Matrix Metalloproteinase-13 (MMP-13): A Novel Tumor Marker for Diagnosis of Breast Carcinoma

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ABSTRACT

Introduction: Matrix metalloproteinases are a family of endopeptidases; they play crucial role in tumor progression and the metastatic process by facilitating extracellular degradation. Experimental evidences shows that MMP-13 or Collagenase 3 overexpresses in breast carcinoma and promote tumor progression. So, it is emerging as a novel tumor marker. But, it is not clear that it can be used as a diagnostic and prognostic marker. Therefore, the aim of this study is to investigate the clinical significance of serum matrix metalloproteinase-13 levels in various stages of breast carcinoma.

Material and Methods: The serum level of MMP-13 was measured with commercially available ELISA kit in 100 healthy controls and 135 breast cancer patients. Other two tumor markers were measured by using ELFA technique on VIDAS family instrument. Independent sample t- test and One-way Anova along with roc curve analysis was done for evaluating results. Box-plots were also generated between the parameters and stages of breast cancer. In order to determine correlation, Pearson correlation was done.

Results: Serum levels of MMP-13 were significantly higher ($p<0.001$) in breast cancer subjects as compared to controls. CA15.3 and CA125 levels were also statistically significant ($p<0.05$) in cases as compared to normal healthy controls.MMP-13 was found highly sensitive (100%) and specific (92.50%) with $p<0.001$ when compared with other tumor markers. There was an increasing trend of MMP-13 levels as the stages advanced in breast carcinoma.

Conclusion: MMP-13 has potential to be used as a diagnostic and prognostic marker for breast carcinoma.

Keywords: Matrix Metalloproteinase-13; CA15.3; CA125; Breast cancer, Tumor marker

INTRODUCTION

Breast cancer is one of the most common and leading causes of cancer death among women worldwide.[1] India has 17 percent of the world's population suffering from breast cancer. Early diagnosis of breast cancer can provide patients a wider range of therapeutic options as well as a higher success rate of therapy that lowers mortality. Quantitative analysis of tumor markers is the most convenient method to screen breast cancer. Various tumor markers are currently available for breast cancer detection including carcinoembryonic antigen, cancer antigen 15.3, and cancer antigen 125 but exhibited certain limitations, like poor sensitivity and specificity which greatly limits the diagnostic accuracy of these markers.[2] Hence, for clinical diagnosis more sensitive and more specific tumor markers are needed. It is difficult to predict the occurrence of distant metastasis because breast cancer is a heterogeneous disease encompassing a variety of pathological entities and a wide range of clinical behaviors, even in patient groups that seem to be clinically similar.[3] Mortality from Breast cancer is due to dissemination of tumor cells and formation of metastasis.[4] Cancer invasion and metastasis requires the degradation of the basement membrane and the extra cellular matrix, which enable tumor cells to migrate. The majority of the destruction of the matrix components during metastasis is carried out by stimulated release of Matrix Metalloproteinases (MMPs).

Matrix Metalloproteinases (MMPs) or matrixins are a family of endopeptidases that can degrade extracellular matrix proteins and promote cell invasion and metastasis. MMPs are differentially expressed and their expressions are often associated with a poor prognosis for cancer patients.[5] In a normal mammary gland, constitutive expression of MMPs is low, except during times of development and pregnancy.[6] Aberrant MMP expression has been observed to be associated with prognosis in breast cancer. MMP-13 (Collagenase-3) EC 3.4.24.22 is the latest human collagenase described in literature. MMP-13 is expressed in a broad range of primary malignant tumors and it is emerging as a novel biomarker.[7] MMP-13 (collagenase-3) is the third member of the collagenase subfamily of MMPs to be identified and has distinct properties compared with the other collagenases. Matrix Metalloproteinase-13 was first identified and cloned from breast cancer tissue in 1994.[8] This enzyme exhibits preference toward cleavage of collagen I, II, III, fibrinogen, gelatin and factor XII. MMP-13 plays important role in cancer invasion, metastasis, growth regulation, immune evasion, apoptosis, and angiogenesis. Elevated levels of MMP13 have been associated with decreased overall survival and lymph node metastasis in breast cancer.[9] So, the aim of our study was to investigate the clinical significance of serum matrix metalloproteinase-13 levels in various stages of breast carcinoma.

MATERIAL AND METHODS

The present study has been carried out in the Department of Biochemistry and Department of Radiotherapy, G.R Medical College and J.A. Group of Hospitals, Gwalior. The prevalence rate of breast cancer in India is around 23% (taking it as a reference) accordingly, the minimum sample size was calculated

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using the appropriate sample size formula -
$n= z^2pq/d^2$ Where z= 1.96 at 95 % confidence interval
p = 0.23 and q= 1-p= 0.77 d= Absolute error 10%
$n= (1.96)^2 \times 0.23 \times 0.77/ (0.010)^2$
Minimum sample size for cases = 100
So, total 235 human subjects were taken in the study. Out of which 100 normal age matched healthy subjects were considered as controls and 135 breast cancer patients subjects as cases which were further divided into their respective stages according to TNM classification. Out of total 135 breast cancer patients there were 40 patients of stage I, 30 patients of stage II, 30 patients of stage III and 35 patients of stage IV.

Inclusion criteria of the study

- Female patients (age >20 years) diagnosed with breast cancer.
- All patients with operable breast lumps and recurrent breast lump in a previously operated case of carcinoma breast.

Exclusion criteria of the study

- Pregnant women.
- Patients with benign breast diseases.
- Patients having other cancer, collagenopathy and all other diseases that affect the level of MMP-13.
- Patients taking chemotherapy and radiotherapy.

Before starting analysis, the written consent from all subjects was taken. The study has been approved by institutional ethical committee and was carried out by keeping all norms in mind. The clinical manifestations of disease, personal history of patients were recorded in study proforma. Blood sample was collected in plain vial and incubated at 37˚C for 30 minutes. After incubation, clot was removed and remaining sample was taken in centrifuge test tube. Samples were centrifuged at 3000rpm for 10 to 20 minutes. Supernatant was collected in clean and dry serum test tube for analysis of matrix metalloproteinase-13, cancer antigen 15.3, cancer antigen 125.

The measurement of serum MMP-13 levels was carried out by ELISA kit (Cloud- Clone Corp. assembled by Uscn Life Sciences Inc. SEA099HU). The microtiter plate provided in the kit has been pre-coated with an antibody specific to MMP13. Standards or samples were then added to the appropriate microtiter plate wells with a biotin-conjugated antibody specific to MMP13. Next, Avidin conjugated to Horseradish Peroxidase (HRP) was added to each microplate well and incubated. After TMB substrate solution was added, only those wells that contain MMP13, biotin-conjugated antibody and enzyme-conjugated Avidin exhibit a change in color. The enzyme-substrate reaction was terminated by the addition of sulphuric acid solution and the color change was measured spectrophotometrically at a wavelength of 450nm ± 10nm. The concentration of MMP13 in the samples was then determined by comparing the O.D. of the samples to the standard curve.

CA 15.3 and CA 125 were analysed on the VIDAS family instruments from human serum. The assay combines a 2-step enzyme immunoassay sandwich method with a final fluorescent detection (ELFA). The Solid Phase Receptacle (SPRI) serves as the solid phase as well as the pipetting device for the assay. Reagents for the assay were ready-to-use and pre-dispensed in the sealed reagent strips. All of the assay steps were performed automatically by the instrument.

STATISTICAL ANALYSIS

The statistical differences between cases and control were determined by student independent sample t-test and one way analysis of variance (ANOVA).Data analyses were performed with the Statistical Package for the Social Sciences, version 21.0 (SPSS, Chicago, Illinois, USA). Box-plots were generated between the parameters and stages of breast cancer. In order to determine correlation, statistical analysis was carried out by using spearman's rank correlation coefficient. Cut off values for tumor markers were calculated on the basis of Receiver operator characteristic curve (ROC) analysis. The p value less than 0.05 were considered as significant.

RESULTS

In our study, the serum level of MMP-13 was found statistically highly significant (149.81± 58.51, $p<0.001$) in breast cancer patients as compared to control healthy subjects. CA15.3 (50.16±19.47) and CA125 (43.78±10.54) levels were also statistically significant ($p<0.05$) in breast cancer patients as compared to control healthy subjects. (Figure 1)

When individual stages were compared with healthy control subjects, we found in Stage I, MMP-13 levels were statistically highly significant (86.00±11.19) as compared to control healthy subjects. In Stage II, MMP-13 levels were highly significant (114.40±11.36) while CA15.3 was statistically significant (40.15±4.21) as compared to control healthy subjects. In Stage III, MMP-13 level was statistically highly significant (181.23± 14.97, $p<0.001$) while CA 15.3(45.66±2.07) and CA125 (41.00±5.6) levels were statistically significant ($p<0.05$) as compared to control healthy subjects. In Stage IV, MMP-13 level was also statistically highly significant (226.17± 16.61, $p<0.001$) while CA 15.3 (75.06±20.39) and CA125 (56.08± 10.08) levels were statistically significant ($p<0.05$) as compared to healthy control subjects.

The one-way anova analysis between the tumor markers and all four stages in breast cancer patients showed that MMP-13 was highly significant (F value 773.70, $p<0.001$) in all stages as compared to other tumor markers.

The correlation of age with tumor markers in breast cancer patients showed that MMP-13 was statistically significantly (r value 0.200, $p<0.05$) positively correlated with the age as compared to other tumor markers.

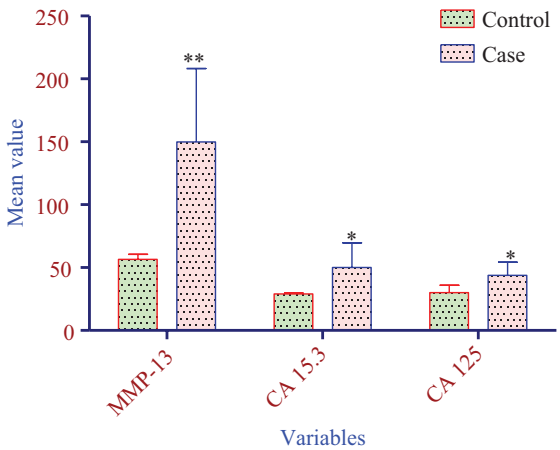

Figure-1: Showing significant changes of tumor marker in healthy control subjects and breast cancer patients (case)

The correlation of all four stages with tumor markers in breast cancer patients showed that MMP-13 was highly significantly (r value 0.964, p<0.001) positively correlated with the stages of breast cancer patients while CA15.3 statistically significantly (r value 0.188, p< 0.05) positively correlated. When we generate box plot we found that there was an increasing trend in the level of MMP-13 with respect to the advancing stages. (Figure 2).The receiver operator characteristic curve (ROC) analysis for tumor markers in breast cancer patients also showed that MMP-13 was highly sensitive (100%) and specific (92.50%) when compared to other tumor markers with p<0.001; cut off value 100ng/ml (Figure 3). Stagewise cutoff value were also determined (Table 1).

DISCUSSION

The breast cancer is diagnosed by the elevation of the tumor markers, histopathological studies and on the basis of clinical manifestations. The MMP-13 is recently added as the diagnostic enzyme along with other conventional tumor markers.MMP-13 or collagenase-3 is a member of the matrix metalloproteinase family that have potent degrading activity for degrading the major protein components of the extracellular matrix and basement membranes of the cells.[10] These proteins are precisely regulated in order to prevent tissue disruption and when this physiological balance is disturbed as in cancer its capability of invading to adjoining tissue increases. The overexpression of this enzyme in the cell has been quoted by many scientists, but in many types of cancer not in breast cancer.[11-14] Decock et al 2008,[15] reported that it may be due to its low sensitivity detection limit by immunoassay. But in our study, we found that the serum MMP-13 level were highly significantly (p<0.001) increased in breast cancer subjects along with CA15.3, CA125 which were also significantly (p<0.05) increased. Many other scientists also reported elevated level of CA15.3, CA125 in breast cancer.[16-18] Among all three tumor markers, MMP-13 plays key role in collagen remodelling and as such to believe in local invasion rather than in metastasis formation. It is therefore showing that MMP-13 resides in the microenvironment in both early and advance stages resulting in low to high levels entering the circulation. Receiver operator characteristic curve (ROC) analysis for tumor markers in breast cancer patients showed that MMP-13 was highly sensitive and specific (p<0.001) when compared to other tumor markers. Wang G et al (2014)[19] also reported sensitivity and specificity of CA15.3 and CA125. Anova analysis also showed that increase in MMP-13 level was highly significant (p<0.001) as compared to other tumor markers. The breast carcinoma cells secrete diffusible factors, including IL-1α and IL-1β, which induce surrounding stromal fibroblasts to express MMP-13[20-21] which is most effective in breaking down type II collagen.[22] The MMP-13 contributes to the formation of a complex microenvironment that promotes malignant transformation in early stages of cancer. Most of the studies done at molecular level showed increased expression of MMP-13 in breast cancer. [2,4,7-9,20,23] Very few studies are available regarding its serum value. The increase in MMP-13 level in serum could be used as a diagnostic marker because in our study we have seen that the level of MMP-13 increases as the stage advances. From individual stagewise studies of tumor markers it has been sought that MMP-13 levels were

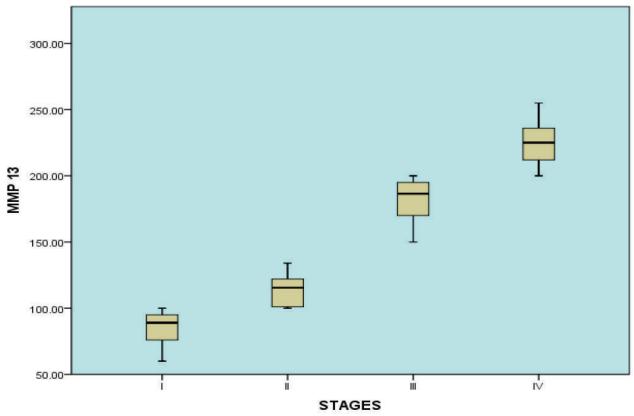

Figure-2: Showing the Box plot between tumor marker- MMP-13 and all four stages in breast cancer patients

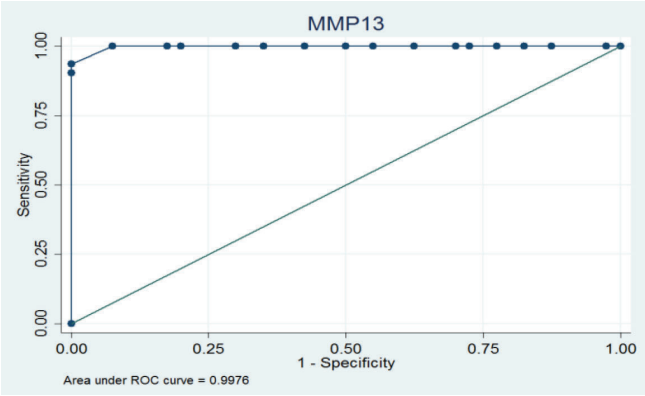

Figure-3: Showing the receiver operator characteristic curve (ROC) analysis for MMP13 tumor marker in breast cancer patients

Subjects	Cut off value of MMP-13
Stage I breast cancer patients	>=66
Stage II breast cancer patients	>=100
Stage III breast cancer patients	>=150
Stage IV breast cancer patients	>=200

Table-4: showing the cutoff value of MMP-13 level in different stages observed in the present study

consistently highly significant (p<0.001) while raise of CA15.3 started from Stage II onwards and CA125 from stage III. So, it was evident that MMP-13 elevation is related to metastasis and progression of breast carcinoma. This may be because in the process of breast cancer turning from low stage to advance stage, MMP13 will break down basement membranes of tissues and release of angiogenic factors to form an invasive carcinoma. This is in agreement with Nielsen et al, 2001.[24]

These serum levels of different stages were further correlated with histopathological studies and we came to conclusion that the increase of the MMP-13 is positively correlated with the various stages of the breast cancer.

The further confirmation requires a study on large number of sample size. We will do comparison of serum MMP-13 level in other carcinomas in future so that we can find out diagnostic accuracy of this novel marker regarding breast carcinoma.

CONCLUSION

The cut-off value of MMP-13 could be used as diagnostic marker for diagnosis of stage I breast cancer because it has role

at different phases of metastatic spread and the measurement of serum MMP-13, could be of clinical value when identifying patient high risk for progression. Lastly, MMP-13 has potential to become a new breast cancer tumor marker when accompanied by current clinical screening methods and could be used as a diagnostic marker in early stage of the breast cancer diagnosis. We have taken the patients of all stages prior to start of chemotherapy and radiotherapy. It may be possible that these therapies can influence their level in serum. The further study requires the effect of chemotherapy and radiotherapy at various stages of breast cancer on serum MMP-13 level.

REFERENCES

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, et al. Global cancer statistics. CA Cancer. J Clin. 2011;61:69-90.
2. Chang HJ, Yang MJ, Yang YH, Hou EJ, Ruslin S. MMP13 is potentially a new tumor marker for breast cancer diagnosis. Oncol Rep. 2009;22:1119-1127.
3. Eiro N, Garcia BF, Gonzalez LO, Vizoso FJ.Clinical Relevance of Matrix Metalloproteases and their Inhibitors in Breast Cancer. J Carcinogene Mutagene. 2013;13:1-13.
4. Balduyck M, Zerimech F, Gouyer V, Lemaire R, Hemon B, Grard G et al. Specific expression of matrix metalloproteinases 1, 3, 9 and 13 associated with invasiveness of breast cancer cells in vitro. Clin Exp Metastasis. 2000,18:171-178.
5. Benson CS, Babua SD, Radhakrishnab S, Selvamurugnac N, Sankara BR. Expression of matrix metalloproteinases in human breast cancer tissues. Dis Markers. 2013;34: 395–405.
6. Morgan H, Hill PA. Human breast cancer cell-mediated bone collagen degradation requires plasminogen activation and matrix metalloproteinase activity. Cancer Cell Int. 2005;5:1-8.
7. Pivetta E, Scapolan M, Pecolo M, Wasserman B, Rumeileh IA, Balestreri L, et al. MMP-13 stimulates osteoclast differentiation and activation in tumor breast bone metastases. Breast Cancer Res. 2011;13:105-120.
8. Freije JM, Itza ID, Balbin M, Sanchez LM, Blascon R, Toliviall J, et al. Molecular Cloning and Expression of Collagenase-3, a Novel Human Matrix Metalloproteinase Produced by Breast Carcinoma. J. Biol. Chem. 1994; 269:16766-16.
9. Zhang B, Cao X, Liu Y, Cao W, Zhang F, Zhang S, et al. Tumor-derived Matrix Metalloproteinase-13 (MMP-13) correlates with poor prognoses of invasive breast cancer. BMC Cancer. 2008;8:83-93.
10. Pendas AM, Uria JA, Jimenez MG, et al An overview of collagenase-3 expression in malignant tumors and analysis of its potential value as a target in antitumor therapies. Clin Chim Acta. 2000; 291:137-55.
11. Jiao XL, Chen D, Wang JG, Zhang KJ. Clinical significance of serum matrix metalloproteinase-13 in patients with esophageal squamous cell carcinoma (ESCC).Eur Rev Med Pharmacol Sci. 2014; 18:509-515.
12. Nikkola J, Vihinen P, Vuoristo MS, Lehtinen PK, Kahari VM and Pyrhonen S. High Serum Levels of MatrixMetalloproteinase-9 and Matrix Metalloproteinase-are associated with Rapid Progression in patients with metastatic melanoma. Clin Cancer Res. 2005;11:5158-5166.
13. Asano Y, Ihn H, Kubo M, Jinnin M, Mimura Y, Ashida R, Tamaki K. Clinical significance of serum matrix metalloproteinase-13 levels in patients with localized scleroderma. Clin Exp Rheumatol. 2006a; 24:394-399.
14. Asano Y, Ihn H, Kubo M, Jinnin M, Mimura Y, Ashida R, Tamaki K. Clinical significance of serum matrix metalloproteinase-13 in patients with systemic sclerosis. Rheumatology. 2006b;45:303-307.
15. Decock J, Hendrickx W, Vanleeuw U, Belle VV, Huffel SV, 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. Park BW, Oh JW, Kim JH, Park SH, Kim KS, Kim JH, et al. Preoperative CA 15-3 and CEA serum levels as predictor for breast cancer outcomes. Ann Oncol. 2008;19:675-81.
17. Callea MR, Giacometti, Marchesini C, Zanin E, Baseggio C, Pasini L. Serum levels of CA15-3, CA125 and CA 19.9 in triple negative breast cancer at time of diagnosis. Presentato al; 15 Simposio annual ELAS-Italia Ligand Bind Assay 2009, Bologna 23-25 November 2009.
18. Qaseem AH, Mahdi NK, Husien AM. The value of CA15-3 in diagnosis and treatment response in women with breast cancer. J Pak Med Assoc. 2013;63:1138-1140.
19. Wang G, Qin Y, Zhang J, Zhao J, Liang Y, Zhang Z, et al. Nipple Discharge of CA15-3, CA125, CEA and TSGF as a New Biomarker Panel for Breast Cancer. Int. J. Mol. Sci. 2014;15:9546-9565.
20. Uria JA, Stahle-Backdahl M, Seiki M, Fueyo A and Lopez-Otin C. Regulation of collagenase-3 expression in human breast carcinomas is mediated by stromal epithelial cell interactions. Cancer Res. 1997;57:4882–8.
21. Balbin M, Pendas AM, Uria JA, Jimenez MG, Freije JP, Lopez-Otin C: Expression and regulation of collagenase-3 (MMP-13) in human malignant tumors. APMIS.1999; 107:45-53.
22. Knauper V, Lopez-Otin C, Smith B, Knight G, and Murphy G: Biochemical characterization of human collagenase-3. J Biol Chem. 1996;271:1544-1550.
23. Shah M, Huang D, Blick T, Connor A, Reiter LA, Hardink JR et al. An MMP13-Selective Inhibitor Delays Primary Tumor Growth and the Onset of Tumor- Associated Osteolytic Lesions in Experimental Models of Breast Cancer. PLoS ONE 7. 2012;1:e29615.
24. Nielsen BS, Rank F, Lopez JM, et al. Collagenase-3 expression in breast myofibroblasts as a molecular marker of transition of ductal carcinoma in situ lesions to invasive ductal carcinomas Cancer Res. 2001;61:7091-7100.

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