วารสารโรคมะเร็ง
THAI CANCER JOURNAL
ปีที่ 37 ฉบับที่ 3
กรกฎาคม-กันยายน 2560

Plasma MMP-13 Level in Patients with Invasive Ductal Breast Cancer

Nuntana Meesiripan[1]
Nonglucksanawan Ritthisunthorn[1]
Anong Tepsuwan[2]
Wassana Tangthai[3]
Thitiluck Swangsri[1]
Tipparat Thiangtrongjit[1]
Jaree Svedginda[4]
Santi Maneewatchararangsri[1]
Porntip Chavalitchewinkoon-Petmitr[5]
Somchai Thanasitthichai[6]
Songsak Petmitr[1*]

Abstract **Matrix metalloproteinases (MMPs) are a family of zinc-proteolytic endopeptidase enzymes that play an important role in extracellular matrix (ECM) degradation in human cancer progression and may be new targets for cancer therapeutics. This study aimed to detect the plasma level of matrix metalloproteinases 13 (MMP-13) in 108 patients with invasive ductal carcinoma (IDC) and 131 healthy controls with commercial enzyme-linked immunosorbent assay (ELISA) kits. The correlations between these protein levels and the clinical characteristics of the patients were also analyzed to elucidate their role in breast-cancer development. The results showed a significantly lower median concentration of MMP-13 in patients compared with controls (89.58 and 289.15 pg/ml, respectively; P=0.000). Receiver-operator characteristic (ROC) curve analysis, to determine a cutoff value for plasma MMP-13 concentration in the patients, had an AUC of 0.98 (95% CI=0.97-0.99), with a sensitivity of 91.6% and specificity of 91.7% at cutoff point 169 pg/ml. The results underline the difference in plasma level MMP-13 concentration in IDC development, and may serve as a biomarker for patients.** *(Thai Cancer J 2017;37:105-113)*

Keywords: invasive ductal breast cancer, MMP-13, plasma biomarker

[1]Department of Molecular Tropical Medicine and Genetics, Faculty of Tropical Medicine, Mahidol University, [2]Biomarker Development Section, Research Division, [3]Out Patient Department, National Cancer Institute, Bangkok, [4]Out Patient Department, Hospital for Tropical Diseases, [5]Department of Protozoology, Faculty of Tropical Medicine, Mahidol University, Bangkok, [6]Department of Surgery, National Cancer Institute, Bangkok

*Correspondence; songsak.pet@mahidol.ac.th

การตรวจวัดระดับ MMP-13 ในพลาสม่าผู้ป่วยมะเร็งเต้านมชนิด Invasive ductal carcinoma

โดย **นันทนา มีศิริพันธุ์[1] นงลักษณวรรณ ฤทธิสุนทร[1] อนงค์ เทพสุวรรณ์[2] วาสนา แตงไทย[3] ฐิติลักขณ์ สว่างศรี[1] ทิพรัตน์ เที่ยงตรงจิตต์[1] จารีย์ เศวตจินดา[4] สันติ มณีวัชระรังสี[1] พรทิพย์ เพ็ชรมิตร[5] สมชาย ธนะสิทธิชัย[6] ทรงศักดิ์ เพ็ชรมิตร[1]**

[1]ภาควิชาชีวโมเลกุลและพันธุศาสตร์โรคเขตร้อน คณะเวชศาสตร์เขตร้อน มหาวิทยาลัยมหิดล [2]งานพัฒนาสารบ่งชี้ทางการแพทย์ กลุ่มงานวิจัย [3]กลุ่มงานผู้ป่วยนอก สถาบันมะเร็งแห่งชาติ กรมการแพทย์ กระทรวงสาธารณสุข [4]กลุ่มงานผู้ป่วยนอก โรงพยาบาลเวชศาสตร์เขตร้อน [5]ภาควิชาพยาธิโปรโตซัว คณะเวชศาสตร์เขตร้อน มหาวิทยาลัยมหิดล [6]กลุ่มงานศัลยกรรม สถาบันมะเร็งแห่งชาติ กรมการแพทย์ กระทรวงสาธารณสุข

บทคัดย่อ Matrix metalloproteinases (MMPs) เป็นสมาชิกในกลุ่มของเอนไซม์ zinc-proteolytic endopeptidases เป็นเอนไซม์ที่มีบทบาทในการทำหน้าที่ย่อยสลายสารเคลือบเซลล์ (extracellular matrix หรือ ECM) ในขบวนการเกิดโรคมะเร็งในคนและอาจใช้เป็นเป้าหมายในการรักษาโรคมะเร็ง การศึกษานี้มีวัตถุประสงค์เพื่อวัดระดับ matrix metalloproteinases 13 (MMP-13) ในพลาสม่าผู้ป่วยมะเร็งเต้านมชนิดลุกลามจำนวน 108 รายและกลุ่มคนสุขภาพปกติ 131 คน ด้วยชุดตรวจ ELISA kit และวิเคราะห์หาความสัมพันธ์ระหว่างระดับโปรตีน MMP-13 กับลักษณะทางคลินิกของผู้ป่วย ผลการศึกษาพบว่าระดับ MMP-13 ในพลาสม่าของกลุ่มผู้ป่วยมะเร็งเต้านมชนิดลุกลามต่ำกว่าในกลุ่มคนสุขภาพดี (89.58 และ 289.15 pg/ml ตามลำดับ, P=0.000) เมื่อวิเคราะห์กราฟ ROC เพื่อหาค่า cut off ของระดับ MMP-13 ในพลาสม่าของกลุ่มผู้ป่วยมะเร็งเต้านมชนิดลุกลามพบว่ามีค่าเท่ากับ 169 pg/ml ซึ่งมีค่าพื้นที่ใต้กราฟหรือ AUC เท่ากับ 0.98 (95% CI=0.97 ถึง 0.99) ค่าความไวเท่ากับ 91.6% และความจำเพาะเท่ากับ 91.7% จากผลการศึกษาพบว่าระดับ MMP-13 ในพลาสม่ามีความแตกต่างกันในกลุ่มของผู้ป่วยมะเร็งเต้านมชนิด IDC กับกลุ่มคนปกติ ดังนั้นอาจนำ MMP-13 มาใช้เป็นตัวตรวจจับทางชีวภาพในผู้ป่วยมะเร็งเต้านมชนิด IDC ได้ *(วารสารโรคมะเร็ง 2560;37:105-113)*

คำสำคัญ: มะเร็งเต้านมชนิดลุกลาม MMP-13 ตัวตรวจจับชีวภาพในพลาสม่า

Introduction

Breast cancer is one of the major health problems in women worldwide. WHO predicts that the incidence of new cases will increase from 1.6 million in 2012 to 2.5 million cases in 2035, and the mortality rate will increase from 521,907 cases in 2012 to 846,587 cases in 2035[1]. The most common histological subtype of breast cancer is invasive ductal carcinoma (IDC), which generally metastasizes to axillary lymph nodes and causes more severe pathology than other histological types[2].

Matrix matelloproteinases (MMPs) are a family of zinc-proteolytic endopeptidase enzymes play an important role in extracellular matrix (ECM) degradation in human cancers progression[3] and may be approached as the new target for cancer therapeutics[4]. Currently, 23 human MMPs have been divided into 6 subgroups including collagenase, gelatinases, stromelysins, matrilysins, membrane type MMPs and other MMPs[5]. Among these, MMP-13 also known as collagenase-3, is a member of MMP family in the collagenase subgroup, play a central role in the MMP activation cascade and has an important role in tumor invasion and metastasis by cleavage type II

collagen[6]. The elevate levels of MMP-13 are associated with aggressiveness and shorter overall survival of several cancers including oral cancer[7], colorectal cancer[8], glioma[9] and breast cancer[10]. Owing to MMP-13 are secretory proteins it may exported from the tumor cells into blood circulation and are able to detect by ELISA method.

This study aimed to determine the plasma levels of MMP-13 in the patients with IDC and healthy women by ELISA method. The correlation between these protein levels to the clinical characteristics of the patients were also analyzed to elucidate their role in breast cancer development.

Materials and methods

Specimens

A total of 108 blood samples from patients diagnosed with IDC, 30-84 years of age, were obtained from the Department of Research and Technology Assessment, National Cancer Institute, Bangkok, Thailand. All patients had not received chemotherapy and/or radiation therapy prior to blood collection. Control group (n=131) constituted Thai healthy women, 36-62 years of age, who had participated in a "Mammogram screening campaign 600 cases for cerebration of the Fifth Cycle Birthday Anniversary of HRH Princess Maha Chakri Sirindthorn" at the Faculty of Tropical Medicine, Mahidol University and had negative mammogram (mammographic BI-RADS category 1 and 2) and clinical examination were within normal range. The study was approved by the Ethics Committee of the National Cancer Institute (101_2015RC_OUT431), Thailand and the Ethics Committee, Faculty of Tropical Medicine, Mahidol University, Bangkok (MUTM 2015-042-01 and MUTM 2015-028-01).

Detection of plasma MMP-13

A 5 ml aliquot of EDTA blood was centrifuged at 1300 g for 10 minutes at 4°C. Plasma was separated and stored at -80°C until used. Plasma MMP-13 content was measured using a human MMP-13 ELISA kit ab100605 (Abcam, England). Plasma MMP-13 concentrations were determined from a calibration curve, linear over the range 8-6000 pg/ml. Each sample was measured in duplicate. Results of the two groups are expressed as median with interquartile range (IQR).

Statistical analysis

Statistical significance of plasma MMP-13 levels between patients and healthy controls were analyzed by independent student *t*-tests. The Kurskal-Willis test was used for analyze the association between plasma MMP concentration and clinicopathologial features of the patients. Odds ratios (OR) and 95% confidence interval (CI) were

Table 1 Clinical characteristic data of all invasive ductal carcinoma breast cancer patients (n=108) and healthy women (n=131)

Characteristics	Number (%)
Patients	
Age (yr)	
≤50	57 (52.8)
>50	51 (47.2)
Histological grade	
Well differentiated	24 (22.2)
Moderately differentiated	45 (41.7)
Poor differentiated	39 (36.1)
TNM stage	
I	25 (23.1)
II	38 (35.2)
III	32 (29.6)
IV	13 (12.1)
Tumor size (cm)	
≤3.0	46 (42.6)
>3.0	20 (18.6)
No data	42 (38.9)
Lymph node metastasis	
Negative	30 (27.8)
Positive	45 (41.7)
No data	33 (30.6)
Organ metastasis	
Negative	37 (34.2)
Positive	14 (13.0)
No data	57 (52.8)
Presence of ER, PR, HER2	
Non-triple negative	79 (73.1)
Triple negative	18 (16.7)
No data	11 (10.2)
Healthy women	
Age (yr)	
≤50	53 (40.5)
> 50	78 (59.5)
Mammographic category	
Category 1	21 (16.0)
Category 2	110 (84.0)

also calculated by multiple logistic regression analysis. Cutoff points of patient plasma MMP-13 levels were determined by receiver-operating characteristic (ROC) curve. All statistical tests were carried out using SPSS statistical software version 20.0 for Microsoft Windows, (IBM's Corp., New York, NY, USA).

Results

Mean±SD age of the patients was 51±12 years, with 53% ≤50 years old. As regards histological feature, 24 (22%) specimens were well differentiated (G I), 45 (42%) moderately differentiated (G II) and 39 (36%) poorly differentiated (G III). Twenty-five (23%) cases were at TNM stage I, 38 (35%) stage II, 32 (30%) stage III, and 13 (12%) stage IV. Mean ± SD age of healthy women control was 54 ± 10 years, with 40% ≤50 years old. (Table 1).

Median plasma MMP-13 level of patients (89.58 pg/ml, IQR = 48.45-126.91 pg/ml) was lower than that of controls (289.15 pg/ml, IQR=230.60 - 339.40 pg/ml (P=0.000, OR=1.60, 95%CI= 1.53-1.68) (Figure 1). ROC curve to determine cut-off value of plasma MMP-13 concentration in patients had an AUC of 0.98 (95%CI=0.97-0.99), with a sensitivity of 91.6% and specificity of 91.7% at cutoff point of 169.36 pg/ml (Figure 2).

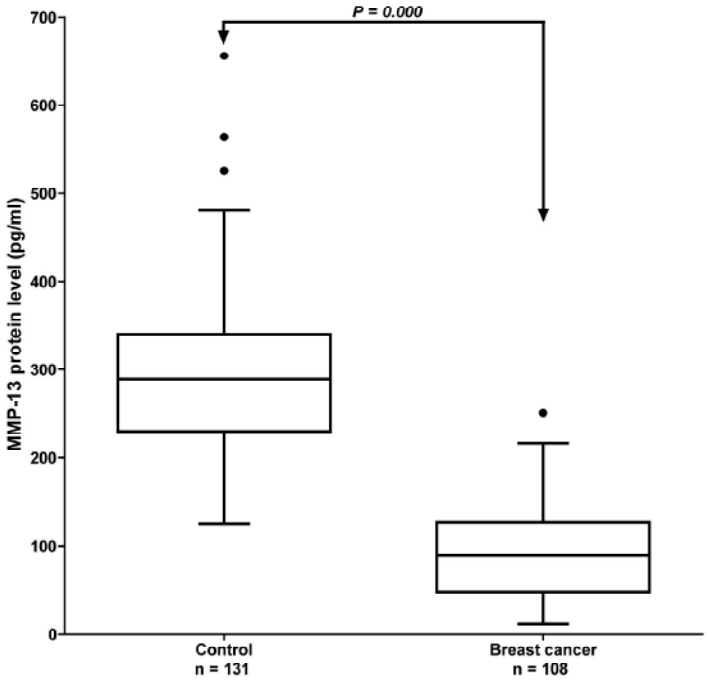

Figure 1 Plasma level of MMP-13. Differences in plasma level of MMP-13 in breast cancer patients and healthy controls. Box plot diagram with median, 1st quartile, 3rd quartile and non-outlier range.

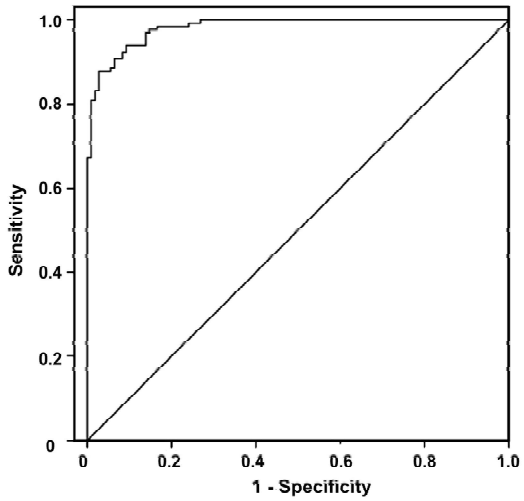

Figure 2 Receiver-operator curves (ROCs) analysis. ROC curve for plasma MMP-13 levels with an AUC of 0.98 (95% CI = 0.97-0.99).

The correlation of MMP-13 in the IDC with various clinical data were demonstrated in Table 2. The plasma MMP-13 level of patients is not significantly associated with all clinical features.

Discussion

Mammography is the best technique for detection of breast cancer[11] but it can only be performed in a hospital setting due to the high operating cost[12]. On the other hand, immunohistochemical technique needs expert pathologist to perform the procedure and to analyze results. Thus, a novel biomarker associated with breast cancer is still needed. This study, the plasma MMP-13 levels in a patients with IDC were measured using commercial ELISA kits.

Little is known for the plasma or serum levels of MMP-13 in several cancer patients. The plasma levels of MMP-13 was higher in prostate cancer patients with metastasis than in the control groups[13], whereas Bonald CM et al[14] reported no difference concentration of plasma MMP-13 in between prostate cancer patients and healthy controls. Moreover, MMP-13 levels could not be detected in both plasma of breast cancer patients and healthy controls because they did not reach the detection sensitivity of the ELISA kits used in the study[15]. However, this study can be detected the MMP-13 concentration in plasma of both two groups. The lower levels of MMP-13 protein in the patients plasma than in the controls found in this study was similar with the previous report in colorectal cancer that the serum MMP-2 levels was

Table 2 Plasma level of MMP-13 in IDC patients in association to clincopathological data

Variables	n	MMP-13 (pg/ml) Median (IQR)	Odds ratio (95%CI)	*P*
Patients				
Invasive ductal carcinoma	108	89.58 (48.45 - 126.91)		
Age (yr)				
< 50	57	88.03 (39.58 - 123.30)		
> 50	51	91.34 (52.59 - 141.75)	0.800 (0.375 - 1.705)	0.563
Histological grade				
Well differentiated	24	95.87 (57.21 - 144.33)		
Moderately differentiated	45	89.06 (46.79 - 122.05)	1.244 (0.449 - 3.447)	0.675
Poor differentiated	39	88.03 (47.42 - 132.06)	1.007 (0.427 - 2.374)	0.988
TNM stage				
I	25	70.51 (39.58 - 102.68)		
II	38	91.86 (50.10 - 142.89)	0.250 (0.060 - 1.048)	0.058
III	32	89.58 (57.62 - 130.42)	0.494 (0.129 - 1.884)	0.306
IV	13	110.93 (84.12 - 126.39)	0.444 (0.113 - 1.743)	0.245
Tumor size (cm)				
≤ 3.0	46	90.20 (56.07 - 127.42)		
> 3.0	20	100.10 (59.68 - 134.64)	0.727 (0.251 - 2.110)	0.558
Lymph node metastasis				
Negative	30	81.33 (50.10 - 107.84)		
Positive	45	88.03 (43.70 - 123.30)	0.915 (0.363 - 2.308)	0.850
Organ Metastasis				
Negative	37	87.00 (52.16 - 110.71)		
Positive	14	114.02 (53.02 - 127.42)	0.340 (0.900 - 1.282)	0.111
Presence of ER, PR, HER2				
Non-triple negative	79	84.12 (42.67 - 126.91)		
Triple negative	18	106.81 (63.51 - 156.29)	0.561 (0.197 - 1.595)	0.278

Non-triple negative = ER+/-, PR+/-, and HER2+

Triple negative = ER-, PR-, and HER2-

lower than that of healthy control[16] and the lower MMP-1 levels in breast cancer patients than that in healthy controls[15]. It was unclear for the unusual lower plasma or serum levels of several MMPs in the cancer patients but Decock J et al[15] hypothesized that this may be reflect the concentration of MMPs in tumor microenvironment. Interestingly, ROC curve analysis to determine cutoff value, sensitivity and specificity of plasma MMP-13 indicate that the potential of this protein may be used as the biomarker for IDC.

In conclusion, the plasma MMP-13 has important roles for IDC development and may be served as a biomarker for IDC. However, further study with larger sample will increase the precision of the data.

Acknowledgments

This study was supported by Mahidol University and the Mammogram Screening Campaign 600 Cases for Cerebration of the Fifth Cycle Birthday Anniversary of H.R.H Princess Maha Chakri Sirindthorn Foundation, Faculty of Tropical Medicine, Mahidol University. The authors thank Ms. Suwapad Junkree for laboratory assistance, Dr. Ngamphol Soonthornworasiri, Ms. Sudaporn Kengkarn for statistical analysis assistance and Prof. Prapon Wilairat, for critical reading of the manuscript.

References

1. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, et al. GLOBALCAN 2012 v1.0, Cancer incidence and mortality worldwide: IARC Cancer Base No.11 [internet]. Lyon, France:International Agency for Research on Cancer; 2013. Available at: http://globocan.iarc.fr. Accessed January 16, 2016.
2. Makki J. Diversity of breast carcinoma: Histological subtypes and clinical relevance. Clin Med Insights Pathol 2015;8:23-3.
3. Yadav L, Puri N, Rastogi V, Satpute P, Ahmad R, Kaur G. Matrix metalloproteinases and cancer-role in threat and therapy. Asian Pac J Cancer Prev 2014; 15:1085-91.
4. Cathcart J, Pulkoski-Gross A, Cao J. Targeting matrix metalloproteinases in cancer: Bringing new life to old ideals. Genes Dis 2015;2:26-34.
5. Verma RP and Hansch C. Matrix metalloproteinases (MMPs): chemical-biological functions and (Q) SARs. Bioorg Med Chem 2007;15:2223-68.
6. Leeman MF, Curran S, Murray GI. The structure, regulation, and function of human matrix metalloproteinase-13. Crit Rev Biochem Mol Biol 2002; 37:149-66.
7. Vincent-Chong VK, Salahshourifar I, Karen-Ng LP, Siow MY, Kallarakkal TG, Ramanathan A, et al. Overexpression of MMP-13 is associated with clinical outcomes and poor prognosis on oral squamous cell carcinoma. Sci World J 2014;897523.
8. Yang B, Gao J, Rao Z, Shin Q. Clinicopathological significance and prognostic value of MMP-13 expression in colorectal cancer. Scan J Clin Lab Invest 2012;72:501-5.
9. Wang J, Li Y, Wang J, Li C, Yu K, Wang Q. Increased expression of matrix metalloproteinase-13 in glioma is associated with poor overall survival of patients. Med Oncol 2012;29:2432-37.
10. Chang HJ, Yang MJ, Yang YH, Hou MF, Hsueh EJ, Lin SR. MMP13 is potentially a new tumor marker for breast cancer diagnosis. Oncol Rep 2009;22:1119-27.
11. Bleyer A and Welch HG. Effect of three decades of screening mammography on breast cancer incidence. N Engl J Med 2012;367:1998-2005.
12. 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.

13. Morgia G, Falsaperla M, Malaponte G, Madonia M, Indelicato M, Travali S, et al. Matrix metalloproteinases as diagnostic (MMP-13) and prognostic (MMP-2, MMP-9) markers of prostate cancer. Urol Res 2005; 33:44-50.
14. Bonald CM, Azzalis LA, Junqueira VBC, de Oliveira CG, Vilas Boas VA, Gáscon TM, et al. Plasma levels of E-cadherin and MMP-13 in prostate cancer patients: correlation with PSA, testosterone and pathological parameters. Tumori 2015;101:185-8.
15. Decock J, Hendrickx W, Vanleeuw U, Van Belle V, Van Huffel S, Christiaens MR, et al. Plasma MMP1 and MMP8 expression in breast cancer: protective role of MMP8 against lymph node metastasis. BMC Cancer 2008;8:77.
16. Groblewska M, Mroczko B, Gryko M, Pryczynicz A, Guzińska-Ustymowicz K, Kędra B, et al. Serum levels and tissue expression of matrix metalloproteinase 2 (MMP-2) and tissue inhibitor of metalloproteinases 2 (TIMP-2) in colorectal cancer patients. Tumor Biol 2014;35:3793-802.