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Original Research Article

Clinical relevance of MMP-9 in terms of neoplasm growth, invasion and metastasis in thyroid, breast and colorectal cancer

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ABSTRACT

Background: Cancer cells invade and metastasis commonly. This poses challenge in cancer management. MMP-9 can be clinically useful to predict the prognosis of the patients. Objective was to study clinical relevance of MMP-9 in terms of neoplasm growth, invasion and metastasis in Thyroid, Breast and Colorectal cancer.
Methods: A hospital based cross sectional study was carried out in Department of General Surgery, Kamineni Institute of Medical Sciences, Narketpally for a period of two years. A total of 30 cases of carcinoma cases were studied. Out of these carcinoma cases, 10 were of gastrointestinal malignancy, 10 were of thyroid malignancy and 10 were of breast carcinoma.
Results: For breast carcinoma, maximum cases were in the age group of 46-50 years (40%), for thyroid carcinoma, maximum cases were seen in the age group of more than 55 years (40%), for colo-rectal carcinoma, maximum cases were above the 50 years of age. For breast carcinoma, maximum cases were in stage III B (70%), for thyroid carcinoma, in III stage (60%) and for colo-rectal carcinoma, in Dukes C1 stage (60%). In breast cancer the positivity of MMP-9 was 66.7% in stage IIIA and it increased to 85.7% in stage IIIB. For thyroid cancer the positivity increased from 33.3% in stage III to 75% for stage IV A. In case of colo-rectal cancer, the positivity of MMP-9 expression increased from 66.7% for stage Dukes C 1 to 75% in stage Dukes C2 stage. Thus, for all types of carcinoma, the expression of MMP-9 increased with advancement of the stage.
Conclusions: MMP-9 is a gelatinase which degrades extra cellular matrix and helps in tumor spread. It is expressed more in advanced stages of the malignant disease. It correlates with prognosis of the patient.

Keywords: Carcinoma, Expression, MMP-9, Prognosis

INTRODUCTION

The extracellular matrix (ECM) is remodeled and broken down by Matrix metalloproteinase (MMPs). MMPs are neutral proteinases. This can occur under various conditions. These can be differentiation, morphogenesis, tissue remodeling as well as during angiogenesis. It also can occur during arthritis, inflammation, diseases of lung, invasion of tumor etc.[1] MMP-9 is also known as 92-kDa gelatinase B type IV collagenase. Macrophages, neutrophils or even transformed cells secrete MMP-9. It has a complex domain structure. Its regulation activity is complex. It is a component of cytoplasmic granules of neutrophils. It is involved in the pathogenesis of many diseases.[2]

When stromal cells and lymphocytes are stimulated by cytokines, they secrete MMP-9. The excessive secretion of MMP-9 damages tissue in the absence or lack of their inhibitors. This damage by MMP-9 leads to degenerative inflammatory disorders. Thus, investigating the role of MMP-9 can be useful to predict the outcome in many

diseased condition especially cancer. This can help early prediction of disease and help therapeutic intervention.[2] In carcinoma management, metastasis and invasion are serious challenges. These events require diverse proteolytic enzymes among which MMP-2 and MMP-9 play a significant role in degradation role in degradation of type IV collagen, the major component of the basement membrane.[3] Present study was planned to study clinical relevance of MMP-9 in terms of neoplasm growth, invasion and metastasis in Thyroid, Breast and Colorectal cancer.

METHODS

A hospital based cross sectional study was carried out in Department of General Surgery, Kamineni Institute of Medical Sciences, Narketpally for a period of two years. A total of 30 carcinoma cases were studied, 10 were of gastrointestinal malignancy, 10 were of thyroid malignancy and 10 were of breast carcinoma. Institutional Ethics Committee approval was obtained before the initiation of the study. Individual consent was also planned from each case.

Inclusion criteria

Patients having breast carcinoma, thyroid carcinoma and gastrointestinal carcinoma were included.

Exclusion criteria

- Patients having benign tumors
- Patients having infective lesions
- Inflammatory swellings.

Demographic details, local findings and clinical stage of the tumor are recorded in the pre-tested, semi structured questionnaire for the present study. Based on physical examination, clinical staging was done. As well as imageology findings were also recorded. Biopsy specimen was collected from tumor and para tumor tissue. Histopathological examination was carried out and pathological staging of the tumor specimen was noted. Immuno-histochemistry staining of all collected specimen were carried out.

First circle tissue section was taken with a diamond pen and kept at 500C overnight. Later deparaffinization was done (2 changes of xylene). Then rehydration by descending concentrations of alcohol was done. Next step was antigen retrieval and then peroxidase block was carried out. Then protein block was done. Primary antibody application for one hour minimum was done and then washed with PBS. Secondary biotin conjugated antibody for minimum 30 minutes was done and then washed with PBS. Strept ABC/HRP complex was done for minimum 30 minutes then washed with PBS. Chromogen for 6-10 minutes minimum and then again washed twice with water. Counterstaining in hematoxylin for 30-60 seconds and then washed twice with water. Finally, dehydration was done in ascending concentrations of alcohol and then mounted. H and E stained section which is serial to the immuno-stained one should be examined to be aware of the morphology of the tissue section under test. True positive staining should be localized in its correct site i.e. membranous, cytoplasmic, nuclear or stromal. Homogenous pale brown or yellow staining of cells is nonspecific. No staining should be demonstrated in the negative control. The positive control should show true positive immuno-staining.

Depending on the staining pattern, scoring can be done. If there is no staining or membrane staining is less than 10% of invasive tumor cells, then it is interpreted as negative and a score of zero is given. If the staining is negative faint or barely perceptive membrane detected in more than 10% of invasive tumor cells, then it is interpreted as negative and a score of +1 is given. If the staining is equivocal weak to moderate complete membrane staining in more than 10% of invasive tumor cells or less, then 30% with strong complete membrane staining then it is interpreted as positive and a score of +2 is given. If the staining is strong and complete or the membrane staining more than 30% of invasive tumor cells, then it is interpreted as positive and a score of +3 is given. The data was entered in Excel sheet and analyzed using proportions.

RESULTS

Table 1: Age wise distribution of study cases.

Type of carcinoma	Age (years)	Number	Percentage
Breast	40-45	02	20
	46-50	04	40
	51-55	01	10
	> 55	03	30
Thyroid	40-45	02	20
	46-50	01	10
	51-55	03	30
	> 55	04	40
Colo-rectal	< 50	02	20
	51-60	04	40
	61-70	04	40

Table 1 shows age wise distribution of study cases. For breast carcinoma, maximum cases were in the age group of 46-50 years (40%) followed by more than 55 years (30%). For thyroid carcinoma, maximum cases were seen in the age group of more than 55 years (40%) followed by 51-55 years (30%). For colo-rectal carcinoma, maximum cases were above the 50 years of age. Table 2 shows stage wise distribution of the study cases. For breast carcinoma, maximum cases were in stage III B (70%). For thyroid carcinoma, maximum cases were seen in III stage (60%). For colo-rectal carcinoma, maximum cases were in Dukes C1 stage (60%).

Table 2: Stage wise distribution of the study cases.

Type of carcinoma	Stage	Number	Percentage
Breast	III A	03	30
	III B	07	70
Thyroid	III	06	60
	IV A	04	40
Colo-rectal	Dukes C1	06	60
	Dukes C2	04	40

Table 3 shows distribution of study cases as per MM-9 expression in different stages. In breast cancer the positivity of MMP-9 was 66.7% in stage IIIA and it increased to 85.7% in stage IIIB. For thyroid cancer the positivity increased from 33.3% in stage III to 75% for stage IV A. In case of colo-rectal cancer, the positivity of MMP-9 expression increased from 66.7% for stage Dukes C 1 to 75% in stage Dukes C 2 stage. Thus, for all types of carcinoma, the expression of MMP-9 increased with advancement of the stage.

Table 3: Distribution of study cases as per MMP-9 expression in different stages.

Type of carcinoma	Stage	MMP-9 expression	Cases		Control	
			Number	Percentage	Number	Percentage
Breast	III A	Positive	02	66.7	00	00
		Negative	01	33.3	03	100
	III B	Positive	06	85.7	01	14.3
		Negative	01	14.3	06	85.7
Thyroid	III	Positive	02	33.3	00	00
		Negative	04	66.7	06	100
	IV A	Positive	03	75	00	00
		Negative	01	25	04	100
Colo-rectal	Dukes C 1	Positive	04	66.7	00	00
		Negative	02	33.3	06	100
	Dukes C 2	Positive	03	75	00	00
		Negative	01	25	04	100

DISCUSSION

For breast carcinoma, maximum cases were in the age group of 46-50 years (40%), for thyroid carcinoma, maximum cases were seen in the age group of more than 55 years (40%), for colo-rectal carcinoma, maximum cases were above the 50 years of age. For breast carcinoma, maximum cases were in stage III B (70%), for thyroid carcinoma, in III stage (60%) and for colo-rectal carcinoma, in Dukes C1 stage (60%). In breast cancer the positivity of MMP-9 was 66.7% in stage IIIA and it increased to 85.7% in stage IIIB. For thyroid cancer the positivity increased from 33.3% in stage III to 75% for stage IV A. In case of colo-rectal cancer, the positivity of MMP-9 expression increased from 66.7% for stage Dukes C 1 to 75% in stage Dukes C 2 stage. Thus, for all types of carcinoma, the expression of MMP-9 increased with advancement of the stage.

Maruyama S et al found that the concentration of MMP-2 in the tissue of papillary carcinoma, benign nodules and normal tissue was 12.1±8.1, 5.7±4.3, and 0.6±0.5 ng/mg tissue protein, respectively, and that of MMP-9 was 4.2±4.1, 2.1±1.7 and 0.4±0.3 ng/mg tissue protein, respectively.[4] Concentrations of both enzymes were significantly higher in the papillary carcinoma tissue. These findings imply that MMP-2 and MMP-9 are related to the degree of malignancy of cancer, especially metastasis.

According to Wang T et al the expression of MMP-9, TIMP-1, VEGF and TGFβ-1 in the tumor tissue of eighty-five cases of papillary thyroid carcinoma and 59 cases of follicular thyroid carcinoma by immuno histochemistry using Envision method and found the expression of MMP-9, TIMP-1, VEGF and TGFβ-1 carries clinical significance in evaluating the degree of differentiation, invasiveness, metastatic potential and prognosis of papillary thyroid carcinoma and follicular thyroid carcinoma.[5]

Mitmaker EJ et al proved there was decreased protein activity of MMP-2 and MMP-9 in human papillary and follicular thyroid cancer cell lines in a dose dependent manner after 48 hours of treatment compared with untreated controls.[6] This resulted in decreased cell invasion. Levy AT et al demonstrated increased tissue levels of MMP-2 in colorectal tumors using immunohistochemical techniques.[7] Zucker S et al demonstrated increased levels of MMP-9 activity in plasma of patients with colon cancer.[8] Onisto M et al used RT-PCR to detect MMP-2 and MMP-9 mRNA in human colon adenocarcinoma. Adenomas are well defined, and the adenocarcinoma can be classified into

distinct biological and prognostic stages.[9] Duke's stage A tumors are limited to the bowel wall; Dukes' stage B tumors have penetrated the muscularis propna, and Duke's stage C tumors have spread to the regional lymph nodes.

The more recent Jass classification system uses four variables selected by means of Cox regression analysis as having an important and independent influence on survival. These are the number of positive lymph nodes with metastatic tumors, the character of the invasive margin, peri-tumoral lymphocytic infiltration and local spread. These variables are used to divide colorectal tumors into four biological and prognostic groups, with a Jass score of one if none or one of these variables occurs at an unfavorable level and a Jass score of four if most of the four factors are unfavorable.[10] Delektorskaga VV et al studied the expression of β-catenin, matrix metalloproteinase 9, collagen IV and laminin in invasive front of primary colon adenocarcinoma and their metastases in lymph nodes and liver.[11] Intensive expression of matrix metalloproteinase 9 in zones of invasive growth of the tumor was associated with enhanced accumulation of β-catenin in the nuclei of tumor cells in peripheral zones of 28% studied tumors.

The presence of nuclear β-catenin and increased content of metalloproteinase 9 in the tumor were associated with abnormal accumulation of laminin in cell cytoplasm and with the absence of collagen IV containing basal membranes. These changes were typical of tumors with high invasive and metastatic potential. Our findings suggest that β-catenin matrix metalloproteinase 9, laminin and collagen IV are important predictors of clinical course of rectal cancer and that the peculiarities of protein expression related to the risk of liver metastases have concordant pattern and are most pronounced in invasive front of the tumor.

Liabakk NB et al in their study found that as the stage of cancer increased, the MMP-2 and MMP-9 levels increased significantly.[12] Dragutinovic V et al concluded that MMP-9 in serum plays an important role in the progression of gastric cancer.[13] Parson SL et al observed that the gelatinase is over expressed in gastrointestinal neoplasia suggesting that these enzymes may have an important role in tumor invasion and metastasis.[14] Kong L et al studied the expression of MMP-9 in gastric cancer by immunohistochemical Envision plus non biotin technique in 94 cases of gastric cancer and peri-carcinoma tissues.[15] The correlation with clinicopathologic characteristic was analyzed. The positive expression rates of MMP-9 protein in gastric cancer were significantly higher than in peri-carcinoma tissues ($p < 0.01$).

CONCLUSION

MMP-9 is a gelatinase which degrades extra cellular matrix and helps in tumor spread. It is expressed more in advanced stages of the malignant disease. It correlates with prognosis of the patient.

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REFERENCES

1. Egeblad M, Werb Z. New functions for the matrix metalloproteinase in cancer progression. Natl Rev Cancer. 2002;2:161-74.
2. Lopez-Otin C, Overall CM. Protease degradomics: A new challenge for proteomics. Natl Rev Mol Cell Biol. 2001;3:509-19.
3. St-Pierre Y. Regulation of MMP-9 gene expression for the development of novel molecular targets against cancer and inflammatory diseases. Expert Opin Ther Targets. 2011;8(5):473-89.
4. Maruyama S, Yamamichi I, Kawata R, Shimada T, Shinomiya T, Murakami Y, et al. Study of matrix metalloproteinase-2 and -9 in thyroid papillary cancer. Nihon Jibiinkoka Gakkai Kaiho 2000;103(5):499-505.
5. Wang T, Jiang CX, Li Y. Pathologic study of expression and significance of matrix metalloproteinases-9, tissue inhibitor of metalloproteinase-1, vascular endothelial growth factor and transforming growth factor beta-1 in papillary carcinoma and follicular carcinoma of thyroid. Zhonghua Bing Li Xue Za Zhi 2009;38(12):824-8.
6. Mitmaker EJ, Griff NJ, Grogan RH, Sarkar R, Kebebew E, Duh QY, et al. Modulation of matrix metalloproteinase activity in human thyroid cancer cell lines using demethylating agents and histone deacetylase inhibitors. Surg. 2011;149(4):504-11.
7. Levy AT, Cioce V, Sobel ME, Garbisa S, Grigioni WF, Liotta LA, et al. Increased expression of the Mr 72,000 type IV collagenase in human colonic adenocarcinoma. Cancer Res. 1991;51:439-44.
8. Zucker S, Lysik RM, Zarrabi MH, Moll U. Collagenase is increased in plasma of patients with colon cancer and breast cancer. Cancer Res. 1993;53:140-6.
9. Onisto M, Garbisa S, Caenazzo C, Freda MP, Di Francesco C, Nitti D, et al. Reverse transcription polymerase chain reaction phenotyping of metalloproteinase and inhibitors involved in tumor matrix invasion. Diagn Mol Pathol. 1993;2:74-80.
10. Dukes EC. The classification of cancer of the rectum. J Pathol Bacteriol. 1932;35:323-32.
11. Delektorskaya VV, Kushlinskii NE. Clinical significance of the content of biomolecular markers in invasive front of colon carcinomas. Bull Exp Biol Med. 2011;150(3):368-71.
12. Liabakk NB, Talbot I, Smith RA, Wilkinson K, Balkwill F. Matrix metalloprotease 2 (MMP-2) and matrix metalloprotease 9 (MMP-9) type IV

collagenases in colorectal cancer. Cancer Res. 1996;56(1):190-6.
13. Dragutinovic V, Izrael-Zivkovic L, Radovanovic N. Relation of matrix metalloproteinase-9 to different stages of tumors in the serum of gastric cancer. Dig Dis Sci. 2009;54(6):1203-7.
14. Parsons SL, Watson SA, Collins HM, Griffin NR, Clarke PA, Steele RJ. Gelatinase (MMP-2 and MMP-9) expression in gastrointestinal malignancy. Br J Cancer. 1998;78(11):1495-502.
15. Kong L, Sun JW, Zhang CH. The expression of integriαvβ6 and MMP-9 in gastric cancer and the correlation with clinicopathologic characteristic. Chinese J Cellular Molecular Immunol. 2011;27(1):92-4.

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