

STUDY OF COMPLEMENT C3 AND C4 LEVELS IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Medicine

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Abstract:

Chronic obstructive pulmonary disease (COPD) is a very common disease encountered in our hospitals. There are several conflicting studies which looked at the association of serum complement levels and various radiological and functional indices reflecting the severity of emphysema in patients with COPD. A few studies showed lower serum levels of complement components C3 and C4 compared to healthy subjects and this may indicate sustained complement activation as a result of recurrent infection of respiratory tract. Some studies showed no correlation at all. We studied the same association in our hospital to find out for ourselves.

Objective: Evaluation of patients of COPD with reference to levels of complement C3 and C4 and correlate with PFT and radiological emphysema score.

Key Words: COPD, C3, C4, FEV1, FVC, PFT

Introduction

Chronic obstructive pulmonary disease (COPD) is defined as a disease state characterized by airflow limitation that is not fully reversible. COPD includes emphysema, an anatomically defined condition characterized by destruction and enlargement of the lung alveoli; chronic bronchitis, a clinically defined condition with chronic cough and phlegm; and small airways disease, a condition in which small bronchioles are narrowed. COPD is also a disease of increasing public health importance around the world. Estimates suggest that COPD will rise from the sixth to the third most common cause of death worldwide by 2020.¹

Although a great deal is known about etio-pathogenesis of COPD, there are still significant lacunae in understanding of

the role of immunity in the part played by recurrent infections in COPD. There are several conflicting results in studies which looked at the association between serum complement levels and various radiological and functional indices reflecting severity of emphysema in patients with COPD.

Patients with COPD have been shown to exhibit lower serum levels of complement components C3 and C4 than healthy subjects, and this may indicate sustained complement activation as a result of recurrent respiratory tract infections.² Activation of complement leads to influx of inflammatory cells into the lung parenchyma with subsequent release of elastases and oxidants that cause damage to elastic lung tissue. It has been postulated that there might be a quantitative relationship between complement consumption and degree of elastic tissue

destruction. There have been studies which demonstrate that COPD patients with lower levels of C4 are those who experience respiratory infections and tend to have more radiological signs of Emphysema and have a predominant small airway resistance.

However, a small study from Turkey and a few other studies elsewhere found no correlation between the level of complement and severity of COPD indicating lack of clear cut knowledge about the complement role in COPD.³ The dichotomy in the results of various studies indicates the present gap in the knowledge about the role of complement in COPD. In view of very few studies carried out in our population regarding this matter hence we took up this study.

Methods

This was hospital-based, prospective, observational, and comparative study of COPD patients with emphysema during last 2year from October 2012 to September 2014.

Thirty five patients were included in study between age group of 40 and 70 years. Exclusion criteria are patients with immunological disorders that might interfere with compliment activation, patient's unable to perform pulmonary function tests PFT, pregnancy, and bronchial asthma. PFT was done by Spiroback-G machine. Global Initiative for Chronic Obstructive Lung Disease's criteria was used to stage the severity of COPD. Blood samples were collected in ethylenediaminetetraacetic impregnated Vacutainer and stored at 2°c until they could be transported and analyzed for C3 and C4 by immunoturbidity method in a lab accredited with NABL.

Cases were divided into three groups based on PFT-Mild (forced expiratory volume in 1 s [FEV₁]/forced vital capacity FVC<0.70 with FEV₁80% normal), moderate (FEV₁/FVC <0.70 with FEV₁ 50-79% normal), severe (FEV₁/FVC <0.70with FEV₁30-49% normal). Based on emphysema score0-6, 7-10, and >10. Controls were divided into different equal number of groups to match with subject cases for comparison.

Statistical quantitative data are represented as mean ± standard deviation. To assess the association among qualitative variables independent sample t-test and one-way ANOVA are used. Differences considered statistically

significant if P< 0.05. Statistical analysis was performed by SPSS version 18.0 for Microsoft Windows. Pearson's correlation co-efficient was used for bivariate correlations.

Observations

This was a prospective study for a period of 2-year (October 2012- September 2014) comprising 35 patients with COPD admitted at Santhiram Medical College and General Hospital.

Out of the total 70 subjects, 35 were cases (50%) and 35 were controls (50%)[Table1 and Graph1].

Gender distribution of cases

Out of the total 35 patients with COPD, 20 patients (57%) were males and 15 patients (43%) were females [Table 2 and Graph2].

Cases were predominantly seen in age groups 61-65 (28%) and 66-70 (22%) years [Table 3 and Graph 3].

Total numbers of controls were 35, in which males were 18 (51%) and females were 17 (49%) [Table 4 and Graph 4].

Comparison of cases with different degrees of obstruction based on PFTs with controls

There was no statistically significant difference (P= 0.97) observed in complement C3 levels and complement C4 levels (P=0.61) between COPD patients with mild PFT obstruction compared to age and sex matched controls [Table 5 and Graph 5].

In this group though there was no statistically significant difference (P=0.662) in complement C3 level, but there is significant difference observed in mean C3 values; and there was statistically significant difference (P=0.004) observed in complement C4 levels [Table 6 and Graph 6].

There was statistically significant difference in mean complement C3 (P=0.017), C4 (P=0.005) values compared to controls in this group [Table 7 and Graph 7].

There was no statistical significance observed among cases

with mild PFT obstruction (P=0.97) and moderate PFT obstruction (P=0.662) compared to controls and statistical significance (P=0.017) observed among patients with severe PFT obstruction compared to age and sex matched controls for complement C3 [Graph 8].

There was no statistical significance observed among cases with mild PFT obstruction (P=0.616) and statistical significance observed among patients with moderate PFT obstruction (P=0.004) and severe PFT obstruction (P=0.005) compared to age and sex matched controls for complement C4 [Graph 9].

When total COPD patients with different degree of obstruction were compared with controls though there was no statistically significant difference (P=0.054) in serum C3 levels, but there was significant difference in mean C3 values observed and there was statistically significant difference (P=0.002) observed in serum C4 levels [Table 8].

Comparison of cases with controls based on emphysema scores

When COPD patients with emphysema score (0-6) were compared with age and sex matched controls, there was no significant difference between serum C3 (P=0.988), C4 levels (P=0.725) observed [Table 9 and Graph10].

When COPD patients with emphysema score (7-10) were compared with age and sex matched controls; there was no statistically significant difference in C3 levels (P=0.412), but there was significant difference in mean vales observed; and there was statistically significant difference (P=0.003) present in serum C4 levels [Table 10 and Graph 11].

When COPD patients with emphysema score (>10) were compared with age and sex matched controls, there was statistically significant difference in both C3 (P=0.017), C4 levels (P=0.023) observed [Table 11 and Graph 12].

There was no statistical significance (P=0.988) observed among cases with emphysema score (0-6) and emphysema score 7-10 (P=0.412) compared to controls, and statistical significance observed among patients with emphysema score (>10) compared (P=0.017) to controls for complement C3 [Graph 13].

There was no statistical significance (P=0.725) observed among cases with emphysema score (0-6) and statistical significance observed among patients with emphysema score 7-10 (P=0.003) and for patients with emphysema score (>10) (P=0.023) compared to controls for complement C4 [Graph 14].

Table 1: Demographic characteristics of the study population	
Total number of subjects	Number
Cases	35
Controls	35

Table 2: Gender distribution of cases	
Gender distribution of cases	N (%)
Males	20 (57)
Females	15 (43)

Table 3: Age distribution of cases (n=35)		
Age distribution of cases (in years)	Number of patients (n)	Percentage
40-45	2	5
46-50	3	8
51-55	5	14
56-60	7	20
61-65	10	28
66-70	8	22

Table 4: Gender distribution of controls (n=35)		
Gender distribution of controls	Number of subjects (n)	Percentage
Males	18	51
Females	17	49

Table 5: COPD patients with mild obstruction compared with age and sex matched controls						
Complement	Mild obstruction	n	Mean complement level in mg/dl	Standard deviation	t value	P value
C3	(A1) Cases	12	120.33	26.925	0.030	0.976
	(B1) Controls	12	120.00	27.555		
C4	(A1) Cases	12	22.75	7.034	0.508	0.616
	(B1) Controls	12	23.92	3.704		

COPD: Chronic obstructive pulmonary disease

Table 6: COPD patients with moderate obstruction compared with controls						
Complement	Moderate obstruction	n	Mean (mg/dl)	Standard deviation	t value	P value
C3	(A2) Cases	10	110.00	51.631	0.445	0.662
	(B2) Controls	10	119.70	45.714		
C4	(A2) Cases	10	17.90	2.601	3.325**	0.004
	(B2) Controls	10	24.00	5.185		

COPD: Chronic obstructive pulmonary disease

Table 7: COPD patients with severe obstruction compared with controls						
Complement	Severe obstruction	n	Mean (mg/dl)	Standard deviation	t value	P value
C3	(A3) Cases	13	86.38	23.8	2.57	0.017
	(B3) Controls	13	117.00	35.7		
C4	(A3) Cases	13	16.00	3.416	3.088	0.005
	(B3) Controls	13	24.08	8.789		

COPD: Chronic obstructive pulmonary disease

Table 8: Comparison of total COPD patients with different degrees of obstruction with controls						
Complement	Study group	n	Mean (mg/dl)	Standard deviation	t value	P value
C3	Total cases	35	104	37.4	1.95	0.054
	Total controls	35	120.2	34.2		
C4	Total cases	35	19.17	5.5	3.12	0.002
	Total controls	35	23.43	5.9		

COPD: Chronic obstructive pulmonary disease

**Table 9:
COPD patients with emphysema scores (0-6) compared with controls**

Complement	Study group	n	Mean (mg/dl)	Standard deviation	t value	P value
C3	Cases(E1)	13	121.85	26.35	0.022	0.988
	Controls (H1)	13	122	27.55		
C4	Cases (E1)	13	22.69	6.7	0.356	0.725
	Controls (H1)	13	23.46	3.9		

COPD: Chronic obstructive pulmonary disease

**Table 10:
COPD patients with emphysema scores (7-10) compared with controls**

Complement	Study group	n	Mean (mg/dl)	Standard deviation	t value	P value
C3	Cases (E2)	11	104.36	48.7	0.83	0.412
	Controls (H2)	11	120	38.92		
C4	Cases (E2)	11	18.27	2.76	3.31	0.003
	Controls (H2)	11	23.9	4.92		

COPD: Chronic obstructive pulmonary disease

**Table 11:
COPD patients with emphysema scores (> 10) compared with controls**

Complement	Study group	n	Mean (mg/dl)	Standard deviation	tvalue	Pvalue
C3	Cases (E3)	11	82.45	23.81	2.59	0.017
	Controls (H3)	11	118.90	40.12		
C4	Cases (E3)	11	15.90	3.56	2.45	0.023
	Controls (H3)	11	22.90	8.76		

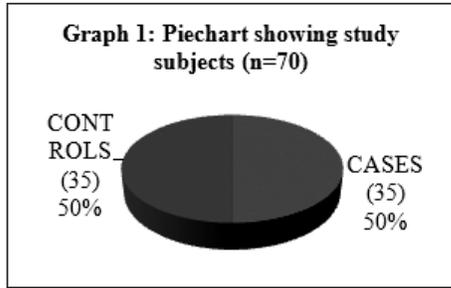
COPD: Chronic obstructive pulmonary disease

**Table 12:
Comparison of various studies exploring relationship of complement levels in COPD**

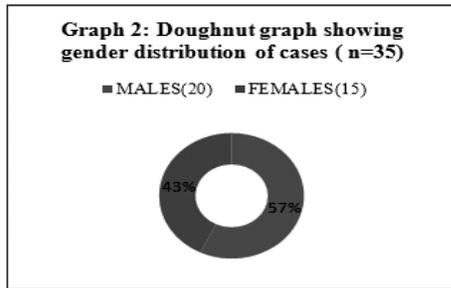
Author	Year	COPD and relationship with C3 and C4 complements
Miller et al. ⁶	1980	Low C3 and C4
Kew et al.	1987	Low C3
Chauhanet al. ³	1990	Low C3 and C4
Kosmas et al. ⁴	1997	Low C4
Fisunet al.	2000	No change in C3 and C4
Matejaet al. ⁹	2004	No change in C3 and C4
Serpilet al. ⁵	2006	Low C4
Present study	2013	Low C3 and C4

COPD: Chronic obstructive pulmonary disease

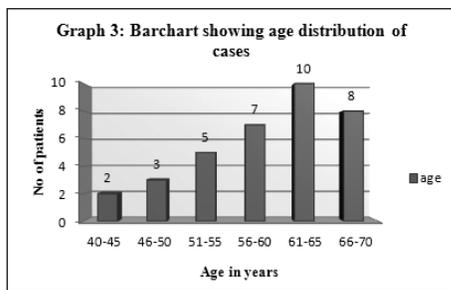
Graph Legends



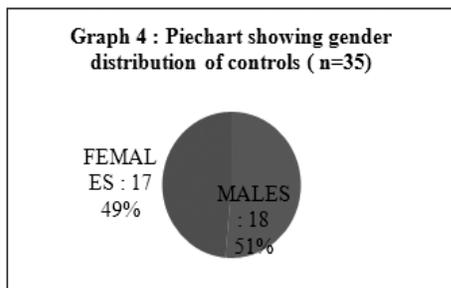
Graph 1: Study subjects (n=7)



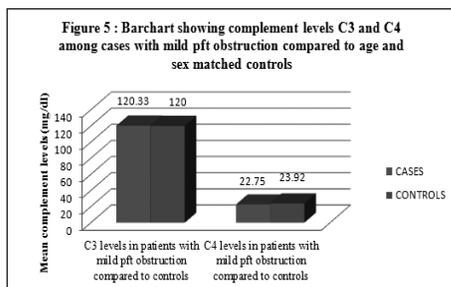
Graph 2: Gender distribution of case (n = 35)



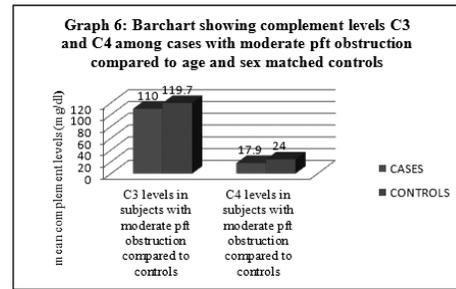
Graph 3: Age distribution of cases



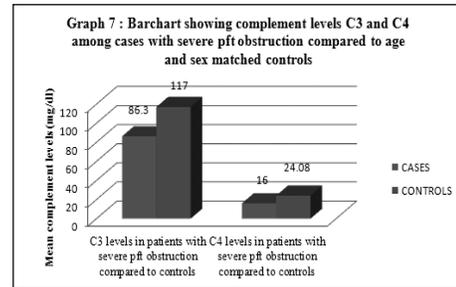
Graph 4: Gender distribution of controls (n = 35)



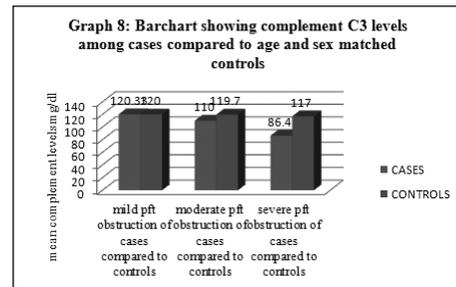
Graph 5: Complement levels C3 and C4 among cases with mild pulmonary function tests obstruction compared to age and sex matched controls



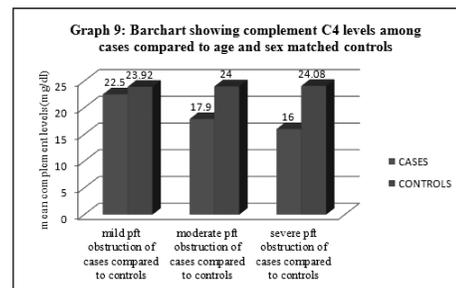
Graph 6: Complement levels C3 and C4 among cases with moderate pulmonary function tests obstruction compared to age and sex matched controls



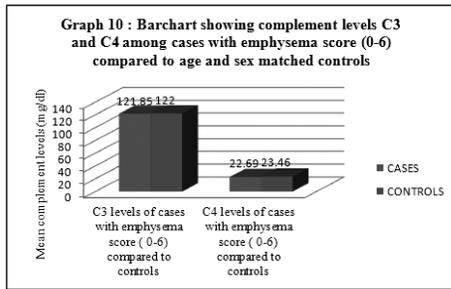
Graph 7: Complement levels C3 and C4 among cases with severe pulmonary function tests obstruction compared to age and sex matched controls



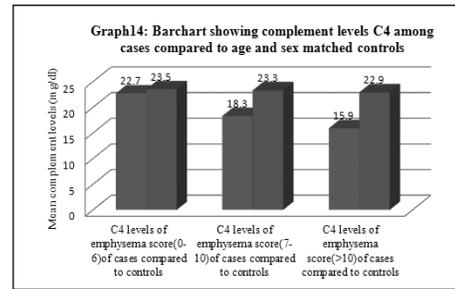
Graph 8: Complement C3 levels among cases compared to age and sex matched controls



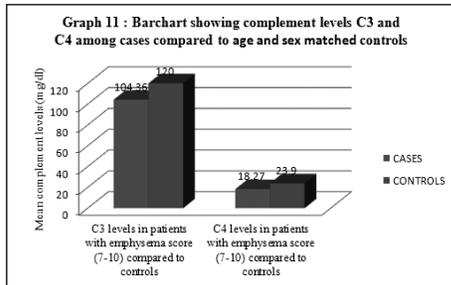
Graph 9: Complement C4 levels among cases compared to age and sex matched controls



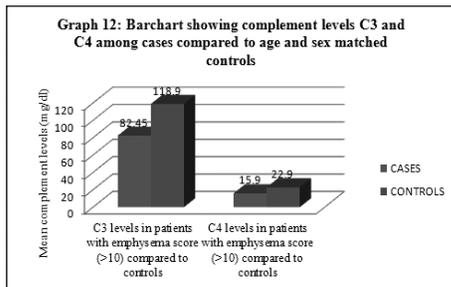
Graph 10: Complement levels C3 and C4 among cases with emphysema score (0-6) compared to age and sex matched controls



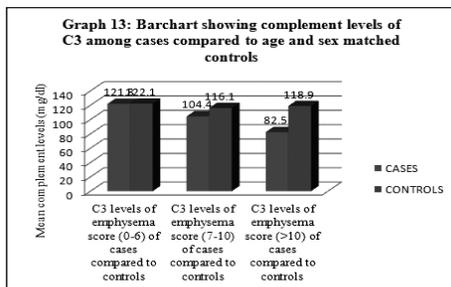
Graph 14: Complement levels



Graph 11: Complement levels C3 and C4 among cases with emphysema score (7-10) compared to age and sex matched controls



Graph 12: Complement levels C3 and C4 among cases with emphysema score (>10) compared to age and sex matched controls



Graph 13: Complement levels of C3 among cases compared to age and sex matched controls

Discussion

In the present study, out of 35 cases 57% (n=20) were males and 43% (n=15) were females. The male to female sex ratio was 1.3:1. This was in accordance with study done by Jindal et al.¹

In the present study, the maximum number of cases of COPD and exacerbations (60%) (n=21) were admitted during rainy and winter seasons (July-December). A similar trend was seen in a study done by Jenkins et al.⁴ Factors potentially contributing to this include increased exposure to viral infections, increased host susceptibility; greater time spent indoors, reduced physical activity, and temperature-related reduction in lung function.

In the present study, subjects with mild, moderate and severe PFT obstruction were compared with the age and sex matched controls. In patients with mild PFT obstruction, mean values of complements C3 and C4 were calculated for both cases and controls. There was no significant difference observed in complement C3, C4 levels between COPD patients with mild PFT obstruction and healthy age and sex matched controls.

Patients with moderate PFT obstruction were also compared with age and sex matched controls. In patients with moderate PFT obstruction, mean values of C3 and C4 were calculated for both cases and controls. Significant difference in mean values of C3 (110 for cases and 119.70 for controls) were observed in both cases and controls. However, statistical significance could not be achieved (P=0.662) for complement C3, and we observed a statistical significance for complement C4 (P=0.004) for both cases and controls. Patients with severe PFT obstruction were compared with age and sex matched controls. C3 and C4 complement levels were calculated for both cases and controls. We observed a significant difference in mean values of complements C3 and C4 for both cases and

controls, and we also observed statistical significance for both C3 ($P=0.017$) and C4 ($P=0.005$). Similar observations were made by Chauhan et al.⁵ where they observed both serum C3 (IU) and C4 (IU) were lower in COPD patients ($C3 = 95.9 \pm 33.11$, $C4 = 113.6 \pm 62.4$) than in control ($C3 = 167.3 \pm 25.42$, $C4 = 205 \pm 76.5$; $P<0.05$).

In other studies done by Mateja et al.⁶ and Fishnet et al.,⁷ there was no significant difference observed in C3 and C4 levels between patients with COPD and healthy control subjects which was in contrast to our study.

When total COPD patients with different degree of obstruction were compared with controls though there was no statistically significant difference in serum C3 levels ($P=0.054$), there was significant difference in mean C3 values were observed, and there was statistically significant difference observed for serum C4 levels ($P=0.002$).

Similar observations were noticed by Kosmas et al.⁸ and Serpiletal.⁹

In this study, COPD patients with emphysema score 0-6 (E1) were compared with age and sex matched controls for complement levels C3 and C4. When COPD patients with emphysema score (0-6) were compared with healthy sex and age matched controls, there was no significant difference between serum C3, C4 levels observed.

When COPD patients with emphysema score (7-10) were compared with controls, although significant difference observed in mean C3 values, these values were statistically not significant ($P=0.412$). However, statistical significant difference ($P=0.003$) observed in C4 values.

When COPD patients with emphysema score (>10) were compared with controls, statistical significance observed in both C3 ($P=0.017$), C4 ($P=0.023$) levels, similar observations were made by Burki and Krumpelman¹⁰ and Chugh et al.¹¹ in their studies.

Findings arising from this study showed that for patients with COPD, particularly moderate to severe degree COPD (moderate to severe obstruction, high emphysema score) had significantly low serum complement C3, C4 levels compared to healthy controls.

Complement proteins are a part of humoral defense and they have the characteristic of interacting with certain antibody molecules once these have combined with antigen.

Quantitatively, C3 and C4 comprise approximately two thirds of the complement system. The classic complement pathway is activated by either antibody-coated targets such as microorganisms or antigen-antibody complexes, while the alternative complement pathway is activated directly by bacterial polysaccharides.

Probable reason for lower serum levels of complement components C3 and C4 in COPD patients compared to healthy controls could be because of the following, sustained complement activation secondary to repeated respiratory infections leading to influx of inflammatory cells into the lung parenchyma with subsequent release of elastases and oxidants that cause damage to elastic lung tissue. This leads to the logical inference that there might be a quantitative relationship between complement consumption and degree of elastic tissue destruction.

Limitations of the study

There are certain limitations in our study, which we would like to put on record.

1. Although more number of patients were enrolled in this study compared to other similar studies, even more cases could have been taken
2. We could have used CT scan to measure the emphysema score instead of chest X-ray, although chest X-ray was also a well-known modality to know the lung destruction.

Strengths of the study

However, it is hereby restated that this study has several strengths, mentioned as follows:

1. More numbers of subjects were included in our study when compared to other studies; hence observed values were more significant.
2. Better method (immune -turbidity) was used to estimate C3 and C4 levels compared to other studies, which has got high sensitivity and specificity.

Summary

The 35 patients with COPD were studied during the period October 2011-September 2013.

The majority of COPD patients were males comprising of 57% (n=20) and females 43% (n=15) and majority of patients (n=18) were above 60 years (50%), 44% (n=15) in between 45 and 60 years age group and only 5% of patients (n=2) in <45 years age group. The maximum number of cases (n=21) of COPD and exacerbations (60%) were seen during rainy and winter seasons (July-December).

The 35 patients with COPD and 35 controls were studied to know the relation between complement components C3, C4 levels and degree of obstruction and lung damage for this we classified cases in to three groups based on degree of airway obstruction and lung destruction with the help of PFTs and emphysema score.

Mean complement C3, C4 levels of the cases were compared with age and sex matched controls.

The observations made were

1. Mean complement component C3, C4 levels were significantly low in patients with moderate to severe PFT obstruction and moderate (7-10) to higher emphysema scores (>10) compared to controls (Table 12).
2. In cases with mild obstruction and low emphysema score (0-6) there was no significant difference observed.
3. It was observed that there was significant decrease in complement C3, C4 levels with increase in severity of obstruction and lung destruction.

Probable explanation for the lower serum levels of complement components C3 and C4 in COPD patients compared to healthy controls could be as follows: Sustained complement activation secondary to repeated respiratory infection leading to influx of inflammatory cells into the lung parenchyma with subsequent release of elastases and oxidants that cause damage to elastic lung tissue, this leads to the inference that there might be a quantitative relationship between complement consumption and degree

of elastic tissue destruction.

Hence, it is postulated that the severity of lung damage and morbidity are related to lower complement C3, C4 levels. These complement levels can be used as marker of severity of COPD. Monitoring of complement C3, C4 levels may help in assessment of severity and progression of COPD along with PFTs and emphysema score.

Conclusions

The analysis of results of the present study leads to the following conclusions:

There was a direct correlation between the severity of COPD as estimated by radiological emphysema score and PFTs with serum complement levels. That is lower levels of complements being seen in progressive severity of COPD. This is in agreement with postulates of other studies, that serum complements levels may well serve as marker for COPD severity, however further large scale studies needed in this regard.

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