



Original Research Article

Complement components C3 and C4 levels in Chronic Obstructive Pulmonary Disease (COPD) patients and their correlation with severity of airflow obstruction

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ABSTRACT

Introduction: Although a great deal is known about etiopathogenesis of Chronic Obstructive Pulmonary Disease (COPD), there are still substantial lacunae in comprehending the role of immunity in the part played by recurrent secondary infections occurring in patients with COPD who have been shown to present decreased serum levels of complement components C3 and C4 than healthy subjects.

Objectives: Measure and compare the levels of complement C3 and C4 in COPD patients with healthy controls and also correlate their levels with severity of COPD.

Materials and Methods: This was a prospective case control study conducted at GMC, Bhavnagar with 75 known cases of COPD patients and 75 controls. Cases were subdivided into three subgroups with Pulmonary Function Test scores. Biochemical investigations from venous blood samples were performed on Mispal i2 specific protein analyzers. Data analysis was done by using unpaired t-test, one-way ANOVA test and post hoc test.

Results: In the present study the levels of complement C3 (Case: 142.23 ± 47.23 , control: 160.48 ± 37.77 and $p < 0.005$) and C4 (Case: 26.51 ± 5.50 , control: 36.13 ± 5.06 and $p < 0.0001$) were significantly decreased in COPD patients in comparison to healthy subjects. In addition to that C4 levels were significantly decreased in between mild to moderate and mild to severe obstructive COPD cases ($p < 0.0001$), While C3 levels were decreased with increasing severity of COPD but they were not statistically significant ($p > 0.05$).

Conclusion: It is suggested from this study that measurement of complement levels may be used to assess severity of obstruction in COPD patients with more emphasis given to C4 levels.

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1. Introduction

Chronic obstructive pulmonary disease (COPD) is a major cause of burden on health care worldwide and one of the leading cause of death that is increasing in prevalence.^{1,2} According to World Health Organization (WHO) estimates, 65 million people have moderate to severe COPD and it is expected to be the third most common cause of death worldwide in the year 2020.³ COPD mortality is very hard to assess because of nomenclature inconsistencies and

COPD not being listed as the cause of death on death certificates; for this reason, COPD mortality is likely to be underestimated.⁴

COPD is a chronic inflammatory condition affecting predominantly lung parenchyma and peripheral airways which leads to progressive and largely irreversible airflow limitation.¹ COPD is not merely a "smoker's cough" but it is an under diagnosed, life threatening lung disease.² Etiopathogenesis of COPD is largely driven by environmental factors, although genetic susceptibility is also an important factor. Tobacco smoking and other hazards for COPD is related to an interaction involving

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genetic factors and various environmental exposures, which could also be affected by comorbid diseases.^{5,6}

The complement system, which includes three independent but interacting pathways, makes up a potent arm of innate immunity. Even though it is a fine-tuned system with innate capacity to distinguish self from non-self along with danger from non-danger signals, an unnecessary activation can however arise which can cause tissue destruction.^{7,8} Activation of complement system promotes incursion of inflammatory cells into the lung parenchyma with ensuing release of elastases and oxidants that can injure and cause damage to elastic lung tissue. It has been postulated that there might be a quantitative relationship among complement consumption and extent of elastic tissue damage in lungs.⁹ Sustained activation of complement pathways due to recurrent respiratory tract infections, demonstrated by the presence of decreased levels of serum C3 and C4, may bear a quantitative relationship to the extent of elastic tissue destruction since quantitatively C3 and C4 combinedly forms major portion of the complement system.⁹⁻¹²

Even though a great deal is known about etiopathogenesis of COPD, there are still substantial lacunae in comprehending the role of immunity in the part played by recurrent secondary infections occurring in patients with COPD who have been shown to present decreased serum levels of complement components C3 and C4 than healthy subjects. Whereas few studies reports no correlation between the level of complement and severity of COPD demonstrating lack of clear cut knowledge about the complement role in COPD.^{9,11} Therefore, we sought to evaluate the levels of C3 and C4 levels in COPD patients and their correlation with disease severity.

2. Materials and Methods

The present study was conducted at Department of Biochemistry, Government Medical College and Sir Takhtsinhji General Hospital, Bhavnagar after prior approval from Institutional Ethics Committee. In this study total 75 known cases of COPD (Group A) between the age group of 35 to 65 years were enrolled. The COPD patients were primarily assessed by clinical examination and Pulmonary Function Tests (PFTs) for the diagnosis of COPD. All cases were out patient department (OPD) based in Pulmonary Medicine Department of Sir Takhtsinhji General Hospital, Bhavnagar. A group of 75 normal healthy individuals, age and sex matched from the same population served as controls (Group B). The subjects having (i) immunological disorders that might interfere with complement activation (ii) associated bronchial asthma and (iii) being unable to perform pulmonary function tests were excluded. For correlation of complement C3 and C4 with severity of disease, Group A (COPD patients) was subdivided into three subgroups according to Global Initiative for Chronic

Obstructive Lung Disease (GOLD) criteria.¹³ Group A1 included 34 patients with mild airflow obstruction (forced expiratory volume in 1 second [FEV₁]/forced vital capacity FVC < 0.70 with FEV₁ ≥ 80% normal), Group A2 included 26 patients with moderate airflow obstruction (FEV₁/FVC < 0.70 with FEV₁ 50-79% normal) and Group A3 included 15 patients with severe airflow obstruction (FEV₁/FVC < 0.70 with FEV₁ 30-49% normal). Informed consents were taken from all the patients and subjects participating in this study.

Venous blood was collected from each patient and analyzed for complement C3 level (Nephelometric immunoassay method)^{14,15} and complement C4 level (Turbidimetric immunoassay method)^{14,15} in Mispai 2 Specific Protein analyzer (Agappe Diagnostics, Switzerland) at Clinical Biochemistry Section, Laboratory Services Sir Takhtsinhji General Hospital, Bhavnagar. Results of the present study were analyzed by using GraphPad InStat version 3.0. In data analysis, comparison of this parameter between COPD patients and controls were carried out by applying unpaired t-test. Correlation of the levels of complement C3 and C4 with severity of COPD was carried out by applying one-way ANOVA test and post hoc test. Interpretation was done according to p-value (p < 0.001 – highly significant, p < 0.05 – significant and p ≥ 0.05 – not significant)

3. Results

In the present study Group A (cases) and Group B (control) each contained 64 male participants and 11 female participants. The majority of the participants were male and the gender ratio was same in both groups. Mean age in study Group A was 55.60 ± 8.58 years while it was 54.37 ± 8.02 years in Group B. Maximum number of patients belonged to age group between 56-65 years in both the groups. (Table 1)

Mean value of serum complement C3 level in COPD patients was 142.23 ± 47.23 whereas in control was 160.48 ± 37.77. Mean value of serum C4 level in COPD patients was 26.51 ± 5.50 whereas in control was 36.13 ± 5.06. (Table 2)

Mean value of serum complement C3 level in Group A1 were 149.29 ± 44.89, Group A2 were 139.00 ± 50.52 and Group A3 were 131.80 ± 47.16. Mean value of serum complement C4 level in Group A1 were 30.09 ± 4.97, Group A2 were 24.39 ± 3.34 and Group A3 were 22.07 ± 4.58. (Tables 3, 4, 5 and 6)

4. Discussion

Most important function of the complement system is to identify and destroy pathogenic microorganisms along with removal of modified self-antigens. In animal models the activation of complement system leads to the accumulation of neutrophils and macrophages into alveoli and pulmonary

Table 1: Age distribution in Group A and Group B

Age in Years	Number of Cases Group A	Percentage (%)	Number of Subjects Group B	Percentage (%)
35-45	14	18.67	15	20.00
46-55	17	22.67	19	25.34
56-65	44	58.67	41	54.67
Total	75	100	75	100
Mean \pm SD	55.60 \pm 8.58		54.37 \pm 8.02	

Table 2: Comparison of biochemical parameters between Group A and Group B

Parameter	Biological Reference Interval ¹⁵	Group A COPD patients			Group B Healthy control			Statistical Significance
		Minimum level observed (mg/dl)	Maximum level observed (mg/dl)	Mean \pm SD	Minimum level observed (mg/dl)	Maximum level observed (mg/dl)	Mean \pm SD	
C3	90-180 mg/dl	42	272	142.23 \pm 47.23	73	305	160.48 \pm 37.77	t=2.614 p=0.0099
C4	10-40 mg/dl	13	42	26.51 \pm 5.50	23	49	36.13 \pm 5.06	t=10.192 p < 0.0001

Note: p < 0.05 – significant, p < 0.001 – highly significant, p \geq 0.05 – not significant

Table 3: Comparison of C3 levels in three subgroups of Group A

	Number of patients	Minimum level observed (mg/dl)	Maximum level observed (mg/dl)	Mean \pm SD
Group A1 (COPD patients with mild obstruction)	34	61.0	262.0	149.29 \pm 44.89
Group A2 (COPD patients with moderate obstruction)	26	42.0	272.0	139.00 \pm 50.52
Group A3 (COPD patients with severe obstruction)	15	76.0	206.0	131.80 \pm 47.16

Table 4: Correlation of C3 levels among three subgroups of Group A

		Mean Difference	Std. Error	Statistical Significance
Group A1 (COPD patients with mild obstruction)	Group A2	10.2941	12.3382	P=0.683
	Group A3	17.4941	14.6797	P=0.462
Group A2 (COPD patients with moderate obstruction)	Group A1	-10.2941	12.3382	P=0.683
	Group A3	7.2000	15.3555	P=0.886
Group A3 (COPD patients with severe obstruction)	Group A1	-17.4941	14.6797	P=0.462
	Group A2	-7.2000	15.3555	P=0.886

Table 5: Comparison of C4 levels in three subgroups of Group A

	Number of patients	Minimum level observed (mg/dl)	Maximum level observed (mg/dl)	Mean \pm SD
Group A1 (COPD patients with mild obstruction)	34	19.0	42.0	30.09 \pm 4.97
Group A2 (COPD patients with moderate obstruction)	26	18.0	31.0	24.39 \pm 3.34
Group A3 (COPD patients with severe obstruction)	15	13.0	32.0	22.07 \pm 4.58

Table 6: Correlation of C4 levels among three subgroups of Group A

		Mean Difference	Std. Error	Statistical Significance
Group A1 (COPD patients with mild obstruction)	Group A2	5.7036	1.1454	p<0.0001
	Group A3	8.0216	1.3628	p<0.0001
Group A2 (COPD patients with moderate obstruction)	Group A1	-5.7036	1.1454	p<0.0001
	Group A3	2.3179	1.4256	p=0.241
Group A3 (COPD patients with severe obstruction)	Group A1	-8.0216	1.3628	p<0.0001
	Group A2	-2.3179	1.4256	p=0.241

capillaries. These complement activated neutrophils causes production of toxic oxygen radicals which leads to intrapulmonary capillary sequestration of neutrophils and vascular injury.^{10,16–18}

In COPD patients recurrent respiratory tract infections is seen very commonly leading to activation of complement system through chemotactically active fragments of complement proteins (C3a, C5a, C567) which causes influx of inflammatory cells into the lung parenchyma with subsequent release of elastases and toxic oxidants that causes damage to elastic lung tissue. This sustained activation of complement pathways with increasing morbidity and lung damage in COPD patients, ultimately leads to lower levels of complement C3 and C4 as they are combinedly comprise of approximately two thirds of the complement system quantitatively.^{9–11,16}

Data analysis showed that decrease in mean value of serum complement C4 level in COPD patients was highly significant (p<0.0001) and in complement C3 level it was significant (p<0.005) as compared to healthy controls (Table - 2). These findings are consistent with the studies conducted by Mahesh M *et al.*,⁹ Rao RP *et al.*,¹¹ Miller RD *et al.*¹² and Chauhan S *et al.*¹⁹ They reported that sustained activation of complement pathways due to recurrent respiratory tract infections in COPD patients leads to lower levels of complement C3 and C4.

On further correlating decrease in levels of Complement C3 and C4 with increasing severity of COPD it was found that statistically significant decrease (p<0.0001) in complement C4 levels in between mild and moderate obstructive COPD cases and also in between mild and severe obstructive COPD cases were present but in case of moderate and severe obstructive COPD cases although there was decrease in the mean value but it was not statistically significant (p=0.241). In contrast to that there was no statistically significant difference (p>0.05) in serum complement C3 levels in between mild, moderate and severe obstructive COPD cases (Table –3,4,5,6). These findings supports the results of previous studies conducted by Kosmas *et al.*¹⁰ and Mahesh M *et al.*⁹ The reason may be that complement C3 appears to be affected in a more complicated manner than the complement C4, as it is engaged in both the classic and alternative complement pathways and it is recognized to be degraded by other factors as well. This fact may be accountable for deranging

the quantitative relationship between severity of COPD and complement C3 serum levels, thus hampering the materialization of tighter correlations.¹⁰

Rao RP *et al.*¹¹ in their study observed that there was significant decrease in both complement C3 and C4 levels with increase in the severity of obstruction and lung destruction which is partially similar to the finding of our study.

5. Conclusion

It is concluded from the present study that complement C3 and C4 levels are decreased significantly in COPD patients compared to healthy subjects. The likely reason for lower complement levels might be complement consumption due to recurrent respiratory infection in COPD patients. In our study complement C4 levels decreased significantly with progressive severity of airflow obstruction in COPD, while only mild decrease was observed in complement C3 levels. It points towards that there may be a direct correlation between the severity of COPD and serum complement levels. Thus measurement of complement levels may be used to asses severity of obstruction in COPD patients and more emphasis can be given to C4 levels.

6. Source of funding

None.

7. Conflict of interest

None.

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