Original research article

Study Of Systemic Inflammatory Parameters In Chronic Obstructive Pulmonary Disease Patients.

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Abstract

Chronic Obstructive Pulmonary Disease (COPD) is a name coined for the diseases previously known as chronic bronchitis and emphysema. COPD is defined as common preventable and treatable disease characterized by persistent airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases. This study was planned to observe the evidence of systemic inflammation in different stages of COPD. The present study was a cross sectional study carried out in 100 stable male COPD patients. Following inflammatory parameters were included in study , C- Reactive Protein (CRP) , Erythrocyte Sedimentation rate (ESR), Differential leucocyte count (DLC): neutrophil count (%) , Total leucocyte count (TLC). 100 Subjects were divided in four groups from mild to very severe (GOLD stage I-IV) according to spirometry values based on FEV1% predicted and FEV1/FVC ratio < 0.7. Then changes in systemic inflammatory parameters in different stages of COPD were observed applying ANOVA test. P value <0.05 was considered statistically significant. TLC was significantly increasing ($p<0.05$) as the severity of COPD increases. Neutrophil count also significantly increased with GOLD, COPD stages ($p<0.5$). Mean values of ESR showed an ascending pattern as the severity of disease progressed but it was not statistically significant ($p >0.05$). CRP level was elevated in most of the patients of severe (III) and very severe (IV) patients suggesting presence of systemic inflammation. As COPD is a multifactorial heterogeneous disease so, for determining the severity of COPD, other parameters such as inflammatory markers which will predict the morbidity as well mortality of the patients should be included.

Key words: COPD, Inflammatory parameters

Introduction

Chronic Obstructive Pulmonary Disease (COPD) is a name coined for the diseases previously known as chronic bronchitis and emphysema. Recently the Global Initiative for Chronic Obstructive Lung Disease (GOLD) defined COPD as "a common preventable and treatable disease characterized by persistent airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases. Exacerbations and comorbidities contribute to the overall severity in individual patient". [(1)] The global prevalence of physiologically defined chronic obstructive pulmonary disease (COPD) in adults aged more than 40 year is approximately 9-10 % .[(2)] In India, the prevalence of COPD is 4.1% with a male-to-female ratio of 1.56:1 in the population of above 35 years of age. [(3)] COPD is the fourth-leading cause of mortality

worldwide. Mortality from COPD is expected to increase further. It will rank at the third position causing 6 million deaths annually in 2020 [4]. According to the Global Burden of Disease Study, it results in 1.68 years of 'living with disability' (YLD) per 1,000 population, representing 1.8% of all YLDs, with a greater burden in men than in women (1.93% vs. 1.42%). [5] The general ageing of the world's population is reinforcing this trend, as the prevalence is higher in age groups more than 50 years and because the incidence remains high in the elderly. The estimated risk of developing COPD for a male aged greater than 55 year, who is free from COPD, over the next 40 years is 24%.[6] There is an overall increase in mortality, morbidity, health care burden and per capita cost for COPD in our country too. Prevalence and morbidity data greatly underestimate the total burden of COPD because the disease is usually not diagnosed until the patients already have had symptoms for some time and the disease is then often already quite advanced. [7] Epidemiological studies and clinical trials conducted in last decade have helped us to understand the pathophysiology of COPD better. In recent years there has been significant uptake of COPD as a systemic inflammatory disease rather than a disease of respiratory system. Evidence prove that COPD is not a disease limited to lungs alone, it often associated with significant extra-pulmonary abnormalities, the so-called "systemic effects" of COPD. These systemic effects are clinically relevant and may contribute to thorough understanding and management of the disease. Evidence of systemic inflammation has been reported in the form of either increased circulating cytokines, chemokines and acute phase proteins, or as abnormalities in circulating cells such as neutrophils, macrophages and a large number of activated T cells. The low-grade systemic inflammation in these patients is due to "spill over" of inflammatory mediators from the inflamed pulmonary compartment. [8] The components of this systemic inflammation may worsen the co-morbid diseases and also might enhance clinical symptoms that causes negative impact on quality of life. [9] Measurement of systemic biomarkers in COPD patients has emerged as a new tool, having many applications. It may be useful for assessment of exacerbation episodes as well as combination of biomarkers and clinical symptoms may identify the etiological origin and degree of severity. It would be also helpful in monitoring and assessing the clinical evolution of the disease, correlating biomarker levels with the response to interventions. These approaches would enable a better risk stratification and correct management of COPD patients. [10] Though COPD is not fully reversible, the negative impact of the systemic inflammation could be reversed by appropriate therapy. The mainstay of treatment for COPD is suppression of inflammation to prevent its consequences. Hence, identification of inflammatory process and estimating its severity is important for clinical evaluation. There has been considerable research being undertaken to identify the nature of systemic inflammation and to predict the clinical outcomes. This may also identify new targets for therapy in the history of COPD. So, the study was planned to observe the evidence of systemic inflammation in different stages of COPD.

Material and Methods

The present study was carried out in department of Physiology in collaboration with Chest and Tuberculosis department. The study protocol was approved by the Institutional Ethics Committee (IEC) of Indira Gandhi Government medical college, Nagpur.

Study design: Cross sectional study.

Study population: 100 Male patients of age group 40-65 years, coming in Chest and Tuberculosis, Out Patient Department, diagnosed as COPD by chest physicians, were included in the study. All patients with COPD were in stable state at the time of study. After registration of the subject, a detailed clinical history was obtained from each patient including history of occupation, smoking per pack-year, disease onset, exacerbations, associated co-

morbidities, and current therapy. Clinical examination was done in all patients and findings were recorded.

Selection Criteria:

1. Male patients of age group 40-65 years.
2. Diagnosed as COPD by chest physicians.
3. Clinically stable on examination without any acute exacerbation.
4. On medication according to the stage of their disease.
5. No self-reported asthma or reversibility >12% of airway obstruction after administration of a β_2 agonist.

Exclusion Criteria:

Persons with following features were excluded from the study:

1. Diagnosed cases of bronchial asthma.
2. Respiratory infection in the last 4 weeks.
3. Active lesion of tuberculosis.
4. Use of systemic corticosteroids
5. Subjects with gross clinical abnormality of vertebral column, thoracic cage, neuro-muscular diseases.
6. Diagnosed cases of malignancy, drug addiction & alcoholism.
7. Subjects who had past abdominal or chest surgeries.
8. Known cases of Diabetes Mellitus, Coronary Artery Disease, Hypertension.
9. Patients with history of unstable angina or a heart attack previously.

Study Protocol:

The selected patients were given appointment from 10: 00 am -12:00 pm in Physiology Department 2 days after their registration in chest OPD. They were asked to keep 4 hours fasting before coming for the study procedure on the day of appointment. They were also told to avoid tea, coffee, alcohol 48 hour prior to the tests. The whole procedure of investigation and purpose of the study was explained to all subjects and informed written consent was obtained. Their queries (if any) were solved to their full satisfaction to decrease the anxiety and apprehension. Following inflammatory parameters were included in study.

- **Acute phase reactants**

- C- Reactive Protein (CRP)
-Erythrocyte Sedimentation rate (ESR)

- **Circulating white blood cells**
  - Differential leucocyte count (DLC): neutrophil count (%)
  - Total leucocyte count (TLC)

Collection of blood: Under all strict aseptic precaution 5 ml of blood was collected from anti-cubital vein by venepuncture. EDTA blood collected in plain vial for TLC, DLC were sent in the central haematology laboratory. The blood sample from another plain vial was then centrifuged to separate serum for estimation of CRP. Citrated anticoagulated blood was used for ESR measurement.

Total leucocyte count (TLC):

Principle: Blood was diluted with an acid solution named Turk's fluid containing glacial acetic acid & gentian violet that removes red cells by haemolysis and accentuates the nuclei of white cells respectively. Counting was done using a microscope under low- power 10X objective and with the knowledge of the volume of fluid examined, the number of white blood cells per mm^3 of undiluted whole blood was calculated by multiplying dilution factor 50 with number of white cells counted under microscope. [(11)] Normal values of TLC in adults:

4,000-11,000/mm^3 of blood. There is no gender variation in TLC. In inflammatory conditions it may climb extraordinarily high levels to 15,000-20,000cells/mm^3 of blood.

Differential leucocyte count (DLC) - neutrophil%:

Principle: A tongue shaped peripheral smear was prepared by using clean, grease free glass-slides. Then the dried peripheral smear was stained with Leishman stain and examined under oil-immersion 100X objective. The different types of White Blood Corpuscles were identified and counted by moving the slide in a zigzag manner. The percentage of neutrophil was determined. (12) The percentage of neutrophils in adult blood is 50-70 percent.

Erythrocyte Sedimentation Rate (ESR): by Westergren's method

The International Committee for Standardization in Haematology (ICSH) recommends the use of the Westergren method to measure the ESR than Wintrobe's method. So, in our study ESR was determined by Westergren's method. (13) Normal values of ESR depends on age and gender, and for men they present themselves as follows: (14)

-15 mm/hour or less for men < 50 years old

-20 mm/hour or less for men > 50 years old

Principle: The ESR reflects red blood cell aggregation and it is measured by fall or setting of a vertical column of erythrocytes within 1 hour when held vibration free at room temperature. Venous blood was diluted in 4:1 ratio where 4 part is blood mixed with one part sodium citrate (3.8%) and placed in a 200-mm glass tube with a 2.5-mm internal diameter. At the end of 1 h, the distance from the meniscus to the top of the column of erythrocytes is recorded as the ESR in units of millimetres per hour.

C- reactive protein (CRP): By Nirmal's latex turbimetric method.

Principle: Polystyrene latex particles coated with anti-CRP monoclonal, IgG stabilized in glycine buffer pH 8.2. Then plasma samples were centrifuged within 30 min at 3000 r.p.m. Serum containing CRP is mixed with the latex a clearly visible agglutinate forms if the CRP concentration between 0.6 to 40 mg/dl. There is no agglutination with CRP concentration below 0.6 mg/dl.

Spirometry: The technique of performing various lung function tests in the present study was based on operation manual of the instrument with special reference of American Thoracic Society. The following parameters of spirometry were recorded:

1) Forced expiratory volume in one sec (FEV1) in % predicted.
2) Forced vital capacity (FVC) in % predicted.
3) FEV1/FVC ratio in % predicted.

The study subjects were staged as per GOLD guidelines, 2013: Post bronchodilator (FEV1/FVC ratio<70% predicted), mild (FEV1≥80% predicted), moderate (50%≤FEV1<80% predicted), severe (30%≤FEV1<50% predicted), and very severe (FEV1<30% predicted) or FEV1<50% predicted plus the presence of signs of chronic respiratory failure. The patients were divided into four groups (I, II, III and IV) according to the severity by GOLD stages.

Statistical Analysis: The data was presented as mean (SD). Statistical significance of differences in all study groups were estimated with one-way Analysis of Variance (ANOVA) with an appropriate post hoc test (Tukey's post hoc test) wherever necessary. For categorical variables Chi-square test was used. Main Analysis was performed using statistical software SPSS version 22; $p<0.05$ considered significant.

Results:

The present study was a cross-sectional study carried out in the department of Physiology in collaboration with Chest and T.B department of our institute. The study population included 100 male, COPD patients who were clinically stable.

Following were the inflammatory parameters assessed in the study:

Systemic Inflammatory Parameters:

- TLC (/mm^3 of blood)
- DLC (Neutrophil %)
- ESR (mm)
- CRP (considered positive when plasma level of CRP elevated >0.6 µl and negative <0.6 µl)

100 Subjects were divided in four groups from mild to very severe (GOLD stage I-IV) according to spirometry values based on FEV1% predicted and FEV1/FVC ratio <0.7. Then changes in systemic inflammatory parameters in different stages of COPD were observed applying ANOVA test. P value <0.05 was considered statistically significant.

On examining the study participants for systemic inflammatory parameters like TLC, neutrophil % and ESR following results were obtained. The mean values of each parameter were subjected to comparison by ANOVA test.

Table 1: Systemic inflammatory parameters of the 100 COPD patients in different stages by GOLD criteria.

Parameter	**I (n = 13)**	**II (n = 20)**	**III (n = 26)**	**IV (n = 41)**	**P value**
TLC (/mm^3 of blood)	7500 (716.47)	9730 (1038.77)	11654.88 (814.84)	13238.46 (782.34)	<0.05
Neutrophil (%)	55.08 (3.22)	60.45 (4.26)	66.51 (3.70)	72.65 (2.80)	<0.05
ESR (mm)	31.42 (8.52)	33.51 (5.02)	35 (11.18)	39.37 (2.98)	>0.05

Data presented as mean (SD). Results of ANOVA with p value <0.05 considered significant; p>0.05 not significant.

Table 1 shows there is significant increase in both TLC and neutrophil as the disease progresses in more advanced stage with p value <0.05. Mean values of ESR also shows a rising pattern as the severity of disease increases but it is not statistically significant (p >0.05).

Table 2: Showing distribution of 100 COPD patients in relation to CRP+/- cases in different stages by GOLD criteria.

CRP(µl)	STAGES OF COPD				
	I n = 13	**II n = 20**	**III n = 41**	**IV n = 26**	$X^{2 =}$ 41.52
Positive (>0.6 µl)	2	5	32	25	Df = 3

Negative (<0.6 µl)	11	15	9	1	P<0.0001; HS

*Chi square test; p<0.0001 highly significant.

Patients were positive for CRP in advanced stages (III and IV). Most of the patients in earlier stages (I and II) were negative for CRP. Maximum CRP positive cases were found in stage III, 32 patients among 41 followed by in stage IV, 25 patients out of 26.

Discussion:

Total Leucocyte Count and Neutrophil %

The result of present study showed mean TLC was increasing significantly with the GOLD stages. The mean values were 7500 ± 716.47 in stage I, 9730 ± 1038.77 in stage II, 11654.88 ± 814.84 in stage III,13238.46 ± 782.34 cells /mm^3 of blood in stage IV ($p<0.05$). The neutrophil % also increased significantly as the disease progressed with p value <0.05. The mean neutrophil % according to the GOLD stages were 55.08 ± 3.22, 60.45 ± 4.26, 66.51 ± 3.70, 72.65 ± 2.80 cells (from stage I –IV) respectively. Several other studies in this field which showed similar results are, Rufino R.et al (2007), Ischaki et al (2009), Devi et. al (2014), Sin D. D. et al (2015). [15,16,17,18] Devi et al (2014) reported there was significant increase in both total leukocyte count as well as absolute neutrophil count with the fall of FEV1. According to Sin D. D. et al (2015) low grade systemic inflammation was present in moderate to severe airflow obstruction. They observed participants mainly in the severe group (stage III) had higher circulating leucocyte and neutrophil count which was similar with our finding. In our study we observed chronic inflammation was characteristically present among all stages of COPD but highest in very severe (IV) patients. Rufino R.et al (2007) have observed neutrophil % and total leucocyte count was significantly higher in induced sputum from patients of COPD compared with smokers and nonsmokers. Ischaki et al (2009) have shown there is significant increase in neutrophil % in induced sputum as the disease progressed to advanced stage with lowest mean in stage I (57 ± 6) and highest in stage IV (67 ± 5). In the literature, there are reports that ongoing inflammation was often determined by evidence of inflammatory cells in the respiratory tract. The possible explanation for this fact referred to the rapid migration of neutrophils from the vessel toward the airways, making it more common to find them in the airways. But in the present study, circulating levels of leukocytes count largely by neutrophilc subpopulation in blood raised in stable COPD which cannot be attributed to pulmonary infection, since the patients were free of exacerbations. We found significant relationship of TLC and neutrophil % with the severity of the disease. The cause may lie in the pathophysiology of COPD itself. Neutrophils release multiple mediators and tissue degrading enzymes such as elastases that orchestrate tissue destruction and chronic inflammation. In COPD the neutrophilic inflammatory response dominates. Some authors also suggested there is increased monocyte count and eosinophilia along with raised circulating CD8+ T cells. [19] The other reasons of high inflammatory cells may be due to use of steroids. Although both TLC and neutrophil% showed significant relationship with COPD staging, the role of systemic inflammatory cells as markers of severity needs further research.

Erythrocyte Sedimentation Rate (ESR) and C-reactive protein (CRP):

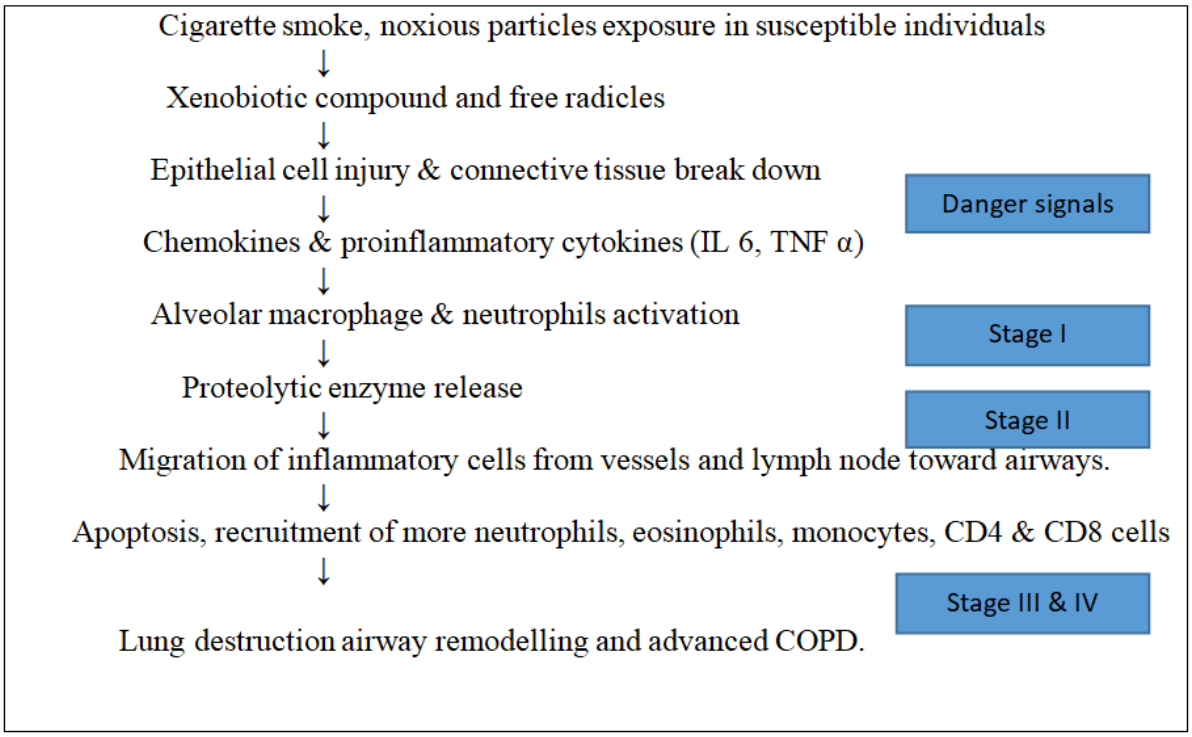
ESR measured by Westergren method shows an ascending pattern as the severity of disease increases but it was not statistically significant ($p>0.05$). Mean ESR in stage I was 31.42 ± 8.52; in stage II, 33.51 ± 5.02; in stage III, 35 ± 11.18 and in stage IV, 39.37 ± 2.98 mm. The mean ESR was 31.46 ± 9.57 mm. in all 100 COPD patients. Another systemic inflammatory

marker we assessed was CRP. It was observed in table-7 as the disease became severe more patients were with elevated CRP. Maximum number of CRP elevated patients were found to be in stage III (32 patients among 41) followed by in stage IV (25 out of 26 patients) which was statistically significant ($p<0.01$). Corsonello A. (2010) evaluated both CRP and ESR in stable COPD patient. They found CRP not ESR, shows a weak correlation with COPD severity. Shameem M. et al (2011) studied strong negative association between CRP level and lung function (FEV1%). Nillawar A. et al (2012) also showed hs-CRP raised significantly in stable COPD patients. They established elevated CRP level in stable COPD is a high risk factor for cardiovascular complication. A meta-analysis conducted by Zhang Y. (2015) showed higher level of CRP concentration might be an indicator of disease severity. (20,21)

ESR is an important marker in the diagnosis of many inflammatory conditions and in the prognosis of non-inflammatory conditions. Current studies suggest that the ESR when elevated remains high until the primary inflammatory process is resolved. (22) So, ESR may be a less expensive alternative to C-reactive protein marker of systemic inflammation in stable COPD. It increases in response to rising serum levels of acute phase proteins, fibrinogen, immunoglobulins, as well as in response to anaemia. This may seem to be an advantage in the assessment of patients with COPD as that disease is mainly connected with haemolytic anaemia. In our study higher level of ESR was found in stage III and stage IV. Hence, ESR can also be considered as a potential marker of COPD severity especially when considering it as a systemic and not only respiratory disease. It is also suggested that such an almost cost-free procedure will be well suited for low-income countries, where the prevalence of COPD is dramatically increasing.

Available literature for raised CRP in stable COPD says that it is a non-specific marker of systemic inflammation which is synthesized by the hepatocytes in response to IL-6. It is a stable molecule used as acute phase reactant with half-life of 18-24h. CRP may deposit directly into the arterial wall during atherogenesis, interacting with other inflammatory mediators to create foam cells, which serve as building blocks of atherosclerotic plaques. Leucocytosis and neutrophilic inflammation may destabilize atherosclerotic plaques, leading to their rupture. Both ESR and CRP can be influenced by several non-pulmonary factors such as age, sex, drugs, and inflammatory process in other anatomical regions. So, as suggested by previous investigators, their clinical application in prediction of severity of COPD is partly limited and the results should be explained with caution.

Flow chart showing possible mechanism of increase in inflammatory cells in COPD:



Courtesy: **Herzog R. and Cunningham-Rundles S. Immunologic impact of nutrient depletion in chronic obstructive pulmonary disease. Current Drug Targets 2011; (12): 489-500.**

CRP levels tend to increase as COPD progresses and conversely nutritional status worsens. This may be associated with a poor prognosis. Treatment in severe COPD should also be directed toward inflammation and nutritional status, as this may improve prognosis. The study shows that increased systemic inflammation is present in COPD patients, and CRP is an important biomarker in reflecting severity of disease. [23] COPD is the result of an abnormal and persistent inflammatory process that damages the lung architecture, resulting in progressive physiological deterioration. Understanding the processes involved can lead to the rational development of new molecular biomarkers that can be used to monitor disease activity to identify individuals at high risk of rapid disease progression.[24]

Conclusion:

Total Leucocyte Count, Differential Leucocyte Count (Neutrophil count), C - reactive protein and ESR were measured to observe systemic inflammation in COPD patients. These markers of inflammation increase with the severity of COPD. Thus, there is a state of chronic systemic inflammation. The easy and cost-effective inflammatory markers such as TLC, neutrophil%, ESR and CRP measurement should be done routinely in the patients of COPD for knowing the level of systemic inflammation. As COPD is a multifactorial heterogeneous disease with multisystem involvement, classification of COPD severity depending on FEV_1% predicted seems inadequate. So, for determining the severity of COPD, other parameters which will predict the morbidity as well mortality of the patients should be included such as inflammatory parameters.

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