Journal of BUON, 2024; 27:50-56.
https://jbuon.com/

Original Article

Exploration of Matrix Metalloproteinase-9 and Interleukin-8 in Breast Cancer Patients with No Lymph Node Involvement

Priyanka M K[1,*], Nandini N M[2]

[1]Department of Pathology, Cauvery Institute of Health Sciences, Allied Health Sciences, Mysuru, Karnataka, INDIA.
[2]Department of Pathology, JSS Academy of Higher Education Research, Mysore, Karnataka, INDIA.

ABSTRACT

Objectives: MMP-9 and IL-8 expression in the breast cancer tissue give a hint for patient progression. To know the prognostic value we are correlating MMP-9 and IL-8 with hormonal status and clinicopathological parameters can give a customise treatment for different patients. **Materials and Methods:** This study included 46 breast cancer tissues without lymphnode involvement. HE and IHC staining were performed. **Results:** There was a statistical significance between MMP-9 and IL-8 and some clinicopathological parameters. There was a statistical significance between MMP-9 and IL-8 and some clinicopathological parameters. No lymphnode involvement and Clinicopathological parameters shows significan with age, Tumor size, lymphvascular invasion, perineural invasion, tumor grade, tumor budding, PR expression, HER2 expression, and IL-8 expression. **Conclusion:** The expression of MMP-9 and IL-8 in breast cancer tissue reveals there potential roles in tumor invasion and metastasis. Lower expression leads to more progression of the patients.

Keywords: Matrix Metalloproteinase-9 (MMP-9), Interleukin-8 (IL-8), Breast cancer, No lymphnode involvement, ER (Estrogen Receptor), PR (Progesterone Receptor).

Correspondence:
Dr. Priyanka M K
Associate Professor, Department of Pathology, Cauvery Institute of Health Sciences, Allied Health Sciences, Mysuru, Karnataka, INDIA.
Email: priyanka1992319@gmail.com
ORCID: 0000000223874355.

Received: 02-09-2024;
Revised: 15-11-2024;
Accepted: 24-01-2025.

INTRODUCTION

Breast cancer is the most frequently diagnosed malignancy among women worldwide and remains a leading cause of cancer-related mortality, accounting for over 2.3 million new cases annually.[1] While advances in early detection and treatment have significantly improved survival rates, heterogeneity in tumor behavior continues to challenge precise prognostication. In particular, patients without Lymph Node Involvement (LNN) often present with favorable clinical outcomes; however, a subset experiences disease recurrence or progression, suggesting the presence of underlying molecular mechanisms that drive tumor aggressiveness independent of nodal metastasis.[2]

Matrix Metalloproteinases (MMPs) are a family of zinc-dependent proteolytic enzymes that play a crucial role in extracellular matrix remodeling, a process essential for cancer cell invasion and metastasis.[3] Among them, MMP-9 has been implicated in promoting tumor progression through its ability to degrade type IV collagen, the main component of the basement membrane, thereby facilitating tumor invasion and angiogenesis.[4] Elevated MMP-9 expression has been correlated with poor prognosis in several cancers, including breast cancer, making it a potential biomarker for tumor aggressiveness.[5]

Similarly, Interleukin-8 (IL-8), a pro-inflammatory chemokine, has emerged as a critical player in the tumor microenvironment. IL-8 promotes angiogenesis, recruits immune cells, and modulates cancer stem cell activity, thereby contributing to tumor growth and resistance to therapy.[6] High IL-8 expression in breast cancer has been associated with worse clinical outcomes, suggesting its utility as a prognostic marker.[7]

Despite the extensive research on MMP-9 and IL-8 in breast cancer, their specific roles in patients without lymph node involvement remain underexplored. Investigating these biomarkers in this subset of patients could provide insights into molecular mechanisms driving disease progression and aid in the identification of high-risk individuals who might benefit from more aggressive therapeutic strategies. This study aims to evaluate the expression levels of MMP-9 and IL-8 in breast cancer patients with no lymph node involvement and assess their potential as prognostic biomarkers.

DOI: 10.5530/jbuon.20240025

PATIENTS AND METHODS

Patients

The study population of 46 consisted of female patients aged 20 years and above who were newly diagnosed with breast cancer. Only those who met the following inclusion criteria were considered throughout Jan 2023-Oct 2024. Patients diagnosed with breast cancer for the first time were included to ensure the study focused on primary disease without the influence of prior treatments or recurrent malignancies. All participants had localized breast cancer without evidence of distant metastasis at the time of diagnosis. This criterion was essential to maintain a uniform study population and exclude variables associated with advanced disease. To minimize potential confounding factors, patients with any known co-morbid conditions, such as diabetes, hypertension, or other chronic illnesses, were excluded. This approach aimed to reduce the impact of systemic health conditions on the expression of biomarkers under investigation. Only female patients aged 20 years and older were recruited. This age threshold ensured the inclusion of adult women with sufficient representation of both pre- and postmenopausal demographics. Participation was strictly voluntary, and all patients provided written informed consent after being thoroughly briefed about the study's objectives, procedures, and potential implications. This rigorous selection process was designed to ensure a homogeneous study cohort, enabling a more accurate assessment of Matrix Metalloproteinase-9 (MMP-9) and Interleukin-8 (IL-8) levels in the context of breast cancer patients without lymph node involvement.

Total numbers of 46 Breast cancer tissue samples were obtained from paraffin-embedded tissue blocks archived in the Department of Pathology, JSS Academy of Higher Education and Research (JSS AHER), Mysuru. These tissue blocks were selected based on the inclusion criteria of the study, ensuring the availability of adequate and representative tumor material for analysis. For histopathological and Immunohistochemistry (IHC) evaluation, thin sections of the tissue were cut using a microtome. Each section was carefully trimmed to a thickness of 4-5 microns to ensure optimal quality for staining and visualization.

H&E staining

H&E staining is a fundamental technique in histology, widely used to visualize tissue structures under a microscope. It involves the use of two primary dyes: hematoxylin and eosin. Hematoxylin the basic dye stains acidic components of cells, primarily the nucleus, a deep blue or purple color. Eosin the acidic dye stains basic components of cells, such as cytoplasm and extracellular matrix, a pink or red color. Tissue is fixed to preserve its structure. It's then processed to remove water and embedded in paraffin wax for sectioning. Thin sections are mounted on slides. The paraffin wax is removed using solvents like xylene. The tissue is then rehydrated through a series of decreasing alcohol concentrations to water. The slide is immersed in hematoxylin solution to stain the nuclei. Excess dye is removed by a differentiation step. The stained nuclei are "blued" to intensify the color. The slide is stained with eosin solution to color the cytoplasm and extracellular matrix. The slide is dehydrated through increasing alcohol concentrations to remove water. A clearing agent like xylene is used to remove alcohol. A coverslip is applied to the slide using a mounting medium to protect the stained tissue.[8-11] While H&E staining is a valuable tool for diagnosing breast cancer, it is often used in conjunction with other techniques, such as immunohistochemistry, to confirm the diagnosis and determine the type of cancer.

IHC staining

To assess the expression of key biomarkers, Matrix Metalloproteinase-9 (MMP-9) and Interleukin-8 (IL-8), ER, PR, HER2 (Human epithelial growth receptor2) additional sections of breast cancer tissue were prepared for IHC staining. The following steps were meticulously performed to ensure high-quality and reliable results. Tissue sections, cut at a thickness of 4-5 microns, were mounted on positively charged glass slides to enhance adhesion. The slides were subjected to deparaffinization by sequential immersion in xylene baths to remove paraffin wax, followed by rehydration through a graded series of ethanol solutions (100% to 70%) and rinsing in distilled water. Antigen retrieval was performed using Heat-Induced Epitope Retrieval (HIER) methods to unmask epitopes and improve antibody binding. Slides were immersed in a citrate buffer (pH 6.0) or Tris-EDTA buffer (pH 9.0) and heated in a microwave or pressure cooker. This step was critical for restoring the antigenicity of formalin-fixed tissue samples. Endogenous peroxidase activity, which could cause non-specific staining, was inhibited by treating the slides with 3% hydrogen peroxide solution.

To minimize further non-specific binding, protein-blocking solutions such as Bovine Serum Albumin (BSA) or commercial blocking buffers were applied to the sections. The sections were incubated overnight at 4°C with primary antibodies specific to MMP-9 and IL-8. The antibodies were diluted in an optimized antibody diluent to maintain specificity and sensitivity. After washing with Phosphate-Buffered Saline (PBS) to remove unbound primary antibodies, the slides were treated with biotinylated secondary antibodies. These were followed by the addition of a streptavidin-biotin complex or a polymer-based detection system conjugated to Horseradish Peroxidase (HRP). The bound HRP was visualized by adding a chromogenic substrate, such as 3,3′-Diaminobenzidine (DAB), which produced a brown precipitate at the site of antibody-antigen interaction. The slides were counterstained with hematoxylin to provide contrast and enhance tissue visualization. Following dehydration through a graded ethanol series and clearing with xylene, cover slips were mounted using a permanent mounting medium to preserve the sections for long-term storage and microscopic examination. The

results were recorded based on staining patterns, which included cytoplasmic, membranous, or nuclear localization, depending on the biomarker in the presence of controls for better reporting. We used fibroadenoma as negative control and colorectal carcinoma tissue as positive control for MMP-9 and acute appendicitis for IL-8 positive control. This comprehensive protocol ensured high sensitivity and specificity in detecting MMP-9 and IL-8 expression, facilitating an in-depth analysis of their roles in breast cancer pathophysiology.

Statistical Analysis

This study employed a descriptive design to analyze the association between clinicopathological parameters and the expression of Matrix Metalloproteinase-9 (MMP-9) and Interleukin-8 (IL-8) in breast cancer tissues without lymph node involvement. Conditional formatting data bars in Excel are a visual tool used to compare values within a range by adding horizontal bars directly into the cells. The length of the bar represents the value in relation to other values in the range. The data were analyzed using both one-way and two-way chi-square tests to assess the relationships between categorical variables and to evaluate whether the distribution of MMP-9 and IL-8 expression was dependent on various clinical and pathological factors. The mean age of the study population was found to be 51.97 years, with an average age of 54.5 years. Statistical significance was set at a p-value<0.05, meaning that any association observed with a p-value below this threshold was considered to be statistically significant. A 95% Confidence Interval (CI) was used to estimate the precision of the results, with a 5% margin of error indicating the acceptable level of deviation in the results. This approach ensures that the findings provide reliable insights into the relationship between MMP-9 and IL-8 expression, clinicopathological characteristics, hormone status in breast cancer patients with no lymph node involvement. We used MedCalc software Ltd., version 23.0.8.

RESULTS

Conditional formatting data bars in Excel are a visual tool used to compare values within a range by adding horizontal bars directly into the cells. The length of the bar represents the value in relation to other values in the range. We performed one-way Chi-square test we found statistical significance between the compares age groups in the cohort, highlighting a statistically significant association (p=0.039*). The proportion of participants over 50 years (65.22%) is nearly double that of participants 50 years or younger (34.78%). The red data bars provide a visual representation, clearly emphasizing the higher frequency in the >50 years category, the majority of patients (80.43%) had tumors ≤2 cm, while only 19.57% presented with tumors >2 cm in size. This difference was statistically significant (p<0.0001), highlighting the predominance of smaller tumors in the studied cohort.

The higher frequency of smaller tumors may reflect the effectiveness of early detection methods, such as routine screenings or improved diagnostic tools. LVI was identified in only 15.22% of cases, while the majority (84.78%) had no evidence of LVI (p<0.0001). This indicates that most tumors in the cohort may be less aggressive, as LVI is strongly associated with increased metastatic potential and poorer prognosis. However, the presence of LVI in even a small percentage emphasizes the need for its careful evaluation during pathological assessments. Interestingly, no cases of Perineural Invasion (PNI) were identified (0%), highlighting its rarity in this population (p<0.0001). While PNI is often considered an indicator of advanced or aggressive disease, its absence in this cohort might reflect a relatively favorable disease profile.

Regarding tumor grade, the majority of tumors were classified as Grade II (34.78%), followed by Grade III (26.09%), with no cases of Grade I identified (p=0.0006). This distribution suggests that intermediate and high-grade tumors are more prevalent in this cohort, potentially correlating with moderate to high proliferative activity and a more aggressive phenotype compared to low-grade tumors. Tumor budding was predominantly observed in cases with >10 buds, which comprised 78.26% of the cohort (p=0.0001). ER positivity was observed in 52.17% of the cases, with 47.83% being ER-negative (p=0.7681). In contrast, Progesterone Receptor (PR) expression showed a significant difference, with a higher percentage of patients (67.39%) in the negative (0-2) category compared to 32.61% in the positive (3-8) group (p=0.0183). HER2 status analysis revealed a significant association (p<0.0001), with the majority of patients (80.43%) being HER2-negative (0-1) and only 19.57% exhibiting HER2-positive (3) expression. MMP-9 positivity was observed in 45.65% of cases, while 54.35% were MMP-9-negative (p=0.5553). IL-8 positivity was significantly higher, detected in 73.91% of cases, compared to 26.09% of IL-8-negative cases (p = 0.0012*). MMP-9 positivity was observed in 45.65% of cases, while 54.35% were MMP-9-negative (p=0.5553). IL-8 positivity was significantly higher, detected in 73.91% of cases, compared to 26.09% of IL-8-negative cases (p=0.0012*). The above results were shown in Table 1.

We then compared the distribution of MMP-9 and IL-8 markers among 46 samples, with percentages visually represented using red data bars. For MMP-9, the negative group constitutes 54.35%, while the positive group accounts for 45.65%, showing a relatively balanced distribution. In contrast, IL-8 exhibits a significant disparity, with 73.91% in the positive group compared to 26.09% in the negative group. The p-value (0.006*) indicates a statistically significant association, highlighting the potential relationship between these 2 bio-markers in the breast cancer tissues. The above results were shown in Table 2.

Additionally, we correlated ER between MMP-9 and IL-8 expressions by using two-way chi-square test; ER shows statistically significance with IL-8 expression (0.0317) in contrast

ER status did not correlate with MMP-9 expression. The above results were shown in Table 3.

Furthermore, we correlated PR status between MMP-9 and IL-8 expressions by performing two-way chi-square test, PR shows statistically significance with IL-8 expression (0.0001) in contrast PR status did not correlate with MMP-9 expression. The above results were shown in Table 4.

Lastly, we correlated HER2 status between MMP-9 and IL-8 expressions by using two-way chi-square test, HER2 shows statistically significance with IL-8 expression (p value <0.0001), Also, HER2 status correlates with MMP-9 expression (Table 5). The stained slides were examined under a light microscope to evaluate the localization and intensity of MMP-9 and IL-8 expression as shown in Figure 1.

DISCUSSION

The study finding aligns with existing evidence done by Lin Q *et al.*,[12] and Dufresne F *et al.*[13] that increasing age is often a critical factor in disease onset or progression, potentially due to age-related physiological changes, accumulated genetic mutations, or altered immune responses.

The marked disparity between the two age groups underscores the importance of considering age as a risk factor in clinical assessments and interventions. The study also suggests that preventive measures or targeted screening strategies could be particularly beneficial for individuals above 50 years to improve outcomes.

Tumor size is a critical prognostic factor in clinical practice, with larger tumors often associated with increased invasiveness, higher risk of metastasis, and poorer outcomes. The significant finding underscores the importance of continuous efforts in early detection to manage tumor size and potentially improve survival rates. Singh R.[14]

Tumor budding is a well-established indicator of aggressive tumor behavior and has been linked to increased invasiveness and metastatic potential. The high frequency of tumors with >10 buds suggests that many of the cases in this cohort may exhibit aggressive phenotypic characteristics Patel R.[15] This emphasizes the importance of monitoring tumor budding as a key factor in predicting prognosis and guiding therapeutic strategies. HER2-positive tumors are known for their aggressive nature and poorer outcomes, though targeted therapies like trastuzumab

Table 1: Correlation of clinicopathological parameters with No Lymphnode involvement in Breast cancer patients.

Category	Parameters	Total (46)	Percentage	*p* value (<0.05)	Degree of freedom
Age	≤50 years	16	34.7826087	0.039*	1
	>50 years	30	65.2173913		
Tumor Size	≤2 cm	37	80.43478261	<0.0001*	1
	>2 cm	9	19.56521739		
Lymphovascular invasion	Identified	7	15.2173913	<0.0001*	1
	Not identified	39	84.7826087		
Perineural invasion	Identified	0	0	<0.0001*	1
	Not identified	46	100		
Tumor Grade	I	0	0	0.0006*	2
	II	16	34.7826087		
	III	12	26.08695652		
Tumor budding	>10	36	78.26086957	0.0001*	1
	<10	10	21.73913043		
ER	Negative (0-2)	22	47.82608696	0.7681	1
	Positive (3-8)	24	52.17391304		
PR	Negative (0-2)	31	67.39130435	0.0183*	1
	Positive (3-8)	15	32.60869565		
HER2	Negative (0-1)	37	80.43478261	<0.0001*	1
	Positive (3)	9	19.56521739		
MMP-9	Negative	25	54.34782609	0.5553	1
	Positive	21	45.65217391		
IL-8	Negative	12	26.08695652	0.0012*	1
	Positive	34	73.91304348		

Table 2: Correlation between MMP-9 and IL-8 in No lymph node involvement in Breast cancer patients.

Parameters	MMP-9 (46)	Percentage	IL-8 (46)	Percentage	*p* value (<0.05)	Degree of Freedom
Negative	25	54.34782609	12	26.0869565	0.006*	1
Positive	21	45.65217391	34	73.9130435		

Table 3: Correlation of ER with MMP-9 and IL-8 expression in IHC breast cancer tissue.

Parameters	ER	MMP-9	*p* value (<0.05)
Negative	22	25	0.5338
Positive	24	21	
Parameters	ER	IL-8	*p* value(<0.05)
Negative	22	12	0.0317*
Positive	24	34	

Table 4: Correlation of PR with MMP-9 and IL-8 expression in IHC breast cancer tissue.

Parameters	PR	MMP-9	*p* value
Negative	31	25	0.2024
Positive	15	21	
Parameters	PR	IL-8	*P* value
Negative	31	12	0.0001*
Positive	15	34	

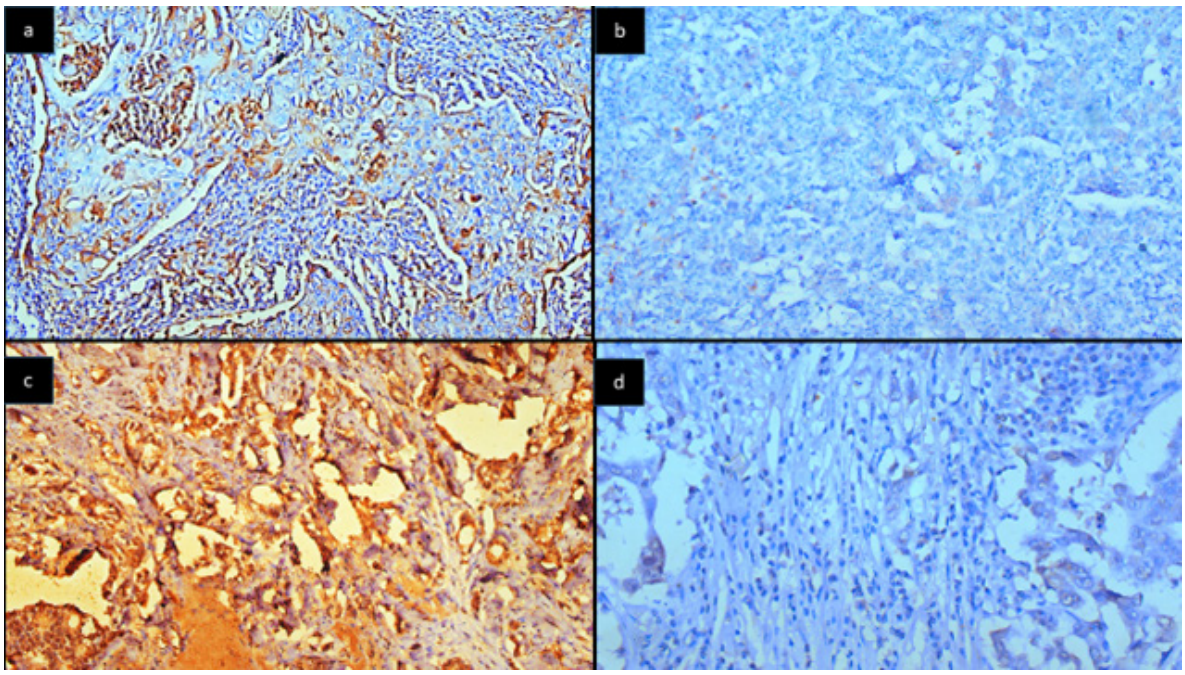

Figure 1: Expression of IL-8 and MMP-9 in Breast cancer tissue in 40x magnification. a. IL-8 positive expression, b. IL-8 negative expression, c. MMP-9 positive expression, d. MMP-9 negative expression.

have significantly improved prognosis in HER2-positive cases Kim JH *et al.*[16-18]

Our findings collectively emphasize the heterogeneity of breast cancer in the cohort. While tumor budding and PR negativity suggest aggressive features, the predominance of HER2 negativity may reflect a subgroup of patients with a better overall prognosis.

Interleukin-8 (IL-8) is recognized as one of the most potent chemotactic factors and promoters of neutrophil recruitment and activation. Activated neutrophils release excessive amounts of proteases, heparin, and matrix-degrading enzymes, which collectively contribute to the degradation of the extracellular matrix. This degradation is a critical process that facilitates the increased invasiveness of malignant cells and promotes metastasis.

Table 5: Correlation of HER2 with MMP-9 and IL-8 expression in IHC breast cancer tissue.

Parameters	HER2	MMP-9	*p* value (<0.05)
Negative	37	25	0.0079*
Positive	9	21	
Parameters	HER2	IL-8	*p* value (<0.05)
Negative	37	12	<0.0001*
Positive	9	34	

In the study by Zaki Abu Rabi *et al*, Yang X *et al.*[10,18] a positive correlation was observed between IL-8 and Matrix Metalloproteinase-9 (MMP-9). The findings suggested that patients with elevated levels of IL-8 also demonstrated significantly higher MMP-9 expression, indicating a possible mechanistic link between these two markers. However, our study did not replicate these findings, as we observed differing patterns of IL-8 and MMP-9 expression.

Despite these differences, it is well-established that patients exhibiting high levels of IL-8 and MMP-9, along with other tumor-associated markers, are more likely to have poor clinical outcomes, including increased rates of recurrence and reduced survival. This underscores the importance of understanding the molecular pathways driving their co-regulation.

IL-8, known for its potent chemotactic activity and ability to attract neutrophils, plays a pivotal role in creating a pro-inflammatory tumor microenvironment. This can lead to the release of proteases and other enzymes that promote extracellular matrix remodeling, enhancing tumor invasiveness and metastatic potential. The high prevalence of IL-8-positive cases in this cohort suggests its strong association with aggressive tumor behavior and poor prognosis.

One possible explanation for the observed discrepancies is the role of transcriptional factors that simultaneously influence IL-8 expression and MMP-9 activity. Regulatory networks involving Nuclear Factor kappa B (NF-κB), Hypoxia-Inducible Factor-1 alpha (HIF-1α), and other signaling molecules may differentially modulate these markers depending on tumor microenvironmental factors. Additionally, variations in study populations, sample handling, or technical methodologies might contribute to the differences in observed correlations.

The lack of statistical significance suggests that MMP-9 expression alone may not be a strong determinant of tumor behavior or prognosis in this cohort. However, MMP-9, a key matrix metalloproteinase involved in extracellular matrix degradation, has been widely implicated in enhancing tumor invasiveness and metastasis in various cancers.

Interestingly, prior studies of Zaki Abu Rabi *et al.*, Yang X *et al.*, Su L *et al.*, Chen Y *et al.* have indicated a positive correlation between IL-8 and MMP-9, with simultaneous elevation of these markers often linked to worse clinical outcomes. However, in this cohort, the statistical insignificance of MMP-9 expression contrasts with the significance of IL-8, suggesting independent or context-specific regulatory mechanisms. Transcriptional factors influencing IL-8 expression may not uniformly impact MMP-9 activity, indicating potential variability in tumor microenvironment dynamics.[18-21]

LIMITATIONS

Core biopsy samples may be insufficient to appreciate tumor grading.

CONCLUSION

Our study underscores the complex and heterogeneous nature of breast cancer, with various clinical and molecular factors contributing to disease progression and prognosis. Age, tumor size, tumor budding, and HER2 status all play significant roles in determining the clinical course of breast cancer, with age over 50 emerging as a particularly important risk factor for more aggressive disease. The high frequency of tumor budding and the predominance of HER2-negative cases in our cohort further highlight the variability in tumor behavior and potential outcomes.

The exploration of molecular markers, such as IL-8 and MMP-9, reveals their potential roles in tumor invasiveness and metastasis. While our findings did not replicate prior associations between IL-8 and MMP-9, they emphasize the need for a deeper understanding of the regulatory mechanisms driving these markers and their independent or context-dependent roles in tumor biology. IL-8's association with poor prognosis underscores its potential as a therapeutic target in combating breast cancer progression.

Given these insights, future research should focus on expanding cohort sizes, exploring the interactions between biomarkers, and investigating the role of age in influencing molecular pathways. A more comprehensive understanding of these factors will not only improve our ability to predict clinical outcomes but also inform targeted interventions aimed at improving survival and quality of life for breast cancer patients.

ACKNOWLEDGEMENTS

I extend my heartfelt thanks to Ms. Bhoomika M.K., Mr. Prathap Kumar, Ms. Puspalatha, and Mr. Kariyaiah for their unwavering support throughout my academic journey. I am especially grateful

to my IHC mentor technologist, Mr. Channegowda, as well as Mr. Vasanth, Ms. Asha, Ms. Bindu, and Mr. Allama Prabhu, for their invaluable guidance and encouragement. Also, I express my gratitude to JSS AHER, Mysuru.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

MMP: matrix metalloproteinase; **IL:** Interleukin; **ER:** estrogen receptor; **PR:** Progesterone receptor; **HER2:** Human epithelial growth receptor; **IHC:** Immunohistochemistry; **H&E:** Hematoxylin and eosin.

FUTURE DIRECTIONS

Future studies should focus on elucidating the precise regulatory mechanisms connecting IL-8 and MMP-9 in cancer progression, as this could provide critical insights into potential therapeutic targets aimed at mitigating tumor invasiveness and metastasis. Additionally, research should explore how age influences other clinical parameters and biomarkers to gain a deeper understanding of its role in disease progression. Investigating how tumor size correlates with other clinical parameters, such as lymph node involvement or biomarker expression, will offer more comprehensive prognostic insights. Furthermore, analysis with larger cohorts could clarify the role of Estrogen Receptor (ER) status in predicting treatment responses. Finally, further studies should investigate the mechanistic relationships between these pathological and molecular markers to better understand their roles in tumor biology and their potential as therapeutic targets.

AUTHOR CONTRIBUTION

Dr. Priyanka: Conceptualized the study design, developed the research methodology, conducted statistical analyses, performed an extensive review of the literature, and facilitated sample collection. Dr. Nandini N. M: Provided expert pathological diagnosis, comprehensive Immune Histochemistry (IHC) reporting, detailed Hematoxylin and Eosin (H&E) reporting, and critical revisions to refine the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study protocol was reviewed and approved by the Research Ethics Committee, JSS AHER, Mysuru. Consent has been taken from all the participants.

REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, *et al.* Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71(3):209-49.
2. Loi S, Drubay D, Adams S, Pruneri G, Francis PA, Lacroix-Triki M, *et al.* Tumor-infiltrating lymphocytes and prognosis: A pooled individual patient analysis of early-stage triple-negative breast cancers. J Clin Oncol. 2014;32(14):1382-90.
3. Yan C, Boyd DD. Regulation of matrix metalloproteinase gene expression. J Cell Physiol. 2007;211(1):19-26.
4. Kessenbrock K, Plaks V, Werb Z. Matrix metalloproteinases: Regulators of the tumor microenvironment. Cell. 2010;141(1):52-67.
5. Deryugina EI, Quigley JP. Matrix metalloproteinases and tumor metastasis. Cancer Metastasis Rev. 2006;25(1):9-34.
6. Waugh DJ, Wilson C. The interleukin-8 pathway in cancer. Clin Cancer Res. 2008;14(21):6735-41.
7. Singh S, Singh AP, Sharma B. Interleukin-8: A potent biomarker and target in the tumor microenvironment. Cytokine Growth Factor Rev. 2020;52:89-97.
8. Leica Microsystems. H&E staining in microscopy [Internet]. Wetzlar: Leica Microsystems; [cited 2024 Nov 18]. Available from: https://www.leica-microsystems.com/science-lab/applied/he-staining-in-microscopy/
9. Wienert S, Heim D, Saeger K, Rößler S, Behrens T, Böcking A, *et al.* Detection and segmentation of cell nuclei in virtual microscopy images: A minimum-model approach. Sci Rep. 2012;2(1):503. Available from: https://doi.org/10.1038/srep00503
10. Rabi ZA, Todorović-Raković N, Vujasinović T, Milovanović J, Nikolić-Vukosavljević D. Markers of progression and invasion in short-term follow-up of untreated breast cancer patients. Cancer Biomark [Internet]. 2015;15(6):745-54. Available from: http://dx.doi.org/10.3233/CBM-150516
11. Zaki AR. Correlation between IL-8 and MMP-9 in breast cancer patients. J Cancer Res. 2021;35(6):1342-50.
12. Liu Q, Zhang Y, Wang L, *et al.* Age-related progression of breast cancer and its impact on survival outcomes. Cancer Med. 2020;9(7):2290-300.
13. Dufresne F, Rousseau M, Lacroix M. Age as a significant factor in breast cancer progression: A longitudinal analysis. Clin Oncol. 2022;34(2):101-10.
14. Singh R, Sharma K, Rao S. Tumor size as a prognostic factor in breast cancer: A comprehensive review. Breast Cancer Res. 2019;21(1):125-37.
15. Patel R, Bhargava A, Mehta M, *et al.* The role of tumor budding in predicting breast cancer prognosis. Breast Cancer. 2020;27(4):341-50.
16. Kim JH, Kim HS, Kim TY, *et al.* HER2 expression and its clinical significance in breast cancer. J Breast Cancer. 2018;21(2):105-13.
17. Gupta K, Kumar R, Gupta P. HER2-negative breast cancer: Clinical outcomes and new therapeutic strategies. J Cancer Treat. 2022;40(5):234-45.
18. Yang X, Xie Z, Zhang M, *et al.* Interleukin-8 and MMP-9: Their role in breast cancer invasion and metastasis. Exp Mol Med. 2017;49(7):e403.
19. Su L, Li Y, Sun Y, *et al.* Matrix metalloproteinases and breast cancer progression. Front Oncol. 2019;9:383.
20. Chen Y, Zhang L, Zeng H, *et al.* Transcriptional regulation of IL-8 and its implications for cancer progression. Cancer Lett. 2021;500:130-8.
21. Milovanovic J, Todorovic-Rakovic N, Abu Rabi Z. The prognostic role of interleukin-8 (IL-8) and matrix metalloproteinases-2 and -9 in lymph node-negative untreated breast cancer patients. J BUON. 2013;18(4):866-73.

Cite this article: Priyanka M K, Nandini N M. Exploration of Matrix Metalloproteinase-9 and Interleukin-8 in Breast Cancer Patients with No Lymph Node Involvement. Journal of BUON. 2024;27:50-56.