Matrix Metalloproteinase-13 Can Be Used As an Independent Diagnostic and Prognostic Marker for Breast Carcinoma

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Abstract: Background & Objectives: Matrix Metalloproteinase-13(MMP-13) or Collagenase3 overexpresses in breast carcinoma and promotes tumor progression. Therefore, this study aims to investigate the clinical significance of serum matrix metalloproteinase-13 levels in various stages of breast carcinoma. Methods: The study was conducted in the Department of Biochemistry, G. R. Medical College, Gwalior using the Case-control study design. 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. Serum levels of MMP-13 were estimated by ELISA technique while CA 15.3 and CA 125 levels by ELFA technique. Biochemical parameters – Alkaline Phosphatase, Acid Phosphatase and Calcium levels were determined by using fully automated analyzer using commercially available kits according to manufacturer instructions. Hematological parameters were determined by using Sysmex automated analyzer. Independent sample t- test and one-way ANOVA in combination with Tukey HSD along with ROC curve analysis was done for evaluating results. Results & Conclusion: Serum levels of MMP-13 were significantly higher ($p<0.001$) in breast cancer subjects as compared to controls. MMP-13 was found highly sensitive (100%) and specific (92.50%) with $p<0.001$ when compared with other tumor markers and biochemical parameters. So, MMP-13 can be used as an independent diagnostic and prognostic marker for breast carcinoma [Shrivastava S NJIRM 2017; 8(2):15-19]

Key Words: Matrix Metalloproteinase-13; breast cancer; cancer antigen 125, cancer antigen15.3.

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Introduction: Breast cancer is one of the most common and leading causes of cancer death among women worldwide.[1] 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.[2] 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 tumours and it is emerging as a novel biomarker.[3] 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.[4] 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. Change in serum levels of some biochemical parameters could assist diagnosis, prediction and follow-up of breast cancer. Hematological investigations are important part and parcel of breast cancer patients. It can help in the assessment of disease progression and the behavior of different malignancies.

Methods: 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 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

- 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

- 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 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.

Biochemical parameters – Alkaline Phosphatase, Acid Phosphatase and Calcium levels were determined by using fully automated analyzer using commercially available kits according to manufacturer instructions. Estimation of parameters was done by Sysmex Automated hematology Analyzer Kx-21. The SysmexKX-21 is an automatic multi-parameter blood cell counter for in vitro diagnostic use in clinical laboratories.

Statistical Analysis: Independent sample t- test and one-way ANOVA in combination with Post hoc Tukey HSD along with ROC curve analysis was done for evaluating results. Box-plots were also generated between the parameters and stages of breast cancer. The p value less than 0.05 were considered as significant.

Results: We found significant changes in hematological parameters in control healthy subjects and breast cancer patients. The data indicated that hemoglobin level, polymorphs, lymphocytes, monocytes and RBC count were statistically reduce ($p<0.05$) while other parameters were non-significant as compared to control healthy subjects. We also found that hemoglobin level and lymphocyte count gradually decreases with stages while other parameters were also deranged but not specific trend observed.

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.

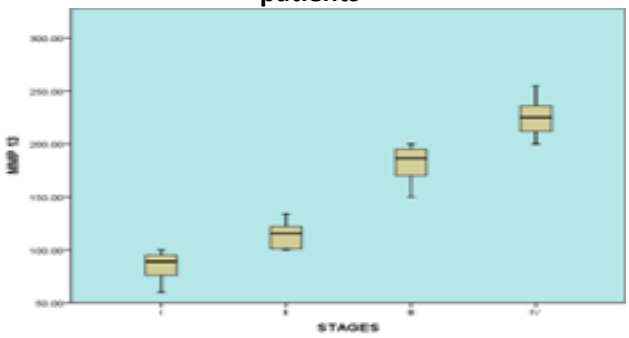
When individual stages were compared with healthy control subjects, we found in Stage I, MMP-13 levels were statistically highly significant (86.00±11.19) as compared to control healthy subjects while other parameters were non-significant. In Stage II, MMP-13 levels were highly significant (114.40±11.36) 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.

Alkaline phosphatase (163.93 ±25.07) was found statistically significant ($p<0.05$) while acid phosphatase and calcium were non-significant in breast cancer patients as compared to control healthy subjects. When all four stages were individually compared with control we found that alkaline phosphatase level was gradually increased with advancing stages. The one-way anova analysis between the tumor markers and biochemical parameters in all four stages of breast cancer patients showed MMP-13 and alkaline phosphatase were highly significant ($p<0.001$) in all stages as compared to other parameters. For further knowing the status of these two significant parameters with stages, we have done Post hoc Tukey HSD analysis.MMP-13 was found highly significant ($p<0.001$) in all stages while ALP was non-significant.

There was increasing trend of MMP-13 level with respect to stages when box graph was plotted

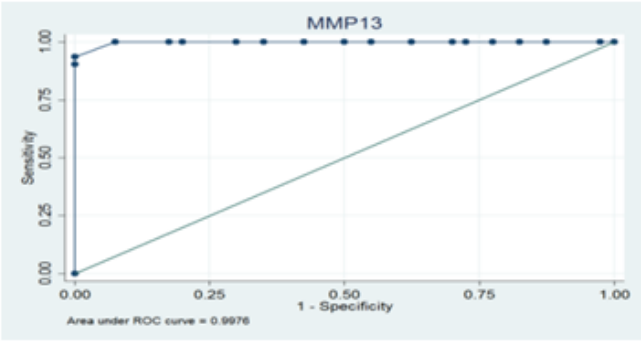
between level of MMP-13 and stages in breast cancer patients. No values were found outliers (Graph 1). Other parameters showed neither increasing nor decreasing trend with respect to advancing stages.

Graph 1: Showing the Box plot between tumor marker- MMP-13 and all four stages in breast cancer patients



MMP-13 was 100% sensitive and 92.50% specific under ROC curve area 0.99 with p value 0.001 i.e. highly significant in breast cancer patients (Graph 2).

Graph 2: showing the receiver operator characteristic curve (ROC) analysis for MMP13 tumor marker in breast cancer patients



Discussion: Breast cancer patients have deranged hematological parameters. We observed anaemia, neutropenia, lymphocytopenia and low monocytic count in breast cancer patients. This is same as quoted by Spivak et al., 2009, Ali, 2014, Ufelle et al., 2012.[5-7] It could be due to increased levels of pro- inflammatory cytokines such as IL-1, IL-6, TNF-α, and INF-δ that induce iron retention by the reticulo-endothelial system, gastrointestinal tract, and liver, thereby exerting an inhibitory effect on erythroid precursors and hematopoiesis.[8-9]

In our study, alkaline phosphatase was found statistically significant ($p<0.05$) while acid phosphatase and calcium were non-significant in breast cancer patients as compared to control subjects. The elevation of serum ALP occurs because of the accelerated denovo synthesis of the enzyme and subsequent regurgitation into the serum.[10-12] The level of ACP did not show any significant increase in our study. Similarly, the level of the calcium also showed non-significant variation. This might be due to diagnostic insensitivity and non-specificity towards breast carcinoma or effect of supplementation by patients.

Serum MMP-13 levels were statistically highly significant ($p<0.001$) while CA15.3 and CA125 were also statistically significant ($p<0.05$) in breast cancer patients subjects as compared to control healthy subjects. This may be because the breast carcinoma cells secrete diffusible factors, including IL-1α and IL-1β, which induce surrounding stromal fibroblasts to express MMP-13.[13-14] We also found CA15.3 significantly ($p<0.05$) in breast cancer patients. This may be because CA15.3 is the product of MUC-1 gene, and mucins which are aberrantly over expressed in many adenocarcinomas in an under-glycosylated form and then shed into circulation.[15] CA125 levels were also significantly high in breast cancer patients. Increase in CA125 level was also caused by the metastatic breast cancer cells because of infiltration of cancer cells into the peripheral tissues. From individual stagewise studies of tumor markers it has been sought that MMP-13 levels were 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.

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.[16-19] 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. 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.[20]

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. We also found cut-off values for the MMP-13 stage wise to make the easy diagnosis of breast cancer which is as follows-

Table 1: showing the cut-off value of MMP-13 level in different stages observed in the present study by roc analysis

Subjects	Cut off value of MMP-13 (ng/ml)
Stage I	>=66
Stage II	>=100
Stage III	>=150
Stage IV	>=200

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. MMP-13 in our study did not found as a prognostic marker but the elevated level of this marker will definitely help in predicting the starting of metastasis. 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

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