n=14)	Total (n=30)
Tumor Subtype			
Hormone Receptor Positive (%)	13 (81%)	9 (64%)	22 (73%)
HER2 Positive (%)	5 (31%)	8 (57%)	13 (43%)
Triple Negative (%)	2 (13%)	4 (29%)	6 (20%)
Stage at Diagnosis			
• Stage I (%)	6 (38%)		6 (20%)
• Stage II (%)	10 (63%)		10 (33%)
• Stage III (%)		6 (43%)	6 (20%)
• Stage IV (%)		8 (57%)	8 (27%)
Histological Type			
• Invasive Ductal Carcinoma (%)	12 (75%)	11 (79%)	23 (77%)
• Invasive Lobular Carcinoma (%)	3 (19%)	2 (14%)	5 (17%)
• Mixed Carcinoma (%)	1 (6%)	1 (7%)	2 (7%)
• Other (%)			
Tumor Grade			
• Grade 1 (Low) (%)	4 (25%)	2 (14%)	6 (20%)
• Grade 2 (Moderate) (%)	8 (50%)	6 (43%)	14 (47%)
• Grade 3 (High) (%)	4 (25%)	6 (43%)	10 (33%)
Tumor Size			
• <2 cm (%)	7 (44%)	2 (14%)	9 (30%)
• 2-5 cm (%)	8 (50%)	9 (64%)	17 (57%)
• >5 cm (%)	1 (6%)	3 (21%)	4 (13%)
Lymph Node Involvement			
• Negative (%)	10 (63%)	3 (21%)	13 (43%)
• Positive (%)	6 (38%)	11 (79%)	17 (57%)
Ki-67 Proliferation Index			
• Low Ki-67 (<15%) (%)	10 (63%)	4 (29%)	14 (47%)
• Moderate Ki-67 (15%-30%) (%)	4 (25%)	7 (50%)	11 (37%)
• High Ki-67 (>30%) (%)	2 (13%)	3 (21%)	5 (17%)

Vimentin Expression In CTCs

Vimentin expression in CTCs was assessed using RT-PCR, with GAPDH as the reference gene. Both early- and advanced-stage breast cancer patients showed significantly higher vimentin expression compared to controls.

Early-stage patients showed a 5.34–12.02-fold increase (mean = 9.3), while advanced-stage patients exhibited a 45.25–60.63-fold increase (mean = 50.4), with an average 5.42-fold difference between the two groups ($p < 0.001$), indicating a strong correlation with disease progression (Figure 1).

Agarose gel electrophoresis confirmed the specificity of vimentin amplification, with no non-specific bands observed (Figure 2).

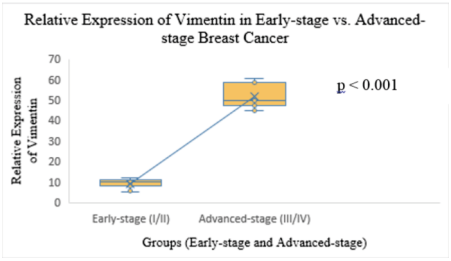

Figure 1: Vimentin expression in early-stage (I/II) vs. advanced-stage (III/IV) tumors. The box plot shows a significant increase in Vimentin expression in advanced-stage tumors compared to early-stage tumors, suggesting its potential role in tumor progression and metastasis.

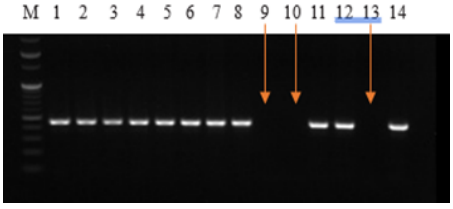

Figure 2: Agarose Gel Electrophoresis image showing vimentin mRNA expression in circulating tumor cells (CTCs) isolated from breast cancer patients. Lane M represents the DNA marker (100 bp ladder). Lanes 1–8 show amplified vimentin mRNA products from different patient-derived CTC samples, demonstrating varying expression levels. Lanes 9, 10, and 13 show no detectable amplification, indicating absence or very low expression of vimentin. In contrast, Lane 14 exhibits strong amplification, suggesting higher expression of the mesenchymal marker vimentin in that particular sample. These findings highlight the heterogeneous expression of vimentin among CTCs from different breast cancer patients.

Association Between Vimentin Expression And Clinical Parameters

Elevated vimentin expression was observed in advanced-stage (III/IV) patients, with a higher fold change in advanced stages compared to early-stage (I/II) patients, indicating a correlation with tumor progression and metastatic potential (Figure 3). Higher vimentin expression was associated with higher tumor grades, particularly Grade III tumors. Grade III tumors, which are more poorly differentiated, showed a greater degree of vimentin upregulation compared to Grade I and Grade II tumors ($r = 0.72$, $p < 0.05$) (Figure 4). A strong positive correlation was found between vimentin expression and the Ki-67 proliferation index ($r = 0.65$, $p < 0.05$) (Figure 5). These findings suggest that tumors with higher Ki-67 expression exhibit enhanced EMT features, further establishing Vimentin as a potential prognostic biomarker for breast cancer progression and metastasis. Although no significant differences were found between tumor subtypes, these findings highlight vimentin as a potential prognostic biomarker for monitoring breast cancer progression and metastasis, especially in advanced-stage disease.

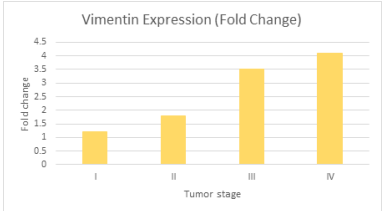

Figure 3: Vimentin expression across tumor stages. Vimentin expression increases progressively from early-stage (I/II) to advanced-stage (III/IV) tumors, indicating its association with tumor progression and metastatic potential.

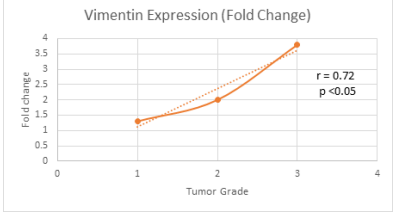

Figure 4: Correlation between Vimentin expression and tumor grade. The graph shows a positive correlation between Vimentin expression (fold change) and tumor grade, with higher expression observed in poorly differentiated Grade III tumors, indicating its potential role in tumor aggressiveness.

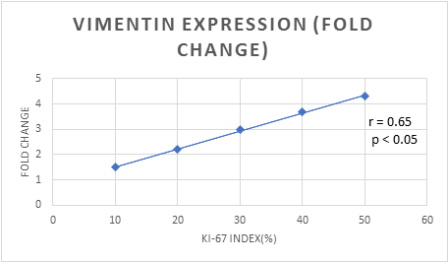

Figure 5: Correlation between Vimentin expression and Ki-67 index. Vimentin expression increases with the Ki-67 proliferation index, indicating a potential link between Vimentin upregulation and tumor proliferation.

DISCUSSION

Breast cancer is a heterogeneous disease, and identifying biomarkers to monitor its progression is vital(Sarhadi & Armengol, 2022). This study evaluated vimentin expression in CTCs across different stages of breast cancer and its association with clinical parameters. Vimentin expression was significantly elevated in both early- and advanced-stage patients compared to controls, with a mean 9.3-fold increase in early-stage and 50.4-fold in advanced-stage cases. The 5.42-fold higher expression in advanced stages supports vimentin's role in EMT, metastasis, and disease progression. These findings suggest vimentin as a potential prognostic biomarker, especially in advanced breast cancer.

Statistical analysis revealed a strong positive correlation between vimentin expression and tumor grade, with the highest expression observed in poorly differentiated Grade III tumors. This suggests vimentin may serve as a marker for tumor aggressiveness and differentiation. Its elevated expression aligns with EMT-associated features of invasive and metastatic cancers. Additionally, vimentin expression correlated positively with the Ki-67 proliferation index, indicating a link between vimentin and tumor cell proliferation. These findings reinforce vimentin's potential as a prognostic biomarker for tumor aggressiveness and metastatic potential(H. Li et al., 2024).

No significant differences in vimentin expression were found across different tumor subtypes (e.g., hormone receptor-positive, HER2-positive, triple-negative), suggesting that vimentin is more closely linked to tumor progression and aggressiveness than to molecular subtypes. Other markers like EpCAM, CK-19, and N-cadherin, which play roles in EMT and tumor invasiveness, may further aid in understanding tumor progression. This study highlights vimentin's potential as a non-invasive biomarker for monitoring disease progression and metastasis, with its elevated expression correlating with clinical parameters like tumor grade, Ki-67, and disease stage. The combination of epithelial and mesenchymal markers could improve monitoring, aid personalized treatments, and enhance patient outcomes.

CONCLUSION

This study highlights vimentin's potential as a biomarker for assessing breast cancer aggressiveness, stage, and metastatic potential, with its upregulation in advanced-stage patients linked to tumor grade, proliferation, and metastasis. However, limitations such as small sample size and lack of other markers should be addressed. Future research should validate these findings in larger cohorts, explore the molecular mechanisms of vimentin in cancer progression, and evaluate its potential as a therapeutic target. Combining vimentin with markers like EpCAM, CK-19, and N-cadherin could enhance understanding of tumor behavior and treatment response.

Conflict Of Interest

The authors declare no conflict of interest.

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