ORIGINAL ARTICLE

Bali Medical Journal (*Bali MedJ*) 2024, Volume 13, Number 1: 337-341
P-ISSN.2089-1180, E-ISSN: 2302-2914

Matrix Metalloproteinase as Predictors of Local Recurrence in Early-Stage Breast Cancer After Mastectomy and Chemotherapy A Cross-Sectional Study in Single Centre

Andreas Andrianto[1], I Ketut Sudiana[2*], Desak G.A Suprabawati[3]

[1]Doctoral Program of Medical Science, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia
[2]Department of Pathology Anatomy, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia
[3]Division of Oncology, Department of Surgery, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

*Corresponding to:
I Ketut Sudiana; Department of Pathology Anatomy, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia;
i-ketut-s@fk.unair.ac.id

Received: 2023-10-10
Accepted: 2023-11-29
Published: 2023-12-28

ABSTRACT

Introduction: The prognosis for early-stage breast cancer is generally favourable. However, in cases when a recurrence manifests after therapy, the prognosis becomes significantly poor. To determine early breast cancer recurrence risk, a marker is needed. Currently, previous tumour markers are used to predict general breast cancer, lacking specificity in the context of early breast cancer. Matrix Metalloproteinase1 (MMP1) is already acquainted with the local recurrence process of invasive ductal carcinoma (IDC). MMP1 expression may better predict early recurrence in early-stage IDC than other approaches. This research examined the potential predictive value of MMP1 expression in early-stage IDC for early local recurrence and its correlation with clinicopathological factors.

Methods: This study was a cross-sectional analysis of a single center's medical records. The sample size was 25 subjects for the group without recurrence and 25 subjects with recurrence. Mastectomy and chemotherapy were performed from January 2014 through December 2019. Using immunohistochemistry, paraffin blocks of surgical specimens were examined for MMP1. The data processing was done utilising the trial version of IBM SPSS 25. and Openepi 3.0 programs and analysed using Bivariate, which is performed using the Chi-square test or Fisher's exact test.

Result: The statistic test showed a significant difference ($p < 0.000$) in the average expression of MMP1 for non-recurrence cases (3,95±5,36) compared to recurrence cases (14,95±5,36). We also found a statistically significant correlation between recurrence and high expression of MMP1 (odd ratio [OR]= 60.38, 95% confidence interval [CI]=10.01-364.3, $p<0.001$). Clinicopathological variables and MMP1 expression did not have a significant correlation.

Conclusions: In early-stage IDC, MMP1 expression had the potential to predict and could be an independent prognostic factor for early recurrence.

Keywords: Ductal Carcinoma, early recurrence, MMP1.
Cite This Article: Andrianto, A.A., Sudiana, I.K., Suprabawati, D.G.A. 2024. Matrix Metalloproteinase as Predictors of Local Recurrence in Early-Stage Breast Cancer After Mastectomy and Chemotherapy. A Cross-Sectional Study in Single Centre. *Bali Medical Journal* 13(1): 337-341. DOI: 10.15562/bmj.v13i1.5035

INTRODUCTION

Worldwide and in Indonesia, breast cancer continues to be a substantial public health concern. Breast cancer is the most commonly detected type of malignancy. The incidence rate in Indonesia stands at 42.1 per 100,000 individuals. The disease in question is correlated with an average mortality rate of 17 per 100,000 individuals.[1] The two most common histological types are invasive ductal carcinoma (IDC) at 80% and invasive lobular carcinoma (ILC) at 15%.[2] Only about 20-30% of breast cancer patients come to the physician at an early stage, while around 50-70% of breast cancer patients come in an advanced stage.[3]

Present breast cancer management is based on the concept of personalised therapy, in which every patient is attended to according to their specific requirements. The selection of therapy modality and time frame is determined by the tumour's characteristics and the particular stage of breast cancer that is being treated. To optimise the management of breast cancer and minimise the risks of inadequate or excessive treatment, several therapy protocols are frequently implemented. Early-stage breast cancer that receives appropriate treatment has a favourable prognosis. However, the prognosis significantly worsens in cases of recurrence following sufficient treatment. The five-year survival rate for recurred patients ranges from 35% to 59%, which is similar to that of patients with distant metastases from breast cancer. Recurrence is most prevalent during the initial two years following the primary surgical procedure.[4] Adjuvant chemotherapy is one endeavour to decrease the probability of recurrence. In early-stage breast cancer, adjuvant chemotherapy is administered to decrease the likelihood of micrometastasis. The Early Breast Cancer Trialists Collaborative Group (EBCTG) study demonstrated that patients with early-stage breast cancer who receive adjuvant chemotherapy have a greater chance of achieving disease-free survival

and overall survival.[5] The chemotherapy regimen usually uses a combination of anthracycline and taxane groups.[6]

To ensure their survival, cancer cells must possess the ability to withstand the body's defence mechanisms and chemotherapy drugs. Cancer cells are capable of changing the tumour microenvironment to favour survival. One marker widely studied for its correlation with recurrence and resistance to chemotherapy drugs is matrix metalloproteinases (MMPs). MMPs are part of the *zinc-dependent* endopeptidase group, which comprises > 21 types in humans. Matrix metalloproteinases (MMPs) are initially produced as biologically inactive zymogens and then undergo activation through proteinase cleavage. MMP activity is regulated by endogenous inhibitors such as α2 macroglobulin, tissue inhibitors of metalloproteinases (TIMPs), and reversion-inducing cysteine-rich protein with kazal motifs (RECK).[7]

The primary function of MMPs is regulating various cell behaviours related to the biological behaviour of cancer cells. Cancer cell functions encompass a range of processes, including proliferation, differentiation, apoptosis, invasion and migration, regulation of tumour angiogenesis, and defence against immune cells of the host. Among the several forms of matrix metalloproteinases (MMPs), MMP1 has been found to exhibit the strongest association with breast cancer.[8] The overexpression of MMP1 has been observed to diminish the efficacy of chemotherapy agents by enhancing the activity of the TGF-β pathway, particularly in conjunction with cancer-associated fibroblasts (CAF). This collaborative impact leads to increased production of collagen IV by cancer cells, ultimately reducing the effectiveness of chemotherapy drugs. MMP1 can induce a more aggressive phenotype by cleaving proapoptotic factors by generating apoptosis-resistant cells.[9]

This study aimed to assess the potential of MMP1 expression as a predictive factor for early local recurrence in early-stage IDC and to explore the correlation between MMP1 and clinicopathological characteristics.

METHODS

A cross-sectional analytical observational study design was used for this research. The research data was obtained from medical record records at the surgical department of dr. H Koesnadi Bondowoso hospital. The samples of this study were patients with ductal-type invasive breast carcinoma who performed modified *radical mastectomy* (MRM) and adjuvant chemotherapy with taxane and anthracycline-based regimen at dr H Koesnadi Bondowoso Hospital. The researcher himself carries out all surgical procedures.

The groups with and without local recurrence sample sizes were 25 for each. The sample size estimation is predicated on the bare minimum of 25 samples required for regression analysis.[10] This research has received ethical approval from the Research Ethics Committee of the Faculty of Medicine, University of Jember, with number 1.537/H25.1.11/KE/2021.

The inclusion criteria of this study are Patients with invasive ductal breast carcinoma in the early stages (*early breast cancer*) who were carried out MRM and received taxan and anthracycline as adjuvant chemotherapy. The exclusion criteria were that the sample was not registered in Dr. H Koesnadi Bondowoso Hospital and that incomplete data was found.

Clinical and pathological data of patients were obtained from medical records and reports of anatomical pathology examination results. The cancer stage is ascertained through clinical examination, radiology, and pathology reports. Following an explanation, all patients who participated in this study signed a consent form and a publication consent form.

Immunohistochemistry (IHC) examination MMP1

The material in this study was a specimen from MRM surgery, which was fixed using a 10% formalin buffer. Anatomical pathology specialists will review paraffin blocks eligible for examination based on previous H&E paintings—immunohistochemical analysis using monoclonal antibodies from MyBioSource with catalog number MBS702551. The assessment of MMP1 expression involved quantifying the number of cells exhibiting positive reactivity to the antibody over ten distinct fields of view by employing a light microscope featuring a 400x magnification. Subsequently, the average values were computed.

Statistical Analysis

The data processing was conducted with the trial version of IBM SPSS 25 and Openepi 3.0 software applications. The bivariate analysis was performed with either the Chi-square or Fisher's exact test. The threshold values for MMP1 expressions are established using the sensitivity and specificity values derived from the receiver operating characteristic (ROC). Logistic regression tests are employed for the multivariate study of clinical and pathological variables. A significance level of <0.05 is used to determine the statistical result that is considered significant.

RESULTS

Characteristics of the patient

The study included a total of 50 research samples, consisting of 25 without recurrence and 25 with recurrence. We found that the mean age of the group with local recurrence (51.00±9.57 years old)

Table 1. The association between the characteristics of the sample and recurrence.

	Without local recurrence (n=25)	With local recurrence (n=25)	p
Age (mean±SD)	50.25±14.93	51.00±9.57	0.920
KGB metastases (mean±SD)	1.25±1.5	3.00±1.53	0.09
Grade (mean±SD)	1.86±0.00	2±0.69	0.61
Hormonal status			
Pre menopause	1	5	0.197
Menopause	3	2	
MMP1 expression (mean±SD)	3.95±5.36	14.95±5.36	< 0.000*

*significant at p<0.05 by independent t-test; SD = standard of deviation; p=p-value

was older than without local recurrence (50.25±14.93 years old). Similar findings were found in KGB metastasis, grading, and MMP1 expression. MMP1 expression in the local recurrence group was 14.95±5.36, and 3.95±5.36 was in the group without local recurrence. According to several variables, we only found that expression had a significant mean difference statistically (p< 0.000). Table 1 provides comprehensive data regarding the outcomes of the bivariate analysis.

Evaluation of Local Recurrence and MMP1 Expression in Early-Stage IDC

The *cut-off* value of the test results was determined based on the sensitivity and specificity graphs of MMP1 (Figure 1). The *cut-off* value for used was 6.55. The MMP1 expression was considered high when the H-Score was more significant than or equal to 6.55 and low when it was less than 6.65.

Association between MMP1 with local recurrence

Most recurrence patients had high MMP1 expression (23 samples), contrasting those with low MMP1 expression. We found that the high MMP1 expression can increase local recurrence in breast cancer patients 60.38 times higher than with low MMP1 levels, with a 95% CI of 10.01-364.3. A significant correlation (p<0.001) has been found between MMP1 expression and local recurrence in patients with early-stage IDC, as determined by the Chi-Square test (Table 2).

Moreover, we classified the sample into two groups according to the MMP1 expression. The mean age of the patients with high MMP-1 levels was 51.96±12.2 years old, while the group with low MMP-1 levels was 49.52±10.5 years old. Regarding the proportion of lymph node metastasis in each group, it was found that most of the samples with high expression of MMP1 had more than three lymph node metastases; results were inversely proportional compared to the group with low expression of MMP1. In this study, the condition of menopause was related to MMP-1 expression. This can be seen from the majority of samples with high MMP-1 expression, which have menopause, while the majority of samples with low MMP-1 expression have not yet experienced menopause. Unfortunately, the study was not statistically significant. Unfortunately, several variables were examined based on the grouping of MMP-1 expression (p>0.05) (Table 3).

MMP1 expression also had a good diagnostic indicator in recurrence cases of breast cancer samples. The sensitivity and specificity of MPP1 expression was 80%, PPV was 85.2%, and NPV was 91.3%. The high PLR and low NLR values suggest that MMP1 expression can be a good diagnostic indicator. This study found that for every one false positive result of the MMP-1 expression test, there will be 7.67 true positive results of the MMP-1 expression test or for every ten false positive results of the MMP-1 expression test, there will be 76.7 true positive results of the MMP-1 expression test. In addition, the NLR score indicated that every 0.9 false negative

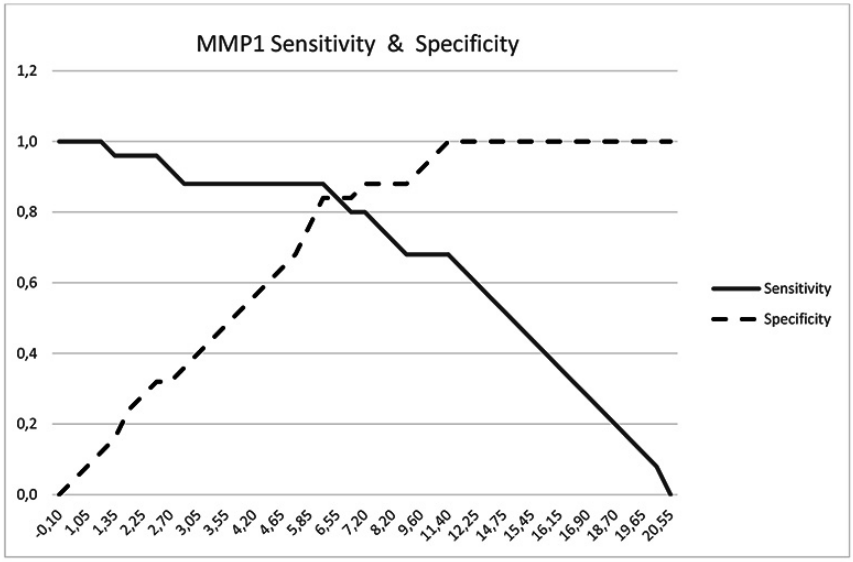

Figure 1. Sensitivity and specificity of MMP1 examination. The *cut-off* value used is 6.55.

Table 2. Relationship between MMP1 expression and recurrence

Type		Recurrence (+)	Recurrence (-)	OR(95%CI)	p
MMP1 Expression	High	23	4	60.38 (10.01-364.3)	<0.000*
	Low	2	21		

*significant at p<0.05 by independent t-test; p=p-value ; OR=Odds Ratio.

Table 3. Bivariate analysis between clinicopathological factors and MMP1 expression

		MMP-1 Expression Low (n=23)	MMP-1 Expression Hight (n=27)	p
Age (years)		49.52±10.5	51.96±12.2	0.474
Age category	≤40	4	5	0.918
	>40	19	22	
Lymph node metastasis	≤3	16	18	0.827
	>3	7	9	
Grade	Low	21	17	0.827
	High	2	10	
Menopause	Pre	13	13	0.554
	Post	10	14	

result of the MMP-1 expression test would get ten true negative results of the MMP-1 expression test (Table 4).

Table 4. Diagnostic indicators of MMP1 expression in recurrence cases

Indicators	(n=50)
Sensitivity	80%
Specificity	80%
Positive predictive value (PPV)	85.2%
Negative predictive value (NPV)	91.3%
Positive likelihood ratio (PLR)	7.67
Negative likelihood ratio (NLR)	0.09
Accuracy	88%

DISCUSSION

Characteristics of the patient

In this study, the mean age of patients with breast cancer recurrence was 51.00±9.57 years, whereas the mean age of patients who didn't have a recurrence was 50.25±14.93 years (p=0.920). Age at breast cancer diagnosis is a significant prognostic indicator. Young breast cancer is more aggressive and often recurs. According to some epidemiological studies, young breast cancer patients have a worse prognosis than their older counterparts.[11]

In this study, the average number of KGB metastases in patients with recurrence was 3.00±1.53, while those without recurrence were 1.25±1.5 (p = 0.09). Regional lymph node status is the single most important prognostic factor in breast cancer. The prognosis of breast cancer is directly proportional to the number of lymph nodes that metastasise. Regional lymph node involvement can also be used as an effective parameter to estimate local recurrence and survival of breast cancer patients. Tonellotto's study found that prognosis was related to the number of metastasised lymph nodes, not the level at which they occurred. In breast cancer patients who experience metastases in lymph nodes as many as 1-3 nodes, the one-year life expectancy drops to 40%, while if the number of nodes that experience metastases is 4-10 pieces, the one-year life expectancy is only 20%.[12]

The degree of tumor malignancy is one of the risk factors for recurrence. Studies conducted by Potter say that there is a relationship between the degree of tumor malignancy and the occurrence of metastasis.[13] In this study, there was no significant difference between patients who experienced relapse and those who did not experience relapse (p = 0.61). In this study, there was also no significant difference in the hormonal status of patients (pre-menopause/menopause) (p = 0.197). Hormonal status is one of the prognostic factors for breast cancer. In premenopausal patients, recurrence occurs earlier when compared to those who have been menopausal.[14]

The present study conducted Bivariate and non-parametric statistical analyses on clinicopathological variables such as age, lymph node metastases, tumor grade, and menopausal status. The results of these analyses did not demonstrate any statistically significant differences. (p>0.05). These results differ from those of other studies, possibly due to the homogeneity of the invasive ductal carcinoma samples used in this investigation. In this analysis, all tumors were classified as T1-2, indicating greater uniformity in size and grading. Research conducted by Vicini demonstrates that lymph node metastases do not substantially affect the prognosis of early-stage breast cancer.[15]

Relationship between MMP1 expression and recurrence

After transforming into CAF, normal fibroblasts will regulate cancer cell invasion and metastasis. CAF will increase the mobility of the cell structure, facilitating migration. To promote the metastasis of cancer cells, CAF will induce the production of MMP1, which degrades the extracellular matrix (ECM) surrounding the cells. MMP1 can make cancer cells avoid apoptosis triggered by collagen type I (COL1). The primary constituent of the extracellular matrix is type I collagen, which serves as the initial barrier preventing the migration of tumour cells after their ability to pass through the basal membrane. Type I collagen activates the discoidin domain receptor, eventually triggering the BCL-2 Interacting Killer (BIK). MMP1 will degrade type 1 collagen so that the COL1/DDR/BIK pathway becomes inactive, and apoptosis does not occur. MMP I can also activate growth factor receptors so that proliferation occurs through the MAPK/ERK pathway.[16]

In this study, a group of patients who experienced local recurrence obtained high MMP1 expression in 23 patients and low in 2 patients. Four patients had high MMP1 expression for the non-recurrence patient group, and 21 patients had low MMP1 expression. Analysis with the Chi-square test shows an odd ratio of 60.38 (10.01-364.3) with 95%CI. These results indicated that breast cancer patients with high MMP1 expression were more likely to experience recurrence. In line with Bostrom's research, Bostrom notes that MMP1 overexpression is associated with accelerated tumor progression and a poor prognosis.[17] Overexpression of MMP1 was positively correlated with the triple-negative breast cancer subtype, which has a poorer prognosis than luminal breast cancer, according to Wang's research.[7] Additionally, Shen et al. noted that elevated MMP1 expression in breast cancer predicts chemotherapy drug resistance.[18]

The results of this study revealed that the expression of MMP1 had the following characteristics: sensitivity of 92%, specificity of 88%, positive predictive value of 85%, negative predictive value of 91%, positive likelihood ratio of 7.67, negative likelihood ratio of 0.09, and accuracy of 82% when used to predict recurrence in the first two years following surgery. Hence, measuring the level of MMP1 expression may serve as a predictive marker of local recurrence in the case of early-stage IDC after chemotherapy and mastectomy.

Our results are equivalent to those of other biomarker predictors of recurrent

breast cancer. A study by Ojha on predicting breast cancer recurrence using data mining techniques with Support Vector Machines (SVM) showed that currently established biomarkers have the highest accuracy of 1%.[19] In another study by Zain et al., predicting the recurrence of breast cancer using principal component analysis (PCA) techniques, it was found that the current biomarkers have an accuracy of 76.1%.[20]

According to our findings, MMP1 expression may serve as a prognostic indicator of local recurrence in breast cancer. This is necessary when surgeons inform patients and their families about disease prognosis and treatment strategies. However, there are still some limitations to this study, such as the use of a cross-sectional design that could not evaluate the progression of the samples, the use of a limited number of samples, and the short follow-up period. In the future, conducting studies with a larger sample size would be more beneficial.

CONCLUSION

Immunohistochemical examination to determine MMP1 expression can also be used as a predictor of recurrence in the first two years after surgery and adjuvant chemotherapy in early-stage breast cancer patients.

ETHICAL APPROVAL

The research was carried out with the approval of The Research Ethics Committee of the Faculty of Medicine, State University of Jember (1.537/ H25.1.11/KE/2021).

FUNDING

This research used independent funds.

CONFLICT OF INTEREST

The researchers confirm that no external factors, including financial, professional, or personal obligations, exerted any influence on the methodology or delivery of the study described in this manuscript.

AUTHOR CONTRIBUTION

AA made contributions to the preparation and editing of the manuscript, concept and design of the study, literature search, data acquisition, and data analysis. The study's conception and design were made by IKS and DGA.

REFERENCES

1. Kemenkes. Panduan Penatalaksanaan Kanker Payudara. Kementerian Kesehatan Republik Indonesia. 2015;1–50. Available from: http://kanker.kemkes.go.id/guidelines/PPKPayudara.pdf
2. Kennecke H, Yerushalmi R, Woods R, Cheang MCU, Voduc D, Speers CH, et al. Metastatic behavior of breast cancer subtypes. Journal of Clinical Oncology. 2010;28(20):3271–7.
3. Rivera-Franco MM, Leon-Rodriguez E. Delays in breast cancer detection and treatment in developing countries. Breast Cancer (Auckl). 2018;12.
4. Al-Hilli Z, Grobmyer SR. Management Strategies for Locally Recurrent Breast Cancer: Redo-Lumpectomy, Redo-Sentinel Node Biopsy, Redo-Radiation. Ann Surg Oncol. 2019;26(10):3018–24. Available from: https://doi.org/10.1245/s10434-019-07545-y
5. Cianfrocca M, Gradishar WJ. Controversies in the Therapy of Early Stage Breast Cancer. Oncologist. 2005;10(10):766–79.
6. Hassan MSU, Ansari J, Spooner D, Hussain SA. Chemotherapy for breast cancer (review). Vol. 24, Oncology Reports. Spandidos Publications; 2010. p. 1121–31.
7. Wang QM, Lv LI, Tang Y, Zhang LI, Wang LF. MMP-1 is overexpressed in triple-negative breast cancer tissues and the knockdown of MMP-1 expression inhibits tumor cell malignant behaviors in vitro. Oncol Lett. 2019;17(2):1732–40.
8. Figueira RCS, Gomes LR, Neto JS, Silva FC, Silva IDCG, Sogayar MC. Correlation between MMPs and their inhibitors in breast cancer tumor tissue specimens and in cell lines with different metastatic potential. BMC Cancer. 2009;9:1–11.
9. Cui Q, Wang B, Li K, Sun H, Hai T, Zhang Y, et al. Upregulating MMP 1 in carcinoma associated fibroblasts reduces the efficacy of taxotere on breast cancer synergized by collagen IV. Oncol Lett. 2018;16(3):3537–44.
10. Jenkins DG, Quintana-Ascencio PF. A solution to minimum sample size for regressions. PLoS One. 2020;15(2):1–15.
11. Gnerlich JL, Deshpande AD, Jeffe DB, Sweet A, White N, Margenthaler JA. Elevated Breast Cancer Mortality in Women Younger than Age 40 Years Compared with Older Women Is Attributed to Poorer Survival in Early-Stage Disease. J Am Coll Surg. 2009;208(3):341–7.
12. Tonellotto F, Bergmann A, de Souza Abrahão K, de Aguiar SS, Bello MA, Thuler LCS. Impact of Number of Positive Lymph Nodes and Lymph Node Ratio on Survival of Women with Node-Positive Breast Cancer. Eur J Breast Health. 2019;15(2):76–84.
13. Allinen M, Beroukhim R, Cai L, Brennan C, Lahti-Domenici J, Huang H, et al. Molecular characterization of the tumor microenvironment in breast cancer [Internet]. Available from: http://www.cancercell.org/
14. Demicheli R, Bonadonna G, Hrushesky WJM, Retsky MW, Valagussa P. Menopausal status dependence of the timing of breast cancer recurrence after surgical removal of the primary tumour. Breast Cancer Research. 2004;6(6).
15. Vicini FA, Kestin L, Huang R, Martinez A. Does local recurrence affect the rate of distant metastases and survival in patients with early-stage breast carcinoma treated with breast-conserving therapy? Cancer. 2003;97(4):910–9. Available from: http://doi.wiley.com/10.1002/cncr.11143
16. Maquoi E, Assent D, Detilleux J, Pequeux C, Foidart JM, Noël A. MT1-MMP protects breast carcinoma cells against type i collagen-induced apoptosis. Oncogene. 2012;31(4):480–93.
17. Boström P, Söderström M, Vahlberg T, Söderström KO, Roberts PJ, Carpén O, et al. MMP-1 expression has an independent prognostic value in breast cancer. BMC Cancer. 2011;11.
18. Shen CJ, Kuo YL, Chen CC, Chen MJ, Cheng YM. MMP1 expression is activated by Slug and enhances multi-drug resistance (MDR) in breast cancer. PLoS One. 2017;12(3).
19. Ojha U, Goel S. A study on prediction of breast cancer recurrence using data mining techniques. In: 2017 7th International Conference on Cloud Computing, Data Science & Engineering - Confluence. 2017. p. 527–30.
20. Zain ZM, Alshenaifi M, Aljaloud A, Albednah T, Alghanim R, Alqifari A, et al. Predicting breast cancer recurrence using principal component analysis as feature extraction: An unbiased comparative analysis. International Journal of Advances in Intelligent Informatics. 2020;6(3):313–27.