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INTEGRATING CANCER GENETICS WITH METASTATIC BIOMARKER DYNAMICS: *MMP-2*, AND *MMP-9* IN BREAST CANCER PROGRESSION AND DISEASE MANAGEMENT

Original Research

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ABSTRACT

Background: Breast cancer is one of the leading causes of cancer-related morbidity and mortality globally, with its metastatic nature contributing significantly to poor clinical outcomes. Identifying reliable plasma biomarkers associated with angiogenesis and metastasis is crucial for improving early diagnosis, disease progression monitoring, and therapeutic interventions. Matrix metalloproteinases, particularly MMP-2 and MMP-9, play pivotal roles in tumor invasion and metastasis, making them potential candidates for biomarker development.

Objective: This study aimed to determine the plasma expression levels of MMP-2 and MMP-9 in breast cancer patients and assess their association with clinicopathological variables to evaluate their potential as biomarkers for advanced disease assessment.

Methods: A total of 116 breast cancer patients and 24 healthy controls were included in this cross-sectional study. Peripheral blood samples (5 ml) were collected in EDTA tubes, followed by RNA extraction using *TRIzol* reagent. High-quality RNA was reverse transcribed into cDNA, and gene expression levels of MMP-2 and MMP-9 were quantified using RT-qPCR. Expression levels were analyzed in relation to tumor grade, molecular subtypes, and disease stages. Statistical analyses were performed using GraphPad Prism 9, with significance set at $p < 0.05$.

Results: MMP-2 and MMP-9 levels were significantly elevated in breast cancer patients compared to healthy controls. MMP-9 expression was highest in grade 3 tumors ($p = 0.0104$) and showed an increasing trend with advanced TNM stages ($p = 0.0345$). MMP-2 expression was significantly associated with stages III and IV ($p = 0.0373$) and was elevated in aggressive subtypes, including *HER2*-enriched and triple-negative breast cancer. Observed expression levels correlated strongly with clinicopathological variables, including tumor grade and molecular subtype.

Conclusion: The increased plasma expression of MMP-2 and MMP-9 highlights their role in breast cancer metastasis and angiogenesis. These biomarkers show potential for use in disease severity assessment and therapeutic targeting, offering prospects for improving breast cancer management.

Keywords: Angiogenesis, Biomarkers, Breast Neoplasms, Gene Expression Profiling, Metastasis, Therapeutics, Tumor Microenvironment

INTRODUCTION

Breast cancer remains one of the most prevalent malignancies affecting women worldwide, with significant global morbidity and mortality. In 2020 alone, an estimated 685,000 deaths and 2.3 million new cases of breast cancer were reported, underscoring its immense health burden (1). While early detection and diagnosis are pivotal for improving patient outcomes, breast cancer management continues to grapple with challenges posed by angiogenesis and metastasis. The dynamic interplay of epithelial cell proliferation, migration, and differentiation is central to the formation of new capillaries from pre-existing ones, a hallmark process of pathological angiogenesis. This phenomenon is further exacerbated by metabolic stressors, including hypoxia and acidosis, as well as inflammatory responses and genetic mutations that perpetuate a pro-angiogenic microenvironment (1, 2). Matrix metalloproteinases, particularly *MMP-2* and *MMP-9*, are zinc-dependent gelatinases that degrade both extracellular and non-matrix proteins, thereby facilitating tumor cell invasion and metastasis. The role of tissue inhibitors of metalloproteinases (*TIMPs*), especially *TIMP-1*, has historically been viewed as tumor-suppressive due to their ability to inhibit metalloproteinase activity. However, recent evidence suggests a more nuanced role for *TIMP-1* in cancer biology. Overexpression of *TIMP-1* in breast epithelial cells has been shown to induce *TWIST1*-mediated suppression of *E-cadherin*, promoting cancer cell depolarization and motility during epithelial-mesenchymal transition (EMT). This dual regulatory role of *TIMP-1*, encompassing both protease-dependent and protease-independent functions, underscores its complex involvement in breast cancer progression (3, 4).

The intricate interconnection between angiogenesis, extracellular matrix remodeling, and metastasis highlights the multifaceted nature of breast cancer progression. Elevated levels of *MMP-2* and *MMP-9* within the tumor microenvironment have been strongly correlated with poor clinical outcomes, increased metastatic activity, and resistance to treatment, making them critical biomarkers for prognostic and therapeutic strategies (5, 6). Dysregulated expression of these biomarkers not only drives aggressive tumor phenotypes but also contributes to suboptimal treatment outcomes, emphasizing the need for their integration into diagnostic and prognostic frameworks (7). Additionally, *TIMP-1's* ability to regulate cancer cell behavior, in conjunction with its metalloproteinase inhibition, adds another layer of complexity to its role in breast cancer dynamics. The challenges posed by therapeutic resistance further complicate breast cancer management. Primary resistance, characterized by the lack of an initial favorable response to therapy, and acquired resistance, driven by alternative pro-angiogenic pathways, limit the effectiveness of current treatments. These mechanisms disrupt drug and oxygen delivery, exacerbating hypoxia and tumor microenvironmental stress, which are central to cancer biology and resistance pathways (9, 10).

A comprehensive understanding of the molecular mechanisms regulating *MMP-2*, *MMP-9*, and *TIMPs* in breast cancer progression offers promising avenues for developing novel therapeutic approaches. By targeting the underlying processes of angiogenesis, extracellular matrix remodeling, and metastasis, future research could significantly improve diagnostic accuracy and therapeutic outcomes. This study aims to rationalize the incorporation of *MMP-2*, *MMP-9*, and *TIMPs* into clinical frameworks, providing a foundation for innovative strategies in breast cancer management.

METHODS

This cross-sectional study included 116 breast cancer patients and 24 healthy controls. Peripheral venous blood (5 ml) was collected from each patient undergoing chemotherapy through venipuncture. The blood samples were immediately preserved in EDTA tubes and transported under controlled conditions to the molecular biology laboratories at affiliated institutions, primarily Lahore College for Women University and Quaid-i-Azam University, Islamabad. Female patients aged 30 years or older with histological evidence of breast cancer subtypes, ECOG performance status of 0 or 1, and those undergoing multimodal therapy, including surgery, radiation, or chemotherapy, were included in the study upon obtaining informed consent. Patients with autoimmune disorders, hematological, hepatic, or renal conditions, other malignancies apart from breast cancer, or those who were pregnant or breastfeeding were excluded. Total RNA was extracted from blood samples using the Invitrogen *TRIzol* reagent (Catalog #15596026, USA), following the manufacturer's protocol. The quality and quantity of the RNA were assessed using a Nanodrop 2000/2000c spectrophotometer (*Thermo Scientific*). RNA samples with a 260/280 and 260/230 ratio greater than 1.5 and clear bands on agarose gel electrophoresis were selected for further processing. RNA samples that failed these criteria were either re-precipitated or re-extracted. Complementary DNA (cDNA) was synthesized using the Thermo Scientific RevertAid First Strand cDNA Synthesis Kit (Catalog #K1622, USA) as per the manufacturer's instructions.

Primers for the target genes were designed using the consensus CDS sequence from the NCBI database. Their specificity and universality were confirmed through Primer-BLAST and BLASTn analysis, respectively. Optimization of primers was performed using a Bio-Rad T100 gradient PCR thermocycler to determine the optimal annealing temperature and amplicon properties. The sequences of the forward and reverse primers for *MMP-2* and *MMP-9* are detailed in Table 1. Quantitative real-time PCR (RT-qPCR) was conducted using Bio-

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Rad's CFX 96 qPCR system to evaluate gene expression levels. The reactions utilized the *ThermoFisher Scientific SYBR Green* qPCR Master Mix (Catalog #4309155, USA) according to the manufacturer's guidelines. Statistical analyses were performed using GraphPad Prism 9 software. Ordinary one-way ANOVA was used for comparing multiple groups, while unpaired Student's t-tests were applied for two-group comparisons. All clinical data were reported as the arithmetic mean of duplicate analyses, expressed as mean ± standard deviation (SD). Results were deemed statistically significant at $P < 0.05$.

Menopausal status and hormonal receptor profiles, including estrogen receptor (ER), progesterone receptor (PR), and *HER2* status, were assessed for all breast cancer patients as part of the inclusion criteria to ensure comprehensive stratification of the study population. For RT-qPCR, gene expression was normalized using *GAPDH* as a housekeeping gene, ensuring accurate quantification and minimizing variability due to sample processing. These additional parameters provided critical insights into the molecular characteristics of the participants, enhancing the reliability and reproducibility of the results.

Table 1: Primers Sequences

Gene Name	Primer Sequence (5'-3')
MMP-2	*5' F- AGTCTGTGTTGTCCAGAGGC 3'*
	5' R-TGAAGCCAAGCGGTCTAAGT 3'
MMP-9	*5' F- GCAACTACGACACCGACGA 3'*
	5' R- ACTGGCAGGGTTTCCCATCA 3'

RESULTS

Out of 140 analyzed samples, the majority of breast cancer patients (68%) were between the ages of 40 and 70, while 32% were under the age of 40. Among the study population, 42.8% were premenopausal, and 57.2% were postmenopausal. All cases included in the study were diagnosed as invasive ductal carcinoma, with luminal A subtype accounting for the highest proportion at 45.7%. Other molecular subtypes included luminal B (22.2%), triple-negative breast cancer (21.4%), and *HER2*-enriched (10.7%).

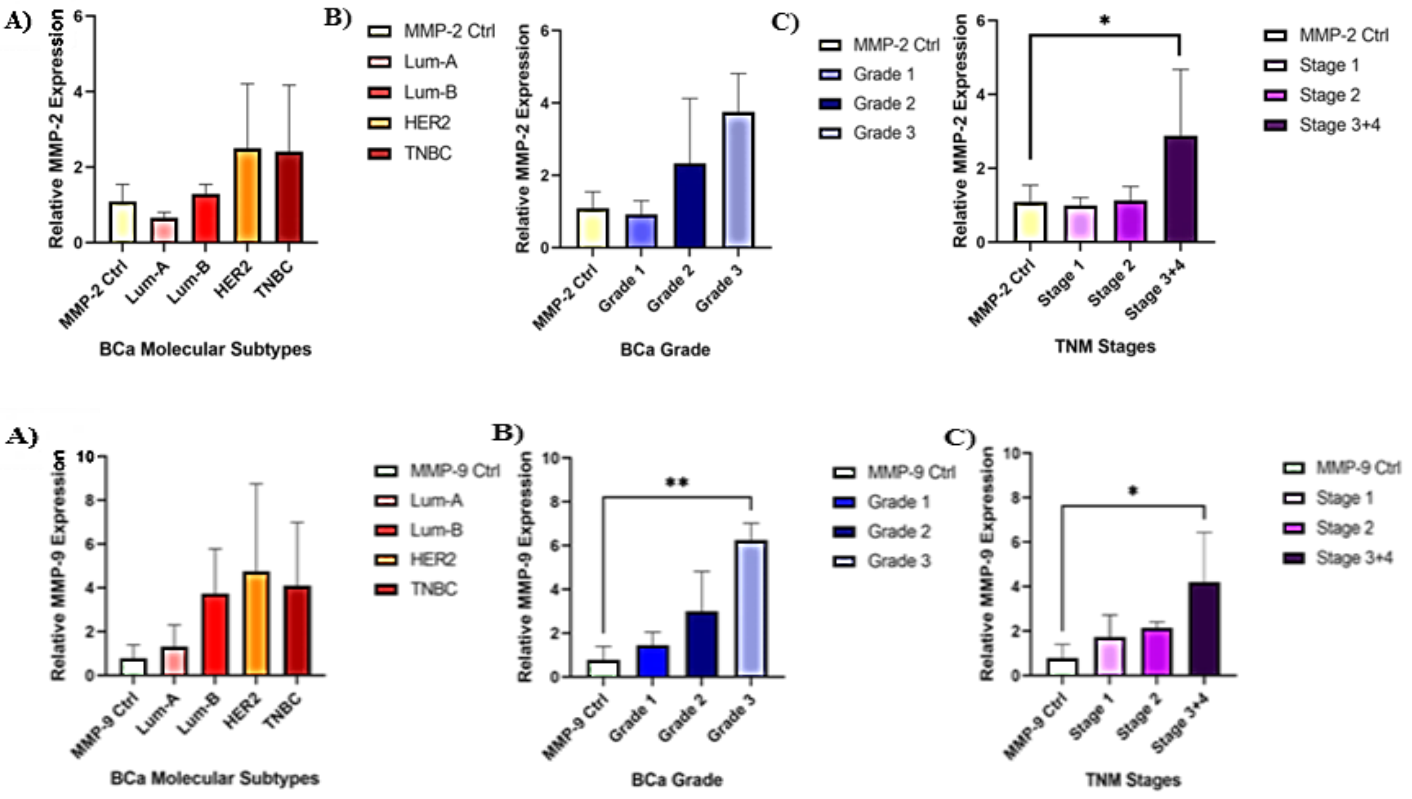
Table 2: Clinicopathological characteristics of study population (n=56)

Characteristics	%
Menopausal Status	
Premenopausal	42.8%
Postmenopausal	57.2%
Luminal Subtypes	
Luminal A	45.7%
Luminal B	22.2%
Her2 enriched	10.7%
Triple Negative	21.4%
Grading	
Grade 1	7.2%
Grade 2	78.5%
Grade 3	14.3%
Stage of disease at diagnosis	
I	11.5%
II	13.5%
III	25%

IV		50%
Metastasis		
No		50%
Yes		50%
If Metastasis Yes, then	Visceral+/- bone	94.7%
	Bone only	5.3%
Therapeutic Setting		
Adjuvant Treatment		46.5%
Neoadjuvant Treatment		53.5%

Grading revealed that most tumors were of grade 2 (78.5%), while grade 1 and grade 3 tumors comprised 7.2% and 14.3%, respectively. At the time of diagnosis, 50% of patients were in stage IV, with the remaining distributed across stages I (11.5%), II (13.5%), and III (25%). Among the metastatic cases (50%), visceral metastases, with or without bone involvement, were most common (94.7%), while bone-only metastases were observed in 5.3%. Regarding therapeutic settings, 53.5% of patients received neoadjuvant treatment, while 46.5% underwent adjuvant therapy. Expression profiling demonstrated that *MMP-2* levels were significantly elevated in more aggressive molecular subtypes, including *HER2*-enriched and triple-negative breast cancer, compared to luminal A and controls. Similarly, *MMP-2* expression correlated positively with higher pathological grades and advanced TNM stages. The increase in *MMP-2* expression in stages III and IV combined was statistically significant ($p = 0.0373$).

MMP-9 expression also showed a strong association with aggressive disease characteristics. Elevated levels of *MMP-9* were observed in *HER2*-enriched and triple-negative subtypes compared to luminal subtypes. Furthermore, *MMP-9* expression progressively increased with tumor grade, with the highest expression observed in grade 3 tumors ($p = 0.0104$). In TNM staging, *MMP-9* levels were significantly elevated in stages III and IV combined, with a statistically significant difference ($p = 0.0345$). Both *MMP-2* and *MMP-9* displayed a pattern of increased expression in molecular subtypes and disease grades associated with poor prognosis, highlighting their role in the pathophysiology of breast cancer.



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DISCUSSION

The findings of this study underscore the critical roles of *MMP-2* and *MMP-9* in the progression and metastasis of breast cancer, particularly in advanced stages and aggressive molecular subtypes. Elevated levels of these biomarkers were observed in late-stage breast cancer (stages III and IV), highlighting their involvement in invasive tumor behavior and metastatic spread. These results align with previous research demonstrating correlations between *MMP* expression, TNM staging, nodal metastases, and poor prognosis (11, 12). Furthermore, the differential expression of these matrix metalloproteinases in molecular subtypes, such as triple-negative and *HER2*-enriched breast cancer, reinforces their relevance as mediators of the metastatic cascade. *MMP-2* and *MMP-9*, as zinc-dependent gelatinases, contribute to tumor invasion by degrading type IV collagen, a major component of the basement membrane, enabling cancer cells to invade adjacent tissues and disseminate through the circulatory system (13). The study observed that *MMP-9* expression was slightly upregulated in early-stage breast cancer (stages I and II), suggesting a role in establishing the pre-metastatic niche during the initial phases of tumorigenesis. In contrast, *MMP-2* expression remained stable in early stages and increased significantly in advanced disease stages, indicating a sequential activation mechanism in the metastatic process. *MMP-9* appears to play a pivotal role in vascular remodeling and the preparation of the metastatic niche, whereas *MMP-2* facilitates tumor invasion in later stages. These findings suggest that *MMP-2* and *MMP-9* serve complementary functions in breast cancer progression and could be valuable surrogate markers for disease staging and progression monitoring (14, 15).

The prognostic value of *MMP-2* and *MMP-9* extends beyond their involvement in tumor invasion. Elevated *MMP-9* levels have been associated with poor overall survival and shorter disease-free intervals, while increased *MMP-2* expression has been linked to lymph node metastases and worse survival outcomes. These findings are consistent with this study, which identified significant associations between the expression of these biomarkers and clinicopathological parameters, such as tumor grade and molecular subtype (16, 19). The role of *MMP-9* in angiogenesis is particularly noteworthy, as it facilitates the degradation of the basement membrane surrounding blood vessels, enabling endothelial cell migration and the formation of new vasculature to sustain tumor growth. This mechanism is especially critical in triple-negative breast cancer, where rapid proliferation and aggressive behavior depend on a robust blood supply (20). The study highlights the heterogeneity of breast cancer and underscores the importance of a personalized approach to treatment. The differential expression of *MMP-2* and *MMP-9* across molecular subtypes presents an opportunity to tailor therapies based on the individual molecular drivers of each patient's disease. For instance, patients with triple-negative or *HER2*-positive breast cancer may benefit from targeted strategies aimed at inhibiting these metalloproteinases, potentially improving treatment outcomes by disrupting key pathways involved in metastasis and progression (21, 22).

Although this study provides valuable insights, its limitations should be acknowledged. The single-center design and relatively small sample size may limit the generalizability of the findings. Furthermore, the study did not explore the genetic and epigenetic regulation of *MMP-2* and MMP-9, which could offer additional insights into their role in breast cancer pathogenesis. Future research should validate these results in larger, multicenter cohorts and investigate the molecular mechanisms underlying MMP regulation through whole-genome sequencing and transcriptomic analyses. Advanced techniques, such as flow cytometry and exosome analysis, should be explored to refine the use of *MMP-2* and *MMP-9* as biomarkers for metastasis. Additionally, expanding biomarker profiling to include other molecular players, such as *SNAIL*, *SLUG*, and *TWIST1*, may reveal further insights into tumor progression. The integration of proteomic and genomic data could facilitate the development of more effective therapeutic strategies. Targeting *MMP-2* and MMP-9, either alone or in combination with other therapies, holds potential to suppress metastasis and improve patient outcomes. A deeper understanding of their roles in the tumor microenvironment will enable the development of personalized therapies for breast cancer patients, addressing the diverse molecular and clinical features of this heterogeneous disease.

CONCLUSION

The findings of this study highlight the significant role of MMP-2, MMP-9, and related biomarkers in the progression and prognosis of advanced breast cancer. These markers demonstrate potential utility in early disease detection, prognostic assessment, and predicting therapeutic responses. Additionally, TIE-2 exhibited a complex, context-specific role, contributing to metastasis while interacting with other molecular pathways. The study underscores the importance of plasma levels of *E-cadherin*, *MMP-2*, *MMP-9*, and *TIMP-1* as promising prognostic tools for monitoring breast cancer metastasis and tailoring treatment strategies. These insights provide a foundation for developing more precise, biomarker-driven approaches to breast cancer management.

AUTHOR CONTRIBUTIONS

Author	Contribution
Maria Sardar	Conceptualization, Methodology, Formal Analysis, Writing - Original Draft, Validation, Supervision
Muhammad Farhan Mukhtar	Methodology, Investigation, Data Curation, Writing - Review & Editing
Ayesha Muddasser	Investigation, Data Curation, Formal Analysis, Software
Akrama Ehsan	Software, Validation, Writing - Original Draft
Muhammad Arsalan	Formal Analysis, Writing - Review & Editing

REFERENCES

1. Madu CO, Wang S, Madu CO, Lu Y. Angiogenesis in breast cancer progression, diagnosis, and treatment. Journal of Cancer. 2020;11(15):4474.

2. Abou Khouzam R, Brodaczewska K, Filipiak A, Zeinelabdin NA, Buart S, Szczylik C, Kieda C, Chouaib S. Tumor hypoxia regulates immune escape/invasion: influence on angiogenesis and potential impact of hypoxic biomarkers on cancer therapies. Frontiers in Immunology. 2021 ;11:613114.

3. Agnello L, d'Argenio A, Caliendo A, Nilo R, Zannetti A, Fedele M, Camorani S, Cerchia L. Tissue Inhibitor of Metalloproteinases-1 Overexpression Mediates Chemoresistance in Triple-Negative Breast Cancer Cells. Cells. 2023;12(13):1809.

4. Guccini I, Revandkar A, D'Ambrosio M, Colucci M, Pasquini E, Mosole S, Troiani M, Brina D, Sheibani-Tezerji R, Elia AR, Rinaldi A. Senescence reprogramming by *TIMP1* deficiency promotes prostate cancer metastasis. Cancer cell. 2021 ;39(1):68-82.

5. Fouad FA, Khali MA, Moaz I, Elmasry H, Gheta N, Abdeen A, Tantawi M, Elkholy G, Rihan S, Kamel MM, El-Kenawy AE. Prognostic impact of matrix metalloproteinase 2 (MMP-2) and matrix metalloproteinase 9 (MMP-9) in Egyptian breast cancer patients. International Journal of Immunopathology and Pharmacology. 2024 ;38:03946320241304911.

6. Jiang H, Li H. Prognostic values of tumoral MMP2 and MMP9 overexpression in breast cancer: a systematic review and meta-analysis. BMC cancer. 2021;21:1-3.

7. Huang Q, Wang J, Ning H, Liu W, Han X. Integrin β1 in breast cancer: mechanisms of progression and therapy. Breast Cancer. 2024:1-7.

8. Bi X, Lou P, Song Y, Sheng X, Liu R, Deng M, Yang X, Li G, Yuan S, Zhang H, Jiao B. Msi1 promotes breast cancer metastasis by regulating invadopodia-mediated extracellular matrix degradation via the *Timp3–Mmp9* pathway. Oncogene. 2021 ;40(29):4832-45.

9. Marra A, Trapani D, Viale G, Criscitiello C, Curigliano G. Practical classification of triple-negative breast cancer: intratumoral heterogeneity, mechanisms of drug resistance, and novel therapies. NPJ breast cancer. 2020 ;6(1):54.

10. Ramadan WS, Zaher DM, Altaie AM, Talaat IM, Elmoselhi A. Potential therapeutic strategies for lung and breast cancers through understanding the anti-angiogenesis resistance mechanisms. International Journal of Molecular Sciences. 2020 ;21(2):565.

11. Lukianova N, Mushii O, Borikun T, Zadvornyi T, Bazas V, Krotevych M, Sivak L, Lyalkin S, Martynyuk O, Hrybach S, Chekhun V. PATTERN OF MMP2 AND MMP9 EXPRESSION DEPENDS ON BREAST CANCER PATIENTS'AGE. Experimental Oncology. 2023;45(1):17-27.

12. Jafari E, Safinejad S, Dabiri S, Naghibzadeh-Tahami A. Study of the relationship between *MMP-2* and *MMP-9* and *Her2*/neu overexpression in gastric cancer: clinico-pathological correlations. Asian Pacific Journal of Cancer Prevention: APJCP. 2021;22(3):811.

13. Shakery T, Safari F. Downregulation of Pinkbar/pAKT and MMP2/MMP9 expression in MDA-MB-231 breast cancer cells as potential targets in cancer therapy by hAMSCs Secretome. Cells Tissues Organs. 2023 ;212(2):155-63.

14. Zhuyan J, Chen M, Zhu T, Bao X, Zhen T, Xing K, Wang Q, Zhu S. Critical steps to tumor metastasis: alterations of tumor microenvironment and extracellular matrix in the formation of pre-metastatic and metastatic niche. Cell & Bioscience. 2020;10:1-9.

15. Barillari G. The impact of matrix metalloproteinase-9 on the sequential steps of the metastatic process. International journal of molecular sciences. 2020 ;21(12):4526.

16. Shoari A. Potential of *MMP-2* and *MMP-9* Gelatinase Blockade as a Therapeutic Strategy in Fibrosarcoma Treatment: A Decadal Review. Targets. 2024;2(2):104-25.

17. Ozdemir K, Zengin I, Guney Eskiler G, Kocer HB, Ozkan AD, Demiray T, Sahin EO. The predictive role of MMP-2, MMP-9, *TIMP-1* and TIMP-2 serum levels in the complete response of the tumor to chemotherapy in breast cancer patients. Journal of InvestIgatIve surgery. 2022 ;35(7):1544-50.

18. Joseph C, Alsaleem M, Orah N, Narasimha PL, Miligy IM, Kurozumi S, Ellis IO, Mongan NP, Green AR, Rakha EA. Elevated MMP9 expression in breast cancer is a predictor of shorter patient survival. Breast cancer research and treatment. 2020;182:267-82.

19. Muhammad KI, Dige MA, Kida ZH. Clinical significance of *MMP-2* and *MMP-9* levels as potential biomarkers in patients with breast cancer in Maiduguri–Nigeria. Journal of Biotechnology. 2023;2(1):34-47.

20. Navasatli SA, Vahdati SN, Arjmand TF, Behboudi H. New insight into the role of the ADAM protease family in breast carcinoma progression. Heliyon. 2024.

21. Alrehaili AA, Gharib AF, Karam RA, Alhakami RA, El Sawy WH, Abd Elrahman TM. Clinical significance of plasma MMP-2 and MMP-9 levels as biomarkers for tumor expression in breast cancer patients in Egypt. Molecular Biology Reports. 2020;47:1153-60.

22. Sebastian A, Hum NR, Martin KA, Gilmore SF, Peran I, Byers SW, Wheeler EK, Coleman MA, Loots GG. Single-cell transcriptomic analysis of tumor-derived fibroblasts and normal tissue-resident fibroblasts reveals fibroblast heterogeneity in breast cancer. Cancers. 2020;12(5):1307.