STUDY OF INFLAMMATORY BIOMARKER (PARC/CCL18) IN NONSMOKERS AND ACTIVE SMOKERS WITH COPD

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ABSTRACT:

BACKGROUND: India 68.6% COPD patients are Non-smokers and Secondhand smoke exposure (SHS) believed to be the important cause for COPD. Serum Cotinine is a biomarker for assessing smoking status of the individual. COPD is an independent risk factor for cardiovascular disease. Serum PARC/CCL18 is a COPD specific biomarker. There is paucity in Literature, revealing the association of Serum PARC/CCL18 with Smoking status of the patient. **OBJECTIVE:** In our study we sub grouped the COPD patients into Non-smokers with COPD and Active smokers with COPD using Cotinine levels and intended to compare the levels of Serum PARC/CCL18 in them. We also wanted to assess the association between PARC/CCL18 and Serum Cotinine levels. **METHODS:** This is a cross sectional study done on n=130 male COPD patients. Anthropometric, basal & Biochemical parameters such as Serum Cotinine and Serum PARC/CCL18 were assessed in them. Later, based on the Serum Cotinine levels the COPD patients were sub grouped into Active smokers with COPD & Non-smokers with COPD. Data was analysed by SPSS 19.0 version software. MannWhitney- U test were used to find any Statistical difference between the groups. Correlations between the variables were done using Spearman correlation test. **RESULTS:** Statistically significant difference in Serum PARC/CCL-18 levels ($p<0.05$) were found between Non-smokers with COPD and Active Smokers with COPD. Statistically significant Correlation was found for Serum PARC/CCL-18 and Serum cotinine levels ($p<0.05$).

Conclusion: Significantly increased Serum PARC/CCL18 levels with high Serum Cotinine levels in the test group indicate that Active smokers with COPD are more susceptible for cardiovascular events in future.

KEYWORDS: Cotinine, Cardiovascular risk, inflammatory marker, Non-smokers, PARC/CCL18

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Introduction

Chronic obstructive pulmonary disease (COPD) is a disease state, characterized by airflow limitation that is not fully reversible.[1] According to WHO, the burden of COPD - 65 million around the world and COPD was predicted to be the third most common cause of death by 2020.[2] In India, the burden of COPD-14.84 million and India contributes 64.7% of COPD mortality - highest in the world. [3-5] Cigarette smoking causes COPD and it is considered as a major risk factor for COPD.[6] Studies proved that non-smokers also develop COPD & may account for one-third of all COPD cases.[7-9] In India 68.6% of COPD patients are Non-smokers and Secondhand smoke exposure (SHS) believed to be the important cause for COPD in nonsmokers.[9,10] It is important to screen them because nonsmokers with COPD could also suffer from COPD induced comorbidities; mainly from cardiovascular diseases.[7] Smoking index & Questionnaires are commonly used to assess the smoking status of COPD patients which would have respondent and recall bias. Moreover, it can't measure SHS and Passive smoking in nonsmokers.[11,12] So COPD in non-smokers go often undiagnosed in India which makes them vulnerable to COPD induced morbidity and mortality.[7] Cotinine, a primary metabolite of nicotine is recommended biomarker for classifying smoking status. Estimated values of Serum Cotinine as given by CDC were: Non-smokers (with SHS)- Serum Cotinine levels <10ng/ml & Active smokers- Serum Cotinine levels >10ng/ml.[13]

COPD is an independent risk factor for Cardiovascular disease and FEV1 is the only

biomarker of COPD widely used in clinical trials but FEV1 is not specific to COPD.[14] PARC - Pulmonary and activation-regulated chemokine (PARC/CCL18) expressed by alveolar macrophages and dendritic cells, in response to helper T-cell type 2 cytokine and it is a very specific inflammatory marker to COPD.[15] Studies have proved that PARC/CCL18 can be used as a specific inflammatory marker of COPD to assess cardiovascular morbidity & mortality.[15,16] So, in our study, we sub-grouped the COPD patients into Non-smokers with COPD and Active smokers with COPD using Cotinine levels and intended to compare the levels of Serum PARC/CCL18 in them. We also wanted to assess the association between PARC/CCL18 and Serum Cotinine levels.

Materials & Methods

Study Design This was a Descriptive study conducted in 130 male COPD patients. It was designed to assess the PARC/CCL18 levels and serum cotinine levels and also to find the association of PARC/CCL18 levels with serum cotinine levels in male COPD patients. The study was conducted in Department of Physiology, JIPMER in Collaboration with Department of Pulmonary Medicine, JIPMER. Before the start of the study, approval from JIPMER scientific advisory committee and Institute ethics committee for human studies were obtained. In the study group, biochemical parameters of PARC/CCL18 & serum cotinine levels were studied. Later they were classified into two subgroups Non-smokers with COPD and Active smokers with COPD based on Cotinine levels.

Selection of Subjects

Male COPD patients attending JIPMER Pulmonology OPD were included in the study. COPD patients (who cannot maintain oxygen saturation above 88%), COPD patients with systemic complications like coronary heart disease, arrhythmia, Stroke, Alcoholics, Diabetic and hypertensive patients &Tobacco chewers are excluded from the study. Subjects were health educated about the disease and are motivated to know their Disease severity & Cardiovascular risk associated with their Disease.

Experimental Design

The study was carried out in Department of Physiology, JIPMER between 9 am to 1 pm. The study involved minimal invasive procedure of collecting 5 ml blood. The subjects were explained clearly about study protocol in their native language and written informed consent was obtained from them.

Statistical Analysis of Data

SPSS version 19 was used for statistical analysis. The data were subjected to Kolmogorov-Smirnov normality test. The continuous data such as age, duration of illness, anthropometric parameters (Ht, Wt, WC, HC, WHR, WhtR), heart rate and blood pressure and cotinine were expressed as mean with standard deviation and the intergroup differences were compared using Oneway ANOVA test. PARC/CCL18 was expressed in median with interquartile range and MannWhitney-U test were used to find any Statistical difference between the groups. Correlations between PARC/CCL18 and cotinine levels were done using Spearman correlation test. The difference was considered statistically significant if probability of chance was less than 0.05

Result

All the anthropometric and biochemical parameters were assessed in 130 COPD patients after obtaining informed consent from them, and the data were analysed.

Comparison of parameters among different stages of COPD:

Demographic characteristics

The mean age, duration of illness, anthropometric indices (height, weight, BMI, waist circumference, hip circumference, waist-hip ratio and waist height ratio) and serum cotinine levels of the study group were given in Table 1.

Heart rate and Blood pressure parameters

The mean Heart rate, SBP, DBP, PP, MAP of the study group were given in Table 2.

No significant difference was noted among the subgroups of COPD.

Biochemical parameters:

Comparison of PARC/CCL18 levels among the subgroups was done using MannWhitney- U test.

Serum PARC/CCL18 levels were significantly high (Table 3) in Active smokers with COPD when

compared to non-smokers with COPD. Significant positive Correlation was found between PARC/CCL18 and cotinine levels.(Table 4).

Table: 1 Demographic characteristics of study participants (n=130)

Variables	Mean ± SD
Age (Years)	53.37±5.65
Duration (Years)	6.92± 2.57
Height (cm)	161.33 ±7.72
Weight (Kg)	55.06± 9.60
BMI (Kg/m^2)	21.15±3.47
Waist (cm)	89.00±8.39
Hip (cm)	108.77± 16.02
Waist Hip Ratio	0.82± 0.09
Waist Height Ratio	0.55±0.04
Serum Cotinine(ng/ml)	12.50±2.4

The values are expressed in mean with SD

Table: 2 Comparison of Basal heart rate and blood pressure among COPD patients

Cardiovascular parameters	Total (n=130)	Non-smokers with COPD (n=46)	Active smokers with COPD (n= 84)	P value*
HR	73.7±9.7	67.41± 3.43	72.72± 3.09	0.102
SBP	110± 13.8	106.25± 4.24	111.53± 4.85	0.091
DBP	70.9± 13.4	66.76± 3.68	71.25± 5.44	0.054
PP	43.34± 9.58	39.49± 4.45	45.28± 6.85	0.066
MAP	83.9± 10.58	79.92± 3.26	85.35± 4.15	0.070

Values are expressed as mean (SD); Comparison of variables between groups done using ANOVA
*p<0.05 is statistically significant among the four groups of COPD
HR: heart rate (bpm); SBP: Systolic blood pressure (mmHg); DBP: Diastolic blood pressure (mmHg); PP: pulse pressure (mmHg); MAP: mean arterial pressure (mmHg)

Table: 3 Comparison of PARC/CCL18 levels among COPD patients

Biochemical parameters	Total (n=130)	Non-smokers with COPD (n=46)	Active smokers with COPD (n= 84)	P value*
PARC/CCL 18 (ng/ml)	50.50(22)	38 (9)	48 (12)	0.002

Values are expressed as Median (Interquartile range); Comparison of variables between groups done using Mann whitney U test
*p<0.05 is statistically significant among the four groups of COPD
PARC/CCl-18: Pulmonary and activation regulated chemokine ligand-18 (ng/ml)

Table 4: Correlation between PARC/CCL18 and Serum Cotinine.

PARAMETERS	SERUM PARC/CCL18	
	Spearman correlation (ř)	P value
Serum Cotinine (ng/ml)	0.414	0.003

Correlation between variables is done using Spearman correlation test. *p<0.05 is statistically significant.

Discussion

This study was conducted in Department of Physiology, JIPMER in collaboration with department of pulmonary medicine from January 2016 to July 2017. 130 male stable COPD patients without any major systemic illness were recruited for the study. Biochemical parameters such as PARC/CCL18 and Serum Cotinine were studied in them. Serum PARC/CCL18, a COPD specific inflammatory biomarker was used to assess cardiovascular risk in COPD patients. We had assessed the levels of PARC/CCL18 in COPD patients and got the mean PARC/CCL18 concentration as 50.50 ng/ml. Serum cotinine was used as the biomarker to assess smoking status of the COPD patients, which will also measure secondhand smoking (SHS); as it is considered to be the important cause of COPD in nonsmokers in India.[9,10] The mean serum cotinine levels in our study group of COPD patients were 12.50 ng/ml.

ECLIPSE study & LHS study were done to evaluate the role of PARC/CCL-18 in COPD.[15] ECLIPSE study showed that serum PARC/CCL-18 concentrations were independently related to COPD and also associated PARC/CCL-18 with total mortality rate caused by COPD.[15] LHS study showed strong independent relationship of PARC/CCL-18 with future risk of cardiovascular hospitalization and mortality.[15] ECLIPSE study & LHS study showed non-smokers with COPD had higher PARC/CCL18 levels than active smokers with COPD and concluded that PARC/CCL18 levels were not significantly associated with smoking status of the patient with COPD.[15] In these studies, total smoking exposure was measured by pack-years of smoking which cannot measure SHS. In our study we measured smoking status objectively using Serum Cotinine levels which will remove respondent and recall bias of the patient. Cotinine levels also measure SHS and passive smoke exposure which are considered to be the important cause of COPD in India. We found that PARC/CCL18 levels higher in Active smokers with COPD than Non-smokers with COPD with positive correlation of PARC/CCL18 levels with Serum Cotinine levels. These findings suggested that the cardiovascular risk was more in Active smokers with COPD than Non-smokers with COPD i.e, cardiovascular risk in COPD depends on the smoking status of the individual.

Conclusion

Active smokers with COPD had increased levels of PARC/CCL18 and were more susceptible for cardiovascular events in future than the Non-smokers with COPD. Our results also demonstrated a positive correlation between Serum PARC/CCL-18 and Serum cotinine levels, which is a marker of Smoking status including SHS. Thus, proving the fact, Non-smokers with COPD who were exposed to SHS are also prone for increased cardiovascular risk.

Acknowledgement

I thank JIPMER for providing me intramural grant to do the study. I would like to thank all the faculty members, technical staffs and my colleagues in JIPMER Physiology and Pulmonology department for helping me to do the study.

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Disclosure: There was no conflict of interest.