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Expression of Matrix Metalloproteinase-1 (MMP1) in Breast Cancer: Integrative Survival Analysis of Tumor Characteristics and Clinical Outcomes

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Abstract **Background:** Breast cancer is the most frequently diagnosed cancer type and the second leading cause of cancer-related deaths among women worldwide. Following a diagnosis of breast cancer, the most immediate challenges in patient management are the determination of prognosis and identification of the most appropriate adjuvant systemic therapy. Determining prognosis can best be addressed with a combination of clinicopathological prognostic factors. **Methods:** A retrospective study was performed on paraffin blocks of Breast carcinoma, histopathological findings were reviewed and recorded, Immunohistochemistry (IHC) for Matrix Metalloproteinase-1 (MMP1) proteins were performed. The investigation incorporated key clinical variables including hormone receptor status (Estrogen Receptor (ER) and Progesterone Receptor (PR), human epidermal growth factor receptor 2 (HER2) expression, tumor laterality, focality and pathological grade. **Results:** Statistical analyses encompassed descriptive statistics, correlation analysis, Cox proportional hazards regression and survival curve estimation using Kaplan-Meier methodology revealed strong intercorrelations between survival metrics and identified matrix MMP1 as a significant negative prognostic indicator across all survival endpoints. Chi-square analysis demonstrated significant association between HER2 and ER status, suggesting distinct biological subtypes with varying therapeutic implications. **Conclusion:** This study identifies MMP1 as a potent independent prognostic marker for poor survival in breast cancer. The results advocate for the integration of MMP1 assessment with standard biomarker profiling to enhance prognostic accuracy and inform clinical decision-making for personalized patient management.

Key Words Prognostic Factors, Breast Cancer, Survival Analysis, Tumor Characteristics, Clinical Outcomes, MMP1

INTRODUCTION

Breast cancer remains one of the most prevalent malignancies worldwide, with survival outcomes significantly influenced by tumor characteristics and molecular biomarkers [1]. The heterogeneity of the disease necessitates comprehensive prognostic models that integrate multiple factors to guide personalized treatment [2]. Prognostic indicators including hormone receptor status human epidermal growth factor Receptor 2 (HER2) expression, tumor grade and pathological features continue to serve as fundamental components in clinical decision-making processes [3]. The integration of multiple survival endpoints, including Disease Progression Free Survival (DPFS), Overall Survival (OS) and Local Progression-Free Survival (LPFS), provides a comprehensive framework for evaluating treatment efficacy and disease progression patterns [4]. These metrics collectively offer

insights into both local disease control and systemic treatment effectiveness, enabling clinicians to make informed decisions regarding therapeutic interventions [5].

Recent advances in biomarker research have highlighted the potential significance of Matrix Metalloproteinases (MMPs) in cancer progression and metastasis [6]. Specifically, MMP1 has emerged as a potential prognostic indicator due to its role in extracellular matrix degradation and tumor invasion processes [7]. Although previous studies have linked MMP1 expressions to poor outcomes in various cancers, its prognostic value in breast cancer particularly in relation to integrative survival analysis incorporating multiple clinical endpoints remains underexplored [8].

The primary objective of this study was to determine whether MMP1 expression is an independent prognostic marker in breast cancer. Secondary objectives included evaluating its correlation with other biomarkers, assessing its impact on different survival endpoints and exploring its potential integration into existing prognostic models.

By focusing on MMP1 within a multidimensional analytical framework, this study aims to provide new insights into its clinical utility and contribute to the evolving paradigm of personalized breast cancer care.

METHODS

This retrospective cohort study was conducted using archived Formalin-Fixed Paraffin-Embedded (FFPE) breast carcinoma tissues from the pathology laboratories of Mansoura University, Egypt and from central lab of Arar Hospital, Saudia Arabia. The study period spanned from September 2023 to September 2024 (ethical approval ref no: 73/44/H).

The available Clinicopathological data was retrieved from the patients' medical records. All data were treated confidentially and clinic-pathological data of these cases were studied regard to age, size, multiplicity, histopathological grade, Metastasis (M) and TNM staging:

- **Inclusion Criteria:** (1) Histologically confirmed primary breast carcinoma, (2) Availability of adequate FFPE tissue blocks and (3) Complete clinicopathological data and follow-up information in the medical records
- **Exclusion Criteria:** (1) Cases with neoadjuvant therapy prior to biopsy, (2) Incomplete medical records or lost to follow-up and (3) Poor-quality tissue samples unsuitable for immunohistochemical analysis

After applying these criteria, a final cohort of 107 patients was included in the analysis. The sample size was determined by the availability of eligible archived specimens and complete clinical records over the defined study period. No formal sample size or power calculation was performed a priori, which may limit the detection of smaller effect sizes and generalizability of the findings.

Cases with any missing data for the key variables listed above were excluded from the final cohort to ensure completeness for statistical modeling.

Serial sections from paraffin embedded blocks of Breast carcinoma tissues were stained with H&E for recording the histopathological features and staging according to WHO 2010 classification and Tumor, lymph Node, Metastasis (TNM 8) system [9].

Immunostaining was performed using Anti- MMP1, mouse monoclonal antibody (1:200, clone, concentrated, California). The stained slides were evaluated blindly to the patients' information. Staining was brown membranous and cytoplasmic and considered positive if >10% of tumor cells or stromal cells showed immunoreactivity. MMP1 expression was evaluated independently by two experienced pathologists blinded to clinical data using standardized scoring criteria. Inter-observer agreement was assessed using Cohen's kappa coefficient. The inter-observer reliability was substantial (κ = 0.76, 95% CI: 0.68-0.84, $p<0.001$), with 88% concordance between observers.

Statistical Analysis

All data analysis were performed using SPSS version 23.0 and R version 3.5.1. A p-value <0.05 was considered statistically significant [10].

Descriptive and Comparative Statistics

Continuous variables (e.g., survival times, age) were summarized using means, medians and standard deviations. Categorical variables (e.g., ER, PR, HER2 status, Tumer grade) were presented as frequences and percentages. Group comparisons for categorical data were made using the Chi-square test or Fisher's exact test, while the independent samples t-test or Mann-Whitney U test was applied for continuous variables, as appropriate.

Survival Analysis

The primary endpoints were Overall Survival (OS), Disease Progression Free Survival (DPFS) and Local Progression Free Survival (LPFS). Kaplan-Meier curves were generated to visualize survival probability over time, with differences between groups assessed via log-rank test. To identify independent prognostic factors, univariate and multivariate Cox proportional hazards regression models were employed, reporting Hazard Ratios (HR) with 95% Confidence Intervals (CI).

Correlation Analysis

Pearson's correlation was used to examine linear relationships between continuous variables, particularly between MMP1 expression levels and the various survival endpoints (OS, DPFS, LPFS), as well as among the endpoints themselves.

Exploration of Variable Relationships

- **Multiple Linear Regression:** We constructed multiple linear regression models to explore the multivariate relationships between several predictor variables (e.g., age, ER, PR, HER2) and key outcomes like tumor size and grade. This approach

was chosen over simple bivariate tests to control for potential confounding and to understand the collective contribution of these factors

- **Hierarchical Cluster Analysis:** As an exploratory technique, average-linkage hierarchical clustering was performed to identify potential natural groupings or patterns within the dataset without a priori hypotheses. This analysis aimed to reveal underlying associations between variables (e.g., MMP1 expression, clinical parameters) that might suggest novel biological relationships for future investigation

RESULTS

The results include 107 patients. Summary statistics for Disease Progression Free Survival (DPFS), Overall Survival (OS), Local Progression-Free Survival (LPFS) and age which is shown in Figure 1.

The DPFS, OS and LPFS average 63.33, 67.24 and 65.76 days suggesting approximately uniformly distributed survival times between the three endpoints. The mean age was 54.65 years. Moderate variability was indicated by the standard deviations for DPFS (41.146), OS (40.391) and LPFS (41.245), while age had a SD of 12.125 year.

Summary statistics of ER, PR, HER2, right or left, focal or multifocal and grade gave an important insight into characteristics of breast cancer. ER was positive in 64% of patients; thus, cancer in the cells had estrogen receptors. PR-positive in 58% of patients, where cancer had the presence of progesterone receptors. Only 19% came back as HER2 positive, which means a lower prevalence of this aggressive growth factor receptor. The focality had a mean of 1.03, hence most of the tumors were localized and focal; grade had a mean of 2.06, meaning cancer cells are moderately differentiated. These were further supported by the low standard deviations across these variables, with little variation in these patient characteristics (Table 1 and 2).

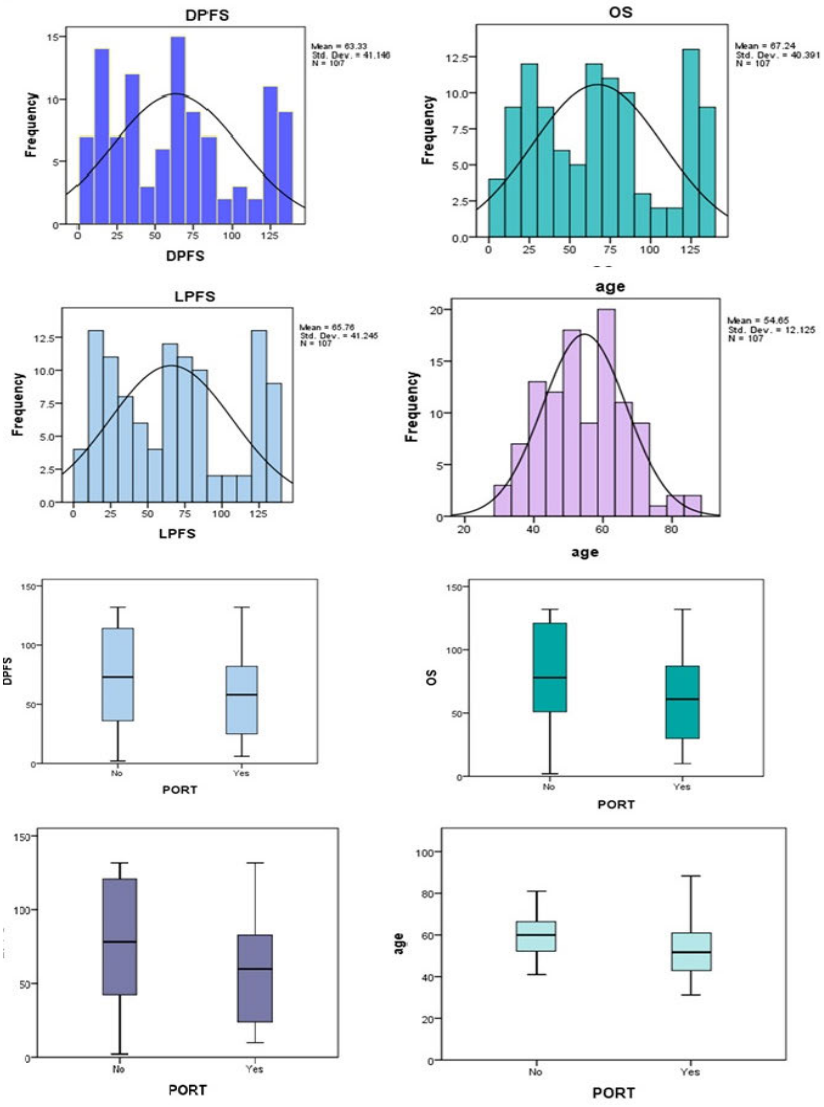

Figure 1: Histograms and Boxplots of All Continuous Variables of the Patients OS = Overall Survival, DPFS = Disease Progression Free Survival, LPFS = Local Progression-Free Survival

Table 1: Summary or Descriptive Statistics for ER, PR, HER2

	ER	PR	HER2	rt_lt	Focal	Grade
N Valid	107.0	107.0	107.0	107.0	107.0	107.0
N Missing	0.0	0.0	0.0	0.0	0.0	0.0
Mean	0.64	0.58	0.19	1.5	1.03	2.06
Median	1.0	1.0	0.0	1.0	1.0	2.0
Std. Deviation	0.484	0.496	0.392	0.502	0.166	0.332
Range	1.0	1.0	1.0	1.0	1.0	2.0
Minimum	0.0	0.0	0.0	0.0	1.0	1.0
Maximum	1.0	1.0	1.0	2.0	2.0	3.0

Er = Estrogen Receptor, PR = Progestron Receptor, HER2 = Human Epidermal Growth Factor Receptor 2, rt lt = right or left

Table 2: Frequency Distribution

		Frequency	Percent	Valid Percent	Cumulative Percent
ER Valid	Negative	39.0	36.4	36.4	36.4
	Positive	68.0	63.6	63.6	100.0
	Total	107	100.0	100.0	
PR Valid	Negative	45.0	42.1	42.1	42.1
	Positive	62.0	57.9	57.9	100.0
	Total	107	100.0	100.0	
HER2 Valid	Negative	87.0	81.3	81.3	81.3
	Positive	20.0	18.7	18.7	100.0
	Total	107	100.0	100.0	
rt_lt valid	LT	54.0	50.5	50.5	50.5
	RT	53.0	49.5	49.5	100.0
	Total	107	100.0	100.0	
Focal Valid	Unifocal	104	97.2	97.2	97.2
	Multifocal	3	2.8	2.8	100
	Total	107	100	100	
Grade valid	1	3	2.8	2.8	2.8
	2	95	88.8	88.8	91.6
	3	9	8.4	8.4	100
	Total	107	100		

Er = Estrogen receptor, PR = Progestron Receptor, HER2 = Human epidermal growth factor receptor 2, rt lt = right or left

Table 3: HER2 and ER Cross Tabulation

	ER Negative	ER Positive	Total
HER2 negative	25	62	87
HER2 positive	14	6	20
Total	39	68	107

Table 4: The Chi-Square Test of Association

	Value	df	Asymp. Sig. (2-sided)	Exact Sig.(1-sided)
Pearson Chi-Square	11.954[a]	1	0.001	0.001
Continuity Correctionb	10.239	1	0.001	
Likelihood Ratio	11.580	1	0.001	
Fisher's Exact Test			0.001	
Linear-by-Linear Association	11.842	1	0.001	
N of Valid Cases	107			

df = degrees of freedom, a = 0 cells (0.0%) have expected count less than 5. The minimum expected count is 7.29

We tested association between HER2 and ER with the help of chi-square test (Table 3 and 4). There was found to be a strong relationship between HER2 and ER biomarkers with 107 patients. HER2 Of these, 62 out of 87 HER2 negative patients had positive ER status, indicative of estrogen-dependent growth. The Chi-Square test gave $\chi^2 = 11.954$, df = 1, p = 0.001. Fisher's Exact Test yielded the same result with p = 0.001\. This would suggest a strong interaction between HER2 and ER status in terms of the different modes of treatment used based on these biomarkers.

Kaplan-Meier Survival Curves

Kaplan-Meier survival curves indicate the percentage of subjects that survived beyond a certain point in time and is a prediction of the survival probability with time. Results showed the estimated Overall Sursvival (OS), Relapse-Free Survival (RFS) and Distant Metastasis-Free Survival (DMFS).

The overall survival curves of the patients described a decrease in survival probability with increasing time (Figure 2). It was very high at 99.1 percent at the beginning of 2 months, then steadily decreasing to 78.5 percent at 18 months and finally to 65.4 percent in 36 months. By 60 months, it had decreased further to 44.9 percent. The declined continued at 108 months to 22.4 percent. Results data showed an abrupt decline in survival at the early stages, followed by further gradual decreases with time. This points out the trend that most events occur in the first few years, but risks run at a lower rate for a longer period. It is these survival curves which highlight the need for long-term monitoring with possible adjustment to treatment strategies for the management of patient outcome.

Cox Regression

A Cox regression model of survival times that included several predictors, including ER, HER2, PR and grade. Detailed results showed that all the predictors were insignificant to OS (Table 5-6 and Figure 3). Specifically, ER had a coefficient of -0.551 with a p-value of 0.246, so this variable did not have a strong impact on survival.

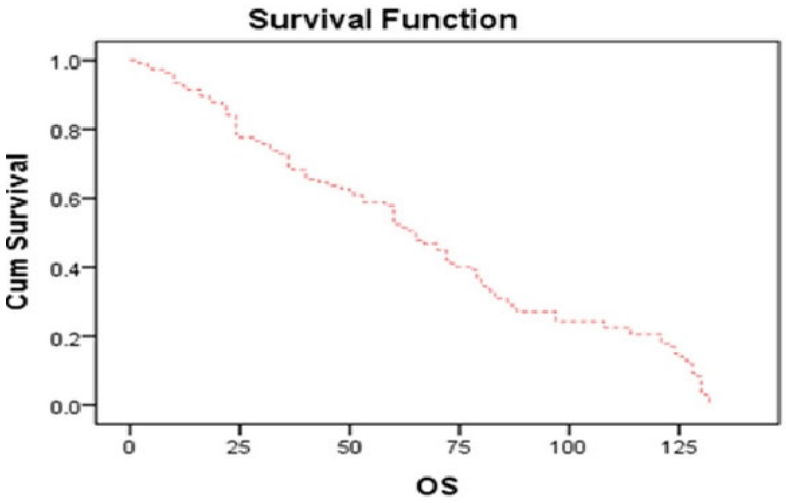

Figure 2: Survival Plots, OS = Overall Survival

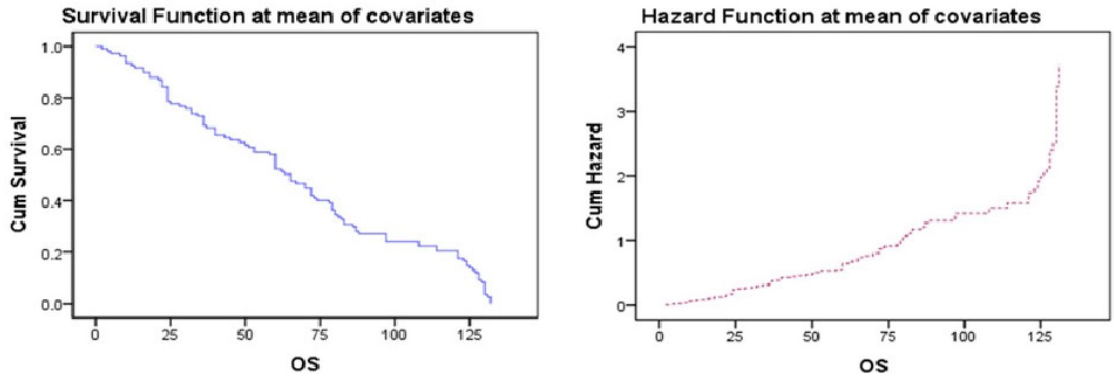

Figure 3: Survival and Hazard Rate Function Curve

Table 5: Variables in the Equation

	Coefficient	SE	Wald	df	Sig.	Exp(B)
ER	-0.551	0.474	1.349	1	0.246	0.577
HER2	0.043	0.284	0.023	1	0.880	1.044
PR	0.338	0.455	0.552	1	0.457	1.402
grade	-0.046	0.292	0.025	1	0.875	0.955

Er = estrogen receptor, PR = Progestron Receptor, HER2 = human epidermal growth factor receptor, SE- Standard Error, Wald = Wald test, df = Degrees of freedom, Sig = Significance, Exp(B)- exponentiated value of the regression coefficient (B)

Table 6: Covariate Means

Covariate	Mean
ER	0.636
HER2	0.187
PR	0.579
Grade	2.056

Er = estrogen receptor, PR = Progestron Receptor, HER2 = human epidermal growth factor receptor

HER2 had a very small coefficient with a p-value of 0.880, which had a very weak effect on OS. Besides, PR with a coefficient of 0.338 and a p-value of 0.457 and grade all did not have significant associations with survival outcome. All the hazard ratios (Exp (B)) did make some sense in terms of potential risks: ER, 0.577, seems to be at risk in a lowered manner; HER2, at 1.044 and PR, at 1.402, showed an increase in risk. Overall, the said model could not finalize any significant predictors for OS, thereby suggesting the need for other variables or added factors, with a view to gaining more insight into survival.

The numerical value represents the relationship was correlation coefficient which always falls between -1 and +1. The correlation coefficient close to ±1 indicate strong correlation (Table 7 and Figure 4).

MMP1 was significantly correlated with all the survival parameters. It reflected a quite high negative correlation with Disease-Progression Free Survival (DPFS): r = -0.433, p<0.001; with Overall Survival (OS): r = -0.379, p<0.001; and with Local Progression-Free Survival (LPFS): r = -0.401, p<0.001). This means that, in general, high levels of MMP1 correlate with poor survival outcomes, pointing to MMP1 measurements being useful as negative predictive markers. In contrast, LPFS, DPFS and OS were very positively correlated with one another.

Table 7: Shows the Degree of Linear Relationship between Two Variables Called Correlations

		MMP1	DPFS	OS	LPFS	Age
MMP1	Pearson Correlation	1	-0.433**	-0.379**	-0.401**	-0.080
	Sig. (2-tailed)		0.000	0.000	0.000	0.412
	N	107	107	107	107	107
DPFS	Pearson Correlation	-0.433**	1	0.970**	0.971**	0.020
	Sig. (2-tailed)	0.000		0.000	0.000	0.841
	N	107	107	107	107	107
OS	Pearson Correlation	-0.379**	0.970**	1	0.989**	-0.022
	Sig. (2-tailed)	0.000	0.000		0.0.000	0.820
	N	107	107	107	107	107
LPFS	Pearson Correlation	-0.401**	0.971**	0.989**	1	-0.010
	Sig. (2-tailed)	0.000	0.000	0.000		0.919
	N	107	107	107	107	107
Age	Pearson Correlation	-0.080	-0.020	-0.022	-0.010	1
	Sig. (2-tailed)	0.412	0.841	0.820	0.919	
	N	107	107	107	107	107

**.Correlation is significant at the 0.01 level (2-tailed), DPFS = Disease-Progression Free Survival (DPFS), OS = Overall Survival, LPFS = Local Progression-Free Survival, p value at <0.05 was set to be significant

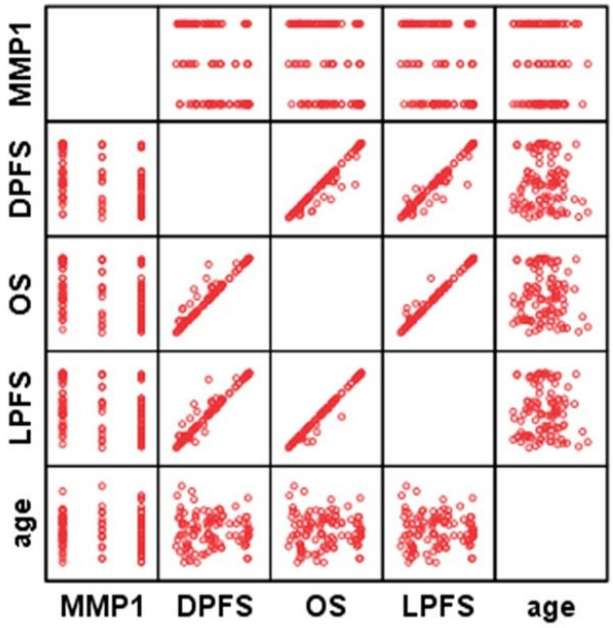

Figure 4: Scatter Plot Matrix. DPFS = Disease-Progression Free Survival (DPFS), OS = Overall Survival, LPFS = Local Progression-Free Survival

To be more specific, LPFS both strongly correlated with DPFS and OS, wherein r = 0.971 and p<0.001 and r = 0.989 and p<0.001, respectively; DPFS was similarly very strongly correlated with OS, wherein r = 0.970 and p<0.001. This establishes a relationship wherein improvements in local progression-free and disease-free survival go with better overall survival. It showed that age is negligibly correlated with MMP1, LPFS, DPFS and OS, suggesting that age is not a key factor that influences these survival metrics.

Cluster Analysis

It is a statistical technique aimed at grouping connected data points into clusters. It tries to maximize the similarity within the clusters and reduces it between the clusters in search of patterns or structures in the data without previous labels or classifications (Table 8 and 9).

Table 8: Case Processing Summary

Cases					
Valid		Missing		Total	
N	Percent	N	Percent	N	Percent
107	100.0	0	0.0	107	100

a. Squared Euclidean Distance used

Average Linkage (Between Groups)

Statistical Inference

Tumor size and MMP1 levels on different outcomes were analyzed (Table 10). In the case of tumor size, no statistically significant difference in Overall Survival, Disease Progression-Free Survival and Local Progression-Free Survival had been shown, with corresponding p-values equal to 0.220, 0.112 and 0.237. This proves that tumor size did not have any effect significantly bearing on these measured survival outcomes. Age, however, has a large p-value of 0.030, indicating that fluctuations in age with different tumor sizes could affect survival outcomes. The analysis done on the MMP1 level showed considerable differences in OS, DPFS and LPFS; p-values all at 0.000, which means that MMP1 levels are strongly relevant towards these survival outcomes. This makes this factor significant in general as a predictor for overall and disease-free survival. On the other hand, age effect was not significant based on MMP1 levels, hence p = 0.643. Thus, while tumor size had limited bearing on survival outcomes, MMP1 had been a crucial factor in predicting survival metrics.

Independent t-Test

An independent sample t-test of OS among ER and focal with large sample size (Table 11).

Independent samples tests for OS by ER and focal demonstrated no significant differences between groups. For ER, with Levene's test p = 0.181 and t-tests p = 0.131 under equal variances assumed and p = 0.144 under unequal variances, it can be shown that different levels of ER did not have a significant effect on OS. For focal status in relation to OS, Levene's test with a p-value of 0.723 and t-tests with p = 0.637 under equal variances assumed and p = 0.738 under unequal variances did not show any significant influence.

Table 9: Agglomeration Schedule

Stage	Cluster Combined		Coefficients	Stage Cluster First Appears		Next Stage
	Cluster 1	Cluster 2		Cluster 1	Cluster 2	
1	2	4	10.000	0	0	2
2	1	2	53.000	0	1	3
3	1	5	108.000	2	0	4
4	1	3	182.500	3	0	8
5	7	8	4123.000	0	0	6
6	6	7	11777.500	0	5	7
7	6	9	207701.000	6	0	8
8	1	6	546121.750	4	7	0

Table 10: ANOVA BY Tumor Size and MMP1

	Sum of Squares	df	Mean Square	F	Sig.
OS Between Groups	7215.754	3	2405.251	1.495	0.220
OS Within Groups	165717.928	103	1608.912		
OS Total	172933.682	106			
DPFS Between Groups	10110.241	3	3370.080	2.050	0.112
DPFS Within Groups	169347.310	103	1644.149		
DPFS Total	179457.551	106			
LPFS Between Groups	7225.908	3	2408.636	1.433	0.237
LPFS Within Groups	173095.775	103	1680.542		
LPFS Total	180321.682	106			
Age Between Groups	1292.497	3	430.832	3.105	0.030
Age Within Groups	14291.709	103	138.754		
Age Total	15584.206	106			
OS (MMP1) Between Groups	24816.665	2	12408.332	8.712	0.000
OS (MMP1) Within Groups	148117.017	104	1424.202		
OS (MMP1) Total	172933.682	106			
DPFS (MMP1) Between Groups	33944.615	2	16972.308	12.130	0.000
DPFS (MMP1) Within Groups	145512.936	104	1399.163		
DPFS (MMP1) Total	179457.551	106			
LPFS (MMP1) Between Groups	29163.851	2	14581.926	10.033	0.000
LPFS (MMP1) Within Groups	151157.831	104	1453.441		
LPFS (MMP1) Total	180321.682	106			
Age (MMP1) Between Groups	131.649	2	65.824	0.443	0.643
Age (MMP1) Within Groups	15452.557	104	148.582		
Age (MMP1) Total	15584.206	106			

Table 11: Independent Samples Test by ER

	OS		
		Equal variances assumed	Equal variances not assumed
Levene's Test for Equality of Variances	F	1.811	-1.478
	Sig.	0.181	
t-test for Equality of Means	t	-1.524	
	df	105	72.285
	Sig. (2-tailed)	.131	0 .144
	Mean Difference	-12.285	-12.285
	Std. Error Difference	8.063	8.311
95% Confidence Interval of the Difference	Lower	-28.272	-28.850
	Upper	3.703	4.281

The mean difference was 11.224 and the confidence intervals were very wide: from −35.850 to 58.298 and −110.778 to 133.227. This means high variability without an obvious effect. Thus, ER or focal status had no significant effect on OS.

Regression

The multiple linear regression models (Table 12), taking tumor size and grade as response variables. If one variable was used as independent variable the regression was known as simple, if more than one variable were involved, the regression was said to be multiple.

A regression analysis predicting tumor size takes HER2, age, PR and ER as predictors. The model R^2 was 0.056 and the adjusted R^2 was 0.019. This shows that only a very small fraction of the variance in tumor size was accounted for by these variables. The results in ANOVA indicated that the overall model turned out to be insignificant, with $F = 1.502$ and $p = 0.207$, which suggests the fact that overall, these predictors do not affect tumor size in a meaningful manner. Considering individual predictors, age and HER2 are insignificant with p-values of 0.683 and 0.556, respectively, thus adding nothing to the prediction of tumor size. In contrast, PR has a highly positive effect, $\beta = 0.489$, at $p = 0.023$, which may be interpreted to associate higher PR levels with larger tumors. ER had a marginal effect of $\beta = -0.380$ at $p = 0.083$, indicating some negative relation with tumor size, though it was not statistically significant. Overall, this model explained little and needs additional factors or other models for the better prediction of tumor size.

Table 12: Model Summary: Grad as Response Variable

	R	R Square		Adjusted R Square	Std. Error of the Estimate
Model	0.236[a]	0.056		0.019	0.576
a. Predictors: Constant, HER2, Age, PR,ERAnova[a]					
Model	Sum of Squares	df	Mean Square	F	Sig.
Regression	1.996	4	0.499	1.502	0.207[b]
Residual	33.892	102	0.332		
Total	35.888	106			
a:Dependent Variable: Tumor Size, b: Predictors: (Constant),					
HER2, age, PR, ER , Coefficient[a], a: Dependent Variable: Grade					
	Unstandardized Coefficients		Standardized Coefficients		
Model	B	Std. Error	Beta	t	Sig.
(Constant)	2.173	0.273		7.960	0.000
age	0.002	0.005	0.040	0.409	0.683
ER	-0.457	0.261	-0.380	-1.751	0.083
PR	0.574	0.249	0.489	2.307	0.023
HER2	0.090	0.152	0.060	0.590	0.556

Table 13: Anova; a: Dependent Variable: Grade, b: Predictors: (Constant), HER2, Age, PR, ER

Model	Sum of Squares	df	Mean Square	F	Sig.
Regression	0.810	4	0.203	1.904	0.115b
Residual	10.853	102	0.106		
Total	11.664	106			
Coefficients[a]					
	Unstandardized Coefficients		Standardized Coefficients		
Model	B	Std. Error		t	Sig.
(Constant)	2.335	0.155	Beta	15.111	0.000
age	-0.006	0.003	-0.204	-2.117	0.037
ER	0.175	0.148	0.255	1.184	0.239
PR	-0.183	0.141	-0.273	-1.299	0.197
HER2	0.117	0.086	0.138	1.355	0.178

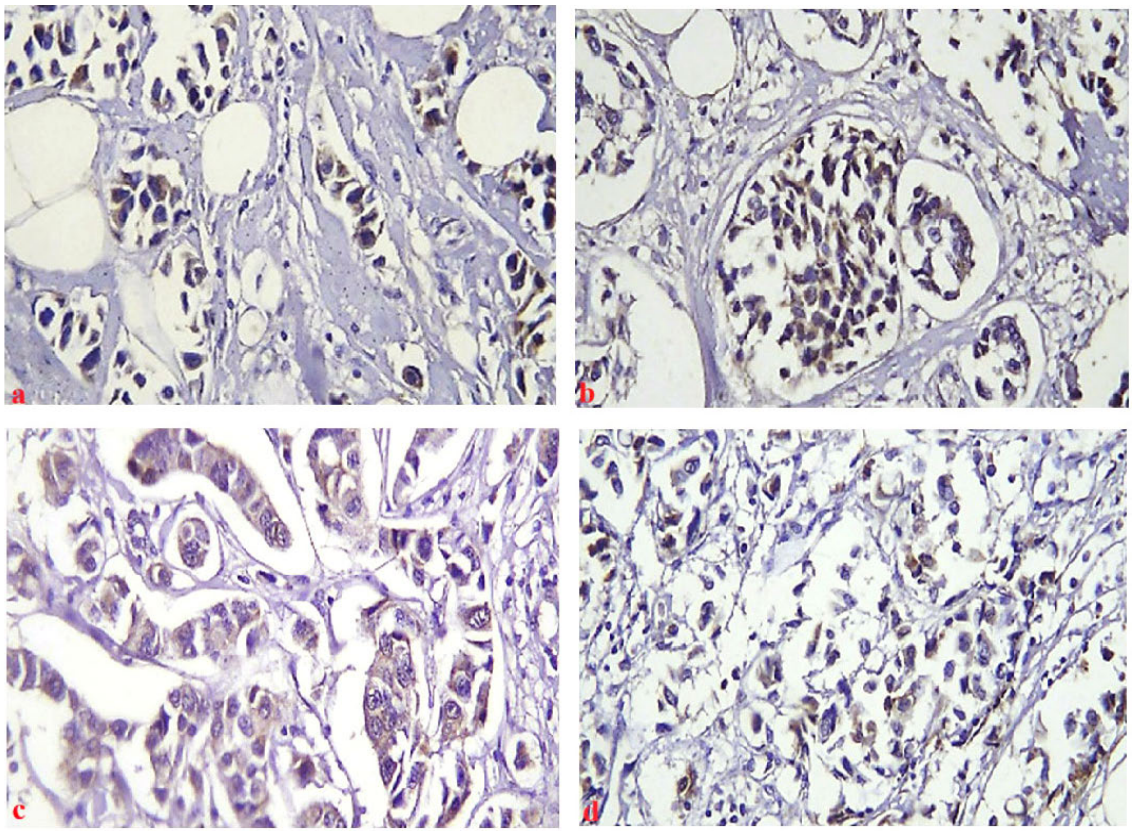

Figure 5(a-d): Immunohistochemical Staining of MMP-1 in Carcinoma Breast (a) Immunohistochemical Staining of MMP-1 in Infiltrating Ductal Carcinoma of Breast. The Staining is observed both in Nuclei and in Cytoplasm. (b) Breast Carcinoma Cells Showing Positive MMP-1 Nuclear Staining (c) Showing Positive Cytoplasm Staining (d) Showing Positive Tumor Cells and Cancer Associated Stromal Cells

The R^2 of the regression analysis for the prediction of the tumor grade using HER2, age, PR and ER as predictors is 0.069 and the adjusted R^2 is 0.033, which means that only a small portion of variance on tumor grade is explained by the model. From the results of the ANOVA (Table 13), this overall model was not significant, $F = 1.904$, $p = 0.115$, indicating that these predictors as a block don't impact tumor grade significantly. Of the individual predictors, only age has a significant effect, negative, with $\beta = -0.204$ ($p = 0.037$), which means older age is related to a lower grade of tumor. On the other hand, ER, PR and HER2 did not have any significant effect since their p-values were above the standard threshold significance level of 0.05, $p = 0.239$, $p = 0.197$ and $p = 0.178$, respectively. In other words, even though age showed some relevance to the grade of the tumor, no other predictor did indeed affect it significantly. The very low explanatory power of the model indicates that more factors should be brought into the model to explain tumor grading.

Immunohistochemical Expression of MMP-1 in Breast Cancer Tissues

MMP-1 staining was assessed from the breast cancer patients' biopsies (Figure 5a–d). Two observers independently determined the number of immunopositive tumor cells per 100 malignant cells (0–100%) from three distinct cell-rich locations using a 40 × objective for MMP-1 expression. Nuclear and/or cytoplasmic staining, or both, were present in tumor cells that tested positive.

DISCUSSION

When predicting the course of breast cancer, a combination of tumor size and grade, the proliferation index Ki-67, hormone receptor status, HER2 expression, lymph node status and patient age are considered. Furthermore, it has been demonstrated that the expression of several markers, such as p53 and bcl-2, is related to survival. Morphological characteristics are the basis for the current classification of breast cancer [2-5]. Five different subclasses have been found using more recent methods of molecular categorization by gene expression profiling associated with the new treatment options and survival outcomes [6, 7].

This study provided valuable insights into the prognostic landscape of breast cancer survival outcomes. The strong inter correlations observed between Disease Progression Free Survival (DPFS), Overall Survival (OS) and Local Progression-Free Survival (LPFS) aligned with established understanding that these survival endpoints are fundamentally interconnected in breast cancer prognosis. This finding corroborates previous large-scale studies demonstrating that disease-free survival serves as a reliable surrogate endpoint for overall survival in breast cancer clinical trials [1,11]. The mean age of 54.65 years (SD = 12.125) aligned with epidemiological data showing breast cancer incidence peaks in the fifth and sixth decades of life. The relatively normal distribution of age in the current study provides a representative sample for generalizing findings to typical breast cancer populations [1-4].

Matrix metalloproteinases play crucial roles in extracellular matrix degradation, facilitating tumor invasion and metastatic spread [12]. The identification of Matrix Metalloproteinase-1 (MMP1) as a significant negative prognostic indicator across all survival endpoints represents a noteworthy finding of this study. The consistent negative correlations observed support the growing body of evidence implicating MMP1 in breast cancer progression and metastasis. Previous studies have demonstrated elevated MMP1 expression in aggressive breast cancer subtypes, with higher levels associated with poor prognosis and increased metastatic potential [13]. Most investigations have evaluated MMP1 in isolation, focused on a single survival endpoint, or lacked integration with a comprehensive panel of clinicopathological variables. This work advances this field by performing an *integrative survival analysis* that simultaneously correlates MMP1 expression with three key clinical endpoints OS, DPFS and LPFS within the same cohort. Furthermore, we contextualize MMP1 within the established prognostic framework by analyzing its relationship with hormone receptor status, HER2, tumor grade and other characteristics. The strong correlation with survival outcomes observed in this cohort suggests that MMP1 expression may serve as a valuable addition to existing prognostic panels. This finding is particularly relevant given the current emphasis on developing personalized treatment strategies based on tumor biology rather than relying solely on traditional staging systems [13,14]. The significant association between HER2 and Estrogen Receptor (ER) status observed in this study reflects the well-established molecular heterogeneity of breast cancer. This finding aligns with the similar studies done earlier which first described the molecular subtypes of breast cancer based on gene expression profiling [15-17]. The inverse relationship between HER2 overexpression and hormone receptor positivity has important therapeutic implications, as HER2-positive tumors typically exhibit different responses to hormonal therapy and targeted agents [18, 19].

The identification of distinct biological subtypes through biomarker profiling has revolutionized breast cancer treatment, enabling the development of targeted therapies such as trastuzumab for HER2-positive disease and endocrine therapy for hormone receptor-positive tumors [20]. The present findings reinforce the importance of comprehensive biomarker testing in routine clinical practice to guide treatment decisions and improve patient outcomes.

The analysis of continuous variables revealed notably similar mean survival times across the three primary endpoints: DFS, OS and LPFS. This convergence suggests a high degree of interdependence among these survival metrics, which was later confirmed through correlation analysis. The prevalence of ER positivity (63.6%) and PR positivity (57.9%) is consistent with global breast cancer statistics, where approximately 60-75% of breast cancers express hormone receptors [21,22]. This finding has

important therapeutic implications, as hormone receptor-positive tumors generally respond to endocrine therapy and often have better prognosis than triple-negative breast cancers. HER2-positive tumors historically represented a more aggressive phenotype; however, the advent of targeted HER2 therapies has dramatically improved outcomes for these patients [23]. Tumor Laterality and Focality. The overwhelming predominance of unifocal tumors (97.2%) versus multifocal tumors (2.8%) has prognostic significance, as multifocal disease often correlates with higher recurrence risk and may influence surgical decision-making [24-26].

Despite the well-established prognostic value of traditional biomarkers such as ER, PR and HER2 status in numerous large-scale studies [23,27], these markers showed limited individual prognostic value in this multivariate analysis. This apparent discrepancy may be attributed to several factors, including the relatively small sample size (n = 107) compared to large registry studies, potential selection bias inherent in single-institution retrospective analyses and the complex interplay between multiple prognostic factors that may mask individual effects in smaller cohorts. The significant inverse relationship between HER2 and ER status corroborates well-established molecular subtypes in breast cancer. The finding that 62 of 87 HER2-negative patients were ER-positive, while 14 of 20 HER2-positive patients were ER-negative, reflects the biological tendency for these pathways to be mutually exclusive. This inverse relationship has important implications for treatment selection, as it helps identify distinct molecular subtypes requiring different therapeutic approaches [28].

This association supports the molecular classification of breast cancer into luminal (ER+/HER2-), HER2-enriched (ER-/HER2+) and other subtypes, each with distinct biological behaviors and treatment responses. The strong statistical significance reinforces the reliability of these biomarkers in clinical stratification [29]. The Kaplan-Meier survival cures reveal a characteristic pattern of breast cancer mortality. The initial high survival probability followed by steady decline reflects the typical trajectory of breast cancer outcomes. The continued decline underscores the chronic nature of breast cancer risk, with late recurrences remaining a persistent concern even years after initial treatment [30,31].

The steeper initial decline followed by a more gradual decrease suggests two distinct phases of risk: an early period of higher vulnerability, likely representing patients with aggressive disease or inadequate treatment response, followed by a prolonged period of lower but persistent risk among survivors. This biphasic pattern has important implications for surveillance strategies and long-term follow-up protocols [32]. In this work hierarchical clustering revealed interesting groupings that suggest underlying biological relationships.

The regression model for tumor grade similarly showed limited explanatory power with age as the only significant predictor. The negative association between age and tumor grade is intriguing, suggesting younger patients may be present with higher-grade tumors. This finding aligns with observations that breast cancer in younger women often exhibits more aggressive features [33,34]. The non-significance of hormone receptors and HER2 in predicting grade may reflect the complex, non-linear relationships between these variables or the limited grade variability in the present study.

The study highlights the complexity of breast cancer prognostication and the limitations of traditional biomarkers when used in isolation. The emergence of MMP1 as a strong prognostic factor suggests the need to expand beyond conventional markers to improve risk stratification. The high interdependence of survival metrics supports streamlined endpoint selection in clinical trials [35-37]. The inverse relationship between HER2 and ER status reinforces the importance of comprehensive biomarker profiling for treatment selection. The lack of significant associations in multivariate models in the present study emphasizes that treatment decisions should consider the full clinical context rather than individual biomarkers in isolation.

The Kaplan-Meier curves in this study showing continued risk even at 108 months underscore the need for long-term surveillance. The biphasic risk pattern suggests surveillance intensity could be tailored to time since diagnosis, with more intensive monitoring in the first 3 years followed by continued but less frequent assessment thereafter [36-38].

The Early Breast Cancer Trialists' Collaborative Group meta-analyses, encompassing thousands of patients, have consistently demonstrated the prognostic significance of hormone receptor status and the predictive value for treatment response [39]. The apparent lack of individual prognostic significance in the current study should not diminish the established clinical utility of these biomarkers but rather highlights the need for larger sample sizes to detect moderate effect sizes in multivariate models.

The findings from this study support the growing trend toward comprehensive molecular profiling in breast cancer management. The emergence of multi-gene assays such as Oncotype DX, Mamma Print and Prosigna has demonstrated the clinical utility of incorporating multiple biomarkers for prognostic assessment and treatment decision-making [37,39-40]. The identification of MMP1 as a potential prognostic marker suggests that expanding current biomarker panels to include novel targets may further refine risk stratification.

Future research directions should focus on validating MMP1 as a prognostic biomarker in larger, multi-institutional cohorts and exploring its potential as a therapeutic target. Recent advances in MMP inhibitor development, despite historical challenges with toxicity and specificity, may provide new therapeutic opportunities for patients with MMP1-overexpressing tumors [41-43].

The current study reinforces the complex, multifactorial nature of breast cancer prognosis while identifying MMP1 as a promising biomarker for risk stratification. The findings support the continued evolution toward personalized medicine approaches in breast cancer management, emphasizing comprehensive biomarker profiling over reliance on single prognostic factors. The significant association between HER2 and ER status underscores the importance of molecular subtyping in treatment planning [35-39].

As precision medicine continues to advance, the integration of novel biomarkers such as MMP1 with established prognostic factors may enhance the ability to predict patient outcomes and optimize treatment strategies.

CONCLUSION

This study demonstrates theat eleveated MMP! Expression is a significant predictor of poor survival outcomes in breast cancer, independent of traditional clinicopathological factors. The integrative analysis of multiple survival endpoints strengthens the evidence for MMP1 as a robust prognostic marker. Further validation in larger, prospective cohorts is essential before MMP1 can be considered for integration into standard clinical prognostic models. Ultimately, this work supports the ongoing pursuit of novel biomarkers to improve personalized risk assessment and treatment strategies in breast cancer.

Limitations

This study has some limitations that should be considered when interpreting the results. First, its retrospetctive design may introduce selection bias and restrict the availability of certain clinical data. Second, the modest sample size (n = 107) and lack of an a priori power calculation may reduce the ability to detect smaller but clinically meaningful effects, particularly in multivariate models. Finally, while immunohistochemistry is a clinically accessible method, semiquantitative scoring of MMP1 expression may be subjected to inter-observer variability, despite efforts to standardize assessment. Future prospective, multi-institutional studies with larger cohorts are needed to validate these findings and explore the potential of MMP1 as a routine prognostic biomarker.

Despite these limitations, the study's strengths include comprehensive survival endpoint analysis, robust statistical methodology employing Kaplan-Meier survival analysis and Cox proportional hazards regression and the identification of a novel prognostic biomarker. The strong correlations between survival endpoints confidence in the reliability of the findings.

Conflicts of Interest

The authors declare no conflict of interest.

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