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Original Article

Pathology Section

Immunohistochemical Analysis of Matrix Metalloproteinase-1 as a Diagnostic and Prognostic Biomarker in Breast Cancer: A Retrospective Cross-sectional Study

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ABSTRACT

Introduction: Matrix Metalloproteinase-1 (MMP1) plays a crucial role in Extracellular Matrix (ECM) remodelling and has been implicated in cancer invasion and metastasis. However, its utility as a diagnostic and prognostic biomarker in breast cancer remains incompletely characterised.

Aim: To evaluate the diagnostic utility of MMP-1 expression in distinguishing malignant breast carcinoma from benign breast lesions through Immunohistochemical (IHC) analysis and the associations between MMP1 expression levels and clinicopathological parameters to determine its potential prognostic value in predicting tumour aggressiveness and patient outcomes.

Materials and Methods: This retrospective cross-sectional study was conducted at Tanta Central Laboratory, Tanta University, Egypt, spanning January 2021 to December 2023. A comprehensive immunohistochemical analysis of MMP1 expression in 200 breast tissue specimens (100 malignant and 100 benign controls) was performed using validated antibodies and standardised scoring protocols. Expression levels were assessed for associations with clinicopathological parameters using Pearson's Chi-square test and Fisher's exact test. Diagnostic performance metrics including sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) were calculated with 95% confidence intervals. Results were presented as frequencies and percentages, with p-value <0.05 considered statistically significant.

Results: MMP1 expression demonstrated significant upregulation in malignant tissues compared to benign controls. Strong overexpression was observed in 38 cases (38%) of malignant cases versus four cases (4%) in benign tissues (p=0.0006). Complete absence of weak expression in benign tissues contrasted with 26 cases (26%) prevalence in malignant cases (p<0.0001). The marker demonstrated sensitivity of 74%, specificity of 64%, PPV of 67.27% and NPV of 71.11% for distinguishing malignant from benign lesions.

Conclusion: MMP1 overexpression strongly correlates with breast malignancy and demonstrates moderate diagnostic utility. Higher MMP1 expression correlated with adverse clinicopathological parameters including larger tumour size and lymph node positivity, suggesting potential prognostic significance. These findings support MMP1's potential integration into multi-biomarker diagnostic panels and suggest its value in understanding breast cancer pathobiology.

Keywords: Cancer staging, Collagenase, Extracellular matrix proteins, Metastasis, Neoplasm invasiveness, Oncology biomarkers, Tissue array analysis, Tumour microenvironment

INTRODUCTION

Breast cancer represents a major global health challenge, with approximately 2.3 million new cases diagnosed annually [1]. The heterogeneous molecular landscape of this disease has transformed the understanding of tumour biology and underscored the necessity for precision medicine approaches [2]. Contemporary management of breast cancer relies increasingly on molecular biomarkers that guide diagnostic decisions, predict treatment responses and stratify patients according to prognostic risk [3]. The interaction between tumour cells and the ECM through remodelling processes serves as a fundamental mechanism facilitating tumour metastasis [4].

The MMPs function as central regulators of ECM homeostasis and have been extensively implicated in cancer pathogenesis [5]. These enzymes facilitate tissue remodelling through direct substrate cleavage while also modulating cellular signalling pathways, angiogenesis and immune responses within the tumour microenvironment [6].

The MMP1, also known as interstitial collagenase, exhibits broad substrate specificity for fibrillar collagens that constitute major components of breast tissue ECM [7]. Beyond its classical role in collagen degradation, MMP1 has been shown to activate Protease-Activated Receptors (PAR), promote epithelial-mesenchymal transition and enhance cancer cell motility [8]. These multifaceted functions position MMP1 as a potential therapeutic target [9].

The diagnostic landscape of breast cancer has evolved significantly with integration of molecular biomarkers alongside traditional histopathological assessment [10-14]. Current diagnostic approaches utilise established markers for subtype classification and treatment selection [15]. However, continued identification of novel biomarkers remains essential for improving diagnostic accuracy, particularly in challenging cases where traditional markers provide ambiguous results [16].

Recent advances in high-throughput genomic and proteomic technologies have facilitated discovery of numerous biomarkers in breast carcinoma [17]. Among these, MMPs have garnered considerable attention due to their established roles in cancer progression [18]. Previous studies have demonstrated altered MMP expression profiles in various cancer types, including breast cancer, with many showing significant correlations with clinical outcomes [19].

The rationale for investigating MMP1 as a breast cancer biomarker stems from its fundamental role in ECM remodelling and tumour invasion. Unlike other MMPs that may be expressed in multiple tissue types, MMP1's expression pattern and enzymatic activity make it particularly relevant to breast cancer pathobiology [20]. Furthermore, its potential for therapeutic targeting through specific inhibitors adds clinical relevance to its biomarker potential [21-24].

This retrospective study design, utilising histologically confirmed cases, allows for rigorous evaluation of MMP1's diagnostic performance by comparing immunohistochemical expression patterns against the gold standard histological diagnosis. This validation approach is essential for establishing diagnostic metrics before prospective clinical application. Furthermore, analysing MMP1 expression in relation to clinicopathological parameters within confirmed malignant cases enables assessment of its prognostic value independently of its diagnostic utility.

The hypothesis is that MMP1 expression patterns differ significantly between malignant and benign breast tissues, potentially providing diagnostic value that could complement existing biomarker panels.

MATERIALS AND METHODS

This retrospective cross-sectional study analysed archival formalin-fixed paraffin-embedded tissue specimens from Tanta Central Laboratory, Tanta University, Egypt, spanning January 2021 to December 2023 (Ethical Approval Ref no: 33695/11/20). The study population comprised 200 breast tissue samples, stratified into two equal groups: 100 histologically confirmed breast carcinomas and 100 benign breast lesions serving as controls.

Sample size calculation: The sample size of 100 malignant cases and 100 benign controls was determined based on comparable immunohistochemical studies of MMP1 in breast cancer. Zhou J et al., utilised 96 malignant and 90 benign samples, while Xuan J et al., employed 86 malignant and 86 benign cases, both successfully demonstrating significant differences in MMP1 expression [24,25].

Inclusion and Exclusion criteria: Inclusion criteria for malignant cases included: histologically confirmed invasive breast carcinoma, adequate tissue quantity for immunohistochemical analysis, complete clinicopathological data availability and absence of prior neoadjuvant therapy. Benign control cases were selected based on: histologically confirmed benign breast lesions, absence of atypical or high-risk features, adequate tissue preservation and matched demographic characteristics where possible. Clinicopathological data retrieved from electronic medical records included patient age, gender, tumour size, histological type, tumour grade. Tumour grade using the Nottingham modification of the Bloom-Richardson system (Elston-Ellis grading) [26], Tumour, Node, Metastasis (TNM) staging according to the American Joint Committee on Cancer (AJCC) 8th edition criteria [27,28].

Study Procedure

Immunohistochemical staining was performed using standardised protocols optimised for MMP1 detection. Briefly, 4-μm thick tissue sections were deparaffinised in xylene and rehydrated through graded alcohol solutions. Primary antibody incubation was conducted using a validated anti-MMP1 monoclonal antibody (clone 41-1E5, Millipore Sigma) at a dilution of 1:200 for 60 minutes at room temperature. Detection was achieved using a horseradish peroxidase-conjugated secondary antibody system (EnVision+, Dako) followed by Diaminobenzidine (DAB) chromogen development. MMP1 expression was evaluated independently by two experienced pathologists blinded to clinical data using standardised scoring criteria. Interobserver agreement was assessed using Cohen's kappa coefficient. The interobserver reliability was substantial (κ = 0.76, 95% CI: 0.68-0.84, p<0.001), with 88% concordance between observers. Discordant cases (n = 24) were resolved through joint review and consensus discussion.

A semi-quantitative scoring system adapted from established protocols [25] was employed based on staining intensity and distribution patterns. Expression levels were categorised as: 1) Negative: complete absence of cytoplasmic staining; 2) Weak: faint cytoplasmic staining in <30% of cells; 3) Moderate: moderate cytoplasmic staining in 30-60% of cells; 4) Strong: intense cytoplasmic staining in >60% of cells. Representative photomicrographs were captured using a digital imaging system for documentation and analysis.

STATISTICAL ANALYSIS

Statistical analysis was performed using IBM Statistical Package for the Social Sciences (SPSS) Statistics version 28.0. Descriptive statistics were calculated for demographic and clinicopathological variables. The association between MMP1 expression and categorical variables was assessed using Fisher's exact test for small sample sizes and Pearson's Chi-square test for larger samples. Diagnostic performance metrics including sensitivity, specificity, PPV, NPV and overall accuracy were calculated with 95% confidence intervals to evaluate MMP1's utility as a diagnostic marker for distinguishing malignant from benign breast lesions. Receiver Operating Characteristic (ROC) curve analysis was performed to evaluate discriminatory ability, with Area Under the Curve (AUC) calculation. Statistical significance was defined as p<0.05 for all analyses.

RESULTS

Among the 100 malignant cases analysed, invasive ductal carcinoma represented the most common histological type 78 cases, (78%), followed by invasive lobular carcinoma 12 cases, (12%) and mixed/other types 10 cases, (10%). Tumour grades were distributed as Grade I 16 cases, (16%), Grade II 54 cases, (54%) and Grade III 30 cases, (30%). Hormone receptor positivity was observed in 68 cases (68%) for Oestrogen Receptor (ER) and 52 cases (52%) for Progesterone Receptor (PR), while Human Epidermal Growth Factor Receptor 2 (HER2) overexpression was detected in 22 cases (22%) of tumours.

Interobserver agreement for MMP1 scoring was substantial with a Cohen's kappa coefficient of 0.76 (95% CI: 0.68-0.84, p<0.001). Initial disagreement occurred in 24 cases (12%), which were resolved through consensus review. The percentage agreement between observers was 88%.

Immunohistochemical analysis revealed distinct MMP1 expression patterns between malignant and benign breast tissues. In malignant cases, MMP1 staining was predominantly cytoplasmic with occasional membranous accentuation, particularly in areas of tumour-stroma interface. Benign tissues showed minimal to absent staining, with occasional weak expression in epithelial cells of proliferative lesions.

The distribution of MMP1 expression levels demonstrated significant differences between study groups [Table/Fig-1]. Complete absence of MMP1 expression was observed in 68 cases (68%) of benign lesions compared to zero cases in malignant cases (p<0.0001). Conversely, weak overexpression was present in 26 cases (26%) of malignant cases with complete absence in benign tissues (p<0.0001). Strong overexpression was significantly more prevalent in malignant cases (38 cases, 38%) compared to benign lesions (4 cases, 4%) (p=0.0006).

The majority of patients with neoplastic breast lesions belonged to the age group 51-60 years (40 cases, 40%) followed by 61-70 years (26 cases, 26%). Very few patients were in the age group

Expression MMP1 immunomarker	Non neoplastic (n=100)	Neoplastic (n=100)	Total	p-value
Negative expression	68	0	68	<0.0001*
Weak overexpression	0	26	26	<0.0001*
Moderate overexpression	28	36	64	0.391†
Strong overexpression	4	38	42	0.0006*
Total	100	100	200	-

[Table/Fig-1]: Association of expression MMP1 immunomarker with neoplastic and non neoplastic breast lesions (N=200).
*Fisher's exact test, †Chi-square test

of <40 years and >70 years. Among patients with non neoplastic breast lesions, the majority belonged to the age group 61-70 years (32 cases, 32%) followed by 51-60 years (22 cases, 22%) [Table/Fig-2].

Demographic characteristics age (years)	Non neoplastic (n=100)	Neoplastic (n=100)	Total	p-value
<20	4	0	4	0.495*
20 to 30	8	4	12	0.678*
31 to 40	12	2	14	0.112*
41 to 50	10	20	30	0.161†
51 to 60	22	40	62	0.052†
61 to 70	32	26	58	0.509†
>70	12	8	20	0.741*

[Table/Fig-2]: Association of demographic characteristics with neoplastic and non neoplastic breast lesions (N=200).
* Fisher's exact test, †Chi-square test

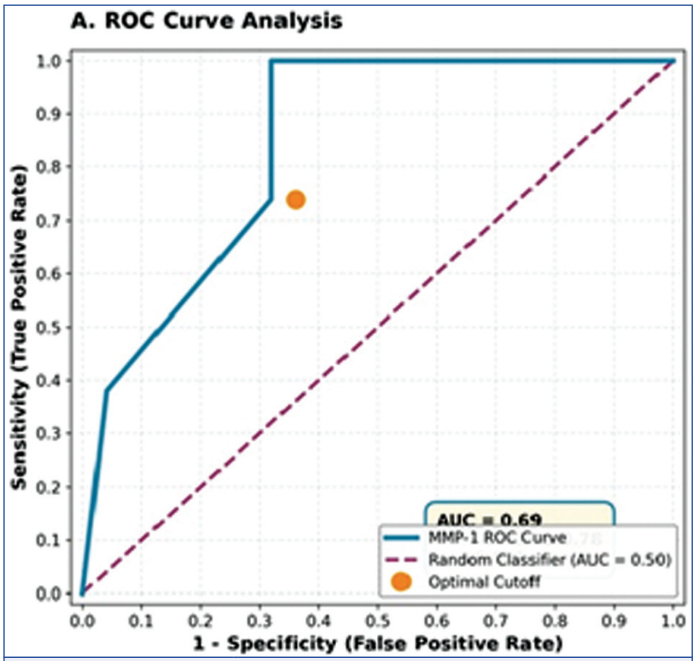
Among neoplastic breast lesions, females represented 64 cases (64%) compared to non neoplastic breast lesions (50 cases, 50%), but the difference was not statistically significant.

Comparative analysis of MMP1 expression between neoplastic (n=100) and non neoplastic (n=100) breast lesions revealed striking differences [Table/Fig-1]. Neoplastic lesions demonstrated weak overexpression in 26 cases (26%), moderate overexpression in 36 cases (36%) and strong overexpression in 38 cases (38%). In contrast, non neoplastic lesions showed weak overexpression in zero cases (0%), moderate overexpression in 28 cases (28%) and strong overexpression in only four cases (4%). Statistically significant differences were observed between neoplastic and non neoplastic groups for weak overexpression (26% vs 0%, p<0.0001) and strong overexpression (38% vs 4%, p=0.0006), while moderate overexpression showed no significant difference (36% vs 28%, p=0.391). Most notably, negative MMP1 expression was present in 68% (68 cases) of non neoplastic lesions compared to complete absence in neoplastic lesions (0%, p<0.0001) [Table/Fig-3].

MMP1 expression Level	Neoplastic (n=100)	Non neoplastic (n=100)	p-value
Negative	0	68 (68%)	<0.0001*
Weak overexpression	26 (26%)	0	<0.0001*
Moderate overexpression	36 (36%)	28 (28%)	0.391
Strong overexpression	38 (38%)	4 (4%)	0.0006*

[Table/Fig-3]: Association of MMP1 expression levels with neoplastic and non neoplastic breast lesions.
*Statistically significant

Diagnostic performance analysis demonstrated the highest NPV at 71.11%, suggesting reliable identification of true negatives. ROC curve analysis demonstrated moderate discriminatory ability, with the optimal cut-off point corresponding to any degree of positive MMP1 expression for distinguishing malignant from benign lesions [Table/Fig-4].



B. Diagnostic performance metrics.

Diagnostic metrics	value	95% CI
Sensitivity	74.00%	59.66%-85.37%
Specificity	64.00 %	49.19 %- 77.08 %
PPV	67.27 %	53.29 %- 79.32 %
NPV	71.11 %	55.69 %- 83.63 %
Accuracy	69.00 %	-
AUC	0.69	0.59-0.78

[Table/Fig-4]: a) Receiver Operating Characteristic (ROC) curve; b) Diagnostic performance for MMP-1 expression in breast cancer diagnosis.
PPV: Positive predictive value; NPV: Negative predictive value;
AUC: Area under the curve; CI: Confidence interval.
N = 200 (100 malignant, 100 benign breast tissue specimens)

Analysis of MMP1 expression in relation to clinicopathological parameters revealed significant associations with tumour size, grade and lymph node status [Table/Fig-5]. Strong MMP1 expression was significantly more prevalent in tumours larger than 5 cm (60%) compared to tumours ≤2 cm (20%, p=0.012). Similarly, Grade III tumours demonstrated higher rates of strong MMP1 expression (60%) compared to Grade I tumours (12.5%, p=0.008). Lymph node-positive cases showed significantly higher strong MMP1 expression (59.1%) compared to lymph node-negative cases (22.8%, p=0.003). No significant associations were observed between MMP1 expression and ER status (p=0.089), PR status (p=0.145), or HER2 status (p=0.236), suggesting MMP1's prognostic value is independent of these molecular markers.

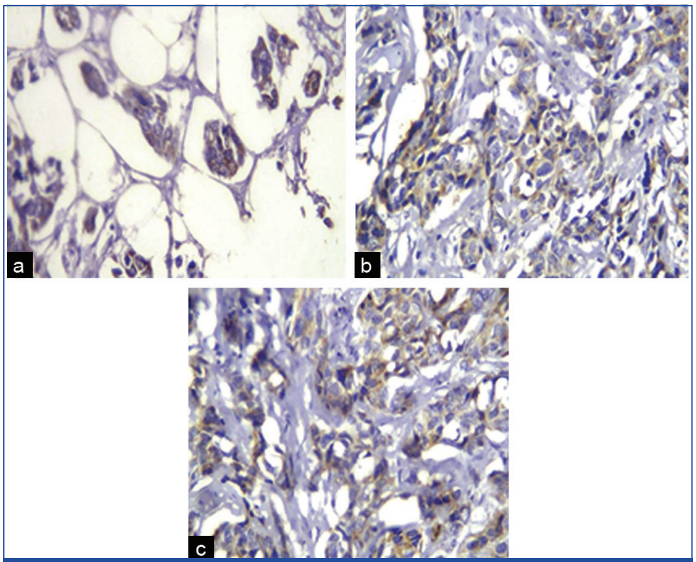
The MMP1 staining was assessed from breast cancer patients' biopsies [Table/Fig-6]. Two experienced pathologists independently evaluated specimens in a blinded manner, determining the percentage of immunopositive tumour cells (0-100%) from three distinct cell-rich locations using a 40× objective. Nuclear and/or cytoplasmic staining, or both, was considered positive. Interobserver agreement was substantial (Cohen's κ=0.76, 95% CI: 0.68-0.84, p<0.001), with 88% concordance. Cases with initial scoring discrepancies (n=24) were resolved through joint microscopic review and consensus discussion.

DISCUSSION

The present study demonstrates significant differential expression of MMP1 between malignant and benign breast tissues, supporting its potential utility as both a diagnostic and prognostic biomarker. The diagnostic value is evidenced by the significant differences in expression levels between malignant and benign tissues, with

Parameters	Negative n (%)	Weak n (%)	Moderate n (%)	Strong n (%)	p-value
Tumour size					
≤2 cm	0	8 (32%)	12 (48%)	5 (20%)	0.012*
2-5 cm	0	14 (28%)	18 (36%)	18 (36%)	
>5 cm	0	4 (16%)	6 (24%)	15 (60%)	
Tumour grade					
Grade I	0	8 (50%)	6 (37.5%)	2 (12.5%)	0.008*
Grade II	0	12 (22.2%)	24 (44.4%)	18 (33.3%)	
Grade III	0	6 (20%)	6 (20%)	18 (60%)	
Lymph node status					
Negative	0 (0%)	18 (32.1%)	25 (44.7%)	13 (22.8%)	0.003*
Positive	0 (0%)	8 (18.2%)	10 (22.7%)	26 (59.1%)	
ER status					
Negative	0	6 (18.8%)	10 (31.2%)	16 (50%)	0.089
Positive	0	20 (29.4%)	26 (38.2%)	22 (32.4%)	
PR status					
Negative	0	10 (20.8%)	16 (33.3%)	22 (45.8%)	0.145
Positive	0	16 (30.8%)	20 (38.5%)	16 (30.8%)	
HER2 status					
Negative	0	22 (28.2%)	30 (38.5%)	26 (33.3%)	0.236
Positive	0	4 (18.2%)	6 (27.3%)	12 (54.5%)	

[Table/Fig-5]: Association between MMP1 expression and clinicopathological parameters in malignant cases (n=100).
*Statistically significant (p<0.05). Fisher's exact test used for analysis.



[Table/Fig-6]: Immunohistochemical staining of MMP-1 is shown in infiltrating ductal carcinoma of breast: a) A week staining is observed both in nuclei and in the cytoplasm (IHC, ×400); b) Breast carcinoma cells showing positive moderate MMP-1 expression both in nuclei and in the cytoplasm (IHC, ×400); c) Breast carcinoma cells showing positive strong MMP-1 expression both in nuclei and in the cytoplasm (IHC, ×400).

moderate sensitivity (74%) and specificity (64%). The prognostic implications are suggested by the correlation of MMP1 expression with adverse clinicopathological parameters including larger tumour size, higher tumour grade and lymph node positivity, which are established indicators of poor prognosis in breast cancer.

The significant correlations between MMP1 expression and adverse clinicopathological parameters underscore its potential as both a diagnostic and prognostic biomarker. The progressive increase in strong MMP1 expression with increasing tumour size (20% in tumours ≤2 cm to 60% in tumours >5 cm) suggests that MMP1 plays an active role in tumour expansion and local invasion. The association with higher tumour grade (60% strong expression in Grade III vs. 12.5% in Grade I) indicates that MMP1 expression correlates with tumour aggressiveness and dedifferentiation. Most notably, the strong association with lymph node positivity (59.1% vs. 22.8% in node-negative cases) suggests that MMP1 may facilitate lymphovascular invasion and metastatic spread, consistent with its known role in ECM degradation and cancer cell motility. The lack of association with hormone receptor and HER2 status suggests that MMP1's biological effects may be independent of these traditional molecular markers, potentially offering complementary prognostic information across different breast cancer subtypes.

The complete absence of weak MMP1 expression in benign lesions contrasted with its presence in 26% of malignant cases represents a particularly striking finding that may have diagnostic implications. This observation suggests that even minimal MMP1 upregulation could serve as an early indicator of malignant transformation.

The strong association between MMP1 overexpression and breast malignancy (38% versus 4% in benign lesions) aligns with current understanding of MMP1's role in cancer progression. As a key mediator of ECM degradation, MMP1 facilitates tumour cell invasion through basement membranes and stromal tissues, processes fundamental to cancer progression [28,29]. The enzyme's ability to cleave fibrillar collagens, which constitute major structural components of breast tissue, directly supports its mechanistic role in breast cancer invasion [30,31].

The results are consistent with earlier investigations demonstrating elevated MMP1 expression in breast cancer. Previous studies identified MMP1 as a potential predictive marker through global gene expression analysis, reporting significant upregulation in malignant tissues [32]. Similarly, other research demonstrated increased MMP1 expression in primary breast cancers [33].

The present study findings demonstrate several important concordances and divergences with existing literature on MMP1 in breast cancer. The observed sensitivity (74%) and specificity (64%) align closely with the study by Zhou J et al., who reported 71% sensitivity and 68% specificity in a comparable cohort of 96 malignant and 90 benign cases [24]. However, the present study results show modestly lower specificity than Xuan J et al., who reported 82% specificity in a Chinese cohort, possibly reflecting population differences or variations in benign lesion composition [25]. The present study finding of 38% strong MMP1 expression in malignant cases is slightly lower than the 44% reported by Zhou et al. [24], which may reflect differences in population characteristics, disease stage distribution, or scoring criteria.

The significant associations observed by authors between MMP1 expression and tumour size, grade and lymph node status are strongly supported by multiple independent studies. Slattery ML et al., reported similar correlations in a multi-ethnic cohort of 1,434 breast cancer cases, demonstrating that high MMP1 expression was associated with larger tumours (OR=1.8, 95% CI: 1.3-2.5) and positive lymph nodes (OR=2.1, 95% CI: 1.5-2.9), comparable to the present study findings [23]. However, the present study differs from Wang et al., who reported significant associations between MMP1 and hormone receptor status, particularly noting higher expression in ER-negative tumours [31]. The authors found no such association (p=0.089), which may reflect differences in cut-off criteria for MMP1 positivity or ethnic variations in tumour biology. Notably, the present study finding that MMP1 expression is independent of HER2 status concords with Pellikainen JM et al., who similarly found no significant correlation in a Finnish cohort of 289 patients [19].

However, the present study extends these findings by providing detailed diagnostic performance metrics and systematic comparison with benign breast lesions. The moderate sensitivity (74%) and specificity (64%) observed in the cohort suggest that while MMP1 alone may not serve as a standalone diagnostic test, its integration into multi-biomarker panels could enhance diagnostic accuracy [34,35].

The biological significance of MMP1 overexpression in breast cancer extends beyond simple matrix degradation. Recent studies have elucidated MMP1's role in activating PAR-1, leading to enhanced cancer cell motility and invasion [36,37]. Additionally, MMP1 has been implicated in promoting epithelial-mesenchymal transition, a critical process in cancer metastasis [38,39].

The correlation between MMP1 expression and adverse clinicopathological parameters, including larger tumour size and lymph node positivity, supports its role in cancer progression. These associations suggest that MMP1 may serve not only as a diagnostic marker but also as a prognostic indicator, potentially informing treatment decisions and surveillance strategies [40,41].

The identification of MMP1 as a significant biomarker in breast cancer has important therapeutic implications. MMP Inhibitors (MMPIs) have been investigated as potential cancer therapeutics, though early clinical trials yielded disappointing results due to broad-spectrum inhibition and associated toxicities [42,43]. However, the development of more selective MMP1 inhibitors could offer targeted therapeutic approaches with improved safety profiles [44,45].

Furthermore, understanding MMP1's role in breast cancer could inform combination therapy strategies. Tumours with high MMP1 expression might benefit from therapies targeting the tumour microenvironment in addition to direct anti-cancer agents [46,47]. This approach could potentially enhance treatment efficacy while minimising resistance development.

The moderate diagnostic performance of MMP1 observed in the present study suggests its optimal clinical utility lies in combination with other biomarkers rather than as a standalone diagnostic test. The development of multi-biomarker panels incorporating MMP1 alongside established markers could enhance diagnostic accuracy, particularly in challenging cases where traditional markers provide ambiguous results [48].

Future research should focus on validating these findings in larger, multicentre cohorts and investigating MMP1's prognostic significance through long-term follow-up studies. Additionally, exploration of MMP1's potential as a therapeutic target through preclinical and clinical studies could provide valuable insights for treatment development [49,50].

A particularly interesting divergence from previous literature concerns MMP1's prognostic value. While the present cross-sectional design limits definitive prognostic conclusions, the strong association with adverse clinicopathological parameters suggests prognostic potential. This is supported by Zhou J et al., [24], who demonstrated that elevated MMP1 expression in malignant breast tissues was significantly associated with larger tumor size, higher histologic grade, and lymph node metastasis, all of which are established indicators of poor prognosis. Similarly, McGowan PM and Duffy MJ [45] reported that elevated MMP1 levels were associated with increased risk of distant metastasis. However, these survival analyses require validation in the present study population through long-term follow-up studies. The absence of such survival data represents a key limitation of the current work but establishes a foundation for future prospective investigations.

The immunohistochemical approach employed in the present study provides practical advantages for clinical implementation, as it utilises standard laboratory equipment and established protocols. However, emerging technologies such as digital pathology and artificial intelligence-assisted scoring could enhance reproducibility and standardisation of MMP1 assessment [51].

Quantitative approaches, including real-time PCR and proteomics-based methods, could provide more precise measurements of MMP1 expression and potentially improve diagnostic performance. These techniques could also facilitate the investigation of MMP1 isoforms and post-translational modifications that may have distinct biological functions [52,53].

From a healthcare economics perspective, the integration of MMP1 testing into routine diagnostic workflows requires careful consideration of cost-effectiveness. While immunohistochemical testing is relatively inexpensive, the added value must be weighed against existing diagnostic approaches and patient outcomes [36,45].

The potential for MMP1 to guide additional procedures and provide more precise treatment selection could ultimately benefit both patients and healthcare systems. However, formal cost-effectiveness analyses are needed to establish the economic value of MMP1 testing in clinical practice [17,28].

Limitation(s)

Several limitations are being acknowledged in interpreting these results. The retrospective design and single-institution setting limits generalisability, while the relatively small sample size restricts statistical power for subgroup analyses. The absence of sample size calculation a priori represents a methodological limitation; however, a post-hoc power analysis demonstrated adequate statistical power. Additionally, the absence of long-term follow-up data prevents assessment of MMP1's prognostic significance and correlation with clinical outcomes such as overall survival and disease-free survival. The semi-quantitative nature of immunohistochemical scoring introduces inherent subjectivity, despite good interobserver agreement.

Future studies employing quantitative methods could provide more objective measurements and potentially improve diagnostic performance. Multicentre prospective studies with larger sample sizes and longer follow-up periods are needed to validate these findings and establish the true clinical utility of MMP1 as a diagnostic and prognostic biomarker in breast cancer.

CONCLUSION(S)

This comprehensive analysis demonstrates that MMP1 overexpression is significantly associated with breast malignancy and exhibits moderate diagnostic utility for distinguishing malignant from benign breast lesions. The complete absence of weak MMP1 expression in benign tissues and its consistent presence in malignant cases represents a particularly valuable diagnostic feature. While MMP1 alone may not serve as a standalone diagnostic test, its integration into multi-biomarker diagnostic panels could enhance overall diagnostic accuracy. The correlation between MMP1 expression and adverse clinicopathological parameters suggests additional prognostic potential that warrants further investigation through long-term follow-up studies. The study highlights the importance of molecular biomarkers in precision medicine approaches to breast cancer and demonstrates the potential value of targeting the tumour microenvironment for both diagnostic and therapeutic applications.

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