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Original Article

Surfactant Protein-D, Fibrinogen, and Interleukin-6 Correlation with Clinical Outcomes in Patients with Chronic Obstructive Pulmonary Disease

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Abstract

Objective: Chronic obstructive pulmonary disease is currently the third leading cause of death worldwide. The current study was designed to see if fibrinogen, interleukin-6 or surfactant protein-D could be used as biomarkers to predict frequency of exacerbations within the frequent exacerbator phenotype.
Study type, settings & duration: This cross-sectional study performed at Institute of Molecular Biology & Biotechnology, The University of Lahore and Mayo Hospital, Lahore from November 2023 to October 2024.
Methodology: Blood samples were collected from 40 patients after informed consent. Simple random sampling was done. Blood sample of 5 ml was taken and centrifuged. Plasma was isolated and ELISA was performed to examine the biomarker panel. Data was analyzed using SPSS version 26.0.
Results: The median±age, CAT score, fibrinogen, IL-6 and SPD were 43.4±9 years, 11.0±5.0, 308.53±215.78, 5.65±3.42 and 8.58±4.27 respectively. The frequency of exacerbations showed a strong reverse correlation with gender (r =-0.36, *p* =0.02) and a positive correlation with IL-6 levels (r =0.32, *p* =0.04) and CAT score (r =+0.44, *p* =0.005). Furthermore CAT score showed a positive correlation with male compared to female patients (r =0.35, *p* =0.03). It did not however, show a significant correlation with age, age gender, interleukin-6 or surfactant protein-D levels.
Conclusion: Results showed a positive link between smoking and disruption of the quaternary structure. There was a significant relationship between SP-D and exacerbation.
Key words: Frequent exacerbator phenotype, exacerbations, COPD, fibrinogen, surfactant protein-D, interleukin-6, ROC curve analysis, biomarker.

Introduction

Chronic obstructive pulmonary disease (COPD) is a debilitating disease which affects millions of individuals.[1] It is the world's third greatest cause of mortality, with a pretty well-understood natural history and a high prevalence of co-morbidities.[2,3] COPD is a chronic inflammatory disease that causes airway constriction and destruction of the alveolar wall. It is produced by an increase in the number of alveolar macrophages and inflammatory mediators in the airways, resulting in inflammation.

COPD is now considered to be a disease encompassing different phenotypes. A phenotype is defined as 'any observed quality of an organism that results from interaction between gene and environment'. The clinicians and researchers are now using a phenotype based approach for COPD. Even 'The Global Initiative for Chronic Obstructive Lung Disease (GOLD)' has used a phenotype-based approach to guide COPD management. One important phenotype based on frequency of exacerbations is the 'frequent exacerbator phenotype'. It is defined as the patient who has two or more exacerbations per year. Frequent exacerbator phenotype is associated with worse

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Authors Contribution

AW & HMU conceptualized the project. AW & MM did the data collection. AW & MA did the literature search. AW, HMU & AW performed the statistical analysis. Drafting, revision & writing of manuscript were done by FH & TM.

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prognosis and increased mortality than the average COPD patient. Hence it is the need of the hour to develop biomarkers for early identification of this phenotype and start aggressive management of the disease. Fibrinogen has emerged as a promising biomarker in COPD, and the US Food and Drug Administration and the European Medicines Agency are now considering it for certification as a drug development tool.[4] Hepatocytes produce fibrinogen, which is then released into the bloodstream in response to IL-6 stimulation.[5] Fibrinogen is a glycoprotein that functions as an acute phase reactant and is an important coagulation agent. The level of fibrinogen is linked to the severity of COPD and the likelihood of exacerbation.

SP-D is a multimeric glycoprotein that belongs to the C-type lectin or collecting family of collagen-containing lectins. It helps maintain pulmonary surfactant equilibrium and is important for pulmonary innate immunity. Chronic inflammatory diseases such as asthma, acute respiratory distress syndrome, interstitial lung fibrosis and COPD have been linked to an increase in serum SP-D.[6] In COPD, the concentration of serum SP-D rises in tandem with the decrease in bronchoalveolar lavage[7,8] The breakdown and alteration of SP-D caused by disease facilitates its systemic leaking from the lungs and circulatory system.[9] SP-D is a lung damage biomarker. Because of their great specificity for lung illness, pneumoproteins (proteins generated primarily in the lungs) are attractive blood biomarkers.[10]

IL-6 is type of cytokine which causes inflammation by activating Janus Kinase – Signal Transducer and Activator of Transcription signalling pathway.[11]

We thus devised a study to investigate the association of COPD frequent exacerbator phenotype with biomarkers SP-D, IL-6 and Fibrinogen. It may be feasible to target these potentially modifiable elements at both the pharmaceutical and non-pharmacological levels if they can be established as biomarkers.[12]

Methodology

It was a cross-sectional study performed at Institute of Molecular Biology & Biotechnology, The University of Lahore and Mayo Hospital, Lahore from November 2023 to October 2024. A sample size of 40 COPD patients was statistically calculated a same number of normal individuals were taken to serve as control. After taking informed consent, 40 patients of both genders diagnosed with stable COPD, aged 30 to 70 years old were included in this study. Other pulmonary diseases such as chest wall deformity, bronchiectasis, pulmonary fibrosis, and lung cancer were eliminated, as well as pregnant or breast-feeding women. History was taken and number of exacerbations overall last 6 months were averaged to obtain information about exacerbations per year.

Data of all 40 study participants including name, sex, age as well as the registration number were recorded on a pre-designed study tool. The results of the preliminary research were documented. A blood sample of 5 milliliters was taken from each patient in a sodium citrate vacutainer for plasma and 5 milliliters in serum vacutainers; centrifugation was done at 5000rpm for 10-15 minutes. Plasma was pipetted out and kept in separate Eppendorf tubes at -80°C until it was needed. Enzyme-linked immunosorbent assay (ELISA) kit was used to examine the biomarker panel.

SPSS-26 was used to enter the information and analyze it. Age, Fibrinogen, Surfactant Protein-D, and IL-6 were reported as Median + IQR as they were skewed in distribution and gender was expressed in percentages. All continuous variables were tested for normality using Shapiro Wilk test. The exacerbation frequency was correlated with age gender, IL-6, SP-D and CAT score using Spearman correlation test. Linear regression was used to check the combined effect of gender, IL-6 and CAT score on exacerbation frequency. For the purpose of linear regression, the scale variables were log transformed.

The ethical approval was obtained from the Ethical Review Committee of The University of Lahore, Lahore vide letter no. IMBB/BBBC/22/10.

Results

A total of 40 patients consisting 32 (80%) males and 8 (20%) females were included as depicted in Figure Ib**.** Male gender remained predominant significantly presenting female to male ratio of 1:4 in this study. There were significant differences in mean age, BMI, baseline CAT score, fibrinogen, IL6, SPD, and E score. In order to further elucidate the findings, the person's correlation test was used to find the correlation. There were 16 (40%) low CAT score, 22 (55%) medium CAT score and only 2 (5.0%) was High CAT score. A remarkable association was found between Exacerbation and CAT score (p <0.05). 2 normal or 4 low or high fibrinogen respondents have no exacerbation, 8 normal and 6 low or high respondents have 1 exacerbation.

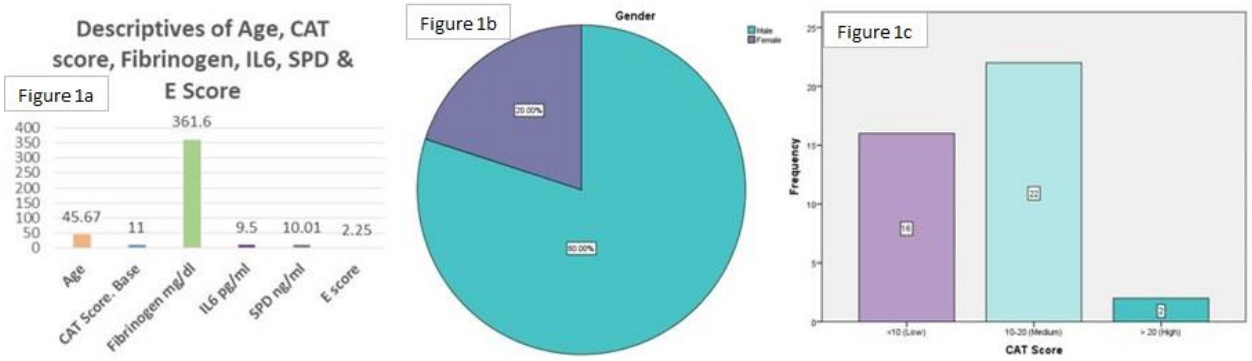

Figure 1: (a) Mean age, CAT score at baseline, fibrinogen, IL6, SPD and E-score levels.
(b) Depiction of CAT Score
(c) Depiction of highest and lowest CAT Scores

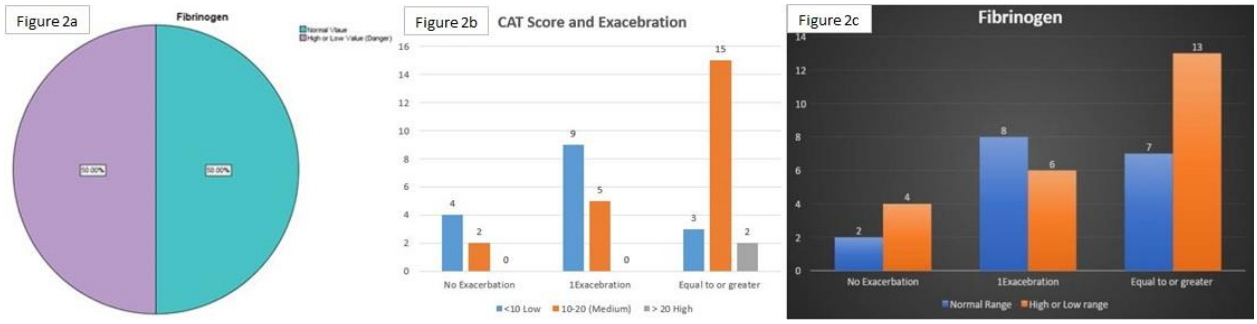

Figure 2: (a) Normal & High levels of fibrinogen
(b) Significant association between CAT score and exacerbation
(c) Non-significant association between Fibrinogen and exacerbation

Figure 3: (a) Strong correlation between IL6 and exacerbation
(b) Correlation between SPD and Exacerbation
(c) Scatter plot showing association between exacerbations and CAT score

Figure-1a shows the mean age, CAT score at baseline, fibrinogen, IL6, SPD and E-score levels as 45.6+10.96, 11.0+4.48, 361.6 +241.5, 9.50+12.79, 10.01+6.21 and 2.25+1.40 respectively, Figure-1c shows low CAT score in 16 (40%),22 (55%) medium CAT score and only 2 (5.0%) had high CAT score. Figure-2a shows 20 (50%) had normal value of fibrinogen and 20 (50%) had high value of fibrinogen. Figure-2b shows a significant association between CAT score and Exacerbation (p ≤0.05) while Figure-2c depicts that fibrinogen and exacerbation are not significantly related (p >0.05).

Figure-3a shows that IL6 and exacerbation are not strongly correlated (p >0.05), Figure-3b demonstrates a moderate correlation between SPD and Exacerbation (p <0.05), while Figure-3c is a Scatter plot depicting association between number of exacerbations and CAT score.

The cohort had a median age of 43.4±9 years. They were all frequent exacerbators according to the definition of GOLD criteria.[5] The mean and median exacerbations per year were 7.95±5.4 and 6±6 respectively. Eighty (80) percent of the cohort were men.

The frequency of exacerbations showed a strong inverse correlation with gender (r =-0.36, *p* =0.02) and a positive correlation with IL-6 levels (r =0.32, *p* =0.04) and CAT score (r =+0.44, *p* =0.005). However, age gender, IL-6, SP-D did not. Furthermore, CAT score exhibited a rising correlation with male individuals as compared to females (r =0.35, *p* =0.03). Correlation with IL-6 approached statistical significance (*p* =0.05) It did not however, show a remarkable correlation with age, IL-6, SP-D levels as well as fibrinogen (Table-1).

Table 1: Spearman's correlation of clinical variables with frequency of exacerbations in frequent exacerbators.

Variable	*Correlation Coefficient 'r'*	*p value*
Age (years)	0.13	0.42
Male	0.39	0.01*
Serum Fibrinogen level	0.10	0.55
Serum SP-D level	-0.03	0.87
Serum IL-6	0.28	0.08
CAT score	0.40	0.01*

*Shows statistically significant results

Multiple linear regression was applied with Frequency of exacerbation as the independent variable and gender, CAT score and IL6 were independent variables. The analysis produced a regression model with model accuracy of *p* =0.003 (ANOVA) and adjusted R2 of 32.3%. It showed that IL-6 and CAT score were strong indicators of exacerbation frequency (*p* <0.05) in frequent exacerbator phenotype. The association of gender was blurred out (*p* >0.05) after adjusting for CAT square and IL-6.

Discussion

The clinical manifestation of COPD is hurdle in air flow in respiratory system, which causes irritation, pathological changes and inflammation in airways and is linked to parenchymal obliteration and the formation of emphysema.[13] However, these patients frequently have systemic symptoms of the illness, which can result in decreased functional ability, severe dyspnea, impairment and poor quality of life, and increased mortality.

This study was aimed at evaluating the association among SP-D, fibrinogen and interleukin-6 with clinical outcomes in COPD patients.

In all smokers, SP-D levels were accordant and linked to the intensity of airway blockage. Furthermore, smoking causes quaternary structure to be disrupted. While in our study there were 2 respondents having low and 6 having high SPD having no exacerbation, 12 low and 14 high SPD respondents having 1 exacerbation. There were 14 low and 20 high SPD have equal to or greater exacerbation. There was near to significant relationship between SPD and exacerbation (P< 0.05).[5,6]

It was discovered that COPD patients' induced sputum had a considerably greater IL-6 levels than asthmatic individuals' sputum, although no significant changes were found in IL-13 levels.[10,11] In individuals suffering from asthma, a consistently lowering correlation between FEV1 or FEV1/FVC or IL-6 level (r =–0.59 and –0.54, respectively) was found as well as a statistically decreasing correlation between FEV1, FEV1 percent, or FVC and IL-6 level in COPD subjects (r =–0.30, –0.30, and –0.38, respectively). There was no link between elevated IL-13 levels and poor lung function. In this study there were 4 respondents having low <14.3 and 2 high IL-6 have no exacerbation, 13 low and 1 high IL-6 respondents have 1 exacerbation. There were 18 low and 2 high IL-6 have equal to or greater exacerbation.

Yet another study revealed that smokers having plasma fibrinogen in upper & middle tertile (> 3.3 and 2.7–3.3 g/L) had 7% (95 percent confidence interval [CI]: 5–8%) and 2% (0–3%) lower percentage displayed FEV1 as compared to smokers with fibrinogen in lower tertile (2.7 g/L). Nonsmokers saw a 6% (4–7%) drop and nonsmokers saw a 0 % (1–2%) decline, respectively. Rate of hospitalization of COPD patients were 93 and 60 / 10K person-years in

Table 2: Contributing factors of exacerbation frequency in 'frequent exacerbator phenotype'.

Contributing Factors	*Unstandardized Coefficients*		*Standardized Coefficients*	*t*	*Sig.*
	B	*Std. Error*	*Beta*		
IL-6 (log)	3.56	1.11	0.47	3.22	.003*
Male Gender	-0.53	2.02	-0.04	-0.26	0.79
CAT score	0.42	0.18*	0.35	2.32	0.03*
a. Dependent Variable: Number of exacerbations per year, ANOVA; *p* =0.03, R^2					

people with plasma fibrinogen in higher and middle tertiles, respectively, compared to 52 per 10K person-years in people with fibrinogen in lower tertile (log-rank: *p* 0.001 and *p* =0.31). After controlling for BMI, age, pack-years, sex and acute infection.[2,6] Our study showed there were 2 normal or 4 low or high fibrinogen respondents have no exacerbation, 8 normal and 6 low or high respondents have 1 exacerbation. There were 7 normal and 13 low or high fibrinogen score have equal to or greater exacerbation. There was no significant relationship found between fibrinogen and exacerbation, ($p > 0.05$).

The above mentioned comparison and data from present study showed that there is significant relation between COPD with these biomarkers (fibrinogen, IL-6 and SP-D) which plays vital part in the severity and staging of COPD.

Current study investigates the prognostic factors within the frequent exacerbator phenotype scarcely found in literature**.** Frequent exacerbations of COPD are related with a faster decrease in FEV1 and other functions and increase in co-morbidities therefore, leading to rapid disease progression and a higher mortality rate. They are also related to a decrease in the quality of life. COPD exacerbations undoubtedly have a dwindling influence on the BODE index as well as its components i.e. exercise capacity, dyspnea, obstruction and body mass index.

The clinical manifestation of COPD is obstruction in air pathways, caused by irritation & modification, which causes inflammation and is linked to parenchymal obliteration and the formation of emphysema. However, these patients frequently have systemic symptoms of the illness, which can result in decreased functional ability, severe dyspnea, impairment and poor quality of life, and increased death rates. The incidence of related cardiovascular illness, undernourishment leading in the destruction and loss of function of body muscles, and other various systemic problems that may lead to lung cancer in the long run are among the top recognized indicators.

Conflict of interest: None declared.

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