RESEARCH ARTICLE

Plasma surfactant Protein-D levels in healthy subjects and COPD patients

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Abstract

Objective: To compare plasma surfactant protein-D levels in healthy smokers and Chronic obstructive pulmonary disease patients.

Methods: The comparative study was conducted at the University of Health Sciences, Lahore, Pakistan, from January to December 2015, and comprised chronic obstructive pulmonary disease patients and healthy smokers of either gender aged 40-80 years. Plasma surfactant protein-D levels of male and female subjects were estimated and compared with lung function and tobacco exposure. Blood samples were collected after complete history, physical examination and spirometry. Plasma levels were measured using enzyme-linked immunosorbent assay. Plasma cotinine levels were also measured for the determination of tobacco as well as biomass exposure along with pack years. SPSS 20 was used for data analysis.

Results: Of the 84 subjects, there were 42(50%) patients and as many controls. Both groups had 21(50%) males and as many females. There was no significant difference in the plasma surfactant protein-D levels of males and females in the patient group compared to their counterparts in the control group ($p>0.05$). Females developed the disease at a younger age compared to males ($p=0.04$). There was no significant difference in terms of pack years and cotinine levels between the groups ($p>0.05$) and lung function showed greater deterioration in the females compared to males with similar tobacco exposure ($p<0.05$).

Conclusion: The gender did not affect plasma surfactant protein-D levels.

Keywords: Surfactant protein-D, COPD, Cotinine, Biomarker, Sex difference. (JPMA 69: 494; 2019)

Introduction

Chronic obstructive pulmonary disease (COPD), which includes chronic bronchitis and emphysema, is characterised by an abnormal inflammatory response of the airways to noxious stimuli. This response may also lead to airflow limitation that is not reversible even after the administration of the bronchodilator.[1,2] Currently, there is a lot of focus in the international community to develop a clinical biomarker of COPD in order to track disease progression. Surfactant protein-D (SP-D) is being proposed as an ideal biomarker for this purpose.[3] However, given the differences between the male and female COPD patients, and reported gender differences in systemic level of SP-D,[4] there is a dire need to assess the efficacy and utility of this biomarker in both genders separately. Such data is not available for any ethnic group or population. SP-D is one of the four members of collagenous subfamily of calcium-dependant lectins (collectins). It is secreted, primarily, by type II alveolar cells, and regulates the innate immune response of the lungs.[5] Studies have shown a direct relation between the serum SP-D levels to smoking and airway obstruction.[6,7] However, no such data is available regarding the female COPD patients despite the fact that COPD is affecting far greater number of women than men worldwide.[8]

Cotinine is the primary metabolite of nicotine which is the main constituent of cigarette smoke. Smoking status of a person cannot only be determined by pack-year history, but also by nicotine and cotinine levels in urine and blood. However, nicotine has a very short half-life (2-3 hours) compared to cotinine (17-20 hours). It has been shown that the cotinine levels in blood or urine are much more reliable markers to assess the active as well as passive smoking and can be used to confirm the smoking history given by the patient.[9,10] The current

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study was planned to estimate SP-D values both in male and female COPD patients with matched tobacco exposure, and to compare these values with those of healthy smokers.

Subjects and Methods

The comparative study was conducted at the University of Health Sciences (UHS), Lahore, Pakistan, from January to December 2015, and comprised healthy smokers (Group I) and COPD patients (Group II). After approval was obtained from the institutional ethics committee, the sample size was calculated using the following formula:

$$n = \frac{(Z_{1-\beta} + Z_{1-\alpha/2})^2(\sigma)^2}{(\mu 1 - \mu 2)^2}$$

Desired power of study=β = 95%; Desired level of significance =α=5%; Mean difference of SP-D = μ1 - μ2 = 29.8 - 19.4 = 10.4[11]; Standard deviation for SP-D of Group I = 15.7; Standard deviation for SP-D of Group II = 13.6; Sample size in each group = 42.

The sample in each group was subdivided into equal number of females (Ia, IIa) and males (Ib, IIb). Controls, recruited from the general population of Lahore, were aged 40-80 years who had an active smoking history (cigarette or huqqa) and did not demonstrate any airflow limitation on spirometry. Cases were recruited from the outpatient departments of tertiary care hospitals of the city. They were age-matched stable COPD patients diagnosed according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria with post bronchodilator Forced expiratory volume in 1 sec / Forced vital capacity (FEV1 / FVC) <70%. Patients who had experienced exacerbation in the preceding four weeks and those with diagnosed asthma or active tuberculosis were excluded. For pulmonary function test (PFT), bronchodilator i.e. 200µg inhaled salbutamol, was given to the subjects 10 minutes prior to the procedure with the help of a spacer (Salbo, Getz Pharma, Karachi, Pakistan). An electronic spirometer (Spirolab 2, SDI Diagnostics, Bristol, MA, USA) was used. All the subjects were ethnically similar i.e. Punjabi, Pakistani. Pack years were calculated for both cigarette and huqqa smokers with the following formulas:

Pack years = No of cigarette smoking per day x No of smoking years / 20.

Pack years of huqqa smokers were calculated using the similar formula, but number of hours of smoking was first converted into number of cigarettes per day.[12]

Five milliliters of blood sample was collected in a sodium citrate vacutainer. For plasma, centrifugation was done at 5000rpm for 10-15 mins. Plasma was pipetted out and stored at -80°C in separate eppendorfs till its use. For detection of SP-D and cotinine in plasma, enzyme-linked immunosorbant assay (ELISA) was carried out using the enzyme-linked immunosorbent kit (Glory Science, Del Rio, TX78840, USA) in the UHS Laboratory of Physiology and Cell Biology, Lahore. The kits were stored at 2-8°C till use and the samples were brought to room temperature before use.

Data was analysed using SPSS 20. Values of plasma SP-D and cotinine were expressed in both mean ± standard deviation (SD) and median with interquartile range (IQR). For the comparison of demographic data (age, weight, height), PFTs and plasma SP-D and cotinine levels both parametric analysis of variance (ANOVA) and non-parametric Kruskal-Wallis tests were used. Post-Hoc testing was done using Tukey's and Mann Whitney-U tests for different comparisons among the groups. P<0.05 was considered statistically significant.

Results

Of the 84 subjects, there were 42(50%) COPD patients and as many controls. Both groups had 21(50%) males and as many females. The age of female in IIa was significantly less compared to males in IIb (p=0.04). Pack-year history showed no significant difference between IIa and IIb groups (p=0.09), but there was significant difference in this regard between females in Ia and males in Ib controls (p=0.02). The FEV1 and FVC values of Ia and IIa females were significantly less than those of Ia and IIa males (p= 0.001 each). No significant difference was seen in the FEV1/FVC ratio between Ia and IIa or when compared with the patients groups (Table 1).

No significant difference was seen in plasma SP-D levels in any of the four groups and no significant difference was seen in the plasma SP-D levels of healthy smokers and stable COPD patients (p>0.05) (Tables 2-3). No significant difference was seen while comparing plasma cotinine levels among the groups (p>0.05), but a significant positive correlation was seen between plasma cotinine and number of cigarettes smoked per day (p<0.05) (Table 4). The correlation values in Ia was 0.424 (p=0.05), IB 0.749 (p=0.001), IIa 0.750(p=0.001) and IIb 0.496(p=0.02).

Table-1: Comparison of demographic and clinical parameters in groups Ia, Ib, IIa & IIb. There were 21 subjects in all the groups.

Parameters		**Ia** (Female smoker)	**Ib** (Male smoker)	**IIa** (Female COPD)	**IIb** (Male COPD)	***p*-value**
Age** (Years)	Mean± SD	44.76 ± 6.17	52.42 ± 9.94	54.80 ± 10.99	63.09 ± 11.97	0.00*
	Median IQR	45 (31-55)	53 (30-71)	55 (37-55)	65 (40-79)	
Weight (Kg)***	Mean±SD	61.09 ± 9.34	69.09 ± 11.48	54.47 ± 11.33	60.09 ± 9.26	
	Median IQR	60 (38-80)	69 (50-98)	56 (38-72)	60 (48-82)	0.00*
Height (cm)**	Mean± SD	155.95 ± 5.74	163.80 ± 6.26	156.71 ± 5.50	168.09 ± 7.72	0.00*
	Median IQR	155 (148-168)	165 (153-175)	156 (145-165)	169 (154-180)	
Pack years	Mean± SD	17.77 ± 26.23	30.71 ± 29.42	79.08 ± 145.38	60.41 ± 108.66	
	Median IQR	7.50 (0.2-120)	22.50 (0.4- 125.5)	10 (0-562)	32 (5-525)	0.01*
FEV_1***(liters)	Mean± SD	1.93 ± 0.56	2.55 ± 0.59	1.43 ± 2.44	1.59 ± 0.49	
	Median IQR	1.86 (1.23-3.67)	2.37 (1.61-3.54)	0.89 (0.22-3.60)	1.63 (0.67-2.53)	0.00*
FVC *** (liters)	Mean± SD	2.33 ± 0.74	3.19 ± 0.80	1.60 ± 0.52	2.64 ± 0.63	
	Median IQR	2.19 (1.36-4.94)	2.93 (1.77-4.90)	1.62 (0.55-2.48)	2.70 (1.40-3.91)	0.00*
FEV_1 / FVC***	Mean± SD	83.51 ± 5.97	80.76 ± 6.83	57.63 ± 9.25	59.30 ± 8.26	
	Median IQR	84.60 (73.50-91.90)	80.50(70.80-91.40)	59.90 (39.60-70.40)	61.40(36.80-67.80)	0.00*

*p-value <0.05 was considered significant, **For normally distributed data single factor analysis of variance (ANOVA) was used, ***For non- normally distributed data Kruskal-Wallis test was used, COPD: Chronic obstructive pulmonary disease, IQR: Interquartile range, SD: Standard deviation, FVC: Forced vital capacity, FEV_1: Forced expiratory volume in 1 second.

Table-2: Comparison of demographic and clinical parameters in group Ia & IIa.

Parameters		**Ia** (Female smoker) n=21	**IIa** (Female COPD) n=21	***p*-value**
Age**(Years)	Mean± SD	44.76 ± 6.17	54.80 ± 10.99	0.00*
	Median IQR	45 (31-55)	55 (37-55)	
Weight (Kg)***	Mean±SD	61.09 ± 9.34	54.47 ± 11.33	
	Median IQR	60 (38-80)	56 (38-72)	0.13
Height (cm)**	Mean± SD	155.95 ± 5.74	156.71 ± 5.50	0.98
	Median IQR	155 (148-168)	156 (145-165)	
Pack years	Mean± SD	17.77 ± 26.23	79.08 ± 145.38	0.96
	Median IQR	7.50 (0.2-120)	10 (0-562)	
FEV_1***(liters)	Mean± SD	1.93 ± 0.56	1.43 ± 2.44	
	Median IQR	1.86 (1.23-3.67)	0.89 (0.22-3.60)	0.00*
FVC *** (liters)	Mean± SD	2.33 ± 0.74	1.60 ± 0.52	
	Median IQR	2.19 (1.36-4.94)	1.62 (0.55-2.48)	0.00*
FEV_1 / FVC***	Mean± SD	83.51 ± 5.97	57.63 ± 9.25	
	Median IQR	84.60 (73.50-91.90)	59.90 (39.60-70.40)	0.00*

*p-value < 0.05 was considered significant, **For normally distributed data single factor ANOVA was used, ***For non- normally distributed data Kruskal-Wallis test was used, COPD: Chronic obstructive pulmonary disease, IQR: Interquartile range, SD: Standard deviation, FVC: Forced vital capacity, FEV_1: Forced expiratory volume in 1 second.

Table-3: Comparison of demographic and clinical parameters in group Ib and IIb.

Parameters		**Ib** (Male smoker) n=21	**IIb** (Male COPD) n=21	***p*-value**
Age**(Years)	Mean± SD	52.42 ± 9.94	63.09 ± 11.97	0.00*
	Median IQR	53 (30-71)	65 (40-79)	
Weight (Kg)***	Mean±SD	69.09 ± 11.48	60.09 ± 9.26	0.03*
	Median IQR	69 (50-98)	60 (48-82)	
Height (cm)**	Mean± SD	163.80 ± 6.26	168.09 ± 7.72	0.13
	Median IQR	165 (153-175)	169 (154-180)	
Pack years	Mean± SD	30.71 ± 29.42	60.41 ± 108.66	
	Median IQR	22.50 (0.4- 125.5)	32 (5-525)	0.12
FEV_1***(liters)	Mean± SD	2.55 ± 0.59	1.59 ± 0.49	0.08
	Median IQR	2.37 (1.61-3.54)	1.63 (0.67-2.53)	
FVC *** (liters)	Mean± SD	3.19 ± 0.80	2.64 ± 0.63	0.05
	Median IQR	2.93 (1.77-4.90)	2.70 (1.40-3.91)	
FEV_1 / FVC***	Mean± SD	80.76 ± 6.83	59.30 ± 8.26	
	Median IQR	80.50(70.80-91.40)	61.40(36.80-67.80)	0.00*

*p-value < 0.05 was considered significant, **For normally distributed data single factor ANOVA was used, ***For non- normally distributed data Kruskal-Wallis test was used, COPD: Chronic obstructive pulmonary disease, IQR: Interquartile range, SD: Standard deviation, FVC: Forced vital capacity, FEV_1: Forced expiratory volume in 1 second.

Table-4: Comparison of Plasma SP-D (ng/ml) and Cotinine (ng/ml) among the groups Ia, Ib, IIa and IIb. There were 21 subjects in all the groups. The data have not been adjusted for age, weight, height, BMI or pack years of smoking.

Parameters		**Ia** (Female smoker)	**Ib** (Male smoker)	**IIa** (Female COPD)	**IIb** (Male COPD)	***p*-value***
Plasma SP-D**	Mean±SD	8.40±2.25	8.43±1.73	8.27±1.18	7.91±0.62	
	Median (IQR)	7.64(7.19-16.09)	7.90(7.26 - 13.96)	7.90(7.19 - 11.75)	7.92(6.89-9.57)	0.71
Plasma Cotinine**	Mean±SD	3653.87±1831.01	3283.49±887.40	3261.68±713.61	3269.26±633.46	
	Median (IQR)	3178.93(2163.15-10569.55)	2995.98(2334.01-6401.82)	3395.02(2112.64-4428.82)	3158.51(2243.69-4686.42)	0.98

*p< 0.05 is considered significant, **For non-normally distributed data Kruskal- Wallis test was applied, COPD: Chronic obstructive pulmonary disease, IQR: Interquartile range, SD: Standard deviation, BMI: Body mass index, SP-D: Surfactant protein-D.

Discussion

In the present study, both female groups (Ia and IIa) were younger and showed greater deterioration of lung functions on spirometry compared to both male groups (Ib and IIb) with similar smoking history. The comparison of plasma SP-D levels of Ia and IIa females with Ib and IIb males had no significant difference, indicating that gender had no effect on plasma SP-D levels. The demographic and spirometric results are in coherence with a Spanish study on 53 COPD men and women which concluded

that women were younger (p<0.05), smoked less (48 pack years vs. 69 pack years; p<0.05) and showed more exacerbation and deterioration of lung functions compared to males.[13] Regarding the basal plasma SP-D levels, a study on European subjects showed similar results as ours. The study also concluded that gender had no effect on the SP-D levels.[14] However, a study in Chinese population concluded that SP-D concentration was significantly higher in males compared to females.[15] The values of plasma SP-D in that study[15] were significantly higher than the values in the current study which can be explained by the fact that ethnical, racial, environmental factors and previous history of exposure to noxious substance may play a significant role in baseline SP-D levels.

A study in Danish population[16] with a sample size of 1476 healthy adults also concluded that baseline SP-D levels were higher in males compared to females, but their baseline SP-D levels were significantly higher compared to the levels of the Chinese population.[15] This difference was explained on the racial and environmental difference in the two studies. Moreover, in Danish population lungs are more sensitive to any noxious stimuli as they have much cleaner environment compared to Chinese or our population. A factor which can influence the SP-D levels is the medium used in the two studies i.e. in the Danish study serum SP-D levels were determined and in Chinese study the medium used was plasma. Zhao et al. in their study also mentioned the difference in the values of SP-D by using two different mediums i.e. serum and plasma for the same sample. It was concluded that SP-D levels were 20-36% higher in serum compared to the levels in plasma.[15,16] Another factor which greatly influences the circulatory levels of SP-D is the circadian variations. Hoegh et al. in 2009 conducted a study on Danish population in which they studied the variations in serum SP-D levels during a single day. Serum SP-D levels were highest during the day around 10 am (1009 ng/ml) and decreased to 867 ng/ml around 10 pm.[17] Therefore, in order to establish SP-D as a biomarker, a standardised approach is required for the conditions of blood sampling, the medium serum or plasma for SP-D detection, and the time of sampling for values that are plausible and comparable.

In the present study, no significant difference was seen in the SP-D levels of male, female smokers and COPD patients. However, a trend appears from the values estimated in various groups of the study. It appears that COPD attenuates the maximum levels. This can clearly be seen from the values obtained by the present study (Table 2). The highest plasma SP-D levels of healthy female smokers were 16.09ng/ml while the same values for female COPD were 11.75ng/ml (27.33% lower). The same trend was seen in the male healthy smokers having highest plasma SP-D levels 13.96 ng/ml compared to 9.57ng/ml in COPD males (30.94% lower). Also, the females had higher values than males both in the current smokers and COPD groups, although the difference was not significant. These levels and trends will have to be confirmed with higher number of samples.

In the present study, no significant difference of plasma SP-D was seen in the control healthy smokers and COPD group which was probably due to the fact that COPD patients were on treatment with oral steroids and were exacerbation-free for more than a month. Lomas et al. in 2009 not only concluded that gender had no effect on the levels of SP-D, but also found that SP-D was highly sensitive to the treatment with steroids. Treatment of COPD patients with 20 mg prednisone greatly decreased the serum SP-D levels from 126 to 82.1 ng/ml due to anti-inflammatory properties of steroids.[14] For these reasons, SP-D is considered a candidate biomarker for exacerbation and anti-inflammatory treatment. More studies are needed in this direction.

There were few limitations of the present study. The sample size was too small to validate the results for generalisation as we still don't have a standard reference value for our population. Lack of availability of diagnosed COPD patients, particularly the female patients with smoking history meant a smaller size.

In future, such a study should be done on newly-diagnosed COPD patients who are not on steroid treatment. Further studies are required in future with a bigger sample size in order to fully understand the role of gender on circulating levels of SP-D.

Conclusion

Gender was found to have no effect on plasma SP-D levels although females showed a significant deterioration of lung functions with similar or less pack-year history. Also, there was no significant difference seen between plasma SP-D levels of stable COPD and control groups.

Disclaimer: The study is part of an M.Phil. thesis.
Conflict of Interest: None.

Source of Funding: University of Health Sciences, Lahore, Pakistan.

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