Original Article

Inflammatory Biomarkers in Chronic Obstructive Pulmonary Disease

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Abstract

Background. The role of systemic inflammation in patients with chronic obstructive pulmonary disease (COPD) remains to be defined. This prospective, observational study was designed to analyse the hypothesis that inflammatory biomarkers in stable COPD can be used as measure of disease severity and risk of exacerbation.

Methods. We conducted a case-control study in a tertiary care, university-affiliated hospital. COPD patients and controls were matched for sex and age in a 1:1 matching ratio. All COPD patients included in the study had to be clinically stable without a history of exacerbation in the last four weeks and should not have been on oral steroid therapy (in the last 4 weeks) or on long-term oxygen therapy. We included only those patients who had quit smoking. Fibrinogen levels and pulmonary function tests were performed in both the groups.

Results. The mean plasma fibrinogen level, total leucocyte count, neutrophil count and erythrocyte sedimentation rate in patients with COPD were significantly higher than that of controls ($p=0.001$). The study also showed a significant association between plasma fibrinogen levels with severity of COPD ($p<0.001$) and number of exacerbations in the previous year ($p=0.001$).

Conclusions. Patients with COPD had significantly higher levels of inflammatory markers than healthy individuals. Plasma fibrinogen level was significantly higher in higher Global Initiative for Chronic Obstructive Lung Disease GOLD class and with more number of prior exacerbations. Hence, fibrinogen may act as a surrogate marker of disease activity in patients with COPD and may help to risk stratify COPD patients for future exacerbations.
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Key words: COPD, Severity, Exacerbations, Inflammatory markers.

Introduction

Chronic obstructive pulmonary disease (COPD) is a common disease of the respiratory system and has extremely high morbidity and mortality rates. At present, COPD is the fourth highest cause of death worldwide and is expected to become the third leading cause of mortality by the year 2020.[1] Exacerbations of respiratory symptoms in COPD are of major importance because of their profound adverse effects, like accelerated deterioration of lung function, poor quality of life, and increased mortality.[2] Forced expiratory volume in one second (FEV_1) is used as a global marker of pathophysiological changes and severity of COPD. However, FEV_1 often fails to reflect the severity of dyspnoea, functional impairment, prognosis, and systemic manifestations of patients with COPD.[3] Hence, there is a constant search for the factors that can predict the exacerbation risk better. One such marker is acute-phase proteins, which has been implicated in both stable and COPD exacerbations. For example, elevated plasma fibrinogen level has been associated with poor prognosis, especially in a severe disease.[4] Similarly, elevated plasma C-reactive protein (CRP) has been recently shown to increase the risk of death only in patients with severe COPD.[5] Plasma CRP level has been found to be associated with the disease severity, quality of life, exercise capacity and response to the treatment.[6] Neutrophils may be mechanistically involved in COPD pathology and these are elevated in the disease, and therefore, make attractive biomarkers for the therapeutic efficacy.[7] It was on this background that the present study was conducted, aimed to examine whether the inflammatory biomarker with stable COPD were a significant predictor of the severity.

Material and Methods

We conducted a prospective, case-control study in a tertiary care, university-affiliated hospital at Thiruvananthapuram, Kerala during the period April

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2012 to April 2013. Patients with COPD and controls were matched for sex and age in a 1:1 matching ratio. COPD was confirmed in patients with a FEV_1/FVC (forced vital capacity) ratio of less than 0.7, measured 20 minutes after the administration of salbutamol and severity of COPD was classified according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines. Patients with COPD visiting the out-patient clinic were sequentially enrolled. The control group consisted of volunteers recruited from the same hospital who visited other out-patient clinics who did not have any comorbidity and had normal spirometry. Patients with COPD with an episode of exacerbation in the last four weeks were excluded. The stable disease group was not on oral steroid therapy (in the last 4 weeks) or long-term oxygen therapy. We also excluded patients and controls diagnosed with asthma in the past five years, current or former smokers with an abstinence time of less than six months, suspected diagnosis of infectious disease, surgery, or trauma in the last 30 days, diabetes, heart failure, chronic renal failure, pregnancy, stroke, malignancy and ischaemic heart disease. The college Ethics Committee approved the research and all enrolled individuals gave written informed consent to participate in the study. The participants were interviewed and data were collected through a standardised questionnaire. Spirometry was performed using a computerised spirometer in both cases and controls and blood sample was collected from all the participants and inflammatory markers were measured using a high sensitivity assay.

Statistical Analysis

Statistical analyses were performed using IBM SPSS (Statistical Package for the Social Sciences) version 20 for Windows. Independent samples 't'-test was performed for continuous variables and was expressed using the mean±standard deviation and the median. Chi-square test was performed for categorical data. Statistical significance was defined as p value <0.05. Univariate analysis was done to determine relative risk of mortality and comparison of means among more than two groups were analysed by one-way analysis of variance (ANOVA). According to the study by Mannino *et al*,[4] the mean and standard deviation of fibrinogen value among patients with COPD and the controls were 3 and 0.9 and 2.3 and 0.5, respectively. Sample size was calculated on the basis of this study using the formula:

$$N = [(Z_{1-\alpha/2} + Z_{1-\beta})^2 (\sigma_1^2 + \sigma_2^2)] / (\mu_1 - \mu_2)^2$$

Here the value for α=0.05 and α=0.2. Sample size calculated according to this formula was 48 in each group. For measuring fibrinogen, blood samples were obtained by venepuncture. The blood was collected into Vacutainer tubes containing sodium citrate. The blood was immediately separated in to plasma and stored at -800 °C. Fibrinogen was estimated using Clauss method[8] using STA-Fib 2 kit in STA-Compact.

Results

Forty-eight patients with COPD were included in the study along with 48 healthy individuals as controls. All the individuals in our study were males. Baseline characteristics of the study participants are presented in table 1. The relationship between the inflammatory biomarker levels and study participants is shown in table 2.

Table 1. Baseline characteristics of the study participants

Features	Cases	Controls
Age (years) (mean ± SD)	62.6±9.8	61.8±8.9
Gender		
Male	48	48
Female	0	0
Occupation		
Manual labour	36 (75%)	28 (58.3%)
Semi-skilled labour	3 (6.3%)	9 (18.8%)
Skilled labour	9 (18.8%)	11 (22.9%)
Smoking status		
Ex-smokers	48 (100%)	0
Non-smokers	0	48 (100%)
Number of exacerbations in last year		
1	18 (37.5%)	0
2	17 (35.4%)	0
3	13 (27.1%)	0
Cyanosis	0	0
Pedal oedema	0	0
Elevated jugular venous pressure	0	0
Systolic blood pressure (mm/Hg)	135.7±21.7	132.0±14.5
Diastolic blood pressure (mm/Hg)	82.3±9.8	82.8±9.4
Body mass index	21.8±2.0	23.7±2.1
Mean FEV_1 (%)	60.33±19.5	83.02±5.30
Mean FEV_1/FVC	0.61±0.06	0.81±0.04
Mean ESR (mm/hr)	20.71±10.77	13.5±8.03
Mean neutrophil count (%)	71.8±12.4	62.8±9.7
Mean leucocyte count (/µL)	9664.2±2656.1	8037.5±1764.0
Mean haemoglobin (g/dL)	13.2±2	13.4±1.0
Mean platelet count (lakhs/µL)	2.2±0.6	2.13±0.4
Mean fibrinogen level (mg/dL)	4.3±0.9	3.3±0.9

Definition of abbreviations: SD=Standard deviation; FEV_1=Forced expiratory volume in one second; FVC=Forced vital capacity; ESR=Erythrocyte sedimentation rate

Table 2. Relationship between the inflammatory biomarker levels and study participants

Category	Mean±SD	t	Significance (2-tailed)
Plasma fibrinogen (g/L)			
Cases	4.3±0.9	5.4	0.001
Controls	3.3±0.8		
Total leucocyte count (/μL)			
Cases	9664.2±2656.1	3.5	0.001
Controls	8037.5±1764.0		
Neutrophil count (%)			
Cases	71.8±12.4	4.0	0.001
Controls	62.8±9.7		
ESR (mm/h)			
Cases	20.7±10.8	3.7	0.001
Controls	13.5±8.0		

Definition of abbreviations: SD=Standard deviation; ESR=Erythrocyte sedimentation rate

Plasma fibrinogen (p<0.001), total leucocyte count (p<0.001), neutrophil count (p<0.001) and erythrocyte sedimentation rate (ESR) (p<0.001) were significantly higher in cases compared to controls. Among inflammatory mediators, there was a significant association of plasma fibrinogen level with severity of COPD (p<0.001) and number of exacerbations in the previous year (p=0.001) (Table 3). Total leucocyte count, neutrophil count and ESR did not have significant association with the severity of COPD or with the number of exacerbations (Table 4).

Discussion

The current and most widely used measure of COPD is FEV_1. However, as a biomarker, FEV_1 does not

Table 3. Relationship between the inflammatory biomarker levels and COPD exacerbations

	No. of Exacerbations in Previous Year	No.	Mean±SD	Minimum	Maximum	Significance
Plasma fibrinogen (g/L)	1	18	3.5±0.8	2.1	4.6	0.001
	2	17	4.5±0.5	3.7	5.2	
	3	13	5.3±0.3	4.4	5.9	
Total leucocyte count (/μL)	1	18	9486.7±2683.9	5100	15000	0.373
	2	17	10352.9±2480.7	7000	16000	
	3	13	9009.2±2835.2	5700	15100	
Neutrophil count (%)	1	18	70.1±11.4	55	88	0.667
	2	17	71.8±12.9	49	94	
	3	13	74.2±13.5	52	92	
ESR (mm/h)	1	18	19.3±11.8	2	50	0.715
	2	17	20.8±9. 7	6	35	
	3	13	22.5±11.3	12	55	

Definition of abbreviations: COPD=Chronic obstructive pulmonary disease; SD=Standard deviation; ESR=Erythrocyte sedimentation rate

Table 4. Relationship between the inflammatory biomarker levels and severity of COPD

	GOLD Class	N	Mean±SD	Minimum	Maximum	Significance
Plasma fibrinogen (g/L)	1	13	3.4108±0.78	2.09	4.32	0.001
	2	24	4.4613±0.77	2.77	5.90	
	3 & 4	11	5.0482±0.53	3.67	5.53	
Total leucocyte count (/μl)	1	13	10112.31±2656.87	6900	15000	0.234
	2	24	9971.67±2965.03	5100	16000	
	3 & 4	11	8463.64±1547.43	5700	11000	
Neutrophil count (%)	1	13	70.85±10.02	55	88	0.748
	2	24	73.17±12.79	52	94	
	3 & 4	11	70.00±14.58	49	92	
ESR (mm/hour)	1	13	16.92±8.92	2	30	0.312
	2	24	22.63±10.77	6	50	
	3 & 4	11	21.00±12.45	12	55	

Definition of abbreviations: COPD=Chronic obstructive pulmonary disease; SD=Standard deviation; GOLD=Global Initiative for Chronic obstructive lung disease; ESR=Erythrocyte sedimentation rate

correlate exceptionally well both with the symptoms and with the measures of the disease, like severity, functional impairment and systemic manifestations. Hence, there is a constant search for biomarkers which can characterise individuals with COPD better and this search for ''biomarkers'' has become a hot area of research in COPD. One such biomarker is acute-phase proteins, which has been implicated in both stable and COPD exacerbations. Persistent low-level systemic inflammation is thought to play a significant pathogenic role in many non-communicable diseases including COPD. Elevated circulating levels of white blood cells, interleukins, fibrinogen and tumour necrosis factor-alpha have been reported in patients with COPD. The present study was performed to evaluate whether biomarkers of systemic inflammation were significant predictors of severity of COPD.

Fibrinogen was the first biomarker to be investigated in the present study. The mean plasma fibrinogen level was significantly higher in patients with COPD than in controls (p=0.001). Many studies have documented a similar finding of elevated plasma fibrinogen levels in patients with COPD.[9,10] As we did not follow up patients, we were not able to find out the risk of future exacerbations. Hence, we compared the biomarker levels with the frequency of exacerbations in the past year. The frequency of past exacerbations was significantly higher in patients with higher fibrinogen level. Studies have shown that there was an increased incidence of hospitalisation and higher frequency of exacerbations in COPD patients with raised levels of fibrinogen.[11,12] Mean plasma fibrinogen level was also statistically significantly higher in advanced GOLD stages (p=0.00). Higher fibrinogen level was inversely associated with lung function at baseline after adjustment for multiple potential confounders and was associated with greater loss of FVC and FEV_1, independent of cigarette smoking, body habitus, baseline lung function and demographic factors.[13-15]

Mean white blood cell count and mean neutrophil count were significantly higher in patients with COPD than the controls. Similar results were seen in the study by Gan *et al*[16] who concluded that raised levels of leucocytes were associated with severe airflow obstruction. The increase in leucocyte count among those with severe airflow obstruction may be largely driven by the neutrophil sub-population of cells. In the present study, leucocyte and neutrophil counts were not significantly associated with the severity of COPD or with the number of past exacerbations. A study by Thomsen *et al*[17] reported that higher leucocyte and neutrophil count was an increased risk of exacerbations in those with elevated fibrinogen level.

Mean ESR was significantly higher in cases compared to controls. However, ESR did not have any statistically significant association with the severity of COPD or with the number of past exacerbations. ESR has not been studied extensively in COPD, and hence, data available on ESR in COPD patients is meagre. Available data does not show significantly elevated levels in COPD nor any relationship between ESR and the severity of COPD.[18,19] However, ESR was significantly elevated in our COPD cohort, and therefore, ESR may be considered as a less expensive alternative marker of systemic inflammation in COPD. This may seem to be an advantage in the assessment of patients with COPD. The prospective of using ESR as a marker should be strengthened by well-standardised and reproducible procedures of measurement. It is also worthwhile mentioning here that such an almost cost-free procedure is well suited for low-income countries, where the prevalence of COPD is dramatically increasing.

In our study, fibrinogen levels, ESR, leukocyte and neutrophil counts were found to be statistically significantly higher in stable COPD patients than in well-matched controls. This finding supports the role of inflammation as a driver of the disease severity and a possible protective role for the elevated markers of tissue repair. There was also a statistically significant correlation between the fibrinogen levels with severity of COPD and past exacerbations. Though ESR, leucocyte and neutrophil count were also significantly higher in patients with COPD, these were not associated with the disease exacerbation or the disease severity. So if plasma fibrinogen is able to predict decline in FEV_1 over time then it may act as a surrogate marker of the disease activity in individuals with COPD. This would be of enormous value in our clinical settings in which we are unable to predict the possibility of future exacerbations. Therefore, it may be recommended to measure the plasma level of fibrinogen in patients with COPD and patients with higher levels of fibrinogen should be considered for a more aggressive treatment. Moreover, newer drugs are under various stages of development and treatment that decrease the level of fibrinogen and other inflammatory markers, like losmapimod may revolutionise COPD treatment.

This study had few limitations. We acknowledge that the study had only male population and selection of biomarkers was incomplete. Sample size was relatively small and follow-up evaluation of these patients was not done.

Conclusions

Biomarker identification in COPD is a developing field, with increasing interest to define clinical phenotypes and monitor response to existing and new therapeutic strategies. Blood biomarkers can be readily measured in patients without the need for invasive procedures. In the present study, patients with COPD had significantly higher levels of inflammatory markers than healthy controls. Plasma fibrinogen level had statistically significant association with GOLD class and with the frequency of past exacerbations. Hence, fibrinogen is likely to be a useful biomarker to assess the severity of COPD and to risk stratifies COPD patients into high or low risk for future exacerbations.

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