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Association between serum cytokine levels and severity of chronic obstructive pulmonary disease in Northern India

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ABSTRACT: The present study was designed to assess the serum cytokines [Interleukin-1β (IL-1β), Interleukin-6 (IL-6), Tumor necrosis factor-α (TNF-α)] levels in chronic obstructive pulmonary disease (COPD) patients and they were correlated with severity of disease by spirometric measurements. Finding of the present study is that among the severity of COPD stages, there was significant ($p<0.01$) difference in the level of TNF-α, IL-6 and IL-1β. TNF-α, IL-6 and IL-1β levels were also significantly ($p<0.05$) higher in serum among the very severe COPD than mild, moderate and severe COPD patients. There was direct correlation in cytokines levels of TNF-α, IL-1β and IL-6 in patients with COPD and their severity. The present study signifies that the levels of TNF-α, IL-1β and IL-6 are directly proportional to the post bronchodilator FEV_1 percentage. Our results provide population-based evidence that COPD is independently associated with low-grade systemic inflammation, with a different inflammatory pattern than that observed in healthy subjects. Overall, these results identify a novel systemic inflammatory COPD phenotype that may be the target of specific research and treatment.

KEYWORDS: Chronic Obstructive Pulmonary Disease, Inflammatory markers, Severity, Spirometric analysis.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is currently defined as "a preventable and treatable disease with some significant extrapulmonary effects that may contribute to the severity in individual patients. Its pulmonary component is characterized by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and associated with an abnormal inflammatory response of the lung to noxious particles or gases" [**1**]. A characteristic symptoms of COPD are chronic and progressive dyspnea, cough and sputum production that can be variable form day to day [**2,3**] A clinical diagnosis of COPD should be considered in patient who has dyspnea, chronic cough and chronic sputum production, and a history of exposure to risk factors for the disease. Spirometry is required to make the diagnosis in this clinical context; the presence of a post bronchodilator $FEV_1/FVC<0.70$ confirms the presence of persistent airflow limitation thus of COPD [**4**]. Episodes of acute worsening of these symptoms (exacerbations) often occur. Prevalence surveys suggest that up to almost a quarter of adults aged 40 years and older have mild airflow obstruction **[5].** COPD is presently the 4th leading cause of death, but WHO predicts that it will become the 3rd leading cause by 2030 **[6].** Mortality owing to cardiac diseases and stroke decreased over the period 1970-2002 but that of COPD doubled over the same period worldwide and the most commonly encountered risk factor for COPD is tobacco smoking **[7]**. Long-term smoking causes airway inflammation characterized by neutrophils, macrophage, and activated T lymphocyte infiltration and by increased cytokines concentrations such as TNF-α,

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IL-6. In many countries, outdoor, occupational and indoor air pollution the latter resulting from burning of biomass fuels are also major COPD risk factors **[8-11].** Although COPD is a lung disease it is associated with systemic manifestations and co-morbid conditions **[12].** Several studies have shown systemic inflammation in COPD patients with increased neutrophils, macrophage and T-lymphocyte numbers and high concentrations of inflammatory mediators in peripheral blood, TNF-α, IL-6, IL-1β **[13-16]**. The levels of many cytokines are known to be raised in serum in COPD **[17]** but their contribution to disease severity is still unknown. Global initiative for Chronic Obstructive Lung Disease (GOLD) laid certain criteria for clinical diagnosis of COPD. The diagnostic tool available is Spirometry. Spirometry is the measurement of the air moving in and out of the lungs during various respiratory maneuvers. The diagnosis made by Spirometry depends on the level of effort made by the patient, which may alter the diagnosis. By exhaustive literature survey we concluded that, the serum level of circulatory cytokines increases in patients with COPD. The inclusion criteria of the study are diagnosed case of chronic obstructive pulmonary disease and exclusion criteria are Patients with acute respiratory distress syndrome, Serious systemic disorders incompatible with the study (either acute or chronic affecting any other target organ in the human body)**,** Patients with history of poorly controlled associated diseases such as heart disease, thyroid disorders, coagulation disorders and hematologic problems etc were excluded from the study. Present study is conducted for establishing the relationship between circulatory cytokines and severity of COPD, so that the clinicians may have a TNF-, IL-1β, IL-6 tests apart from spirometry which are not subjective but are objective.

MATERIALS & METHODS

STUDY DESIGN AND PARTICIPANTS

The present study is cross-sectional and was conducted at a tertiary care teaching hospital of North India. Total 100 newly diagnosed patients from 30-80 years suffering from COPD attending the department of pulmonary medicine as per GOLD guidelines 2012 were included in the study. The written informed consent was obtained fulfilling the inclusion and exclusion criteria of the study. The study was approved by the institutional ethics committee.

METHODS

Participants diagnosed as COPD were selected for the systemic serum cytokines levels. 5 ml of venous blood were collected from each patient in a plain vial and centrifuged for 10 minutes at 2655g at 4^0 C, serum collected; it was stored at -80° C for further analysis. Any sample showing haemolysis was discarded. TNF-α, IL-6, IL-1β were determined with a high sensitivity Enzyme-Linked Immunosorbent Assay using respective estimation kits recommended. The kits were obtained from BECTON, DICKINSON and COMPANY BD BIOSCIENCES. SAN JOSE, CA 95131 USA. Absorbance was measured at λ=450 nm on a microplate ELISA reader (BIO-RAD, i-MARK).

STATISTICAL ANALYSIS

Statistical analysis was carried out by using SPSS 16.0 version (Chicago, Inc., USA). The results are presented in mean±SD and percentages. The Kologomorove test was used to test the pattern of TNF-α, IL-6 and IL-1β for normal distribution. The test showed non-normal pattern of these parameters. Hence, Kruskal-Wallis test followed by Tukey's post-hoc comparison tests was used to compare the levels of TNF-α, IL-6 and IL-1β among different severity COPD. The p-value<0.05 was considered significant.

RESULTS

Table-1 shows the distribution of demographic profile and addiction habit of the patients. About one third of the patients were in the group 61-70 years (32%) and 51-60 years (31%). Majority of the patients were males (90%) and were ex-smokers (68%).

Table-1: Distribution of demographic profile and addiction habit of the patients

Demographic profile and addiction habit	No. (n=100)	%
Age in years		
30-40	10	10.0
41-50	21	21.0
51-60	31	31.0
61-70	32	32.0
71-80	6	6.0
Mean ± SD	56.45±10.35	
Gender		
Male	90	90.0
Female	10	10.0
Height in cms	163.24±8.42	
Weight in kgs	53.43±13.89	
BMI in kg/mtr^2	19.89±4.32	
Smoking habit (duration in years)		
Current smoker	5	5.0 (24.00±5.47)
Ex-smoker	68	68.0 (25.59±7.16)
Passive smoking	4	4.0 (21.25±6.29)
Non-smoker	23	20.1

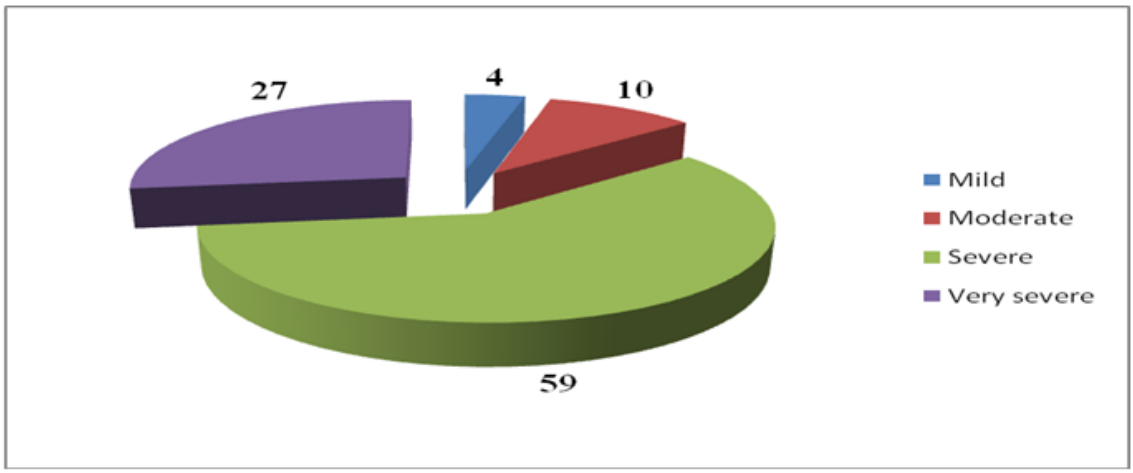

Fig:1 Distribution of severity of COPD patients according to GOLD Stages

More than half of the patients were severe (59% followed by very sever (27%), moderate (10%) and mild (4%) (Fig.1)

Table 2: Correlation of cytokines levels with COPD stages

	Severity of COPD stages according to gold criteria 2012 and cytokines levels				p-value[1]
Biochemical parameters	**Mild N=4**	**Moderate N=10**	**Severe N=59**	**Very severe N=27**	
Tnf –α (pg/ml)	58.81±6.88 [a]	65.92±32.46 [b]	77.89±34.74 [c]	186.09±63.14 [a,b,c]	0.0001*
IL-6 (pg/ml)	120.83±26.33	175.59±109.36	183.33±91.82 [a]	251.52±106.56 [a]	0.009*
IL-1β (pg/ml)	4.76±5.03 [a]	7.66±3.88 [b]	9.02±4.74 [c]	12.92±3.17 [a,b,c]	0.0001*

[1]*Kruskal-Wallis test, *Significant, [a,b,c] p<0.05 (Post-hoc multiple comparisons tests)*

The comparison of biochemical parameters according to severity of COPD are presented in the Table-2. There was significant ($p<0.01$) difference in the level of TNF-α, IL-6 and IL-1β among the severity of COPD. The post-hoc analysis showed that the TNF-α was significantly ($p<0.05$) higher among the very severe COPD than mild, moderate and severe COPD patients. Similar observation was also found for IL-6. However, IL-6 was significantly ($p<0.05$) higher among very severe than severe COPD patients. There was significant ($p=0.0001$) difference in the level of IL-1β among the different severity of COPD. The post-hoc comparison test showed that IL-1β was significantly ($p<0.05$) lower among the mild COPD than very severe. The IL-1β was also significantly ($p<0.05$) lower among the moderate and severe than very severe.

DISCUSSION

COPD is a slowly progressive disease induced primarily by smoking tobacco. FEV_1 is measured that is a spirometric predictor of severity in patients of COPD till date. Factor that affect the decline of FEV_1 have a prognostic importance in COPD **[18].** These results depicted that there was a positive correlation between serum levels of TNF-α, IL-6 and IL-1β and severity of COPD so we can say that as the serum levels of TNF-α, IL-6 and IL-1β increases the severity of COPD also increases. Population based evidence provides that COPD patients have a pro-inflammatory state with increased circulating levels of many inflammatory cytokines. The main finding was that among the severity of COPD stages there was significant ($p<0.01$) difference in the level of TNF-α, IL-6 and IL-1β. TNF-α, IL-6 and IL-1β levels were significantly ($p<0.05$) higher in serum among the very severe COPD than mild, moderate and severe COPD patients. These results correlates with **Vernooy et al., 2002** who declared that several inflammatory markers and mediators have been shown to be increased in the plasma or serum of COPD patients **[19]**. COPD is associated with low-grade systemic inflammation as demonstrated by an increase in blood leukocytes, acute-phase proteins C-reactive protein (CRP) and fibrinogen and inflammatory cytokines as TNF-α, also, during acute exacerbations of COPD, higher levels of interleukin-6 as well as acute-phase proteins CRP, fibrinogen, and lipopolysaccharide binding protein (LBP) have been demonstrated **[20]. Victor pinto-plata et al., 2007** also showed increased serum levels of TNF-alpha among the severity of COPD stages **[21]**. **Nervana Samy et al., 2010** showed significant increase in serum inflammatory markers IL-6 and TNF-α in stage I, II and III COPD and this increment was significantly higher than those of the control and stage 0 groups. Also patients with severe COPD (stage III) showed highly significant elevation in of these biomarkers in comparison to stage 0, I and II but level of TNF-α were more raised in corresponding stages in present study **[22]**. This significance raised value of TNF-α in corresponding stage of COPD may be due to race or genetic factor. Likewise IL-1β levels were significantly ($p<0.05$) higher in serum among the very severe COPD than mild, moderate and severe COPD patients and this was similarly reported by **B. Singh et al., 2010** showed increased serum levels in severity of COPD stages **[23]**. **Akbulut H H et al., 2009** also showed increased serum levels of IL-6 among the severity of COPD stages **[24]**. **Sapey et al., 2009** who demonstrated significantly high levels of IL-1 beta in serum of the COPD patients as compared to the healthy controls and also proved that IL-1 beta plays critical role in COPD where it was found to correlate significantly with FEV_1 suggesting its role in clinical aspects of disease severity **[25]**. **Schmidt Loanas M et al., 2006** reported that the sputum levels of cytokines were significantly increased as compared to serum levels **[26]**. In the study by **Kochetkova E A et al., 2004** changes in the cytokine status in COPD patients were established and an increase in pro-inflammatory cytokines. There was hyperproduction of serum pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) dependent on FEV_1 **[27]**. Firstly, our COPD patient sample does not turn out to be necessarily representative of the COPD regularly seen in clinical practice. Due to the design of the present study, and to avoid the confusion by comorbidities, patients with several associated illness were excluded from the analysis. Present study is conducted in limited sample size i.e. 100 patients of COPD at tertiary care centre. If such type of study conducted at multi centric level the results may be more appropriate.

CONCLUSION

Despite the limitations mentioned, the present study reinforces the view that systemic inflammation is an important phenotypic feature of COPD. Future prospective studies should investigate if these markers will give important prognostic information in relation to disease progression and severity in COPD. Cytokine levels of TNF-α, IL-1β and IL-6 levels were substantially increased in patients with COPD and well co-related with severity.

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