

## ORIGINAL ARTICLE

# Assessment of Serum Levels of the Matrix-Metalloproteinase-9, Prostaglandin E-2 and Cyclo-Oxygenase-2 as a Candidate Biomarker of Chronic Obstructive Pulmonary Disease (COPD)

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**Background:** Chronic obstructive pulmonary diseases are one of the crucial and increasing global health complications. It has highest prevalence not only in Pakistan but also the large cases of COPD are reported world widely.

**Objective:** The study was carried out to find the relationship between elevated serum level of Prostaglandin E2, Matrix-metalloproteinase-9 and Cyclo-oxygenase-2 and Chronic obstructive pulmonary diseases.

**Study design:** It is a case control study conducted at department of biochemistry, Islam Medical College, Sialkot for the duration of six months from February 2022 to July 2022.

**Material and Methods:** The patients suffering from chronic obstructive pulmonary disease were selected for the study. Two groups were made, control group contained 41 healthy individuals and diseased group contained 39 patients. All the patients that were not according to the inclusion criteria were excluded from the study.

**Results:** Average age of patients was calculated and it came out to be 57 years in case of control group and 61 for COPD patients. There were almost similar numbers of male and female members in both groups. The body mass index was also calculated and it was found that 15 participants from the COPD group fell under 23-25 kg/m<sup>2</sup> group.

**Conclusion:** The level was found to be high for Matrix-metalloproteinase-9, Prostaglandin E-2 and Cyclooxygenase- 2 in the serum of patients suffering from COPD. The level was increasing as the GOLD grading was increased. Smoking is positively related to the severity of chronic obstructive pulmonary diseases. The level of, PGE-2 and COX-2 showed a positive association with the chronic obstructive pulmonary diseases patients.

**Keywords:** biomarker and Chronic obstructive pulmonary diseases.

**INTRODUCTION**

Chronic obstructive pulmonary diseases (COPD) are one of the crucial and increasing global health complications. It has highest prevalence not only in Pakistan but also the large cases of COPD are reported world widely. It is considered to be the most prevalent disease that account for the third most common cause of the mortality around the globe. COPD is marked by an enhanced lungs inflammatory response to inhalation of toxic respiratory pollutants<sup>1-3</sup>. There are many environmental and occupational hazards subsidize to the pathology of COPD. Tobacco smoking is the primary one. It leads to the fixed narrowing of small airways and alveolar wall destruction. The number of alveolar macrophages, neutrophil, and cytotoxic T-lymphocytes, and release of multiple inflammatory mediators increased. Oxidative stress level also increase. MMP-9 are the class of proteases that regulate extracellular matrix during COPD lung tissue remodeling. It is also known as gelatinase B. It is secreted by neutrophil, eosinophil, alveolar macrophages and mast cells. Various MMP play a crucial role in the lungs pathology. MMP-9 is an early marker for the detection and staging of COPD by assessing mRNA level, mRNA level of MMP-9 increased two fold in severe COPD as compared to non-COPD smokers<sup>4-6</sup>. Cyclooxygenase- 2 is the rate-controlling enzyme it form arachidonic acid from prostaglandin and thromboxane. COX-2 is undetectable in normal conditions but when subjected to the inflammatory stimuli it is rapidly induced in cells. The COX-2 has a crucial role in the production of versatile prostaglandins during inflammatory responses<sup>7-9</sup>. The increased COX-2 leads to an increase in the synthesis of PGE2. PGE2 stimulates cell invasion, angiogenesis, and the formation of metastasis and cell survival. By a different aggravation like cytokines, growth factors, and tumor promoter. PGE2 is a prostanoid lipid mediator. The arachidonic acid forms it through the COX pathway. Prostaglandins are the main factor in the regulation of inflammation. Paracrine and autocrine signaling are activated proagladins. In the pathogenesis of inflammation in COPD, PGE-2 and COX-2 are essential players<sup>10</sup>. The study was carried out to find the relationship between elevated serum level of Prostaglandin E2, Matrix-metalloproteinase-9 and Cyclo-oxygenase-2 and Chronic obstructive pulmonary diseases.

**MATERIAL AND METHODS**

It is a case control study conducted at department of biochemistry, Islam Medical College, Sialkot for the duration of six months from February 2022 to July 2022. The study was carried out on patients suffering from chronic obstructive pulmonary disease. Two groups were made, control group contained 41 healthy individuals and diseased group contained 39 patients. All the patients that were not according to the inclusion criteria were excluded from the study. There were almost similar numbers of male and female members in both groups. The study approval was taken from the health and review board committee of the hospital. The patients were aware of the study objective and written consent was taken.

Smoking habits of the patients were evaluated. All the patients that were not according to the inclusion criteria were excluded from the study. The patients who had the history of the respiratory tract disease, pregnant women and patients that were diagnosed with the heart disease, asthma and pulmonary tuberculosis were excluded from the study. GOLD guidelines were used for the diagnosis of COPD. The blood sample from peripheral venous blood were collected and centrifuged to separate the serum. The serum was stored in the micro centrifugation tubes for the latter use. The Enzyme linked immunosorbent assay was used to measure the serum level of MMP9 and others. The data was collected and SPSS was used for the statistical analysis. The results were analyzed and presented in the form of tables.

**RESULTS**

The study was carried out on patients suffering from chronic obstructive pulmonary disease. Two groups were made, control group contained 41 healthy individuals and diseased group contained 39 patients. The clinical profile of the patients is described in table no.1. Average age of patients was calculated and it came out to be 57 years in case of control group and 61 for COPD patients. There were almost similar numbers of male and female members in both groups. The body mass index was also calculated and it was found that 15 participants from the COPD group fell under 23-25 kg/m<sup>2</sup> group. Smoking habits were

evaluated as there were 26 patients that showed no smoking profile. Most of the patients belonged to middle class families.

Table 1: Clinical features of the studied patients

Features	Control group n=41	COPD patients n=39
Age in years	57.22 ± 8.9	61.23±8.9
Gender		
Male	21	20
Female	20	19
BMI		
18-23 kg/m <sup>2</sup>	15	14
23-25 kg/m <sup>2</sup>	20	15
>35 kg/m <sup>2</sup>	6	10
Smoking habit		
Ex-smoker	6	4
No smoking	25	26
Smoker	10	9
Socio-economic status		
Upper class	5	5
Middle class	30	30
Lower middle class	6	4
Clinical history (years)		
<10		3
10-20		15
>20		21
GOLD grading *		
1		12
2		7
3		13
4		7
CAT score*		
<10		25
>10		14

After the diagnosis, patients were distributed into different GOLD categories. GOLD (global initiative for chronic obstructive lungs disease) grading was done on base of FEV1 percentage. The predicted FEV1 percentage used was >80% for GOLD 2 category. For GOLD 3, < 80% was used and for GOLD 4, FEV1 percentage < 30% was used. CAT test score was also calculated.

Table 2: Mean level of MMP-9, in patients and control group

	Control group n=41	Patients n=39	P value
MMP-9 (pg/mL)	90.11	180.2	0.005
Gold grade 1	90	150	0.005
Grade 2	90	160	0.005
Grade 3	90	170	0.001
Grade 4	90	310	0.00
CAT score			
Control			
<10	160	210	0.005
>10	160	240	0.001

Table 3: Results clearly stated that the value of these compounds is high in the participants of diseased group as compared to the control.

	Control group n=41	Patients n=39	P value
COX-2 (pg/mL)	110	300	0.006
Gold grade 1	100	150	0.005
Grade 2	100	190	0.001
Grade 3	100	290	0.003
Grade 4	100	420	0.02
CAT score			
<10	210	290	0.000
>10	210	320	0.000

Table 4: Mean level of PGE-2, in patients and control group

	Control group n=41	Patients n=39	P value
PGE-2 (pg/mL)	130	410	0.005
Gold grade 1	140	210	0.004
Grade 2	140	240	0.000
Grade 3	140	380	0.000
Grade 4	140	680	0.03
CAT score			
<10	310	410	0.000
>10	310	430	0.001

Table 2,3 and 4 shows mean level of matrix-metalloproteinase-9 and prostaglandin E-2 in patients and control

group. The results were compared to find the increased level of these compounds in the serum of patients. Table no.3: Mean level of COX-2, in patients and control group

## DISCUSSION

The results demonstrated that the serum level of MMP-9, PGE-2 and COX-2 is high in the blood serum of COPD patients significantly. There was a pattern in the increase of the serum level of these compounds ranging from non-smoker to smoker category. The serum level was found to be high of MMP-9, PGE-2 and COX-2 in the GOLD level 1 to 4. High level of CAT score was also found in the results. Matrix-metalloproteinase -9 plays a key role in the obstruction of airflow so it can be linked to the possible reasons of COPD. As per previous study MMP-9 has a negative correlation with the airflow obstruction, it was found that MMP-9 was linked to cough and low level of FEV1 (Forced expiratory volume in 1s)<sup>11</sup>. In another study carried out to find the link of MMP-9 to COPD, it was found that the patients who had high serum level of MMP-9 had more protein proteolysis which ultimately caused severity of the disease<sup>12-13</sup>. The level of MMP-9 in our study came out to be 150 pg/mL, 160 pg/mL, 170 pg/mL and 310 pg/mL in GOLD 1,2,3 and 4 categories respectively. Which showed that the level of MMP-9 is increasing as the GOLD level is increasing. Similarly, the CAT score was also measured and it was 210 and 240 in case of <10 and >10 groups. A similar analysis was found by a previous study as well where the CAT score came out to be 230 and 240. The statistical significance was also calculated and the results were statistically significant. As per literature the serum level of MMP-9 and MMP-8 was found to be high in peripheral blood in patients suffering from COPD<sup>14</sup>. And the similar study reported that the level of MMP- was significantly increasing as the GOLD grading was increased<sup>15</sup>. The studies are going on to find that either the level of MMP in urine and blood is linked to each other or not. And which level of MMP-9 is linked to increased severity of disease<sup>16</sup>. As a study was carried out and the level of MMP-9 was measured in blood, urine and in the airway. The study was done on patients who were admitted in the hospital for COPD. The results showed that the level of MMP-9 in the serum and urine was increased and MMP-8 was not significantly increased in the serum<sup>17</sup>.

Mean level of COX-2 was also analyzed and the data revealed that the level of COX-2 dramatically increased in GOLD grade 4 with a value of 420 as compared to the value of control which was 100. The CAT score was found to be 290 and 320 for <10 and >10 groups. The study conducted to find the level of COX-2 in the sputum of patients suffering from COPD showed that as compared to the non-smoker group, the patients who had history of smoking showed high level of COX-2<sup>18</sup>. Not only the level was increasing but also there was a gradual increase in the blockage of airflow in smoker patients.

The mean level of PGE-2 came out to be 680 in the fourth grade of GOLD which shows that the level of PGE-2 has increased significantly in the fourth grade. The CAT score was also significantly high in <10 and >10 groups as 410 and 430 respectively. As per study the exhaled breath condensate showed of patients had elevated level of PGE-2 as compared to the control group<sup>19-20</sup>. The percentage of COX-2 in the immunostained cells in the COPD patients was higher. According to literature the expression level of COX-2 and MMP-9 was calculated and there was a significant increase in expression as the severity of COPD disease was increased<sup>21</sup>. As per evidence the smoking was found to be one of the main factors triggering and increasing the severity of COPD. The blockage of airflow passage and the enhanced inflammation was observed more in case of smoker patients. However, the study had one limitation the size of diseased group is small if data was taken from different hospitals a more diverse study could be made.

## CONCLUSION

The level was found to be high for Matrix-metalloproteinase-9, Prostaglandin E-2 and Cyclooxygenase- 2 in the serum of patients

suffering from chronic obstructive pulmonary disease. The level was increasing as the GOLD grading was increased. Smoking is positively related to the severity of COPD. The level of Matrix-metalloproteinase-9, Prostaglandin E-2 and Cyclooxygenase- 2 showed a positive association with the chronic obstructive pulmonary disease patients. These factors can be used as a marker for management and progression of COPD.

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