ORIGINAL ARTICLE
PLASMA SURFACTANT PROTEIN-A LEVELS IN HEALTHY SUBJECTS AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE PATIENTS

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Background: Chronic Obstructive Pulmonary Disease (COPD) is the leading cause of morbidity and mortality across the globe. Currently, there is a dearth of biomarkers which can accurately diagnose and evaluate the prognosis of the disease. Systemic Surfactant Protein- A (SP-A) levels are generally higher in smokers compared to non-smokers as well as elevated in COPD patients as compared to controls. The objective of the study was to estimate and compare plasma surfactant protein-A levels in male and female COPD patients and healthy subjects and to evaluate the role of SP-A as a possible bio-marker for COPD patients. Methods: A Comparative study, conducted at the department of Physiology & Cell Biology, University of Health Sciences, Lahore between August 2013 and April 2015. A total of 84 subjects of both sexes between 30-80 years of age were included in this study. Subjects were taken from local community and were divided into four groups (A- D). COPD was diagnosed on the basis of relevant history and spirometry showing post bronchodilator FEV1/FVC <0.70. Results: Plasma SP-A levels were not different between controls and COPD patients and between male and female COPD patients. However, SP-A levels were directly correlated with cotinine levels (r= 0.503, p=0.001). Female patients were usually more symptomatic than males and developed COPD at an earlier age compared with male patients. Conclusion: Plasma SP-A levels were not significantly different between groups. Plasma cotinine levels (an indication of the tobacco use) were positively correlated with plasma SP-A levels in study subjects. Female patients developed COPD at an early age compared to male counterparts with similar tobacco exposure.

Keywords: Chronic Obstructive Lung Disease, Surfactant Protein A, Cotinine, Spirometry, Smoking

INTRODUCTION
Chronic Obstructive Pulmonary Disease (COPD) is a major cause of morbidity and mortality across the globe. Prevalence of COPD is rapidly increasing in developing countries and it is projected to be the 3rd leading cause of mortality in developing counties by 2030.1 COPD has been characterized as a preventable and treatable disease state characterized by irreversibility of the airflow obstruction.2 Risk factors for COPD include genetic as well as environmental factors. Tobacco smoking and air pollution such as the use of biomass i.e. burning of wood, coal and animal dung for cooking and heating are identified as two of the most common causes of COPD.2 In addition to this, fumes from factories, occupational dust and smoke from vehicles are also among the causative factors.3 Previously COPD was considered as the disease of the male, but due to increasing trend of smoking in children, adolescents and females and increased exposure to second hand smoke, the number of female COPD patients is increasing day by day.3

Due to heavy existing disease burden and rapid increase in number of COPD patients, there is dire need to develop biomarkers to assess the disease progression at an early stage. C-reactive Protein (CRP), fibrinogen, Krebs von den Lungen-6 (KL-6), Matrix metalloproteinases MMPs and surfactant proteins have been evaluated as potential candidate biomarker for COPD but still there is lack of data regarding normal levels of various biomarkers implicated for diagnosis of COPD.6 Lungs become more permeable with infection and injury which leads to increase leakage of various proteins across the blood-lung barrier. Due to this leakage, systemic SP-A levels are generally higher in active smokers compared to non-smokers as well as elevated in COPD patients as compared to controls.7,8 The present study was designed to compare SP-A levels in male and female COPD patients as well as between COPD and control subjects.

MATERIAL AND METHODS
This was a comparative study which was conducted at the Department of Physiology & Cell Biology, University of Health Sciences, Lahore from August 2013 to April 2015. The approval of the study was taken from Institutional Ethical Research Committee and Review Board after which sampling was done. Sample size was calculated by using (Sample Size determination in Health Version 2.0.21, World Health Organization) the following formula keeping the power of study equal to 95% and level of significance equal to 5%.
\[ n = \frac{(Z_1 - \beta + Z_1 - \alpha/2)^2 (\sigma)^2}{(\mu_1 - \mu_2)^2} \]

A total of 84 subjects of both sexes (male or female) were included in this study. Study subjects were taken from nearby Government Hospitals and local community and were divided into four groups (A-D). Group “A” included male current smoker controls that had not developed COPD. Group “B” included male patients having diagnosis of COPD on spirometry. Group “C” included female current smoker controls and Group “D” included female patients having COPD. COPD was diagnosed on the basis of relevant history and spirometry showing post bronchodilator FEV1/FVC <0.70.

Written informed consent was taken from all the subjects and Socio-demographic information (name, age, sex, occupation, full address etc.) was obtained along with other relevant clinical information. Height was measured in centimeters from the highest point of the vertex when the subject was standing in the anatomical position. Weight was measured in kilograms, while all the subjects were bare-footed and wearing minimal clothing. After that, spirometry procedure as standardized by American Thoracic Society was clearly explained to all the subjects and then demonstrated for proper understanding by the subjects. The subjects were informed about possible risks/discomfort associated with spirometry. They were also briefed about the purpose of the study. Subject’s age, sex, weight, and height was recorded and entered into the spirometer so that predicted curves and values can be calculated by the spirometer. Spirometry of all the recruited subjects was performed early in the morning at around 9° clock. 200 µg of inhaled salbutamol was administered in all male and female COPD patients having airway obstruction as defined by the Global Initiative for Chronic Obstructive Lung Disease. Spirometry was then again performed in COPD patients after 15–20 minutes of salbutamol inhalation to confirm the irreversibility of the test.

Blood sample was taken either from medial cubital vein or dorsal hand veins after cleansing the skin with disinfectant solution (70% isopropyl alcohol) in a circular manner moving outwards. Five (5) ml of the blood was collected using a sterile 5ml syringe and was then put in a vacutainer® tube containing Na-citrate as an anti-coagulant.9 The vacutainer tubes were centrifuged at 3000 rpm for 10 minutes to separate the plasma. The plasma was then pipetted out in aliquots which were then stored at -80 °C till used for testing. Plasma levels of surfactant protein-A and cotinine were measured using commercially available ELISA kits manufactured by Glory Science, Texas, USA.

All the data were entered and analyzed using SPSS version 20. Shapiro-Wilk test was used to test the normality of the data. Normally distributed quantitative variables were described using Mean±S.D. while non-normally distributed variables were described using median (IQR). Parametric (two-tailed independent sample t-test & ANOVA) and Non-parametric (Mann-Whitney U & Kruskal-Wallis) tests were used to compare various study parameters between groups. Pearson correlation was applied to observe correlations between parameters. A p-value of ≤0.05 was considered as statistically significant.

## RESULTS

A total of 84 subjects were divided into four equal groups. Summary of physiological, anthropometric and biochemical parameters is detailed in Table-1.

Plasma cotinine (\(p=0.854\)) and SP-A levels (\(p=0.629\)) were not statistically different between control and COPD patients (Table 2). Plasma cotinine levels showed a positive correlation (\(p=0.001\)) with plasma surfactant protein-A levels (Figure-1). Female patients were more symptomatic (69 female vs 49 male) and develop COPD at an earlier stage when compared to male counterparts using independent sample “t” test (\(p \leq 0.009\)). The most frequent complaint from all study groups was dyspnea (\(n=48, 57.1\%\)) followed by cough (\(n=28, 33.3\%) and sputum production (\(n=20, 23.8\%\)) respectively (Figure-2).

<table>
<thead>
<tr>
<th>Study parameters</th>
<th>Group A (Male Smoker)</th>
<th>Group B (Male COPD)</th>
<th>Group C (Female Smoker)</th>
<th>Group D (Female COPD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anthropometric parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)**</td>
<td>52.00 (38.00–55.00)</td>
<td>65.00 (56.00–76.50)</td>
<td>45.00 (40.00–50.00)</td>
<td>55.00 (45.00–65.50)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Height (m)*</td>
<td>1.69±0.25</td>
<td>1.80±0.22</td>
<td>1.62±0.25</td>
<td>1.76±0.17</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Weight (Kg)**</td>
<td>73.24±13.70</td>
<td>61.29±10.63</td>
<td>61.76±9.67</td>
<td>54.62±11.45</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Body Mass Index (Kg/m²)**</td>
<td>26.16±3.75</td>
<td>21.86±3.14</td>
<td>25.64±3.88</td>
<td>22.30±5.08</td>
<td>0.001*</td>
</tr>
<tr>
<td><strong>Biochemical Parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surfactant Protein-A (ng/ml)**</td>
<td>70.35 (58.28–77.42)</td>
<td>77.42 (63.16–88.19)</td>
<td>80.53 (56.22–105.17)</td>
<td>75.08 (62.55–84.78)</td>
<td>0.675</td>
</tr>
<tr>
<td>Cotinine (ng/ml) ***</td>
<td>3.19 (2.74–3.69)</td>
<td>3.32 (3.09–4.05)</td>
<td>3.50 (2.97–3.89)</td>
<td>3.27 (2.84–3.50)</td>
<td>0.428</td>
</tr>
</tbody>
</table>

*\(p \leq 0.05\) is considered statistically significant. **One-way ANOVA test was used for normally distributed values. ***Kruskal-Wallis test was used for non-parametric values.
Table-2: Comparison of various study parameters between control Subjects and COPD patients

<table>
<thead>
<tr>
<th>Study parameters</th>
<th>Control (n=42)</th>
<th>COPD (n=42)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)***</td>
<td>47.00</td>
<td>59.50</td>
<td>0.001*</td>
</tr>
<tr>
<td>(40.00–53.25)</td>
<td>(50.75–70.00)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anthropometric parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height (m)***</td>
<td>2.60±0.28</td>
<td>2.63±0.26</td>
<td>0.624</td>
</tr>
<tr>
<td>Weight (Kg)**</td>
<td>67.50±13.07</td>
<td>57.95±11.42</td>
<td>0.001*</td>
</tr>
<tr>
<td>Body Mass Index (Kg/m²)**</td>
<td>22.08±6.17</td>
<td>&lt;0.001*</td>
<td></td>
</tr>
<tr>
<td>Biochemical Parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surfactant Protein-A (ng/ml)***</td>
<td>71.93</td>
<td>76.64</td>
<td>0.629</td>
</tr>
<tr>
<td>(58.90–84.39)</td>
<td>(63.16–84.58)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cotinine (ng/ml)***</td>
<td>3.30</td>
<td>3.35</td>
<td>0.854</td>
</tr>
<tr>
<td>(2.84–3.75)</td>
<td>(2.95–3.67)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Degree of freedom (df) = 82. * p<0.05 is considered statistically significant. **Two tailed independent sample “t” test was used for normally distributed values. ***Mann-Whitney U test was used for non-parametric values.

Table-3: Comparison of various study parameters between Male and Female COPD Patients

<table>
<thead>
<tr>
<th>Study parameters</th>
<th>Male COPD (n=21)</th>
<th>Female COPD (n=21)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) **</td>
<td>64.95±11.58</td>
<td>55.29±11.10</td>
<td>0.099*</td>
</tr>
<tr>
<td>Anthropometric parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height (m) **</td>
<td>2.80±0.22</td>
<td>2.46±0.17</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Weight (Kg) **</td>
<td>61.29±10.63</td>
<td>54.62±11.45</td>
<td>0.058</td>
</tr>
<tr>
<td>Body Mass Index (Kg/m²) **</td>
<td>23.19</td>
<td>23.19</td>
<td>0.930</td>
</tr>
<tr>
<td>Biochemical Parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surfactant Protein-A (ng/ml) **</td>
<td>73.94±21.67</td>
<td>74.67±17.53</td>
<td>0.905</td>
</tr>
<tr>
<td>Cotinine (ng/ml) **</td>
<td>3.44±0.58</td>
<td>3.24±0.45</td>
<td>0.217</td>
</tr>
</tbody>
</table>

Degree of freedom (df) = 40. * p< 0.05 is considered statistically significant. **Two tailed independent sample “t” test was used for normally distributed values. ***Mann-Whitney U test was used for non-parametric values.

DISCUSSION

The results of the present study show that COPD patients were significantly older in age than controlled group. Various anthropometric measurements e.g. age, weight and body mass index were significantly different in control and COPD groups. The same findings were reported by other scholars while comparing control, asthmatic and COPD subjects. Mean age for the male COPD patients was 64.95 yrs which was significantly higher (P<0.05) than the average age (55.29 yrs) recorded for female COPD patients (Table-1). Our findings suggest that females develop COPD at an earlier age than males even with lower smoking history. Baig et al in a study comparing frequency of post-tuberculosis COPD patients in Rawalpindi, Pakistan reported 56.4 years and 44.2 years as the average age for males and females respectively. The lower age reported by Baig et al may be due to the fact that tuberculosis makes the patients more prone to develop COPD. Results from a large study from Ottawa, Canada also indicated younger age group for controls as compared to COPD patients. Mean ages comparable to our results were also reported by other researchers for the same local study population from which the present study subjects were recruited. Development of COPD in females at a younger age may be attributed to smaller lung volume in women, possibly exposing them to a
greater amount of tobacco smoke per unit lung tissue compared to men when they smoke the same or higher number of cigarettes. Moreover, in the lower social strata of population of the third world countries, there is generally one room available to the whole family which suffices for all daily chores like kitchen, sitting and bed room. This room is invariably less ventilated thus exposing the children also to the smoke of cigarette and smoke coming from the cow dung and other bio-material burning used for cooking. Exposure to second hand smoke due to burning of biomass fuel is another probable cause of this difference.14-16 Studies from Pakistan, India and other under-developed countries have already reported respiratory problems in females due to the use of biomass fuel for cooking in rural areas. Finally, genetic susceptibility to smoke and hormonal factors may also come into play for this variation between male and female groups.17-21

The most frequent symptom reported by all the study subjects was dyspnea (57.1%) followed by cough (33.3%) and sputum production (23.8%) respectively. The prevalence of the symptoms in COPD patients was higher, i.e., dyspnea (78.6%), cough (50%) and sputum production (28.6%) moreover, the prevalence was higher in females compared with male counterparts. Previously, the prevalence of these symptoms was reported from Brazil in the same order but with higher percentages.22 In a pan-European cross-sectional study breathlessness (72.5%), sputum production (63.6%) and cough (58.7%) was experienced by COPD patients in 7 days prior to interview.23 This large difference in %age of patients complaining dyspnea may be due to difference in smoking habits, exposure to second hand smoke and various levels of pollution in the environment of these different countries. The other possibility may be due to proportion of chronic bronchitis and emphysema variant of COPD patients in study populations both of which show different symptoms.24,25

Median value for plasma surfactant protein-A levels in all females included in the present study, was higher than males but this difference was not significant statistically (Table-1). Similarly, the median Plasma SP-A levels in COPD patients was higher than control subjects but it was also not significant (p=0.629) (Table-2). Due to much variability in SP-A values in our study subjects which points to the difference in their background and smoke exposure, the relevant data were not normally distributed and therefore values are also given as median (inter-quartile range). Significantly elevated SP-A levels were reported in smokers as compared with non-smokers.7 In the present study, no significant difference was seen between male and female smokers. Our results are consistent with previously reported trends in SP-A levels where researchers reported no significant difference in SP-A levels between male and female smokers.26 The higher levels in females in our study were possibly due to the increased smoke exposure because of cigarette and second hand smoke. Our results are consistent with the Mutti, et al27 but contrary to previously reported studies by Ohlmeier et al28 and Ishikawa et al29 comparing SP-A levels between controls and COPD patients.27-29 Scientists have reported varying degree of elevation in SP-A levels in various lung pathologies, in different population and even in sub-type of the same disease.29,30 Studies have reported elevated SP-A levels in various fluids in smokers as well as in non-smokers having various lung pathologies.7,8,31 Elevation in SP-A levels in some lung pathologies may be extreme while in others there may be only mild to moderate elevation. The inconsistency in response to injury to the respiratory epithelium may partly be explained on the basis of gender differences, genetic, susceptibility of the individuals towards harmful effects of smoking and variable bronchodilator response.32,33 This variability in SP-A level is in accordance with previous studies reporting very high variability in SP-A levels among study subjects from different populations.7,34 In the present study, the median plasma cotinine level in control group (smokers only) was not different from the COPD patients and also between males and females. This difference may be explained because of poor smoking history, social constraints, variability in daily smoking and preference of smoking brand.35,36

The results of the present study clearly show that there are differences between earlier studies and this study. There can be many reasons for this which include race, genetic back-ground, smoking history, pack years, exposure to the second hand smoke, biomass smoke, general environmental conditions etc. Another possible factor may be the fact that COPD patients were on steroid treatment and exacerbation free at least for last one month. Although no effect of steroids has been discovered on SP-A levels but due to anti-inflammatory properties of the steroids it may be deduced that they can decrease SP-A levels in patients. Finally, the very limited number of the patients and controls included in this study are probably a major deficiency. Therefore, we recommend that a large-scale study with good numbers of subjects and other related parameters.
may be designed and executed, so that meaningful influences are deduced.

CONCLUSION

Plasma surfactant protein-A levels are higher in females compared to their male counterparts but are not statistically significant. No significant difference in cotinine and plasma SP-A levels was found between control and COPD subjects. Plasma cotinine levels are positively correlated with plasma SP-A levels in study subjects. COPD patients are older than controls and female patients develop COPD at a younger age than males with the same exposure to smoke and were more symptomatic.

Limited sample size due to resource constraints was the main limitation of the study. The others include lack of availability of diagnosed female COPD patients. Studies with larger sample size should be done to confirm and validate the results of the present study in entire population. There is dire need of assigning definite cut of SP-A values to control, smokers and COPD patients. Similarly, no definite cut-off values have been assigned to cotinine in local population.

ACKNOWLEDGEMENT

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AUTHORS’ CONTRIBUTION

HMW: Conceived the idea and designed the study. Recruited subjects, did spirometry and clinical analysis. Statistical analysis, manuscript preparation and editing. Read and approved final manuscript.
RAK: Recruited subjects, did spirometry and clinical analysis. Manuscript preparation and finalization.
KPL: Supervisor of the overall project. Edited, finalized and approved final manuscript.

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