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RESEARCH ARTICLE

The role of serum surfactant protein D as a biomarker of exacerbation of chronic obstructive pulmonary disease

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ABSTRACT

Background: The exacerbation of chronic obstructive pulmonary disease (COPD) is a major factor for the high mortality associated with the disease. There is a paucity in the lung-specific biomarkers which diagnose these exacerbations. Surfactant protein D (SP-D) is a promising biomarker in predicting clinical outcomes for patients with COPD, is lung-specific and can be detected in serum. However, the profile in which serum concentrations of SP-D change during acute exacerbation is still unclear. This study aims to estimate and compare the concentrations of serum SP-D in patients with stable disease and during the exacerbation.

Methods: A cross-sectional study was conducted which composed of apparently healthy individuals (n = 28), which included 14 smokers and 14 nonsmokers, patients with stable COPD (n = 28), and patients experiencing acute exacerbations (n = 28). Pulmonary functions were performed for all groups. Serum SP-D concentrations were measured using enzyme-linked immunosorbent assay (ELISA). These concentrations were compared by analysis of variance.

Results: Serum SP-D levels were significantly elevated in patients with acute exacerbations (508.733 ± 102.813 ng/ml) compared to patients with stable COPD (337.916 ± 86.265 ng/ml) and healthy subjects (177.313 ± 46.998 ng/ml; $p < 0.001$). Serum SP-D levels correlated inversely with lung function parameters including FEV1%pred, FVC%pred and FEV1/FVC.

Conclusion: Serum SP-D levels are raised early on during acute exacerbations of COPD, which could be a potential early diagnostic biomarker for COPD exacerbations.

Keywords: chronic obstructive pulmonary disease (COPD), serum biomarker, COPD exacerbations, COPD diagnosis, surfactant protein D (SP-D)

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is an inflammatory systemic disease,[1] and an increasing global health problem.[2]

The exacerbation of COPD is an acute event which is identified as worsening of the patient's respiratory symptoms. The diagnosis of exacerbations depends on the clinical presentation of the patient complaining of an acute change in symptoms.[3] There are no published studies on the incidence of exacerbations of COPD in the Syrian population, even though acute exacerbations are a common cause for hospitalization, and confirmed as an important cause for morbidity and high mortality associated with COPD worldwide.[4,5]

Although forced expiratory volume in one second (FEV1) is the most widely used marker for COPD severity and progression, it does not reflect the underlying disease activity.[6,7] FEV1 correlates poorly with symptoms and impairment of health status.[3] Furthermore, it does not differentiate between the causes of airflow obstruction i.e., small airways disease or emphysema.[6] Surfactant protein D (SP-D), which is mainly produced in type II pneumocytes and clara cells in the lungs, is one of the few lung specific proteins that can be assessed in the peripheral blood, and may be a useful marker in tracking the progress of the disease and health status of patients with COPD.[8,9]

SP-D is a multimeric glycoprotein, which is a member of collagen-containing C-type lectin or collectins.[9,10,11] It contributes in pulmonary surfactant homeostasis and plays a critical role in pulmonary innate immunity.[12,13,14] The elevation of serum SP-D has been associated with smoking and chronic inflammatory conditions such as asthma, interstitial pulmonary fibrosis, acute respiratory distress syndrome and COPD.[10,11,15]

SP-D is thought to play an important role in the pathogenesis of COPD, by protecting the lungs against oxidative and inflammatory stress by affecting efferocytosis and removing pathogenic microbes as part of the host's innate immune system.[10,16] Genetic association studies have showed associations between single nucleotide polymorphisms (SNPs) in the SP-D gene (SFTPD) and COPD. Serum SP-D concentration also exhibits an increase together with the decrease in bronchoalveolar lavage in COPD.[9,17] SP-D has been associated with the progressive decline in lung function, and higher serum SP-D levels have been found in severe COPD cases with worsening health status.[8,9]

Most patients with COPD are identified when they ask for medical help with persistent symptoms (cough and/or dyspnea), or frequent respiratory infections.[18] In some cases, patients with reduced lung function may be asymptomatic or may ignore their symptoms until they are identified only when they have been hospitalized with an acute exacerbation.[18,19] Since spirometry is not recommended during an exacerbation because it can be difficult to perform and measurements are not accurate enough,[3] the status of these patients cannot be determined.

The severity of airway inflammation during acute exacerbations of chronic pulmonary disease (AECOPD) is associated with the elevation of a variety of systemic markers in the circulation. However, these inflammatory markers are synthesized largely by extrapulmonary organs and their relationship with COPD is uncertain.[8,11,15] There is a lack of lung specific biomarkers that diagnose exacerbations of COPD and track their progression. Thus, the present study aimed to estimate serum SP-D levels at the onset of exacerbations, and compared these levels with patients with stable disease to explore the utility of SP-D as a biomarker for COPD exacerbations.

MATERIALS AND METHODS

A cross-sectional study of 84 volunteers who were recruited from outpatient and inpatient departments of Al Assad University Hospital, Damascus, Syria was conducted between November 2014 and April 2015. The study was approved by the institutional ethics committee of Damascus University. Informed consent was obtained from each participant.
The study subjects were divided into the following groups: Patients with COPD: this group was composed of 56 subjects diagnosed with COPD according to the GOLD guidelines.[3] The group was further subdivided into two groups: Patients with stable disease (SCOPD): 28 outpatients who had respiratory symptoms of chronic cough, sputum production and/or dyspnea and demonstrated airflow obstruction on spirometry. The spirometric criterion for airflow limitation uses a fixed ratio of the

postbronchodilator forced expiratory volume in one second, to forced vital capacity (FEV1/FVC) of less than 0.7.[3] Some patients were recently diagnosed whereas others came for follow-up of their disease. Patients were excluded if they had changes in respiratory symptoms in the past month and had seen a doctor or were hospitalized for exacerbations, or if they had taken any medication for exacerbations (oral corticosteroids or antibiotics).

Patients experiencing episodes of acute exacerbations (AECOPD): 28 patients with acute exacerbations were diagnosed by the worsening of the patient's respiratory symptoms (dyspnea, sputum volume and/or sputum color) that are beyond normal day-to-day variations and on clinical examination when they first presented to the hospital. Blood sampling was done immediately when they had been diagnosed and hospitalized for acute exacerbation. Some patients were experiencing their first exacerbation and had not previously been diagnosed with COPD. Others were previously diagnosed and may have experienced several episodes during the last year (exacerbation frequency was 2 (1 – 6); median (min-max)). According to patients' files, 15 patients had exacerbations due to bacterial infections and 8 due to viral infections, 3 patients exacerbated from occupational exposure and 2 patients had unknown cause of exacerbations.

Control subjects: this group included 28 apparently healthy subjects, who were recruited from the general population around the hospital and were invited to the outpatient clinic for clinical assessment and to perform spirometry. They were age-matched individuals (14 smokers - 14 nonsmokers), with a normal spirometry (FEV1/FVC $\geq$ 0.7 and FEV1 $\geq$ 85% pred.).

We excluded patients who had asthma or other respiratory diseases, associated hepatic or renal diseases, recent surgery or malignancy and patients who were under 40 years of age.

All the enrolled participants had their medical history taken, and the following data were gathered: age, gender, smoking status, smoking index, spirometry, O_2 saturation, and exacerbation frequency during the last year.

A 5 ml sample of venous blood was collected from both patient and control groups by venipuncture in plain tubes. The samples were allowed to clot at room temperature for at least 30 minutes, then centrifuged at 2500 rpm for 15 minutes at room temperature. The serum was frozen in $-80°C$ until analyzed.

Blood sampling and spirometry were done at the same time for patients with stable COPD and healthy controls, in order to accurately correlate lung function with serum SP-D levels. For the hospitalized patients experiencing acute exacerbations, blood collection was on the first day of hospitalization and spirometry was performed 7 to 10 days after the onset of exacerbation when the patients were stable enough to perform the spirometric maneuver, although we know that spirometry is not recommended during an exacerbation.[3]

Serum SP-D was determined by using a commercially available human surfactant protein D ELISA kit (Sunred Biological Technology) according to the procedure as described by the manufacturer.

STATISTICAL ANALYSIS

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS version 22) software. The data were normally distributed and expressed as mean $\pm$ standard deviation (SD). Independent student's t-test was done for comparison between COPD patients and controls. Comparisons between study groups (control; SCOPD; AECOPD) of serum SP-D levels were conducted by one-way ANOVA and post hoc testing for multiple comparison with Bonferroni. Multiple linear regression modeling was performed to determine the independent relationship between exacerbation and SP-D with adjustments for covariates including age, gender, smoking status and smoking index. Pearson's and Spearman's correlation analysis were used to evaluate the correlations between different parameters.
A 'p' value of less than 0.05 was considered statistically significant. The threshold value for optimal sensitivity and specificity of the serum SP-D was determined by the receiver operating characteristics (ROC) curve.

RESULTS

The demographic data for all volunteers are shown in Table 1.

The serum SP-D concentrations were higher in COPD patients (425.718 $\pm$ 126.279 ng/ml; mean $\pm$ SD) than compared to the control group (177.313 $\pm$ 46.998 ng/ml; mean $\pm$ SD, $p < 0.001$).

Table 1. The demographic data of study groups

Variable	Healthy controls (n = 28)	Patients with SCOPD (n = 28)	Patients with AECOPD (n = 28)
Age (years)	58.71 ± 7.65	58.78 ± 8.34	64.53 ± 8.17
Gender male/female	23/5	22/6	23/5
Nonsmokers	14	3	1
Current smokers	14	16	16
Former smokers	0	9	11
Smoking index (pack/year)	30.48 ± 27.61	46.89 ± 27.98	49.59 ± 40.25
FEV1% pred.	101.31 ± 8.04	53.07 ± 13.26	36.64 ± 14.59
FVC% pred.	110.94 ± 7.56	75.35 ± 19.71	51.22 ± 18.14
FEV1/FVC	0.78 ± 0.04	0.57 ± 0.11	0.58 ± 0.09
O_2 saturation%	97.11 ± 0.99	94.21 ± 1.39	83.57 ± 5.15
Exacerbation frequency*	–	1 (0–3)*	2 (1–6)*
SP-D ng/ml	177.313 ± 46.998	337.916 ± 86.265	508.733 ± 102.813

AECOPD = acute exacerbation of COPD; SCOPD = stable COPD; FEV1 = forced expiratory volume at 1 s; %pred. = percent of predicted value; FVC = forced vital capacity; SP-D = surfactant protein D; Values are mean ± SD; *median (min-max).

The serum SP-D levels were highest in the AECOPD group, followed by the SCOPD group, and lastly, the control group (508.733 ± 102.813 ng/ml, 337.916 ± 68.265 ng/ml, 177 ± 46.998 ng/ml; mean ± SD, respectively: $p < 0.001$). Table 2 shows pairwise comparison of serum SP-D mean differences between these three groups. The effect size of this ANOVA was $\eta^2 = 0.738$, Cohen's $\int = 1.68$, so the sample size of 15 patients was adequate for a power of 0.999.

Multiple regression model analysis revealed exacerbation to be the best significant predictor of serum SP-D levels (beta coefficient = 0.474, $p < 0.001$) after adjustment for smoking status, smoking index, age and gender. Age also showed association (beta coefficient = 0.397, $p < 0.001$); (model summary: R = 0.814, R Square = 0.663, Adjusted R Square = 0.629).

A receiver operating characteristic curve was used to estimate the ability of serum SP-D levels to distinguish between patients with COPD and healthy controls involving all subjects of the study. The total area under the curve was 0.985 (95% CI, 0.965, 1.000; $p < 0.001$). The best cutoff of 100% specificity was at an SP-D concentration of 294.55 ng/ml, while the sensitivity was 82% (see Fig. 1).

Then a ROC curve including only COPD patients was used to estimate the ability of serum SP-D levels to differentiate patients with acute exacerbation or stable disease. The total area under the curve was 0.883 (95% CI, 0.798, 0.967; $p < 0.001$), and the optimum cutoff of sensitivity and 1-specificity was at an SP-D concentration of 446.55 ng/ml, as sensitivity and specificity were 71.4% and 85.7% respectively (see Fig. 2).

There were significantly negative correlations between serum levels of SP-D and pulmonary functions in controls and patients with stable disease, including FEV1%pred. ($r = -0.902$; $p < 0.001$), FVC%pred. ($r = -0.848$; $p < 0.001$) and FEV1/FVC ($r = -0.664$; $p < 0.001$). Serum SP-D levels was also inversely correlated with O_2 saturation ($r = -0.805$; $p < 0.001$), and positively correlated with exacerbations frequency during the last year ($r = 0.812$; $p < 0.001$). The oxygen saturation was positively correlated with FEV1%pred., FVC%pred and FEV1/FVC ($r = 0.762$, $r = 0.784$, $r = 0.472$ respectively; $p < 0.001$).

Table 2. Multiple comparison of serum SP-D mean differences between each two of the three study groups (controls; SCOPD; AECOPD)

Study group		Mean difference	p-value
AECOPD	Controls	331.420*	<0.001
	SCOPD	170.817*	<0.001
SCOPD	Controls	160.604*	<0.001

AECOPD = acute exacerbation of COPD; SCOPD = stable COPD.

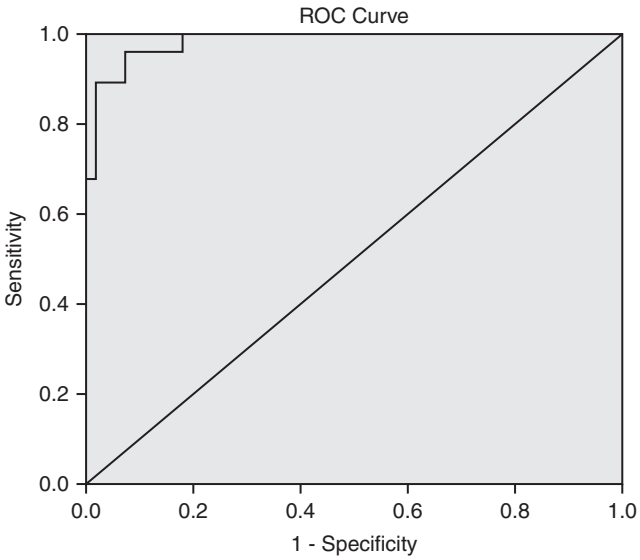

Figure 1. ROC curve with all subjects involved to estimate SP-D levels for COPD diagnosis (AUC = 0.985).

DISCUSSION

This study is the first to be conducted in Syria on the role of serum SP-D as a candidate biomarker of exacerbation of COPD, as no studies have previously been published on the role of serum SP-D in any lung disease in the Syrian population.

In the current study, the mean serum SP-D levels were significantly elevated in patients with COPD compared to healthy smoker and nonsmoker subjects. Serum SP-D was also inversely correlated to their pulmonary function tests. But the most important findings in this study was the significant increase of serum SP-D levels in patients experiencing acute exacerbation episodes compared to patients with stable disease, and the correlation of SP-D levels with exacerbation frequency during the last year and O_2 saturation. In previous studies, Shakoori et al.,[20] and Ju et al.,[15] reported that serum SP-D was significantly the highest in the AECOPD group, followed by SCOPD and lastly the control group, which is in agreement with the present study.

The serum SP-D levels were also significantly raised in patients with SCOPD compared with those of the controls. This finding was consistent with ECLIPSE,[21] a large study reported by Lomas et al., who found higher serum SP-D levels in patients with SCOPD than in healthy smoking controls.

The best cutoff value of SP-D concentration was 294.55 ng/ml, which may distinguish between patients with COPD and healthy controls with 100% specificity to reduce false positives and exhibits good sensitivity (82%). However, spirometry remains the most reproducible and objective measurement of airflow limitation available.[3] While the optimum cutoff value of serum SP-D concentration was 446.55 ng/ml to differentiate between COPD patients with acute exacerbation and stable disease, with 71.4% sensitivity and 85.7% specificity. There is a clear difference between cutoff values of serum SP-D concentration that distinguishes COPD patients and patients with acute exacerbation, which may raise the possibility for serum SP-D to be used as a biomarker for exacerbation.

The precise mechanism of elevated serum SP-D levels is still unclear.[12] Serum SP-D is increased in the circulation in response to lung pathology,[22] which may explain the higher levels during acute exacerbation episodes as a result of increased intrapulmonary inflammation, since previous studies have shown that the severity of exacerbations of COPD correlates with airway inflammation.[21]

Previous studies have found a reverse association between SP-D in circulation and bronchoalveolar lavage fluid (BAL), although the mechanism for this remains unclear. Sin et al., have suggested that reduced levels of BAL SP-D may be due to increased transmigration of SP-D from the lungs into the blood. This may be due to degradation of multimeric clusters of SP-D in the lung into lower molecular weight products that can leak more easily into the circulation,

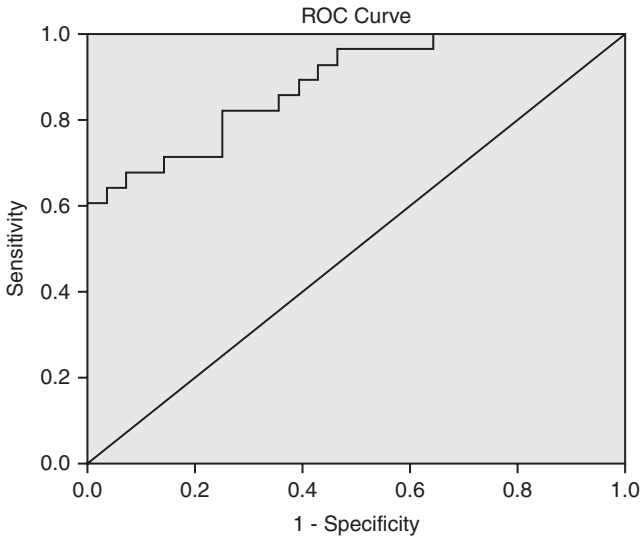

Figure 2. ROC curve with only patients included to evaluate best cutoff of serum SP-D level in predicting exacerbations of COPD (AUC = 0.883).

or as a consequence of increased vascular permeability of the lung associated with inflammation. However, there are few data to support or contradict this theory.[9,11]

There is another explanation for the elevated serum SP-D levels during the exacerbation episodes resulting from its important role in innate immunity. SP-D is responsible for pulmonary host defenses against microorganisms by promoting opsonization, neutralization, agglutination, and enhanced phagocytosis or lysis of inhaled pulmonary pathogens. It also modulates lung inflammation by reducing oxidative radical formation and enhancing apoptotic and necrotic cell clearance.[9,10,16] Thus, the pulmonary expression of SP-D may increase to protect the lung against pathogens and to regulate the inflammatory response in the airways, where high serum levels of SP-D may reverberate the raised lung expression.[20] Lung expression of SP-D during acute exacerbations however was not analyzed as more comprehensive studies are required to evaluate this theory.

Consequently, the elevated levels of serum SP-D in patients with COPD of this study may be associated with the most common theory, which hypothesizes that SP-D, the hydrophilic protein, translocates from lung compartments into the systemic circulation. This process is likely regulated by changes in alveolar-capillary permeability.[12,16,21]

Our data together with previous findings suggests that serum SP-D levels may reflect the disease activity. Since SP-D is produced predominantly in the lungs and translocates into the systemic circulation when the alveolar-capillary barrier permeability is perturbed, serum SP-D has been suggested as a potential biomarker for the epithelial integrity in COPD and tracking disease progression.[12,16,21]

We found an inverse correlation between serum SP-D concentrations and pulmonary functions (FEV1%pred.; FVC%pred.; FEV/FVC), and also with O_2 saturation. Surfactant proteins are suggested to diffuse from the alveolus into the circulation in a way that reflects blood oxygenation and lung injury.[23]

The best predictor of having frequent exacerbation (≥ 2 exacerbations per year) is a history of previous treated episodes.[3] We found a positive correlation between serum SP-D levels and exacerbations frequency during the last year. Lomas et al., reported an association between high serum SP-D levels and an increased risk of COPD exacerbations.[21] However, there is a weak correlation between FEV1 and impairment of patient's health related-quality of life.[3] Therefore, serum SP-D could be associated with COPD severity and the poor health status of patients. These findings are in line with Winkler et al.,[12] who reported that serum SP-D negatively correlate with FEV1%pred and FEV1/FVC in patients and healthy smokers, Sin et al.,[8] showed an inverse correlation between circulating SP-D and FEV1 in patients with advanced COPD, Shakoori et al.,[20] also found a negative correlation with FEV1%pred and FEV1/FVC, Ju et al.,[15] showed a negative correlation with FEV1% pred in patients with stable disease, and El-Deek et al.,[24] concluded an inverse correlation with FEV1% pred., FVC%pred., FEV1/FVC and O_2 saturation in patients with stable COPD. On the other hand, Lomas et al.,[21] did not demonstrate an association between serum SP-D and FEV1, this may due to the fact that patients in the present study had more severe COPD than those in the ECLIPSE study and the number of our study patients was limited compared to that study.

There are several important limitations to the current study. Firstly, the sample size is relatively small, further studies with larger number of subjects will be required to prove the role of SP-D in the exacerbations of COPD. Secondly, for patients with AECOPD, we only had serum at the onset of exacerbation. We couldn't estimate the dynamic change of serum SP-D concentrations during and after the exacerbation. Thirdly, also for patients with AECOPD, we didn't have the serum and pulmonary function tests at the same time since spirometry couldn't be performed during exacerbation. However, blood sampling several days into exacerbation may lead to underestimation of serum SP-D levels, and serum SP-D correlates significantly with pulmonary function tests for patients with SCOPD and controls where the blood sampling and spirometry were done at the same time. Fourthly, some patients with SCOPD were on a variety of inhalers that can modify serum SP-D expression, as it is thought that inhaled steroids seem to decrease serum SP-D concentrations.[16,21,22] Finally, all the patients were of the same ethnicity so the data cannot be generalized to other populations, whereas the ethnicity may affect SP-D expression.[23]

Both pulmonary and systemic inflammation are increased during acute exacerbations of COPD. However, systemic markers are not lung-specific,

do not correlate with respiratory health outcomes or rate of decline in lung function and may increase in most conditions associated with infection or inflammation.[8,15,20] Thus, we concluded that SP-D would be characterized as a lung specific protein which can be assayed in the blood as a candidate biomarker for early diagnosis of acute exacerbations of COPD, and may reflect the disease severity. We look forward to conduct larger future studies to estimate how changes in serum SP-D levels can predict severe exacerbations and its association with BAL SP-D, and how SP-D can be used in clinical COPD practice.

CONCLUSION

Serum surfactant protein D was markedly increased in patients with COPD. Furthermore, it was raised early on during acute exacerbation episodes. The serum SP-D levels were correlated with pulmonary functions. The present study suggests that serum SP-D may be a promising marker in early diagnosis of acute exacerbations of COPD and associated with disease severity and poor health status of patients, but additional studies will be needed to establish the role of SP-D in COPD and to elucidate its mechanism.

CONTRIBUTIONS

Conception and design: All authors. Collection of study subjects, assembly of data, analysis of samples, data analysis and interpretation, manuscript writing: Alaa Zien Alaabden. Supervision, manuscript revision for important intellectual content and final approval of manuscript: Yousser Mohammad and Sahar Fahoum.

CONFLICTS OF INTEREST DECLARATION

None of the authors have any potential conflict of interest to declare in this article.

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