Matrix Metalloproteinase-13 (MMP-13): A proposed Tumor Marker in Breast Carcinoma

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Abstract:

Breast cancer is estimated to account for 31% new onset female cancers. Matrix metalloproteinases (MMPs) are extracellular zinc-dependent endopeptidases involved in the degradation and remodeling of extracellular matrix in physiological and pathological processes**.** In this study, we aimed to evaluate the use of matrix metalloproteinase-13 (MMP-13) as a potential tumor marker in breast cancer and we also aimed to investigate the role of infection as a cause of carcinogenesis by estimating the incidence of anti-cytomegalovirus IgG antibodies in breast cancer patients.

Subjects and Methods:

Serum CA15.3, anti-CMV IgG and MMP-13 were measured in 50 breast cancer female patients, 20 female patients with fibroadenoma of breast and 20 healthy age-matched female volunteers.

Results:

MMP-13 levels showed no significant difference between breast cancer patients and controls; on the other hand MMP-13 levels were significantly higher in fibroadenoma patients compared to controls. CA15.3 levels were significantly higher in breast cancer cases compared to both fibroadenoma and healthy controls.

Conclusion:

The results of this study show that MMP-13 level is highest among control group, while fibroadenoma group had the lowest MMP-13 level. CA15.3 is still the most useful serum tumor marker in patients with breast cancer.

1) Introduction

Breast cancer is one of the most common and leading causes of cancer death among women worldwide **(Power *et al.*, 2018).** 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 **(Chang *et al.,* 2009).** 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 **(Eiro *et al.,* 2013).**

Cancer invasion and metastasis requires the degradation of the basement membrane and the extra cellular matrix, which enable tumor cells to migrate **(Balduyck *et al.,* 2000)**. The majority of the destruction of the matrix components during metastasis is carried out by stimulated release of matrix metalloproteinases (MMPs). MMPs are a family of endopeptidases that can degrade extracellular matrix proteins and promote cell invasion and metastasis **(Benson *et al.,* 2013).** In a normal mammary gland constitutive expression of MMPs is low, except during times of development and pregnancy **(Morgan and Hill, 2005).** 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 **(Pivetta *et al.,* 2011).** 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 **(Freije *et al.,* 1994).** This enzyme exhibits preference toward cleavage of collagen I, II, III, fibrinogen, gelatin and factor XII. MMP-13 plays important roles 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 **(Zhang *et al.,* 2008).** So, the aim of our study was to investigate the clinical significance of serum matrix metalloproteinase-13 levels in breast carcinoma by comparing its level with those found in fibroadenoma patients and healthy controls.

2) Subjects and Methods

The study was conducted at Clinical Pathology Department, Ain Shams Specialized Hospital. Ninety females were enrolled from the outpatient clinic and oncology unit. Subjects were classified into three groups: group 1 included 50 breast cancer female patients at

different clinical and histological grades, group II included 20 female patients with fibroadenoma of breast and group III included 20 healthy age-matched female volunteers (as a control group). All females in the study were subjected to full history taking and thorough clinical examination, measuring of serum CA 15.3 by electro-chemiluminescence technique "ECLIA" on Cobas e411 automated immunoanalyzer (Roche, Germany), measuring the level of Anti-CMV IgG by enzyme linked immunosorbent assay (ELISA) (Foad company) and measuring of serum MMP-13 by ELISA (Sigma).

3) Results:

Table (1): Descriptive statistics of the three studied groups:

	Group I (n=50)		**Group II (n=20)**		**Group III (n=20)**	
	Median	**IQR**	**Median**	**IQR**	**Median**	**IQR**
Age (years)	47	37.7-52.2	47	40-51	45	31-55.7
MMP-13 (Pg/mL)	270	24-3300	19.5	10-29.5	510	180-1890
CA15.3 (U/mL)	206.2	111-413.4	16.4	11.95-22.57	13.85	10.8-17.47

IQR: Interquartile range

A highly significant difference was detected between the three groups regarding MMP-13 (<0.001) and CA15.3 (<0.001). Statistical analysis revealed that group III (control group) had the highest MMP-13 (median=510 Pg/mL) while group II (fibroadenoma group) had the lowest MMP-13 (median=19.5 Pg/mL) **(Table 1)**.

A significant statistical negative correlation was found between CA15.3 levels and MMP-13 levels in group I (breast cancer group) (R= -0.32, P=0.02). No significant statistical correlation was found between CA15.3 levels and MMP-13 levels in either group II (fibroadenoma group) (R= -0.12, P=0.6) nor group III (control group) (R= -0.10, P=0.65) **(Table 2).**

Table (2): Correlation between MMP-13 and CA15.3 in the three studied groups:

	CA15.3 U/Ml					
	Group I (n=50)		**Group II (n=20)**		**Group III (n=20)**	
	R	**P**	**R**	**P**	**r**	**P**
MMP-13 Pg/mL	-0.32	0.02	-0.12	0.6	-0.10	0.65

P: Probability value, S: Significant, NS: Non Significant, P<0.05 was considered significant

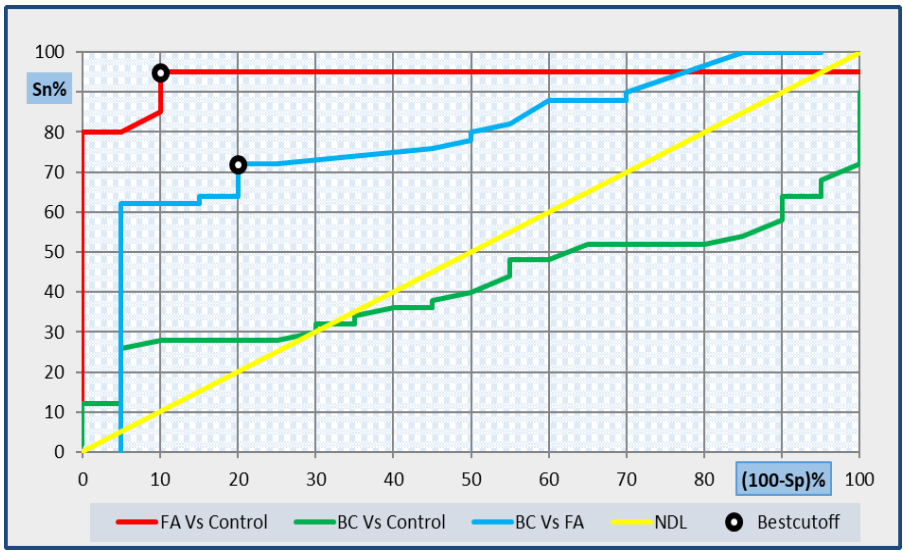
Receiver Operating Characteristics (ROC) curve analysis was applied to examine the diagnostic performance (sensitivity, specificity, negative predictive value, positive predictive value and efficacy) of CA15.3 in the studied groups.

Regarding MMP-13, the best cut off point that discriminate between fibroadenoma and control groups was found at 130 pg/mL with an AUC = 0.935, sensitivity =90%, specificity= 95%, negative predictive value= 94.7%, positive predictive value= 90.5% and efficacy= 92.5%. While, the best cut off point that discriminate between breast cancer and control groups was found at 30 pg/mL with an AUC = 0.393, sensitivity =48%, specificity= 45%, negative predictive value= 25.7%, positive predictive value= 68.6% and efficacy= 47.1%. And the best cut off point that discriminates between breast cancer group and fibroadenoma group was found at 31 pg/mL with an AUC= 0.780, sensitivity =80%, specificity= 72%, negative predictive value= 53.3%, positive predictive value= 90% and efficacy= 74.3% **(Table 3 and Figure 1).**

Table (3): Diagnostic performance of MMP-13 levels:

	Sensitivity (%)	**Specificity (%)**	**Negative Predictive Value (%)**	**Positive Predictive Value (%)**	**Efficacy (%)**	**AUC**
FA Vs Control Best cut off (130 pg/mL)	90%	95%	94.7%	90.5%	92.5%	0.935
BC Vs control Best cut off (30 pg/mL)	48%	45%	25.7%	68.6%	47.1%	0.393
BC Vs FA Best cut off (31 pg/mL)	80%	72%	53.3%	90%	74.3%	0.780

BC: Breast cancer, FA: Fibroadenoma, Vs: Versus, AUC: Area under the curve



AUC	
FA Vs Control	0.935
BC Vs Control	0.393
BC Vs FA	0.780

Figure (1): ROC curve analysis showing the diagnostic performance of MMP-13 for discriminating groups from each other. (BC: Breast cancer, FA: Fibroadenoma and Vs:Versus).

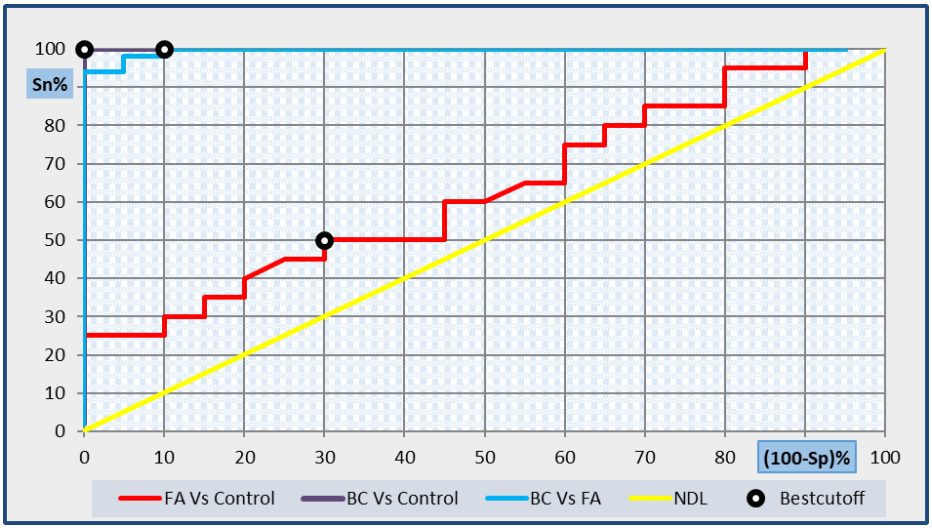
Results in **(Table 4 and figure 2),** regarding CA15.3, the best cut off point that discriminate between fibroadenoma (FA) and control groups was found at 16.16 U/mL with an AUC= 0.626, sensitivity =70%, specificity= 50%, negative predictive value= 58.3%, positive predictive value= 62.5% and efficacy= 60%. While, the best cut off point that discriminate between breast cancer group and control group was found at 22 U/mL with an AUC=1.00, sensitivity =100%, specificity= 100%, negative predictive value= 100%, positive predictive value= 100% and efficacy= 100%. And the best cut off point that discriminates between breast cancer group and fibroadenoma group was found at 30.7 U/mL with an AUC= 0.994, sensitivity =90%, specificity= 100%, negative predictive value= 100%, positive predictive value= 96.2% and efficacy= 97.1%.

Table (4): Diagnostic performance of CA15.3 levels:

	Sensitivity (%)	**Specificity (%)**	**Negative Predictive Value (%)**	**Positive Predictive Value (%)**	**Efficacy (%)**
FA Vs Control Best cut off (16.16 U/mL)	70%	50%	58.3%	62.5%	60%
BC Vs	100%	100%	100%	100%	100%

control Best cut off (22 U/mL)					
BC Vs FA Best cut off (30.7 U/mL)	90%	100%	100%	96.2%	97.1%

FA: Fibroadenoma, BC: Breast Cancer



AUC	
FA Vs Control	0.626
BC Vs Control	1.000
BC Vs FA	0.994

AUC: Area under the curve

Figure (2): ROC curve analysis showing the diagnostic performance of CA15.3 for discriminating patient groups from each other.

4) Discussion:

New cases (910,000) of breast cancer appear globally every year, and 376,000 people die as a result**.** 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 **(Jung *et al.*, 2015)**.

Currently, mammography and tumor markers are the most commonly used screening tools for breast cancer, including: CEA, CA15.3 and CA 27 **(Autier and Boniol, 2018).**

MMP-13 or collagenase-3 is a member of the matrix metalloproteinase family that has potent degrading activity for the major protein components of the extracellular matrix and basement membranes of the cells. 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 **(Pendas *et al.*, 2000).**

Studies analyzing expression of different genes in breast cancer tissue and normal tissue have shown that MMP-13 was a commonly overexpressed gene in the tumor tissue of

breast cancer patients. Therefore, MMP-13 has tremendous potential to serve as a biological marker to assist in the clinical diagnosis of breast cancer **(Chang *et al.*, 2009).**

In the current study, we aimed to investigate the role of MMP-13 as a marker of breast cancer. We assessed serum levels of MMP-13 in 50 breast cancer patients, 20 fibroadenoma patients and 20 healthy age-matched controls using enzyme linked immunosorbent assay.

Our study showed that the control group had the highest MMP-13, while fibroadenoma group had the lowest MMP-13. Comparing breast cancer patients to control group, there was no statistical significant difference between both groups regarding MMP-13 levels. However, comparing fibroadenoma patients to control group, there was a highly statistical significant difference between both groups regarding MMP-13 levels which was higher in control group than in fibroadenoma patients.

In contrast to our results, several studies have reported increased expression of MMP-13 mRNA, detected by polymerase chain reaction, in breast cancer cells **(Balduyck *et al.*, 2000; Nielsen *et al.*, 2001; Zhang *et al.*, 2008; Chang *et al.*, 2009).**

The presence of alternative MMP-13 proteins, that exist as a result of the presence of the different MMP-13 transcripts, might explain the results of our study. Our failure to detect higher serum levels of MMP-13 in breast cancer patients, despite the fact that several authors do report overexpression of MMP-13 mRNA, might be attributed to failure of the ELISA kit used in our study to detect all alternative MMP-13 proteins. Unfortunately, we have no data on the epitope detected by the ELISA kit used in our study and whether this epitope is shared between different alternative MMP-13 proteins or is missing. Also, no data is yet available in the literature regarding which alternative MMP-13 proteins are present in breast cancer patients and whether these alternative proteins differ from those present in healthy subjects.

So, one important limitation of our study is the lack of data regarding the epitopes detected by the capture and detection antibodies in the supplied kit. Further studies are needed to investigate the appropriate technique and appropriate antibodies to be used for MMP-13 detection that could detect all alternative MMP-13 proteins.

Regarding CMV IgG status only one subject of the control group was negative for CMV IgG while all other subjects in the three studied groups were sero-positive for CMV IgG.

Conclusively, the results of this study show that MMP-13 level is highest among the control group, while fibroadenoma group had the lowest MMP-13. CA15.3 is still the most useful serum tumor marker in patients with breast cancer. Serial determination of this marker may be beneficial for early detection of recurrence or metastasis. We found CMV in all subjects of the study denying a possible role in breast cancer association.

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ملخص اللغة العربية

ماتريكس ميتالوبروتينيز-١٣ : دلالة الورم المقترحة في سرطان الثدي

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يقدر سرطان الثدي بحوالي ٣١ بالمئه حالات جديدة من الاناث مصابه بالسرطان٠ و يعطي التشخيص المبكر لمرضي سرطان الثدي مجموعة واسعه من الخيارات العلاجية و كذلك معدل نجاح اعلي للعلاج الذي يقلل من نسبة الوفيات٠ يعتبر الماتريكس ميتالوبروتينز هو الاندوبتيداز الموجود خارج الخليه المعتمد علي الزنك التي تشارك في هدم و اعادة بناء الماتريكس الخارجي للخليه في العمليلت الفسيولوجية والباثولوجية٠وكان الهدف من هذه الدراسة هو التحقيق في دور العدوي بفيروس CMV كأحد أسباب حدوث سرطان الثدي من خلال تقدير حالات الأجسام المضادة لل CMV في مرضي سرطان الثدي٠وتهدف الدراسة أيضا إلي تقييم استخدام MMP-13 كأحد دلالات الأورام لسرطان الثدي و لربط مستوياتها مع تلك الموجودة في مرضي الورم الليفي و الاصحاء٠