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Original Article

Biochemistry Section

Matrix Metalloproteinase-2: A Possible Marker for Non-Small Cell Lung Carcinoma

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ABSTRACT

Introduction: Lung carcinoma is the foremost cause of death among cancer related deaths globally. Matrix Metalloproteinase's (MMPs) are Ca^{+2} and Zn^{+2} dependent endopeptidases which are involved in degradation of Extracellular Matrix (ECM) and have been widely associated with the development of various diseases, including cancer. Matrix metalloproteinase-2 (MMP-2, gelatinase A) the main mediator of ECM degradation, is important in cell proliferation, apoptosis and angiogenesis. MMP-2 exhibits a high level of expression in many human tumours; plays a role in cancer initiation and development.

Aim: To evaluate the level of serum MMP-2 in Non-Small Cell Lung Carcinoma (NSCLC) patients and to establish the clinical significance of this biomarker in NSCLC patients.

Materials and Methods: One hundred and twenty newly diagnosed patients of NSCLC of both sexes and 60 age and sex matched healthy control were taken in this study. Serum MMP-2 level was evaluated in NSCLC patients and healthy controls by ELISA method. The independent t-test was used to compare the level of MMP-2 in healthy controls and NSCLC patients and also in different group of NSCLC patients.

Results: Serum MMP-2 level was found significantly higher in NSCLC patients when compared with healthy controls ($p<0.001$). Significantly, high MMP-2 level was also found in stage III and IV NSCLC patients compared to those in stage I and II NSCLC patients ($p<0.001$).

Conclusion: Present study suggests that serum MMP-2 level can be used as a marker for NSCLC along with other investigations. As MMP-2 is more feasible and economical than already established methods for diagnosis of NSCLC, and many broad spectrum oral MMP inhibitors have been developed which has very potent anti-metastatic effects in lung cancer, hence estimation of MMP-2 could be very beneficial as a marker and for better treatment of NSCLC patients.

Keywords: Biomarker, Enzyme linked immuno sorbent assay, Lung cancer

INTRODUCTION

Lung cancer is broadly divided into NSCLC and Small-Cell Lung Cancer (SCLC) with NSCLC representing the majority of diagnosis. A 75-80% of newly diagnosed lung cancer patients are of NSCLC [1]. The prognosis rate improvement is essential as five years survival rate for NSCLC patients is approx. 13%. This improvement may be attained with early detection of cancers through blood based screening of markers. [2,3,4].

MMP are Zn^{+2} and Ca^{+2} dependent enzymes which are involved in degradation of ECM proteins. The MMP family of enzymes is characterised by the presence of a zinc ion at the catalytic domain and is responsible for the proteolytic degradation of the ECM [5,6]. Expression of MMPs is involved in physiological processes like embryogenesis and tissue repairing after injury as well as in pathological processes which involve tissue damage such as arthritis, cancer, and osteoporosis [7].

MMPs are involved in initial stages of cancer development mediating signaling pathways which are related to cell migration, differentiation, proliferation, apoptosis, angiogenesis and inflammatory reactions through the activation of growth factors, cytokines and other membrane proteins [8]. MMP-2 holds a role in lung cancer angiogenesis mediated by vascular endothelial growth factor expression [9] and also in the invasive behaviour of tumour cells [10]. To limit these effects in malignant tumors, MMP inhibitors have been developed.

MMPs are involved in malignant pathogenesis by targeting basement membrane, migration of tumour cells from circulation or developing tumour microenvironment. Type IV collagen, one of the main composition of basement membrane is targeted by MMP-2 and-9. MMP-2 or Gelatinase A secreted by fibroblasts further targets ECM leading to its degradation and promoting cell proliferation, apoptosis, angiogenesis etc. Thus MMP-2 plays a vital role in initiation and progression of tumours. Specifically in lung cancers, MMP-2 attains resistance to apoptosis by Fas mediated death signalling. MMP-2 and TIMP levels act as markers for advanced stages of cancer by correlating with tumor size and nodal metastasis [13]. Various MMPs as MMP-1,2,7,9,10,11 act as vital diagnostic and prognostic biomarkers for lung cancers. Detailed and thorough understanding of mechanism and pathophysiology of MMPS is required to decode the treatment strategies [19].

Hence in the present study serum level of MMP-2 was quantified in different stages of NSCLC patients to establish the clinical significance and changes of this biomarker during NSCLC disease progression.

MATERIALS AND METHODS

Study Design and Participants

The present cross-sectional, comparative study was conducted in the Department of Biochemistry, in association with the Department of Medical Oncology at SMS Medical College and Hospitals, Jaipur, Rajasthan, India. One hundred and twenty newly diagnosed NSCLC patients of different stages were recruited from July, 2017 to March, 2019 in the Medical Oncology OPD, SMS Hospital; 60 age and sex matched healthy controls were also included in this study. The patients recruited were diagnosed on the basis of histological and cytological examinations.

The study protocol was approved by the institutional CTSC (Clinical Trial and Screening Committee) and Ethics Committee with number: 2157, MC/EC/2016. Informed written consent was obtained from all the study subjects.

Inclusion Criteria

Newly diagnosed patients with NSCLC of both sex on the basis of histological and cytological examinations.

Healthy individuals who willingly participated were enrolled in the study.

Exclusion Criteria

Patients with NSCLC of both sexes who have received CT/RT or surgery for their disease.

Patients with severe cardiovascular, hepatic, renal diseases or uncontrolled infection.

Pregnant patient.

Chronic inflammatory conditions like connective tissue disorders, autoimmune disorders, granulomatous conditions (TB sarcoidosis).

Total 180 study subjects were grouped into three groups:

- Healthy controls
- Group 1: Consist of stage I and stage II NSCLC patients.
- Group 2: Consist of stage III and stage IV NSCLC patients.

The lung cancer patients were staged according to the 7th edition of the International Staging of Lung Cancer, 2009 [20]. Samples were collected from patients and healthy controls by venipuncture. Serum was separated and stored at -80°C for analysis of MMP-2. MMP-2 was measured by ELISA technique on Mindray ELISA analyser.

STATISTICAL ANALYSIS

The presentation of the results is in the form of mean±standard deviation. SPSS for windows (version 15, Chicago, IL, USA) was used for the analysis of data collected. The independent sample t-test was used to compare the means of different variables in the two groups. For all statistical assessment a value of p <0.001 was accepted to be significant.

RESULTS

Demographic Characteristics

A total of 180 subjects were included in this study. Of these 146 were males and 34 were females [Table/Fig-1]. It was observed that 48.89% subjects were of 61-70 years age group and 33.33% were of 51-60 years age group [Table/Fig-2].

[Table/Fig-3] shows the serum MMP-2 concentration in NSCLC patients and healthy controls. The mean value of serum MMP-2 in NSCLC patients and healthy controls was 106.86±100.05 (High standard deviation in this study may be due to the inclusion of study patients with all stages of NSCLC, MMP-2 level is found very low in stage I (i.e., 3.6) and very high in stage IV (i.e., 320) and 7.17±2.92 ng/mL, respectively. The serum level of MMP-2 in NSCLC patients was significantly high than in healthy controls (p<0.001).

	Cases		Control		Total	
Gender	No	%	No	%	No	%
Female	16	13.33	15	25.00	34	18.89
Male	104	86.67	45	75.00	146	81.11
Total	120	100.00	60	100.00	180	100.00

[Table/Fig-1]: Distribution of the subjects according to gender.

	Cases		Control		Total	
Age groups	No	%	No	%	No	%
<40	4	3.33	1	1.67	5	2.78
41 to 50	15	12.50	5	8.33	17	9.44
51 to 60	37	30.83	20	33.33	60	33.33
61 to 70	54	45.00	34	56.67	88	48.89
>70	10	8.33	0	0.00	10	5.56
Total	120	100.00	60	100.00	180	100.00

[Table/Fig-2]: Distribution of the subjects according to age.

MMP-2 (ng/mL)	N	Mean	SD	Minimum	Maximum	p-value
Healthy control	60	7.17	2.92	2.6	16.3	<0.001
NSCLC cases	120	106.86	100.05	3.6	320	

[Table/Fig-3]: Comparison of MMP-2 between NSCLC patients and healthy controls; p<0.001(significant).

[Table/Fig-4] shows the serum MMP-2 concentration in healthy controls and group 1 NSCLC patients (stage I and stage II) and group 2 NSCLC patients (stage III and stage IV). The serum level of MMP-2 was significantly high in group 1 and group 2 patients than in healthy controls (p<0.001). The serum level of MMP-2 was also significantly higher in group 2 NSCLC patients than group 1 NSCLC patients (p<0.001).

MMP-2 (ng/mL)	N	Mean	SD	p-value 1 vs. 2	p-value 1 vs. 3	p-value 2 vs. 3
Control	60	7.17	2.92	<0.001	<0.001	<0.001
Group 1 (stage I and stage II)	55	21.07	15.2			
Group 2 (stage III and stage IV)	65	179.46	82.1			

[Table/Fig-4]: Comparison of MMP-2 between healthy controls and group-1 and group-2; p<0.001 (significant).

DISCUSSION

Cancers are a serious epidemiological, therapeutic and social problem [21], so new markers which may be helpful in diagnosis and prognosis of cancer patients are searched. MMPs have been proved to affect all the stages of carcinogenesis [22]. MMPs influence cell signaling pathways, reduce genome stability, errors during cell division, stimulate processes connected with Epithelial-Mesenchymal Transition (EMT). MMP-2 has capacity to degrade type IV collagen which leads to the breach of basement membrane continuity. Therefore, MMP-2 plays a significant role in the growth and invasion of tumours and formation of metastasis [23].

In the present case control study, the level of MMP-2 was compared in NSCLC patients with healthy controls and furthermore MMP-2 level was compared between group-1 (stage I and stage II) and group-2 (stage III and stage IV) and healthy controls. Serum level of MMP-2 was significantly high [Table/Fig-3] in NSCLC patients than in healthy controls which shows that MMP-2 is associated with the development of NSCLC. These findings are supported by the study of Hrabec E et al., they found that expression of active MMP-2 is 17 fold higher in lung cancer patients than in normal lung parenchyma [23].

Further, MMP-2 level was significantly higher [Table/Fig-4] in group-2 patients than in group-1 patients which shows that serum MMP-2 is also associated with invasion and metastasis of NSCLC. These results are also supported by study of Turpeenniemi-Hujanen T, which states that MMP-2 and MMP-9 have been associated with increased tumour spread and poor prognosis in lung cancer [24]. Qian Q et al., and Kaji M et al., also state that in NSCLC patients MMP-2 expression shows prognostic value and involved in metastasis of lung cancer into lymphnodes [25,26]. Expression of MMP-2 due to genetic polymorphism play role in sensitivity to lung cancer [27]. Results of present study and previous studies are in favor of MMP-2 can be a useful marker for detection of NSCLC and also for severity and stages of NSCLC.

Limitation(s)

This study was performed with limited number of patients. In previous studies MMP-2 expression in NSCLC patients was mostly observed in tissue samples. Studies of MMP-2 level in serum of NSCLC patients are still in limited number. So further studies are required in large study groups to establish MMP-2 as a useful marker for NSCLC.

CONCLUSION(S)

Serum MMP-2 level can be used as a marker for NSCLC along with other investigations. MMP-2 levels are significantly higher in later stages of NSCLC which suggest that MMP-2 is also involved in disease progression of NSCLC. As MMP-2 is more feasible and economical than already established methods for diagnosis of NSCLC such as tissue biopsy and genetic markers, MMP-2 could be very beneficial as a diagnostic and prognostic marker. Many broad spectrum oral MMP inhibitors have been developed which have very potent anti-metastatic effects in lung cancer, so estimation of MMP-2 could be beneficial for targeted treatment of NSCLC patients also.

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