PLASMA MATRIX METALLOPROTEINASE-9 LEVEL IN INVASIVE DUCTAL BREAST CARCINOMA PATIENTS

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Abstract. Matrix metalloproteinases (MMPs) are members of a family of zinc-endopeptidases, which play a key role in extracellular matrix degradation in human cancer progression including breast cancer. Plasma MMP-9 level should provide a marker for differentiation between benign and malignant breast cancer. Here, plasma MMP-9 levels of invasive ductal breast cancer (IDC) patients (n = 108)and those of volunteers with mammography BIRADS categories 1-4 (n =211) were determined using an ELISA technique. Median [25-75% interquartile range (IQR)]concentration of plasma MMP-9 of IDC patients is significantly higher than those of volunteers in all BIRADS categories (13 (10-15) *vs* 9 (7-11) ng/ml) (p-value <0.001). In addition, MMP-9 level of IDC patients is also significantly higher than that of volunteers with mammography BIRADS category 1-4 (9.1 (8.7-9.3), 8.8 (7.9-9.9), 9.7 (8.4-10.5), and 9.8 (7.8-11.9) ng/ml, respectively) (p-value <0.001). Receiver-operator curve analysis for MMP-9 concentrations between IDC patients and volunteers in all BIRADS categories showed an area under curve of 0.829 (95% confidence interval: 0.783-0.869) (p-value <0.001) and a cutoff value of 10.5 ng/ml (76% sensitivity and 82% specificity); however, association between plasma MMP-9 levels and clinicopathological parameters of IDC patients is not significant, and a larger cohort is needed to determine the potential usefulness of such association studies. In conclusion, plasma matrix metalloproteinase-9 level serves as a convenient biomarker for distinguishing between women with benign and malignant breast cancer but association of MMP-9 levels with clinicopathological parameters needs further investigation.

Keywords: biomarker, breast cancer, invasive ductal breast carcinoma, plasma matrix metalloproteinase-9

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INTRODUCTION

Breast cancer is one of the major health problems in women worldwide including Thailand. In 2020, globally female breast cancer has the highest prevalence of 2.3 million, accounting for 11.7% of all cancer cases in women, with 685,000 deaths, the fifth leading cause of cancer mortality (Sung *et al*, 2021). In 2020, Thailand, with a female population of 35,833,918, recorded 22,158 new cases of breast cancer and 8,266 breast cancer deaths (Sung *et al*, 2021).

Metrix metalloproteinases (MMPs), members of zinc-endopeptidase family, play a key role in degradation of extracellular matrix (EMC) associated with human cancer progression (Yadav *et al*, 2014) and are targets of cancer therapeutics (Cathcart *et al*, 2015). There are 23 different human MMPs, divided into six subgroups, namely, collagenase, gelatinase, matrilysins, membrane type MMP, stromelysin, and others. MMP-9 (gelatinase B) is involved in cancer invasiveness and metastasis through digestion of basement membrane type IV collagen (Gioia *et al*, 2009), and high levels of plasma or serum MMP-9 are observed in several type of cancers, such as colorectal (Zhang *et al*, 2015), gastric (Liu *et al*, 2015) and lung (El-Badraway *et al*, 2014).

MMP-9 levels in women with various breast imaging report and data system (BIRADS) categories and invasive ductal cancer (IDC) remain unelucidated. Here, plasma MMP-9 levels in women with BIRADS categories 1-4 and IDC were measured and correlations among enzyme levels, BIRADS categories and clinicopathological characteristics were evaluated to determine the significance of MMP-9 levels in breast cancer development and progression.

MATERALS AND METHODS

Specimens collection

Blood samples from patients diagnosed with IDC during 2011-2012 were kindly provided by the Department of Research and Technology Assessment, National Cancer Institute, Bangkok, Thailand. Patients had not received chemotherapy and/or radiation therapy prior to blood collection. Blood samples were also collected from female volunteers who participated in a "Mammogram Screening Campaign of 600 Women to Cerebrate the Fifth Cycle Birthday Anniversary of HRH Princess Maha Chakri Sirindthorn" at the Faculty of Tropical Medicine, Mahidol University, Bangkok during 2015-2016 and undertook a clinical examination and mammography, with BIRADS category

(Balleyguier *et al*, 2007) assigned by a certified radiologist.

The research protocol was approved by the Ethics Committees of the National Cancer Institute of Thailand (Approval No. 001_2020RB_IN644) and of the Faculty of Tropical Medicine, Mahidol University, Bangkok (Approval No. MUTM 2016-066-01).

Measurement of plasma MMP-9 level

A 5-ml aliquot of EDTA blood sample was centrifuged at 1,300 g for 10 minutes at 4°C and plasma stored at -80°C until used. Plasma MMP-9 concentration was measured in duplicate using a human MMP-9 ELISA kit (Abcam, Cambridge, UK) and a calibration curve constructed from MMP-9 standards (Abcam) with a dynamic range of 1.0-10.0 ng/ml. Results are expressed as median and interquartile range (IQR).

Statistical analysis

Statistical significance of plasma MMP-9 levels between IDC patients and volunteers with mammography BIRADS categories 1-4 were analyzed using an independent Student's t-test. Binary and multinomial logistic regression analyses were performed to determine the association between plasma MMP-9 concentrations and clinicopathological features of IDC patients. Odds ratios (OR) and 95% confidence interval (CI) were calculated using a multiple logistic regression analysis. Cutoff points of MMP-9 levels in volunteers and IDC patients were determined from a receiver-operator characteristic (ROC) curve. A p-value <0.05 is considered significant. All statistical tests were carried out using a Statistical Package for the Social Sciences (SPSS) software version 20.0 for Microsoft Windows (IBM Corp, New York, NY).

RESULTS

Characteristics of IDC patients and volunteers who had a mammography

Mean ± SD age of IDC patients (n = 108) was 51 ± 12 years of age, with a little over half ≤50 years of age (Table 1). Histological examination of tumor tissues revealed 42% were moderately differentiated, with poorly differentiated tissues constituting 36% (Table 1). According to a classification of malignant tumors system based on tumor, lymph node and metastasis (TNM) (stages I-IV) (Amin *et al*, 2017), the majority of cancer cases (36%) were at TNM II stage.

Mean ± SD age of volunteers who had undergone a mammography and been assigned a BIRADS classification (categories 1-4) (n = 211) was 54 ± 10 years old, with over half >50 years of age (Table 2). A little over half of the volunteers were assigned a mammography BIRADS category of 2.

Plasma MMP-9 levels in IDC patients and volunteers assigned a BIRADS classification

In order to evaluate the usefulness of plasma MMP-9 for diagnosis of IDC, plasma MMP-9 levels were compared between IDC patients and volunteers

Table 1

Association between plasma matrix metalloproteinase 9 (MMP-9) levels with clinicopathological parameters of patients with invasive ductal breast carcinoma (IDC), National Cancer Institute, Bangkok, Thailand (2011-2012)

Characteristics	Number of patients	Number of patients with plasma MMP-9 level>10.5 ng/ml (%)	Odds ratio (95% CI)	*p*-value*
IDC patients	108	82 (76)		
Age group				
<50 years old	57	47 (82)		
>50 years old	51	35 (69)	0.47 (0.19-1.15)	0.100
Histological grade				
Well differentiated	24	23 (96)		
Moderately differentiated	45	34 (76)	0.13 (0.02-1.11)	0.030
Poorly differentiated	39	25 (67)	0.09 (0.01-0.72)	0.004
TNM stage				
I	25	23 (92)		
II	38	24 (63)	0.15 (0.03-0.73)	0.010
III	32	26 (81)	0.38 (0.07-2.05)	0.250
IV	13	9 (69)	0.20 (0.03-1.26)	0.070
Tumor size				
≤3.0 cm	45	30 (67)		
>3.0 cm	21	16 (76)	1.60 (0.49-5.21)	0.430
No data	42	-		

Table 1 (cont)

Characteristics	Number of patients	Number of patients with plasma MMP-9 level>10.5 ng/ml (%)	Odds ratio (95% CI)	*p*-value*
Lymph node metastasis				
Negative	26	17 (65)		
Positive	46	34 (74)	1.50 (0.53-4.25)	0.440
No data	36	-		
Organ Metastasis				
Negative	14	9 (64)		
Positive	94	73 (78)	0.52 (0.16-1.71)	0.280
No data	57	-		
Presence of ER, PR, HER2				
Non-triple negative[a]	79	63 (80)		
Triple negative[b]	10	5 (50)	0.25 (0.07-0.99)	0.040
No data	19			

*Significant when $p < 0.05$

[a]ER+/-, PR+/- and HER2+; [b]ER-, PR- and HER2-

CI: confidence interval: cm: centimeter; ER: estrogen receptor; HER2: human epidermal growth factor receptor 2; PR: progesterone receptor; TNM: tumor, lymph node and metastasis

Table 2

Plasma matrix metalloproteinase 9 (MMP-9) levels in invasive ductal breast carcinoma (IDC) patients, National Cancer Institute (2011-2012) and female volunteers with breast imaging report and data system (BIRADS) categories 1 to 4, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand (2015-2016)

Characteristics	Number (%)	Plasma MMP-9 median (IQR) (ng/ml)
IDC patient (*n* = 108)	-	13 (10-15)
Volunteer (*n* = 211)	-	9 (7-11)
Age group		
≤50 years old	90 (43)	
>50 years old	121 (57)	
BIRADS category		
1	21 (10)	9.1 (8.7-9.3)
2	110 (52)	8.8 (7.9-9.9)
3	60 (28)	9.7 (8.4-10.5)
4	20 (10)	9.8 (7.8-11.9)

IQR: interquartile range; ng/ml: nanogram/milliliter

who had undergone mammography. Median (IQR) of plasma MMP-9 level of IDC patients is significantly higher than that of volunteers assigned a mammography BIRADS category (*p*-value <0.001) (Table 2). AUC of a ROC of plasma MMP-9 concentrations in IDC patients compared to volunteers was 0.829 (95% CI: 0.783-0.869; *p*-value <0.001) (Fig 1A). Youden index (0.5839) indicated a cut-off value of plasma MMP-9 concentration discriminating between patients and volunteers of 10.5 ng/ml, with sensitivity and specificity of 76 and of 82% respectively.

Plasma MMP-9 level and BIRADS category

Median MMP-9 levels among women volunteers in the four BIRADS categories are not significantly different (Table 2). AUC of a ROC of plasma MMP-9 concentrations in IDC patients compared to volunteers according to BIRADS category 1 to 4 was 0.857 (95% CI: 0.785-0.912; *p*-value <0.001), 0.849 (95% CI: 0.794-0.893; *p*-value <0.001), 0.808 (95% CI: 0.741-0.865; *p*-value <0.001), and 0.757 (95% CI: 0.673-0.828; *p*-value <0.001), respectively (Figs 1B-E). Youden index of BIRADS categories 1 to 4 (0.778, 0.632, 0.580, and 0.459, respectively)

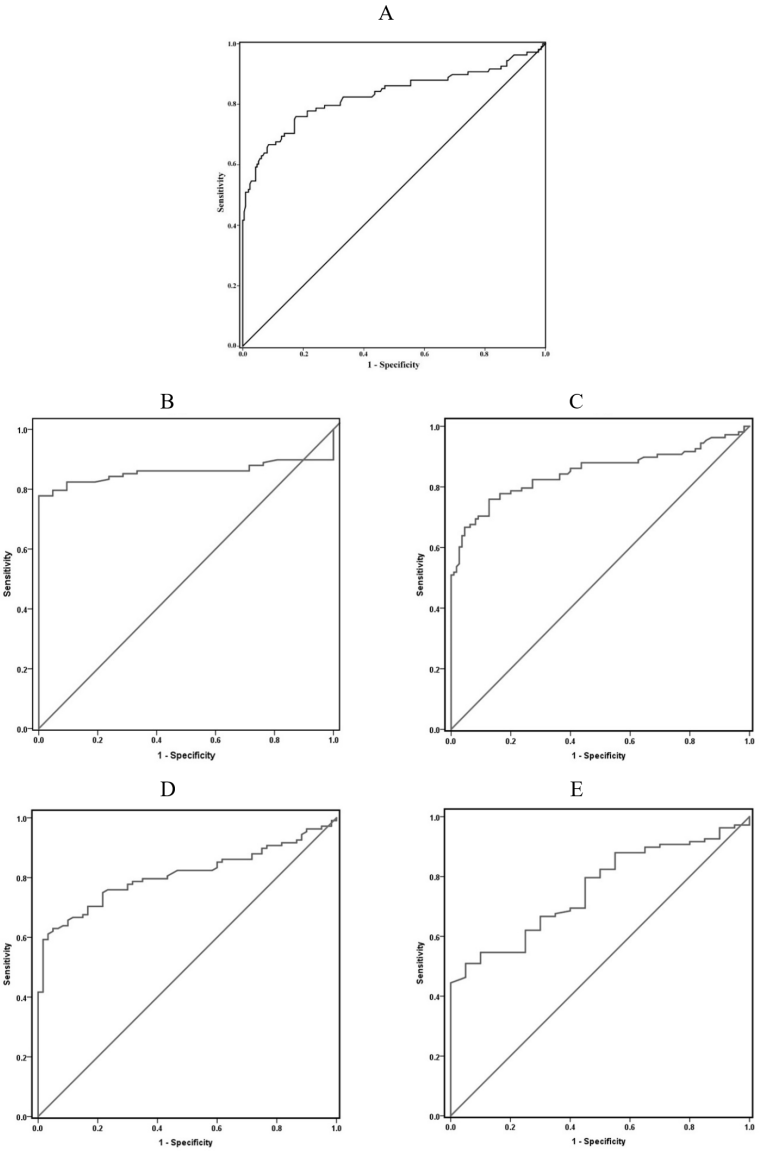

Fig 1 - Receiver-operator curve of plasma matrix metalloproteinase-9 concentrations of female volunteers (A) in all breast imaging report and data system (BIRADS) categories (n = 211), (B) with BIRADS category 1 (n = 21), (C) with BIRADS category 2 (n = 110), (D) with BIRADS category 3 (n = 60), and (E) with BIRADS category 4 (n = 20) compared to IDC patients (n = 108)

BIRADS categories of volunteers were determined at the Faculty of Tropical Medicine, Mahidol University, Bangkok (2015-2016) and IDC patients attended the National Cancer Institute, Bangkok, Thailand (2011-2012).

indicated a cut-off value of plasma MMP-9 concentration discriminating between patients and volunteers of 10.3, 10.46, 11.6, and 12.4 ng/ml, respectively (p-value <0.001 for all comparisons). Sensitivity of BIRADS category 1 to 4 compared with IDC patients was 78, 76, 63, and 51%, respectively and specificity was 100, 87, 95, and 95%, respectively. BIRADS category 1 showed highest sensitivity and specificity.

Association between plasma MMP-9 levels and clinicopathological characteristics in IDC patients

A comparison of plasma MMP-9 levels with clinicopathological parameters of IDC patients, based on the cutoff level of 10.5 ng/ml, indicated >95% of IDC patients with well differentiated histological type had plasma MMP-9 levels higher than the cutoff value, as did those with TNM stage I (92%) and III (81%), and those with the immunohistochemical markers such as estrogen receptor (ER)+/-, progesterone receptor (PR)+/-, and human epidermal growth factor receptor 2 (HER2)+ (Table 1). IDC patients with other clinicopathological parameters had a significantly lower number with plasma MMP-9 levels greater than the cut-off value.

DISCUSSION

Early detection is the most promising prevention strategy to reduce morbidity and mortality of breast cancer (Smith *et al*, 2006) with mammography being the best method (Bleyer *et al*, 2012), but it can only be performed in a hospital setting due to the high operating cost (Leung *et al*, 2014). Whole genome expression study in Thai IDC breast cancer patients indicates an up-regulation of *MMP-9* (Arnutti *et al*, 2013). An ELISA method was chosen for MMP-9 measurement as the assay is rapid to perform and determines both pro- and active forms of the enzyme. Although blood samples of both IDC patients and women volunteers undergoing mammography were collected at different times, it should not affect plasma MMP-9 concentration as the enzyme is very stable and its level shows no difference even after nine years of cryopreservation at -80°C (Jonsson *et al*, 2018).

BIRADS categorization (categories 1-6) of the American College of Radiology is widely used for classification of mammography screening for breast cancer: categories 1 and 2 indicating normal, typical benign or need of regular follow-up mammography; category 3 benign but need of a short follow-up mammography; category 4 suspicious abnormality and need of biopsy or surgery for confirmation; and categories 5 and 6 malignancy (Balleyguier *et al*, 2007). Our results show median plasma MMP-9 level in IDC patients is significantly higher than that of subjects with BIRADS categories 1 to 4. Elevated plasma MMP-9 levels have been reported in breast cancer patients (Susskind *et al*, 2003; La Rocca *et al*, 2004; Talvensaari-Mattila and

Turpeenniemi-Hujanen, 2005; Somiari *et al*, 2006) as well as in benign cases (Wu *et al*, 2008). Serum MMP-9 cutoff value of 315 ng/ml has been shown to provide the best discrimination between breast cancer patients and normal, healthy women similar in age and menopausal status, with sensitivity and specificity of 80% (Patel *et al*, 2011). Our study shows a much lower cutoff value (10.5 ng/ml), with sensitivity and specificity of 76 and 82% respectively. Using this cut-off, we were able to detect IDC among subjects with BIRADS category 1 with 78 and 100% sensitivity and specificity respectively. Apart from plasma MMP-9 level, other tumor markers, such as serum CEA and CA 15-3, demonstrated specificity of 95%, while CEA and CA 15-3 18 and 32% sensitivity respectively in detecting breast cancer, with slightly lower sensitivity for benign breast cancer types (Zeleski *et al*, 2018).

In addition to breast cancer in women elevated serum MMP-9 level has been applied in gastric cancer (ROC demonstrating 85 and 65% sensitivity and specificity respectively using at cutoff value of 60 ng/ml) (Wu *et al*, 2007), cervical cancer employing a plasma MMP-9 cutoff value of 103.8 ng/ml to distinguish squamous carcinoma and high-grade cervical intraepithelial neoplasia with 75.6 and 68.9% sensitivity and specificity respectively (Yang *et al*, 2007), and prostate cancer patients with metastasis (Morgia *et al*, 2005). Furthermore, high MMP-9 expression in tissue samples significantly correlates with tumor aggressiveness and poor prognosis (Li *et al*, 2004; Pellikainen *et al*, 2004; Cid *et al*, 2018). Likewise, melanoma patients with high serum MMP-9 levels have significantly poorer overall survival compared to patients with lower levels (Nikkola *et al*, 2005).

Plasma MMP-9 level of IDC patients was negatively associated with histological grading of tissue differentiation status. Breast cancer patients including those with IDC who had well-differentiated tumor have significantly higher serum MMP-9 activity than those with moderately (p-value = 0.01) or poorly differentiated tumor (p-value = 0.04) (Somiari *et al*, 2006). Moreover, an immunological study confirmed there is no significant association between MMP-9 immunostaining score values and histological grade, stage or node status of IDC patients except for tumor size (p-value <0.05) (Vizoso *et al*, 2007). On the other hand, significantly elevated levels of serum MMP-9 in breast cancer patients with advancement in TNM staging, histological grading, lymph node status, and metastasis were reported (McGowan and Duffy, 2008; Patel *et al*, 2011). However, levels of serum MMP-9 are significantly elevated in only breast cancer patients with metastasis presentation within one year compared to those who presented with a disease history of more than one year (Patel *et al*, 2011).

Our results demonstrate elevated plasma MMP-9 levels (>10.5 ng/ml) of IDC patients were not associated with triple-negative (ER-, PR-, and HER2-) histology, in agreement which was similar with a previous study of patients with ER- and PR- status (Wu *et al*, 2008), but contrary to the study of Patel *et al* (2011) who observed a significantly higher serum MMP-9 concentration was in ER-, PR- patients (p-value < 0.05). Despite reports of higher serum MMP-9 levels in node-positive compared to node-negative breast cancer (Wu *et al*, 2008; Heo *et al*, 2014), this association was not found in our study. These discrepancies might be due to differences in ethnic groups and duration of disease history of patients among the literature and our studies. A multi-center study involving larger cohorts are needed to obtain a more definitive conclusion.

In summary, our work reveals plasma MMP-9 level is a key biological marker in IDC breast cancer in assisting discrimination of benign (mammography BIRADS categories 1 to 4) and malignant breast cancer (BIRADS >4). In addition, laboratory detection of plasma MMP-9 level should be helpful in health settings which lack both expensive equipment and availability of radiologists. However, further multi-center studies with larger cohorts are needed to validate the association of plasma MMP-9 levels and clinicopathological parameters in breast cancer.

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CONFLICTS OF INTEREST DISCLOSURE

The authors declare no conflicts of interest.

REFERENCES

Amin MB, Edge S, Greene F, *et al*, editors. AJCC Cancer Staging Manual. 8th ed. Chicago (IL): Springer International Publishing; 2017.

Arnutti P, Kotepui M, Asanprakit W, *et al*. Determination of whole transcription profiles and specific pathways in invasive ductal breast carcinoma. *Int J Clin Exp Pathol* 2013; 6: 1112-20.

Balleyguier C, Ayadi S, Nguyen KV, Vanel D, Dromain C, Sigal R. BIRADS™ classification in mammography. *Eur J Radiol* 2007; 61: 192-4.

Bleyer A, Welch HG. Effect of three decades of screening mammography on breast cancer incidence. *N Engl J Med* 2012; 367: 1998-2005.

Cathcart J, Pulkoski-Gross A, Cao J. Targeting matrix metalloproteinases in cancer: bringing life to old ideals. *Genes Dis* 2015; 2: 26-34.

Cid S, Eiro N, Fernández B, *et al*. Prognostic influence of tumor stroma on breast cancer subtypes. *Clin Breast Cancer* 2018; 18: e123-33.

El-Badraway MK, Yousef AM, Shaalan D, Elsamanoudy AZ. Matrix metalloproteinase-9 expression in lung cancer patients and its relation to serum mmp-9 activity, pathologic type and prognosis. *J Bronchology Interv Pulmonol* 2014; 21: 327-34.

Gioia M, Monaco S, Van Den Steen P, *et al*. The collagen binding domain of gelatinase A modulates degradation of collagen IV by gelatinase B. *J Mol Biol* 2009; 386: 419- 34.

Heo DS, Choi H, Yeom MY, Song BJ, Oh SJ. Serum levels of matrix metalloproteinase-9 predict lymph node metastasis in breast cancer patients. *Oncol Rep* 2014; 31: 1567-72.

Jonsson A, Hjalmarsson C, Falk P, Ivarsson ML. Stability of matrix metalloproteinase 9 as biological marker in colorectal cancer. *Med Oncol* 2018; 35: 50.

La Rocca G, Pucci-Minafra I, Marrazzo A, Taormina P, Minafra S. Zymographic detection and clinical correlations of MMP-2 and MMP-9 in breast cancer sera. *Br J Cancer* 2004; 90: 1414-21.

Li HC, Cao DC, Liu Y, *et al*. Prognostic value of matrix metalloproteases (MMP-2 and MMP-9) in patients with lymph node-negative breast carcinoma. *Breast Cancer Res Treat* 2004; 88: 75-85.

Leung J, Mckenzie S, Martin J, McLaughin D. Effect of rurality on screening for breast cancer: a systematic review and meta-analysis comparing mammography. *Rural Remote Health* 2014; 14: 2730.

Liu Y, Liu H, Luo X, Deng J, Pan Y, Liang H. Overexpression of SMYD3 and matrix metalloproteinase-9 are associated with poor prognosis of patients with gastric cancer. *Tumour Biol* 2015; 36: 4377-86.

McGowan PM, Duffy MJ. Matrix metalloproteinase expression and outcome in patients with breast cancer: analysis of a published database. *Ann Oncol* 2008;19: 1566-72.

Morgia G, Falsaperla M, Malaponte G, *et al*. Matrix Metalloproteinases as diagnostic (MMP-13) and prognostic (MMP-2, MMP-9) markers of prostate cancer. *Urol Res* 2005; 33: 44-50.

Nikkola J, Vihinen P, Vuoristo MS, Kellokumpu-Lehtinen P, Kähäri VM, Pyrhönen S. High serum levels of matrix metalloproteinase-9 and matrix metalloproteinase-1 are associated with rapid progression in patients with metastatic melanoma. *Clin Cancer Res* 2005; 11: 5158-66.

Patel S, Sumitra G, Koner BC, Saxena A. Role of serum matrix metalloproteinase-2 and -9 to predict breast cancer progression. *Clin Biochem* 2011; 44: 869-72.

Pellikainen JM, Ropponen KM, Kataja VV, Kellokoski JK, Eskelinen MJ, Kosma VM. Expression of matrix metalloproteinase (MMP)-2 and MMP-9 in breast cancer with a special reference to activator protein-2, HER2, and prognosis. *Clin Cancer Res* 2004; 10: 7621-8.

Smith RA, Caleffi M, Albert US, *et al*. Breast cancer in limited-resource countries: early detection and access to care. *Breast J* 2006; 12 (Suppl 1): S16-26.

Somiari SB, Somiari RI, Heckman CM, *et al*. Circulating MMP2 and MMP9 in breast cancer-potential role in classification of patients into low risk, high risk, benign disease and breast cancer categories. *Int J Cancer* 2006; 119: 1403-11.

Sung H, Ferlay J, Siegel RL, *et al*. Global Cancer Statistic 2020: GLOBALCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2021; 71: 209-49.

Susskind H, Hymowitz MH, Lau YH, *et al*. Increased plasma levels of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 in lung and breast cancer are altered during chest radiotherapy. *Int J Radiat Oncol Biol Phys* 2003; 56: 1161-9.

Talvensaari-Mattila A, Turpeenniemi-Hujanen T. Preoperative serum MMP-9 immunoreactive protein is a prognostic indicator for relapse-free survival in breast carcinoma. *Cancer Lett* 2005; 217: 237-42.

Vizoso FJ, González LO, Corte MD, *et al*. Study of matrix metalloproteinases and their inhibitors in breast cancer. *Br J Cancer* 2007; 96: 903-11.

Wu CY, Wu MS, Chiang EP, *et al*. Plasma matrix metalloproteinase-9 level is better than serum matrix metalloproteinase-9 level to predict gastric cancer evolution. *Clin Cancer Res* 2007; 13: 2054-60.

Wu ZS, Wu Q, Yang JH, *et al*. Prognostic significance of MMP-9 and TMP-1 serum and tissue expression in breast cancer. *Int J Cancer* 2008; 122: 2050-6.

Yadav L, Puri N, Rastogi V, Satpute P, Ahmad R, Kaur G. Matrix metalloprotenases and cancer- role in threat and therapy. *Asian Pac J Cancer Prev* 2014; 15: 1085-91.

Yang SF, Wang PH, Lin LY, *et al*. A significant elevation of plasma level of matrix metalloproteinase-9 in patients with high-grade intraepithelial neoplasia and early squamous cell carcinoma of the uterine cervix. *Reprod Sci* 2007; 14: 710-8.

Zaleski M, Kobilay M, Schroeder L, *et al*. Improved sensitivity for detection of breast cancer by combination of miR-34a and tumor markers CA 15-3 or CEA. *Oncotarget* 2018; 9: 22523-36.

Zhang CG, Chen R, Xie JB, Liu CB, Jin Z, Jin C. Immunohistochemical expression of Notch1, Jagged1, NF-κB, and MMP-9 in colorectal cancer patients and the relationship to clinicopathological parameters. *Cancer Biomark* 2015; 15: 889-97.