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ORIGINAL ARTICLE

Inflammatory biomarkers in chronic obstructive pulmonary disease

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KEYWORDS

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Abstract *Background:* Chronic obstructive pulmonary disease (COPD) is a multicomponent disease. There is a need for biological markers for better evaluation of patients with COPD.

Objective: To test the hypothesis that elevated levels of inflammatory biomarkers fibrinogen, C-reactive protein (CRP) and leukocyte count (WBC) in individuals with stable COPD are associated with an increased risk of exacerbation.

Patients and methods: Ninety-eight COPD patients diagnosed and classified as COPD and 30 age and gender matched healthy subjects with normal pulmonary function were observed. Patient follow-up was performed to evaluate the strength of the associations between inflammatory biomarker levels and future outcome.

Results: Inflammatory biomarkers increase with exacerbation compared to the remission state, mean WBC, CRP and fibrinogen at 12.212×10^9/L, 39.462 mg/L and 5.09 g/L in exacerbation respectively compared to 7.877×10^9/L, 4.142 mg/L and 2.299 g/L in remission with P values < 0.001, < 0.001 and < 0.004, respectively.

Statistically significant correlation was noticed between the levels of fibrinogen and the % predicted FEV_1 ($r = 0.209$, $P = 0.038$) however CRP and WBC did not correlate with % predicted FEV1 ($r = -0.031$, $P = 0.765$) for CRP, and that for WBC ($r = 0.125$, $P = 0.221$).

Conclusion: Elevated levels of CRP, fibrinogen and leukocyte count in individuals with COPD were associated with increased exacerbation risk. Fibrinogen in particular has emerged as a potentially useful biomarker and requires further investigation.

Introduction

Chronic obstructive pulmonary disease (COPD) is a major cause of chronic morbidity and mortality; it is currently the fourth highest cause of death in the world, and is predicted to be the third leading cause of mortality worldwide by the year 2020 [1]. COPD is identified mainly according to the Global Initiative

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for Chronic Obstructive Lung Disease (GOLD) guidelines; [post bronchodilator forced expiratory volume in 1 s (FEV_1)/forced vital capacity ratio less than 0.7; severity determined by FEV1 alone] combined with a history of exposure to risk factors [2]. There is considerable evidence of under diagnosis, especially in the mild and moderate groups [3]. The clinical presentation, disease severity and progression are quite heterogeneous and maybe the result of diverse pathogenic processes that involve abnormalities in different pathogenic pathways. Exacerbations of respiratory symptoms in COPD are of major importance because of their profound and long lasting adverse effects on patients [4]. Frequent episodes accelerate loss of lung function, affect the quality of life of patients, and are associated with poor survival. Acute-phase proteins have been implicated in both stable and exacerbating COPD. For example, fibrinogen has emerged as a promising biomarker in COPD. It is an acute phase soluble plasma glycoprotein, synthesized primarily in the liver and converted by thrombin into fibrin during blood coagulation. Normal fibrinogen levels in blood are between 1.5 and 3.5 g/L but can increase threefold during acute phase stimulation in response to increased IL-6 production [5]. Elevated plasma fibrinogen has been associated with poor prognosis, especially in a severe disease [6]. Similarly, plasma C-reactive protein (CRP) has generally been found to be elevated in stable COPD, although not in every study; elevated CRP was recently shown to increase the risk of death only in severe COPD [7]. Plasma C-reactive protein (CRP) level has been found to be associated with disease severity, quality of life, exercise capacity and response to treatment [8]. Also; neutrophils may be mechanistically involved in COPD pathology and they are elevated in the disease and therefore make attractive biomarkers for therapeutic efficacy [9]. It was on this background that the present study was conducted, which aimed to examine whether the Inflammatory biomarker tested (fibrinogen, C-reactive protein (CRP) and leukocyte count) levels in patients with stable COPD were a significant predictor of prognosis. Also, we review the clinical evidence linking them with COPD and discuss its potential utility as a biomarker.

Patients and methods

Study design and patients

This is a prospective controlled study carried out in the Department of Chest diseases and Tuberculosis and Department of Medical Microbiology & Immunology; Faculty of Medicine, Sohag University during the period from January 2013 to March 2014 with a follow-up period of 12 months. The institutional Ethics Committee approved the study. Informed consent was obtained from patients and healthy controls. Ninety eight COPD patients (Group I) were clinically diagnosed and classified as COPD according to the medical history, current symptoms and available pulmonary function tests following Global Initiative for Chronic Obstructive Lung Disease guidelines [2]. The mean age of patients including 82 males (83.7%) and 16 (16.3%) females was 62.29 ± 7.032 years. To exclude patients with asthma, subjects with a history of allergic rhinitis or an improvement in FEV_1 of >12% from the predicted values following inhalation of a bronchodilator, were not included. Patients with evidence of extensive pulmonary tuberculosis, malignancy or who were suffering from psychosis were excluded from the study. All patients with COPD were clinically stable and none had a history of respiratory infection for at least a 4-week period preceding the study. Thirty healthy individuals age- and gender-matched with normal pulmonary function and without evidence of chronic inflammatory disease; 23 males (76.7%) and 7 (23.3%) females have been considered as the control group (Group II). All participants filled out a questionnaire reviewed by an examiner at attendance, had spirometry performed, and had blood samples analyzed.

Spirometry

Lung function data were collected using *the Master Screen PFT Erich JAEGER* Spirometer, (*GmbH*, *Wuerzburg/Germany*). Lung function was measured before and 15 min after administration of 200 μg of albuterol/salbutamol. Spirometry measures reported here include the FEV1 and FVC, as well as the FEV1/FVC ratio. FEV1% predicted, although not reported separately, was used to stage COPD [2]. Participants were grouped according to Global Initiative for Chronic Obstructive Lung Disease (GOLD) grades 1 through 4 for airflow limitation and the recent GOLD grades A through D for assessing both symptoms and risk. Correlations between percent predicted FEV1 and each biomarker value were analyzed.

Procedures

Whole blood was collected by venipuncture into vacutainer tubes. For serum preparation, the blood was allowed to clot for 30 min and serum was obtained by centrifugation at 1500 rpm for 10–15 min. For plasma preparation, whole blood was collected into vacutainer tubes containing EDTA (ethylenediaminetetraacetic acid anticoagulant). Plasma was obtained by centrifugation at 2000 rpm for 10–15 min. Serum and plasma samples were stored at −20 °C until analyzed.

Measurement of CRP levels

The measurement was done using AVITEX CRP; a rapid latex agglutination test kit for the detection of C-reactive protein in human serum. The AVITEX CRP latex particles are coated with antibodies to human CRP. When the latex suspension is mixed with serum containing elevated CRP levels on a slide, clear agglutination is seen within 2 min. (AVITEX® CRP *OMEGA DIAGNOSTICS Ltd. Omega House*, *Scotland*, *United Kingdom*).

1. Using isotonic saline prepare serial dilutions of patient's serum (1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and so on).
2. Transfer one drop (50 μl) of each serum dilution to the test circle on the slide.
3. Shake the latex reagent, then using the dropper provided, add one drop of suspension to the test circle.
4. Mix the drops using a disposable stirrer ensuring coverage of the test circle with the mixture.
5. Gently and evenly, rock and rotate the test slide for 2 min while examining the test slide for agglutination.
6. The test slide was examined under a strong light source after 2 min. A positive result was indicated by the obvious agglutination pattern of the latex, in a clear solu-

tion. A negative result was indicated by no change in the latex suspension on the test slide. AVITEX CRP has a detection limit of 6 mg/L of CRP in patient's serum. Positive results will be obtained at a CRP serum concentration above 6 mg/L and negative results will be obtained at 6 mg/L and below.

7. The serum CRP concentration can then be calculated approximately by multiplying the dilution factor (i.e. 2, 4, 8 or 16) by the detection limit, i.e. 6, to give the number of mg/L concentration e.g. if the agglutination titer appears at 1/8 the approximate serum CRP concentration is $8 \times 6 = 48$ mg/L.

Measurement of fibrinogen levels

The measurement was done using Sysmex® CA-1500 System and the used fibrinogen reagents are Multifibren® U (Siemens health care diagnostic products GmbH Macburg/Germany).

Measurement of white blood cells (WBCs)

Total leukocyte count was detected by an automated method; by using CELL-DYN® 3700 (*Abbott GmbH & Co. KG; Abbott Diagnostics Europe*).

Statistical analysis

Statistical differences were determined by using an analysis of variance (ANOVA) and Student's *t*-test. Results were expressed as mean ± standard deviation of the mean (SD). Qualitative data were compared using either Chi square test or fisher's exact test. The probability (*P value*) was considered significant if *P value* was <0.05, highly significant if *P value* was <0.01 and *P value* < 0.001, and insignificant if *P value* was > 0.05. The relationship between the studied measures was assayed by Pearson's linear correlation coefficient. Pearson correlation is considered negligible if $r < 0.2$, weak if between 0.2 and 0.4, moderate if between 0.4 and 0.7 and strong if $r > 0.7$.

Results

The present study was carried out in the Sohag University Hospital during the period from January 2013 to March 2014. Ninety eight COPD patients (Group I) were included in our study in addition to 30 healthy individuals as the control group (Group II). Baseline characteristics of the study participants are shown in Table 1.

The relationship between the inflammatory biomarker levels and disease activity: (Table 2)

Level of WBCs in the COPD patients and in the control group

The level of WBCs showed highly statistically significant increase in COPD patients at exacerbation ($12.212 \pm 6.175 \times 10^9$/L) compared to patients in remission ($7.877 \pm 2.118 \times 10^9$/L) ($P < 0.001$). Also; the level of WBCs showed a highly statistically significant increase in COPD patients at exacerbation ($12.212 \pm 6.175 \times 10^9$/L) compared to the control group ($7.943 \pm 2.295 \times 10^9$/L) ($P < 0.001$). (Fig. 1).

Serum level of CRP in the COPD patients and in the control group

The serum level of CRP showed a highly statistically significant increase in COPD patients at exacerbation (39.462 ± 65.816 mg/L) compared to patients in remission (4.142 ± 1.134 mg/L) ($P < 0.001$). Also the serum level of CRP showed a statistically significant increase in COPD patients at exacerbation (39.462 ± 65.816 mg/L) compared to the control group (4.2 ± 1.11 mg/L) ($P = 0.004$). (Fig. 2).

Serum level of fibrinogen in the COPD patients and in the control group

The serum level of fibrinogen showed a highly statistically significant increase in COPD patients at exacerbation (5.09 ± 1.861 g/L) compared to patients in remission (2.299 ± 0.571 g/L) ($P < 0.001$). Also the serum level of

Table 1 Baseline characteristics of the study participants with COPD and the control group.

		Group		Total	Chi square	*P* value
		COPD	Control			
Gender	Female	16	7	23	0.765	0.382 (NS)*
	Male	82	23	105		
Current smoker	No	74	25	99	0.802	0.370 (NS)
	Yes	24	5	29		
X smoker	No	40	23	63	11.811	<0.001 (HS)*
	Yes	58	7	65		
Non smoker	No	82	13	95	19.534	<0.001 (HS)
	Yes	16	17	33		
Inhaled B2 agonist	No	65	30	95	13.611	<0.001 (HS)
	Yes	33	0	33		
Inhaled steroid	No	36	30	66	36.809	<0.001 (HS)
	Yes	62	0	62		
Theophylline	No	8	30	38	92.803	<0.001 (HS)
	Yes	90	0	90		
Inhaled ipratropium	No	77	30	107	7.690	0.004(S)*
	Yes	21	0	21		
Total		98	30	128		

* HS, highly significant; NS, non significant; S, significant.

Table 2 The inflammatory biomarker levels in COPD patients at exacerbation in comparison to patients in remission and controls.

	Cases at exacerbation (1)	Cases at remission (2)	Controls (3)	*P* value		
				1 vs 2	1 vs 3	2 vs 3
FEV1 (% predicted)	53.41 ± 7.469	58.58 ± 7.927	88.4 ± 5.137	< 0.001 (HS)	< 0.001 (HS)	< 0.001 (HS)
WBCs (10^9/L)	12.212 ± 6.175	7.877 ± 2.118	7.943 ± 2.295	< 0.001 (HS)	< 0.001 (HS)	0.882 (NS)
CRP (mg/L)	39.462 ± 65.816	4.142 ± 1.134	4.2 ± 1.11	< 0.001 (HS)	0.004 (S)	0.805 (NS)
Fibrinogen (g/L)	5.09 ± 1.861	2.299 ± 0.571	2.073 ± 0.575	< 0.001 (HS)	< 0.001 (HS)	0.061 (NS)

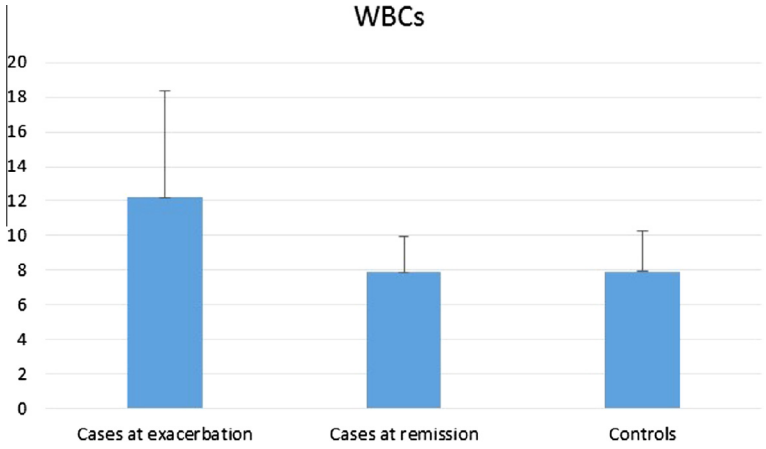

Figure 1 Level of WBCs in COPD patients and in the control group.

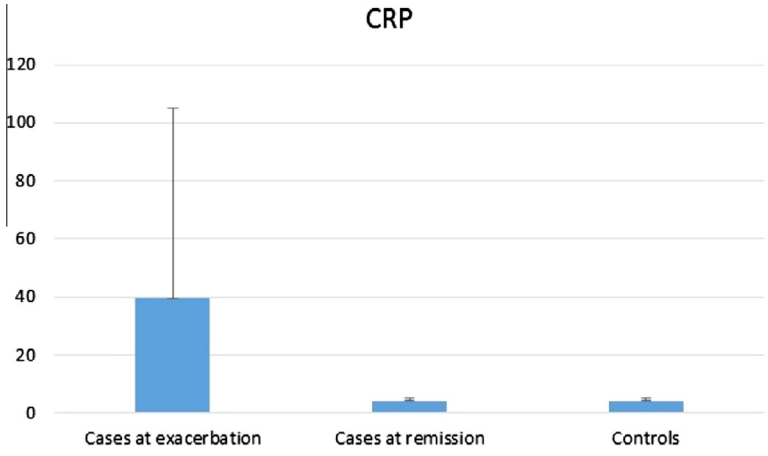

Figure 2 Level of CRP in COPD patients and in the control group.

fibrinogen showed a highly statistically significant increase in COPD patients at exacerbation (5.09 ± 1.861 g/L) compared to the control group (2.073 ± 0.575 g/L) ($P < 0.001$). (Fig. 3).

Correlation of the inflammatory biomarker levels with the lung function decline in COPD patients

There was non-significant correlation between the levels of CRP and the % predicted FEV_1 ($r = -0.031$, $P = 0.765$) and there was non-significant correlation between the leukocyte count and the % predicted FEV_1 ($r = 0.125$, $P = 0.221$) while there was statistically significant correlation between the levels of fibrinogen and the % predicted FEV_1 ($r = 0.209$, $P = 0.038$). So fibrinogen was the only inflammatory biomarker tested associated with disease severity and can be used as a predictor of lung function decline (Table 3).

Discussion

The global burden of COPD is large, with more than 600 million people affected worldwide and nearly 3 million dying from this disorder annually [10]. ''Biomarkers'' have become a hot topic in the study and treatment of COPD. In simple terms, a biomarker is a measurable characteristic that reflects the

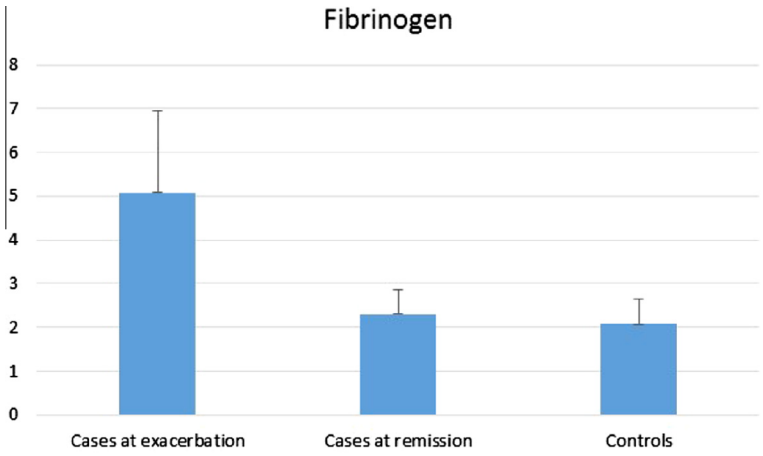

Figure 3 Level of fibrinogen in COPD patients and in the control group.

Table 3 Correlation between FEV1 and the inflammatory biomarker levels.

The variables	At exacerbation		At remission		Control group	
	r	*P*	*r*	*P*	*r*	*P*
WBCs	0.125	0.221 (NS)	0.179	0.077 (NS)	0.014	0.943 (NS)
CRP	−0.031	0.765 (NS)	0.091	0.375 (NS)	0.132	0.485 (NS)
Fibrinogen	0.209	0.038 (S)	−0.148	0.147 (NS)	−0.127	0.503 (NS)

presence, severity, or state of a disease [11]. Biomarker identification in COPD is still a developing field, with increasing interest to aid diagnosis, define clinical phenotypes and monitor response to existing and new therapeutic strategies. Furthermore, blood biomarkers can be readily measured in patients without the need for invasive procedures [5]. Agusti et al. suggested a novel COPD phenotype characterized by persistent systemic inflammation, based on five classic circulating inflammatory biomarkers, namely CRP, IL-6, IL-8, fibrinogen and TNF-α [12]. They identified an increased risk of all-cause mortality associated with this phenotype highlighting the role of COPD biomarkers in clinical practice.

The present study was performed to evaluate whether levels of CRP, fibrinogen and leukocyte count; biomarkers of systemic inflammation were significant predictors of future COPD outcomes. CRP was the first biomarker to be investigated in COPD. Most studies have shown that CRP levels are elevated in these patients [8]. Liu et al. determined that a serum CRP concentration of >3 mg/L was a poorer prognostic variable of COPD compared with a CRP concentration of $\leqslant 3$ mg/L [13]. CRP assays are inexpensive and convenient, it is important for clinicians to use CRP values in stable COPD patients [1]. Furthermore, fibrinogen is likely to be a useful biomarker to stratify individuals with COPD into those with a high or low risk of future exacerbations. It is an ideal blood biomarker for the existence of systemic inflammation. The levels are easily measured and are already integrated into clinical diagnostic practice. Fibrinogen has also been well studied and associated with survival, risk of exacerbation and poor clinical outcome [6]. In our study, levels of CRP, fibrinogen and leukocyte count in individuals with COPD were found to be highly statistically significantly higher in stable COPD patients than in well-matched healthy control subjects and yet further rose during exacerbations. This pattern supported the role of inflammation as one driver of disease severity and a possible protective role for elevated markers of tissue repair. Our results that simultaneously elevated levels of CRP, fibrinogen, and leukocytes were associated with an increased risk of frequent exacerbations in individuals with stable COPD were consistent with those of previous studies, indicating the presence of systemic inflammation in patients with stable COPD [14]. Also; in the present study there was a statistically significant correlation between the levels of fibrinogen and the FEV_1 ($r = 0.209$, $P = 0.038$). Using this FEV1-based comparator we can develop biomarkers that better correlate with clinical phenotypes better than FEV1. However; while the levels of CRP and leukocyte count were also associated with disease exacerbation they did not predict lung function decline, as there was non-significant correlation between the levels of CRP ($r = -0.031$, $P = 0.765$) and the leukocyte count ($r = 0.125$, $P = 0.221$) and FEV_1. So if plasma fibrinogen is able to predict decline in FEV1 over time then it may act as a surrogate marker of disease activity in individuals with COPD. This would be of enormous value in our clinical setting in which we are unable to predict those who will remain stable and those who are likely to deteriorate rapidly. However; this study had some limitations; we acknowledge that the selection of biomarkers was incomplete. But the selection of the markers in our study was based on previous studies that included a high throughput proteomic analysis of 147 markers in stable and unstable conditions and smoker and non-smoker controls [15].

Conclusion

In conclusion, the present study confirmed that there were elevated levels of CRP, fibrinogen and leukocyte count in individuals with COPD than in healthy individuals and they were a

significant long-term predictor of future COPD outcomes in individuals with airway obstruction. There was an evolving evidence that fibrinogen was a useful biomarker in COPD, particularly in defining lung function decline in COPD patients and in acting as a surrogate marker of treatment success.

Conflict of interest

None declared.

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