

**Original Article**

## **CORRELATION OF INFLAMMATORY CYTOKINE INTERLEUKINE (IL-4) AND MATRIX-METALLOPROTEINASE-9 IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)**

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### **ABSTRACT**

**Introduction:** Chronic Obstructive Pulmonary Disease (COPD) presents with respiratory symptoms caused by airflow obstruction. Parenchymal abnormalities, constricted airway and inflammation are major causative factors associated with COPD. Proteases such as matrix metalloproteinases (MMP) and a pleiotropic cytokine Interleukin-4 (IL-4) play an important role in lung tissue remodeling. However, there is a dearth in studies correlating the levels of MMP9 and IL-4 in patients of COPD. This study aims to evaluate the serum levels of MMP-9 and IL-4 in COPD and correlate with clinical characteristics of COPD patients.

**Materials and methods:** This case control study pre-screened 134 participants of which 40 were healthy and 94 were COPD patients from the Department of Pulmonary Medicine at the Prasad Institute of Medical Sciences Lucknow.

**Results:** Significantly increased level of Serum MMP-9 ( $212.6 \pm 75.5$  ng/ml) were detected in COPD group as compared to the control group ( $97.5 \pm 45.6$  ng/ml). Also, the IL-4 levels were significantly different in the control group

**Conclusions:** Detection of higher levels of MMP9 and IL-4 in patients suffering from COPD in the present study implies that these biomarkers could serve as markers for understanding the depth of COPD development and therapy.

**Keywords:** Matrix metalloproteinases (MMP), Interleukin-4 (IL-4), COPD

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## **INTRODUCTION**

Chronic obstructive pulmonary disease (COPD) is characterised by persistent respiratory symptoms and restricted airflow. Shortness of breath and a cough that may or may not produce mucus are the prominent symptoms [1]. As COPD progresses, simple tasks like dressing or walking become more challenging. [2] Although COPD cannot be cured, it can be prevented and treated. Airflow blockage, a characteristic of COPD, a diverse lung disorder, is not entirely reversible. The main causes of airflow restriction in COPD are thought to be structural abnormalities in the lung parenchyma, tiny airways, and inflammation [3]. Smoking, which is a major contributor to COPD, is a risk factor for the disease. In addition to genetics, environmental variables can have an impact on COPD. (4)

In COPD lung tissue remodelling, the extracellular matrix renewal is known to be regulated by a class of proteases called matrix metalloproteinases (MMP) [5] Gelatinase B, commonly known as MMP9, has a molecular weight of 92kD. It is secreted by alveolar macrophages, neutrophils, eosinophils, mast cells, and bronchial epithelial cells during the inflammatory events in COPD. Numerous MMPs have been found to play a role in the lung pathology, MMP-9 is the predominant protease in alveolar tissue and because of its easy detection and quantification, it has attracted the attention in MMPs. Inflammation modulates the protease/antiprotease balance leading to progressive airway destruction as well as remodelling. MMP 9 is not produced in healthy lung tissue, however, during the inflammatory events of COPD, alveolar type II cells, bronchial

epithelial cells, clara cells, endothelial cells, fibroblasts and smooth muscle cells produce MMP-9, as well as the leukocytes in the lung. [6] MMP-9 degrades elastin and promotes further lung damage which can promote inflammation in COPD [6]. Overall, these mechanisms support the role of MMP-9 as a key mediator in COPD.

A pleiotropic cytokine is interleukin (IL)-4, commonly referred to as B-cell-stimulating factor. It primarily stimulates the growth of T cells and causes B cells to produce antibodies. It also causes fibroblasts, endothelium, and epithelial cells to proliferate, differentiate, and become activated. It also boosts the recruitment of inflammatory cells (7). However, few studies investigated the inflammatory cytokine levels and MMP 9 level in patients with COPD. In the present study, we aimed to evaluate the serum levels of MMP-9 and IL-4 in COPD and correlate with clinical characteristics of COPD patients.

## **MATERIALS AND METHODS**

We have 134 participants that were pre-screened for this case-control study, wherein 40 healthy controls and 94 COPD patients were drawn from the Department of Pulmonary Medicine at the Prasad Institute of Medical Sciences Lucknow after taking due permissions. Prior to enrolment, all participants were provided written information and their consent was taken. The study's methodology was approved by the University Ethical Committee of Subharti University, Meerut, where the study was registered.

**The inclusion criteria for COPD patients and Controls were as follows**

**COPD** - Age 40 – 75 years and Patient who has symptoms of a persistent cough, sputum production, or dyspnea, and/or a history of exposure to risk factors for the disease.

**Controls** - Healthy volunteers who will be non-COPD based on their medical-history, clinical examination

Non-tobacco users, non-occupational exposure

**Severity Grading of COPD**

Patients who had a persistent cough, dyspnoea, a history of exposure to COPD risk factors, a forced expiratory volume in one second (FEV1) to forced vital capacity (FVC) ratio of less than 0.7, and reversibility by inhaled bronchodilators in FEV1 of less than 12 percent or 200 mL after two puffs of 200 mg salbutamol administered with a pressure metered-dose inhaler with spacer were considered to have COPD. GOLD guidelines [10] were used to establish the diagnostic criteria for COPD. For GOLD-1, the predicted FEV1 percentage is  $\geq 80\%$ ; for GOLD-2, the predicted FEV1 percentage is between  $50 - 79\%$ ; for GOLD-3, the predicted FEV1 percentage is  $30 - 49\%$ ; and for GOLD-4, the predicted FEV1 percentage is less than  $30\%$ .

**RESULTS**

Forty healthy control and 94 COPD patients were included in the present study. Mean age of the control group was  $52.6 \pm 7.46$  and the COPD group was  $61.3 \pm 7.64$ . In the control

group 21 (52.5%) were male and 19 (47.5%) female. In the COPD group 68 (72.3%) males and 26 (27.6%) females were enrolled. In the COPD group 13 patients were current smoker and 32 were non-smoker, while the rest were ex-smokers. COPD patients were divided into GOLD grade 1 (n=18), grade 2(n=68), grade 3(n=6), grade 4 (n=2). (Table 1)

Significantly increased level of Serum MMP-9 ( $212.6 \pm 75.5$  ng/ml) were detected in COPD group as compared to the control ( $97.5 \pm 45.6$  ng/ml) and also significantly increased level of IL-4 ( $136.3 \pm 20.1$  ng/l) were present in COPD group ( $213.79 \pm 100.52$  ng/ml) as compared to the control group ( $51.1 \pm 7.5$  ng/l) (Fig. 1). The levels of MMP 9 and IL-4 increased significantly as the grading increased from 1 to 4. ( $p=0.001$ )

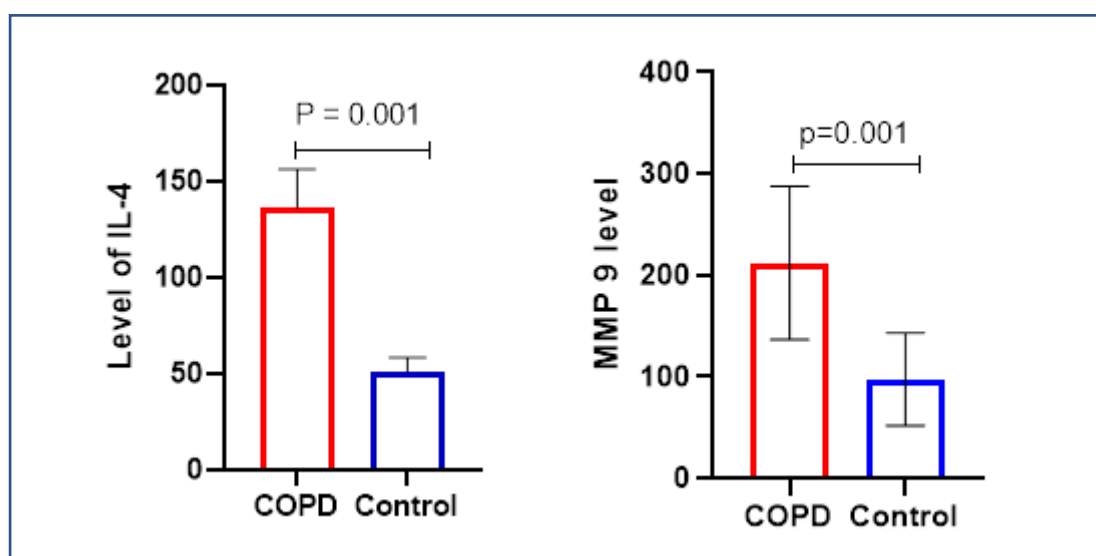
The level of IL-4 positively correlated with FEV1/FVC ( $r=.217$ ,  $p= 0.036$ ) and those of MMP 9 also positively correlated with FEV1/FVC ( $r=.252$ ,  $p= 0.014$ ). (Fig. 2)

**DISCUSSION**

In this population-based study, COPD patients had significantly higher serum MMP-9 and IL 4 compared to the healthy control subjects. MMP-9 may play a fundamental role in the aetiology of COPD. According to a prior study, MMP-9 was adversely linked with the degree of airway blockage (11). A population-based COPD cohort study found a correlation between MMP-9 and productive cough and decreased FEV1 (forced expiratory volume in first sec). Furthermore, the burden of tobacco smoking exposure, assessed as number of pack years, was associated with increasing MMP-9/TIMP-1-ratio in both COPD and non-COPD, indicating

|                               |               |
|-------------------------------|---------------|
| Parameter                     | N= 94         |
| Age mean ( minimum – maximum) | 61.3( 21- 72) |
| Gender                        |               |
| Male                          | 68(72.3%)     |
| Female                        | 26(27.6%)     |
| GOLD grading                  |               |
| 1                             | 18 ( 13.4%)   |
| 2                             | 68(50.7%)     |
| 3                             | 6(4.5%)       |
|                               | 2(1.5%)       |
| Socio economic status         |               |
| Lower                         | 58(61.7%)     |
| middle                        | 36(38.2%)     |

**Table 1 . Demographical and Clinical characteristics of the COPD population.**



**Figure 1. Correlations between plasma inflammatory cytokine levels and MMP-9 and pulmonary function indexes**

a tobacco smoke-induced increase in proteolytic activity, independent of sex, age and FEV1. According to a recently published study of 80 women with COPD and 40 controls, not only smoking, but also exposure to biomass combustion, was related to differences in metalloproteinases, including increased MMP-9 and MMP-9/TIMP-1 ratio among those with

COPD [12]. Small observational studies have demonstrated increased MMP-9 in COPD patients compared to controls, both in analyses of sputum [13], lung parenchyma [14] and serum [15].

To the best of our knowledge, this is the first study, in which serum MMP-9 has been analysed and also proved to be increased in a

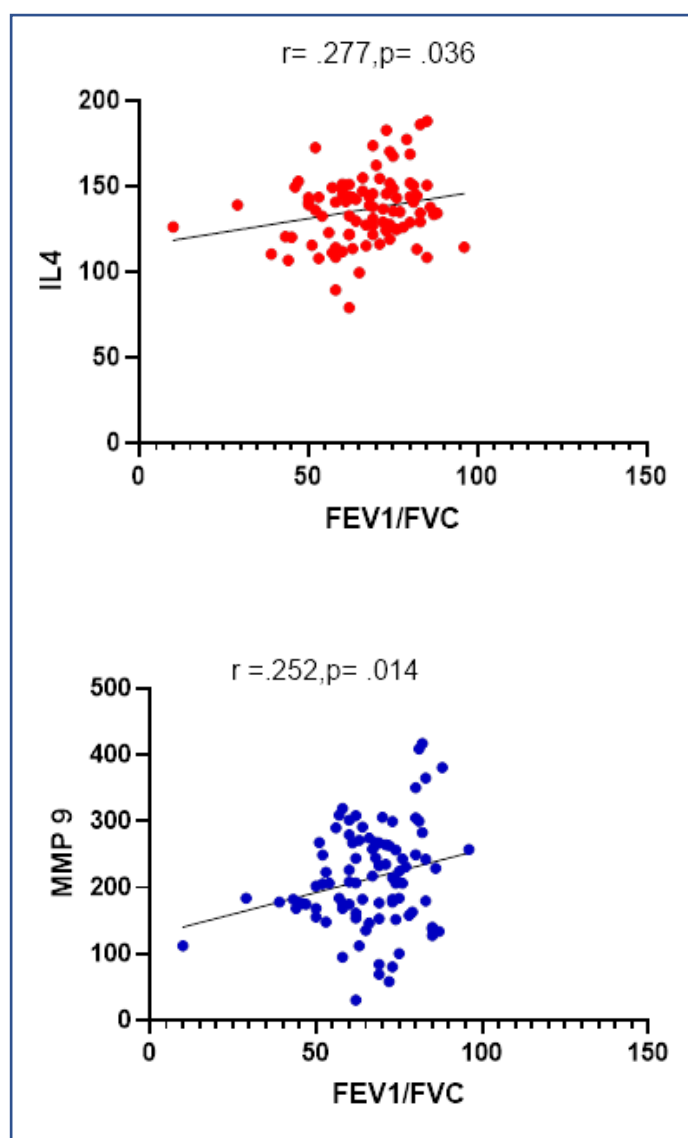


Figure 2. Correlations between plasma inflammatory cytokine levels (IL4) and MMP-9 and pulmonary function indexes

population-based COPD cohort. Furthermore, the association between serum MMP-9 and impaired lung function, assessed as FEV1, in COPD show that MMP-9 is related to disease severity which could indicate that MMP-9 is involved in the disease process in COPD.

### Inflammatory cytokines

The result showed that the IL-4 levels were significantly different in the control group. As a

pleiotropic cytokine, IL-4 plays a crucial role in type 2 T-helper responses and isotype class switching of B cells to IgE synthesis, and it has thus been suggested that IL-4 may have an important role in COPD pathogenesis [16,17] Serum MMP-9 and IL-4 expression rose in the current experiment, and this trend was similar with GOLD grade. All of these markers have a favourable correlation with COPD patients.

These findings indicate that MMP-9, and IL 4 are closely related to the pathogenesis of inflammation and airflow limitation in the progression of COPD.

## CONCLUSION

Patients with COPD had higher serum levels of MMP-9 and IL 4. With the GOLD Grading the elevated levels of MMP-9, IL 4 were discovered, demonstrating the correlation between these biomarkers and the severity of airway limitation in COPD. These findings imply that they could serve as markers for understanding the depth of COPD development and therapy.

## REFERENCES

1. Global Strategy for Prevention, Diagnosis and Management of COPD: 2021 Report (PDF). Global Initiative for Chronic Obstructive Lung Disease. 25 November 2020. Retrieved 28 June 2021.
2. Global Strategy for Prevention, Diagnosis and Management of COPD: 2021 Report (PDF). Global Initiative for Chronic Obstructive Lung Disease. 25 November 2020. Retrieved 28 June 2021.
3. Chen Y, Chen P, Hanaoka M, Droma Y, Kubo K. Enhanced levels of prostaglandin E2 and matrix metalloproteinase-2 correlate with the severity of airflow limitation in stable COPD. *Respirology*. 2008;13(7):1014–21.
4. Uysal P, Uzun H. Relationship between circulating serpin3g, matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 and-2 with chronic obstructive pulmonary disease severity. *Biomolecules*. 2019;9(2):62.
5. Churg A, Zhou S, Wright JL. Matrix metalloproteinases in COPD. *Eur Respir J*. 2012;39:197–209.
6. Abd El-Fatah MF, Ghazy MA, Mostafa MS, El-Attar MM, Osman A. Identification of MMP-9 as a biomarker for detecting progression of chronic obstructive pulmonary disease. *Biochem Cell Biol*. 2015;93(6):541–7.
7. Shen H, Xia L, Lu J: Interleukin-4 in rheumatoid arthritis patients with interstitial lung disease: A pilot study. *Indian J Med Res*, 2013; 138: 919–21.
8. Despotovic M, Stoimenov TJ, Stankovic I et al: Gene polymorphisms of tumor necrosis factor alpha and antioxidant enzymes in bronchial asthma. *Adv Clin Exp Med*, 2015; 24: 251–56
9. Jundi K, Greene CM: Transcription of interleukin-8: How altered regulation can affect cystic fibrosis lung disease. *Biomolecules*, 2015; 5: 1386–98.
10. Jiang S, Shan F, Zhang Y, Jiang L, Cheng Z. Increased serum IL17 and decreased serum IL-10 and IL-35 levels correlate with the progression of COPD. *Int J Chron Obstruct Pulmon Dis*. 2018;13:2483.
11. Beeh KM, Beier J, Kornmann O, Buhl R. Sputum matrix metalloproteinase-9, tissue inhibitor of metalloproteinase-1 and their molar ratio in patients with chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis and healthy subjects. *Respir Med*. 2003;97(6):634–9.

12. Montaña M, Sansores RH, Becerril C, Cisneros J, González-Avila G, Sommer B, et al. FEV1 inversely correlates with metalloproteinases 1, 7, 9 and CRP in COPD by biomass smoke exposure. *Respir Res.* 2014;15:74.
13. . Simpson JL, McDonald VM, Baines KJ, Oreo KM, Wang F, Hansbro PM, et al. Influence of age, past smoking, and disease severity on TLR2, neutrophilic inflammation, and MMP-9 levels in COPD. *Med Inflamm.* 2013;2013:462934.
14. Kang MJ, Oh Y-M, Lee JC, Kim DG, Park MJ, Lee MG, et al. Lung matrix metalloproteinase-9 correlates with cigarette smoking and obstruction of airflow. *J Korean Med Sci.* 2003;18:821–7.
15. Brajer B, Batura-Gabryel H, Nowicka A, Kuznar-Kaminska B, Szczepanik A. Concentration of matrix metalloproteinase-9 in serum of patients with chronic obstructive pulmonary disease and a degree of airway obstruction and disease progression. *J Physiol Pharmacol.* 2008;59:145–52.
16. Miravittles M. Cough and sputum production as risk factors for poor outcomes in patients with COPD. *Respir Med.* 2011;105:1118–28.
17. Omachi TA, Omachi TA, Eisner MD, Eisner MD, Rames A, Rames A, et al. Matrix metalloproteinase-9 predicts pulmonary status declines in  $\alpha$ 1-antitrypsin deficiency. *Respir Res.* 2010;12:35–5.