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MMP-2 LEVELS EVALUATION AND THEIR RELATIONSHIP WITH BREAST CANCER PROGRESSION.

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ABSTRACT… Matrix metalloproteinases (MMPs) play significant part in the tumor development, metastasis and invasion by proteolytic degradation of basement membrane and extracellular matrix. Hence MMPs can serve as good biomarkers for carcinoma breast particularly MMP-2 in our population. **Objectives:** To measure the concentrations of MMP-2 in plasma of breast cancer patients and establishing their correlation with stage and grade of the disease. **Study Design:** Comparative cross sectional. **Setting:** Surgery department Madinah Teaching Hospital (MTH) and the Pathology department, The University of Faisalabad (TUF), Faisalabad. **Period:** 1st August, 2017 to 31st December, 2017. **Material & Methods:** Only females with diagnosed breast cancer disease along with staging and grading were included in the study. Females with incomplete reports, on chemotherapy or radiotherapy or having other diseases like oral squamous cell carcinoma, rheumatoid arthritis etc were excluded. ELISA kit was applied to measure the concentrations of MMP-2 in plasma of the participants. OD values were calculated at 450 nm by ELISA reader placed at Post Graduate Research Laboratory, The University of Faisalabad, Faisalabad. Data was gathered and results were finalized by using SPSS 22 for statistical assessment. **Results:** Age of 64(72%) breast cancer patients was within range of 30-76yrs and age for 25(28%) healthy females as controls range was 18-70yrs. Difference between ages of cases and controls using t-test was statistically highly significant. Ninety five percent cases showed invasive ductal carcinoma (IDC) as histological type. Range of MMP-2 levels in cases was 80-690 ng/mL with mean 451.02 while range of MMP-2 levels in controls was 65-830ng/mL with mean 329.72 showing statistically significant difference. Relationship of different groups of MMP-2 levels in cases with stages of breast cancer was established using chi-square test which was statistically significant, stage IIB (37.5%) and IIIA (34.4%) tumors showed higher values of MMP-2. Relationship between MMP-2 levels, grade, tumor size and node invasion was nonsignificant statistically. **Conclusion:** MMP-2 levels were raised in cases in comparison to healthy controls. Age of majority patients was > 45 yrs while the invasive ductal carcinoma was the main histological type. MMP-2 levels were associated with stage of the disease.

Key words: Breast Cancer Pathogenesis, MMP-2 Levels, Role of Matrix Metalloproteinases in Breast Cancer.

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INTRODUCTION

It became evident during past decade that MMPs play a significant part in the tumor development, metastasis and invasion. This proteolytic degradation is due to action of different matrix metalloproteinases. MMPs can serve as good biomarkers for carcinoma breast.[1]

Breast cancer is the commonest malignancy all over the world and also in Pakistan with considerable regional and ethnic variation. It includes all the women of different geographic areas, races and ethinicities.[2,3,4] In Pakistan its incidence is 2.5 times more than in India and Iran. Per year app. 1 million cases of breast carcinoma are diagnosed in females mostly in developed countries.[5]

One of the important process involved in metastasis of breast cancer is degradation of basement membrane and extracellular matrix specially collagen IV.[6] This proteolytic degradation is due to action of different MMPs. Role of MMPs is not only in metastasis rather they are involved in

tumor growth, angiogenesis and vasculogenesis also.[1] Their levels are also raised in other conditions like oral squamous cell carcinoma, rheumatoid arthritis, pleural effusion and acute myocardial infarction.[7,8,9,10] Many researches have been conducted in the past for the evaluation of the levels of MMPs in plasma of breast cancer patients and their relation with different prognostic factors of the disease showing different results. A significant correlation was observed between the plasma levels of MMP-2 with stage and grade of tumor showing raised levels with increasing grade and stage in study conducted by Vasaturo et al in 2013 in Italy and also by Jinga et al in 2006 in Romania.[6,11] Liu et al also showed that MMP2 levels were higher with grade 3 and 4 than in grade1 and 2 carcinoma breast patients of Taiwan in 2006.[12] Some results were contradictory. MMP9 but not the MMP2 levels were significantly higher in breast carcinomas than fibroadenomas. MMP-2 levels were not different in breast carcinoma and fibroadenomas in a study conducted in Dublin, Ireland in 2000.[13] In other studies i.e. in Romania, Germany and Kingdom of Saudi Arabia MMP levels were significantly raised in carcinoma breast patients.[11,14,15] Previous data enlightens MMPs role in carcinoma breast development and further growth so there are many clinical researches going on emphasizing the role of MMP-2 levels in etiopathogenesis of carcinoma breast.[16]

In Pakistan, no studies have been conducted yet regarding different MMP levels in carcinoma breast patients. In this study, MMP-2 levels were measured and then related to different prognostic factors i.e. tumor size, node invasion, stage and grade of tumor to know the pattern of the disease in our region.

MATERIAL & METHODS

After approval by the ethical committee of the hospital. Pre-operative data and clinical information was collected and entered into proforoma after informed consent taken from patient fulfilling the inclusion criteria with diagnosed biopsy reports of CA breast, presenting to the department of surgery through OPD. Complete history was taken with examination of patients for excluding other conditions related to raised MMP levels i.e. oral squamous cell carcinoma, rheumatoid arthritis, pleural effusion, acute myocardial infarction. Staging of breast cancer was done on OPD basis in surgical department before admission. Five stages of breast cancer are presented by American Joint Committee on Cancer (AJCC) including T: tumor, N; lymph node, M: metastasis.[17] Histopathology reports were collected on follow up visits for grade and type of tumor. The Nottingham Histological score also referred to as Scarff-Bloom-Richardson grading system was used.[18] Peripheral venous blood was drawn, plasma was recovered after centrifugation at 1000 rpm and stored at -20^{o} C until analysis. Plasma concentrations of MMP-2 were evaluated by using ELISA kit according to manufacturer's instructions.

Statistical Analysis

All quantitative variables like age, tumor size and concentrations of MMP-2 were presented as mean ± standard deviation. The correlation between MMP-2 and other parameters like age, histological type, grade and stage of tumor were analyzed by using chi-square test using SPSS 22. Qualitative variables like histological type and groups of MMP-2 were presented as frequency and percentages. P-Value $\leq$ 0.05 was considered statistically significant.

RESULTS

Past studies has suggested important activities of MMP-2 for disease development depicting parallel or opposite association with the disease progression in different regions of the world. In this study MMP-2 levels were correlated with the disease process progression showing the parallel results. First the ages of cases and controls were compared. MMP-2 levels were compared in cases and controls then their relationship was established with stage, grade, tumor size and node invasion. Histological type of tumor was also observed.

Age of breast cancer patients was within range of 30-76yrs with mean and S.D 49.70± 11.42 and age for healthy females range was 18-70yrs with mean and S.D 37.64± 16.16 as given in Table-I.

Difference between ages of cases and controls using t-test was statistically highly significant i.e. p-value= 0.000.

Plasma levels of MMP-2 were presented as mean ± SD with their ranges. Levels within cases and controls were compared with the help of student's t-test. The p–value was calculated which was<0.05 making it statistically significant as shown in Table-II. There was significant difference between levels of MMP-2 in cases versus controls.

Relationship of different groups of MMP-2 levels in cases with stages of breast cancer was established using chi-square test which was statistically significant i.e. P-Value = 0.015 as shown in Table-III. The levels of MMP-2 were significantly related with increasing stage of tumor.

No statistically significant relationship was found between grade of tumor and levels of MMP-2 using Chi-square test as p-value for MMP-2 was 0.192 shown in Table-IV. MMP-2 levels >601 ng/mL were related to grade II tumor in 26.6% cases.

Cases were also distributed according to their histological types with their frequencies and percentages given in Table-V showing majority cases of IDC.

Relationship between tumor size i.e. in diameter and levels of MMP-2 was established using Chi-square test which was nonsignificant statistically i.e. p-value was 0.630 as shown in Table-VI.

Relationship between node invasion (N) and levels of MMP-2 was established using Chi- square test which was statistically nonsignificant i.e. p-value was 0.086 given in Table-VII. Tumors with node invasion present (68.8%) showed levels of MMP-2 between 201-400 ng/mL within 23.4% cases while the absent node invasion tumors (31.3%) showed > 601ng/mL levels in 17.2% cases.

Only stage of tumor showed association with raised MMP-2 levels while other parameters were not associated significantly with these levels.

Age	Cases	Controls
N (sample size)	64	25
Range	30-76	18-70
Mean	49.70	37.64
Std. Deviation	11.42	16.16
Std. Error Mean	1.43	3.23
t-value	3.693**	
Prob.	0.000	

Table-I. Comparison between cases and controls respondents regarding their age.

MMP-2(ng/mL)	Cases	Controls
Patients	64	25
Range	80-690	65-830
Mean	451.02	329.72
S.D	218.73	213.65
S.E	27.341	42.729
t. value	2.366*	
Prob.	0.02	

Table-II. MMP-2 levels.

			MMP-2 (ng/mL)				Total
			≤ 200	201 – 400	401 – 600	>601	
Stage	I	Count	0	0	0	3	3
		% of total	0.0%	0.0%	0.0%	4.7%	4.7%
	IIA	Count	0	5	2	2	9
		% of total	0.0%	7.8%	3.1%	3.1%	14.1%
	IIB	Count	5	5	3	11	24
		% of total	7.8%	7.8%	4.7%	17.2%	37.5%
	IIIA	Count	1	6	10	5	22
		% of total	1.6%	9.4%	15.6%	7.8%	34.4%
	IIIB	Count	2	3	1	0	6
		% of total	3.1%	4.7%	1.6%	0.0%	9.4%
Total		Count	8	19	16	21	64
		% of total	12.5%	29.7%	25.0%	32.8%	100.0%

Table-III. Relationship between stage and MMP-2 levels. Chi-square value = 24.912*, P-Value = 0.015

			MMP2 (ng/mL)				Total
			≤ 200	201 – 400	401 – 600	> 601	
Grade	II	Count	6	11	8	17	42
		% of total	9.4%	17.2%	12.5%	26.6%	65.6%
	III	Count	2	8	8	4	22
		% of total	3.1%	12.5%	12.5%	6.3%	34.4%
Total		Count	8	19	16	21	64
		% of total	12.5%	29.7%	25.0%	32.8%	100.0%

Table-IV. Relationship between grade and MMP-2 levels.
Chi-square value = 4.734, p-value = 0.192

H.Type (Cases)	Frequency	Percent	Valid Percent	Cumulative Percent
IDC	61	95.3	95.3	95.3
ILC	2	3.12	3.12	98.42
Malignant phylloide	1	1.56	1.56	100
Total	64	100	100	

Table-V. Distribution of cases with respect to histological type.

			MMP2 (ng/mL)				Total
			≤ 200	201 – 400	401 - 600	> 601	
Tumor size (cm)	≤ 3	Count	1	6	4	7	18
		% of total	1.6%	9.4%	6.3%	10.9%	28.1%
	4 – 6	Count	6	8	6	8	28
		% of total	9.4%	12.5%	9.4%	12.5%	43.8%
	> 7	Count	1	5	6	6	18
		% of total	1.6%	7.8%	9.4%	9.4%	28.1%
Total		Count	8	19	16	21	64
		% of total	12.5%	29.7%	25.0%	32.8%	100.0%

Table-VI. Relationship between tumor size and MMP-2 levels
Chi-square value = 4.345, P-Value = 0.630

			MMP2 (ng/mL)				Total
			≤ 200	201 - 400	401 – 600	> 601	
Node invasion (N)	Absent	Count	2	4	3	11	20
		% of total	3.1%	6.3%	4.7%	17.2%	31.3%
	Present	Count	6	15	13	10	44
		% of total	9.4%	23.4%	20.3%	15.6%	68.8%
Total		Count	8	19	16	21	64
		% of total	12.5%	29.7%	25.0%	32.8%	100.0%

Table-VII. Relationship between node invasion and MMP-2 levels.
Chi-square value = 6.593, P-Value = 0.086

DISCUSSION

Our results were consistent with the previous studies showing the increased levels of MMPs in breast cancer patients in comparison with healthy controls. Serum levels of MMP-2 were significantly raised in breast cancer patients of India and Italy than in control groups.[19,20] After evaluating the different values of MMPs in cancer as for diagnosis and prognosis of the disease showed raised levels of these in cancer development and progression.[21] Multiple and significant association between the raised expression levels of different MMPs were noted in breast cancer patients of General Hospital, Oviedo, Spain.[22] In our study the mean for age of cases was 49.7. The new female breast cancer estimated cases according to their age in United States showed most cases above the age of 50years along with

the probabilities of developing breast cancer in healthy females increasing with the age of the females gradually.[23] Most cases of breast cancer fall into elder age groups mentioned by European Society of breast cancer specialists in a review.[24]

We also noticed that the invasive ductal carcinoma (IDC) histological type was present in 95% cases depicting data in parallel with the past studies. 91% cases had invasive ductal carcinoma in 10yrs registered data collection in Institute of Nuclear Medicine and Oncology Lahore (INMOL).[25] In a study conducted in Egypt for evaluation of expression of different proteins in breast cancer patients also showed that 81% cases with disease are of invasive ductal carcinoma (IDC) histological type[26] and 72% cases are of IDC.[20]

Serum levels of MMP-2 were higher in breast cancer patients of New Delhi, India as compare to controls while their values were positively correlated with advancing stage of the disease. 54% cases with higher levels of MMPs were with stage IV disease, 28% cases showed higher levels with stage II and 18% showed higher levels with stage I disease.[19] The activity levels of this gelatinase in sera of breast cancer patients were higher than in healthy controls in another study but there was no correlation found between the raised levels with stage of tumor.[27]

Limitations of the Study

MMP-2 plasma levels of only those patients were quantified whose staging and grading reports were available. Careful history was taken and examination was done to rule out other conditions related to raise MMP levels other than carcinoma breast.

CONCLUSION

MMP-2 is related to carcinoma breast in our region. Their levels were raised in patients of breast cancer in comparison to healthy controls relating to the stage of the disease. Further evaluation is needed for better understanding of the disease process, its development and progression which can guide for the synthesis of new drugs modifying MMPs actions leading to new treatment options accordingly.

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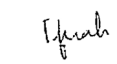
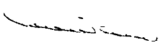
AUTHORSHIP AND CONTRIBUTION DECLARATION

Sr. #	Author(s) Full Name	Contribution to the paper	Author(s) Signature
1	Ifrah Yaqoob	IY did data collection and manuscript writing along with statistical analysis and editing of manuscript and also takes the responsibility and is accountable for all aspects of the work in ensuring that questions related to the acuracy or integrity of any part of the work appropriately investigated and resolved.	
2	Muhammad Saeed	MS conceived the idea of the manuscript and provided the guidelines for practical research work.	
3	Aysha Azhar	AA facilitated in sorting out the technical issues regarding this project.