

C3 and C4 in Patients Suffering from Lung Diseases

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ABSTRACT

Background: Respiratory diseases are among the leading causes of death worldwide. Aim: The aim of this study is to estimate changes in the levels of complement components (C3 and C4) related to inflammatory lung diseases. **Subjects and Methods:** Blood sample from 49 patients were randomly selected from chest hospital in Zagazig, Sharqiyya, Egypt; Alhusayn and Alsayyid Jalaal hospitals, Al Azhar University, Cairo, Egypt. Serum levels of C3 and C4 were measured by radial immunodiffusion technique. **Results:** Chronic Obstructive Pulmonary Disease (COPD) only has shown low activity of complement C3 or C4 or both. Pulmonary fibrosis; Asthma; Lung cancer and Pneumonia have shown moderate activity of complement. Bronchiectasis and Hemoptysis have shown high activity of complement. Both classical and alternative pathways activity of complement was noticed in Hemoptosis while only classical pathway activation was indicated in Bronchiactosis. **Conclusion:** The complement proteins C3 and C4 can be important biomarker in the differential diagnosis of lung diseases.

Key words: Blood, patients, chest hospital, Lung Diseases

Introduction

The role of the complement as a system merging early-phase innate immunity with later-phase acquired immunity has been established. C3 is a key protein of the complement system. Activation of C3 results in a variety of immunologic reactions such as immune adherence, phagocytosis, antibody response, cytolysis, inflammation, and killing of pathogenic microorganisms (Sakamoto *et al.*, 1998).

Complement component C4 plays a central role in classical and lectin pathways of complement. There are two isotypic forms of C4, C4A and C4B that differ in their chemical and serological properties. A C4 deficiency is often seen in association with infection diseases (Jaatinen *et al.*, 2002).

Respiratory diseases are among the leading causes of death worldwide. Lung infections (mostly pneumonia and tuberculosis), lung cancer and chronic obstructive pulmonary disease (COPD) together accounted for 9.5 million deaths worldwide during 2008 (European lung white book, 2016).

Activation of complement pathways, in response to bacterial or viral respiratory infection, induces influx of macrophages and neutrophils through chemotactically active fragments of complement proteins (C3a, C5a, and C5). Activation and thus consumption of complement can be inferred by the presence of low levels of specific complement components, such as C3 or C4. Aggregation of inflammatory cells acting as phagocytes in distal airways and pulmonary microvasculature in response to chemotactic complement components results in the release of elastases and oxygen radicals that are known to play a significant role in the pathogenesis of pulmonary diseases (Kosmas *et al.*, 1997).

The aim of the study is to estimate changes in the levels of complement components (C3 and C4) related to inflammatory lung diseases in Egypt. Studying the role of Complement in inflammatory lung disease might help in combat of these lung diseases.

Patients and Methods:

Patients:

Forty nine patients (28 males and 21 females) were randomly selected from chest hospital in Zagazig, Sharqiyya, Egypt; Alhusayn and Alsayyid Jalaal hospitals, Al Azhar University, Cairo, Egypt. The age of patients ranged from 25 to 75 years old. All patients in the study were diagnosed for lung diseases.

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The lung diseases of patients in the study included: Lung cancer, Asthma, Chronic Obstructive Pulmonary Disease (COPD), Bronchiectasis, Pneumonia, Hemoptysis and Pulmonary fibrosis.

Collections of samples:

The samples were collected from October, 2014 until October, 2015. Consent was taken from all patients before blood sampling. A sample of blood consisting of 5ml was obtained from standard radial vein by a sterile disposable syringe from each patient. The blood sample was poured into clean test tube without anticoagulant and left for 2-3 minutes in water bath (37°C), then centrifuged at 3000 rpm for 6-10 minutes. The serum was separated and transferred to label multiple clean eppendorf tubes with patient full information then stored at -20°C until used.

Radial immunodiffusion (RID) plate:

Levels of C3 and C4 were measured according to the standard procedure provided with the kits supplied from Biocientifica S.A. Argentina. Radial Immunodiffusion (RID) plates were used for determination of Immunoglobulin and other proteins in biological fluids (Strunk *et al.*, 1977).

The clinical application of Diffu-plate is the measurement of proteins in serum and other biological fluids within the range indicated in the reference Table. For concentrations out of this range, samples should be properly diluted or concentrated.

Presentation of kit: RID plate for 12 tests containing monospecific antiserum "directed-against the protein –listed on the label in agarose gel layer. The procedure consists in an immune precipitation in agarose between an antigen and its homologous antibody. It is performed by incorporating one of the two immune reactants (usually antibody) uniformly throughout a layer of agarose gel, and then introducing the other reactants (usually antigen) into wells duly punched in the gel. Antigen diffuses radially out of the wall into the surrounding gel-antibody mixture, and a visible ring of precipitation forms where the antigen and antibody reacted.

Result

Confirmed diagnosis of lung diseases was included in this study. Blood Samples from 49 Patients were collected, depending on the results of clinical examination test for lung diseases, as follows: Lung cancer, Asthma, Chronic Obstructive Pulmonary Disease (COPD), Bronchiectasis, Pneumonia, Hemoptysis and Pulmonary fibrosis.

Levels of C3 and C4 that have been detected in serum samples by Immune-diffusion method using diffu-plates and shown in Table 1 and Fig 1 were as follows:

C3 and C4 in different lung diseases:

Lung cancer:

Out of 7 cases, four cases (57.14 %) had normal C3 and C4 while 3 cases (42.85 %) had abnormal (either high or low) levels of C3 and C4.

Asthma:

Three cases (42.85 %) were normal out of 7 studied cases. In four cases (57.14 %) C3 and C4 were abnormal. In two cases (28.57%) either C3 or C4 was high or low. Other two cases (28.57 %) only C4 was abnormal.

COPD:

Out of 7 cases, four cases (57.14 %) had normal C3 and C4 while 3 cases (42.85 %) had abnormal levels of C3 and C4. Two of these cases were abnormal in C4 and only one case was abnormal in C3.

Bronchiectasis:

Only one case (14.28 %) was normal out of 7 studied cases. The other six cases (85.71 %), C3 and C4 were abnormal. In 4 of these cases (57.14%), both C3 and C4 were either high or low. While the other two cases (28.57 %) only C4 was abnormal.

Pneumonia:

Four cases (57.14 %) were normal out of 7 studied cases. In the other 3 cases (42.85 %), C3 and C4 were abnormal. In two of these cases (28.57%), both C3 and C4 were low. The other one case (14.28 %) only C4 was abnormal.

Hemoptysis:

Only one case (14.28 %) was normal out of 7 studied cases. In the other six cases (85.71 %), C3 and C4 were abnormal. In 4 of these cases, both C3 and C4 were either high or low. The other two cases (28.57 %) only C3 was abnormal.

Pulmonary fibrosis:

Out of 7 cases, three cases (42.85 %) had normal C3 and C4 while the other 4 cases (57.14 %) had abnormal levels of C4 only.

Table 1: Normal and abnormal levels of C3 and C4 in 49 patients having pulmonary diseases (7 patients for each disease type: Lung cancer, Asthma, COPD, Bronchiectasis, Pneumonia, Hemoptysis and Pulmonary fibrosis)

Disease	C3		C4	
	Normal	Abnormal	Normal	Abnormal
Lung cancer	57.14%	42.85%	57.14%	42.85%
Asthma	71.42%	28.57%	42.85%	57.14%
COPD	85.71%	14.28%	71.42%	28.57%
Bronchiectasis	42.85%	57.14%	14.28%	85.71%
Pneumonia	71.42%	28.57%	57.14%	42.85%
Hemoptysis	14.28%	85.71%	42.85%	57.14%
Pulmonary fibrosis	100%	0%	42.85%	57.14%

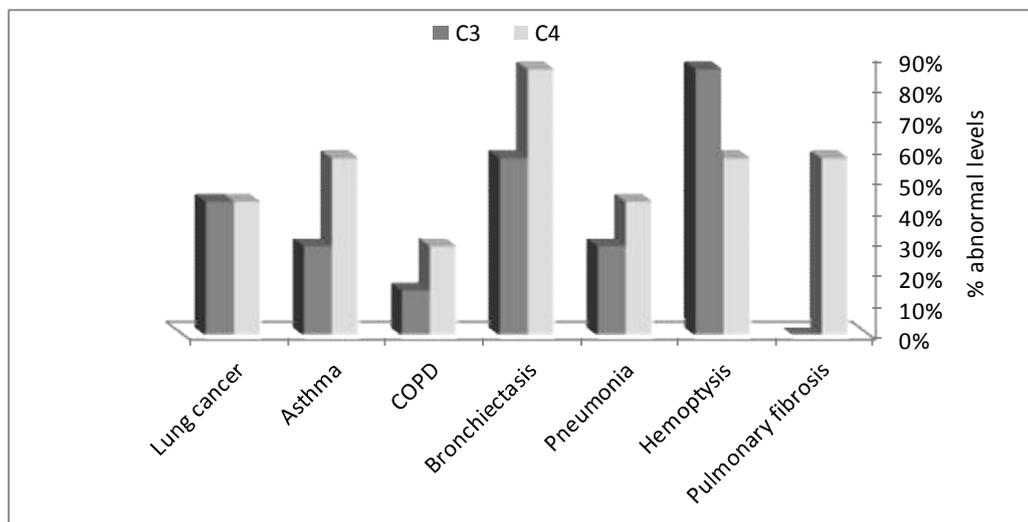


Fig. 1: Percentage abnormal levels (either high or low) of C3 and C4 in pulmonary diseases (Lung cancer, Asthma, COPD, Bronchiectasis, Pneumonia, Hemoptysis and Pulmonary fibrosis)

Discussion

In the present study the increase and decrease in the levels of complement proteins C3 and C4 indicated activation of the complement system in the patients. The abnormal levels of C4 indicate the classical pathway activation of the complement system, when C3 is either normal or abnormal. The abnormal levels of only C3 when C4 is normal indicate alternative pathway activation of complement.

Complement C3 and C4 in Lung Cancer:

This activity of complement was recorded in 42.9% of lung cancer patient. Levels of both C3 and C4 were high in 28.6% and low in 14.3% of patients. These abnormal levels of C4 and C3 indicate classical pathway activation of complement in lung cancer.

Results were in agreement with the findings of Ferda *et al.* (2004) which confirm the hypothesis that malignant tumors contribute to elevation of complement components levels. Complement components (C3 and C4) levels were elevated in cancer patients with different cell types compared with levels in the control group. Also Gmiński *et al.* (1992) reported that serum of complement components (C3 and C4) were significantly elevated in almost all patients from the tumor group as compared with the levels in the control group. Rutkowski *et al.* (2010) found that, the assumption of the complement system facilitates innate immune attack against cancer cells through cytotoxic and lytic effects.

The present study indicated that, the normal value of complement (inactive complement) of C3 and C4 were recorded in 57.1 % of the lung cancer patients. However, there is not any author who has found similar findings. This could be because these patients are subjected for chemotherapy.

Complement C3 and C4 in Asthma:

The results revealed activation of the complement system in 57.1% of asthma patients as respects C4 (14.3% high and 42.8% low) and in 28.6% of patients (14.3% high and 14.3% low) concerning C3. The abnormal levels of C4 indicate the classical pathway activation of the complement system, when C3 is either normal or abnormal.

The obtained result was in agreement with Mosca *et al.* (2011) who observed an increase in the serum levels of the C3 and/or C4 components of the complement system in the majority of the patients with intermittent atopic asthma studied here, when compared with the results for children in the same age group without asthma. They conclude that the presence of elevated C3 and/or C4 complement components could represent a biomarker for diagnosis of intermittent atopic asthma.

These results were nearly similar with those of Abdel Fattah *et al.* (2010) who found that serum levels of C3 are elevated in children with stable asthma, and there is a positive correlation between serum C3 and severity of asthma. There were no similar observations as regards to serum C4. Results also were in agreement with those of Lee *et al.*, (2006) who found the significantly elevated levels of C3a and C4a in the plasma of patients with Aspirin-induced asthma. Whereas, Najam *et al.* (2005) mentioned that, asthmatic patients had higher serum C3 level while C4 level was within normal range. The elevated C3 level was possibly due to induction by pro-inflammatory cytokines such as tumor necrosis factor- α (TNF- α) and interleukin-1 (IL-1). The probable mechanisms of C3 involvement in the pathophysiology of bronchial asthma were discussed. Nakano *et al.* (2003) showed that concentrations of C3a in plasma were significantly higher than normal. Concentrations of C3a, which can induce airway inflammation and bronchoconstriction, were associated with differences in response to emergency treatment of severe asthma exacerbation. Moreover Kurimoto (1975) exhibited that C3 and C4 were elevated in bronchial asthma. Onyemelukwe (1989) reported that C3 mean level was higher than in controls while C1q and C4 mean levels were lower than in controls of asthmatic patients.

The present study revealed that the normal value of complement (inactive complement) was recorded in 71.4 % of the asthma patients as for C3 but it was found in 42.8% of the asthma patients concerning C4.

This result was in agreement with Hutchcroft and Guz, (1978) who found no evidence of complement activation, Yalcin *et al.* (2012) who recorded that complement 3 and 4 levels were normal in all asthma patients, Durham *et al.* (1984) who found no appreciable changes in serum C3 and C4 up to 24 hr after challenge in subjects with late-phase responses in asthma patient, Anderson *et al.* (1983) who reported that levels of total hemolytic complement, C3 and C4 were normal and Davies *et al.* (1976) and Anderson *et al.* (1980) who recorded that serum concentrations of C3 and C4 did not alter during the recurrent nocturnal asthmatic reactions.

Complement C3 and C4 in Chronic Obstructive Pulmonary Disease (COPD):

The results revealed activation of the complement system in 42.9% of chronic obstructive pulmonary disease (COPD) patients as concerns C4 (28.6% high and 14.3% low) and in 14.3% of patients (0% high and 14.3% low) as regards C3. The abnormal levels of C4 indicate the classical pathway activation of the complement system, when C3 is either normal or abnormal.

The present study were in agreement with Marc *et al.* (2004) who found that the levels of complement factors C3a, C4a, and C5a are elevated at the site of inflammation in patients with obstructive pulmonary disease (COPD). Dasgupta *et al.* (1998) showed that the complements are activated and they take part in immune complex formation. The complements were usually depressed in most of these COPD patients. Moreover, Kosmas *et al.* (1997) reported that complement proteins are part of humoral defense and they have the characteristic of interacting with certain antibody molecules once these have combined with antigen. 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. The complement components C3 and C4 are decreased in COPD patients and more so in patients with chronic bronchitis. Also Chauhan *et al.* (1990) recorded that both serum C3 and C4 were lower in COPD patients than in control. Miller *et al.* (1980) found significantly lower blood levels of C3 and C4 in patients with COPD.

The current work indicated that the maximum normal value of complement (inactive complement) in C3 was 85.7% and C4 was 57.1% in COPD patients.

The obtained result was in agreement with those reported by Marinov *et al.* (1986) who found no complement fractions in patients with COPD.

Complement C3 and C4 in Bronchiectasis:

The present work revealed that the normal value of complement (inactive complement) was recorded in 42.8 % of the bronchiectasis patients as for C3 but it was found in 14.3 % of the patients as concerns C4.

The present study was in agreement with King *et al.* (2006) who studied complement levels in 103 adult patients with bronchiectasis. Complement levels were normal. It also agreed with Gibb *et al.* (1987) who reported that serum complement levels of C3 and C4 were normal and C1q binding immune complexes were absent in bronchiectasis patients. Moreover, Chandra (1978) found that the complement system in bronchiectasis patients was normal.

The results revealed activation of the complement system in 85.7% of bronchiectasis patients as concerns C4 (14.3% high and 71.4% low) and in 57.1% of patients (14.3% high and 42.8% low) as regards C3. The abnormal levels of C4 indicate the classical pathway activation of the complement system, when C3 is either normal or abnormal. However, there are not any authors who agreed with this result.

Complement C3 and C4 in Pneumonia:

The present study revealed activation of the complement system in 42.9% of pneumonia patients for both C3 and C4. The abnormal levels of C4 indicate the classical pathway activation of the complement system, when C3 is either normal or abnormal. However, level of C3 was high in 14.3% and low in 28.6% while level of C4 was high in 0% and low in 42.9%.

Similar observations were reported by Carceller *et al.* (2010) who evaluated the clinical presentation and prognosis of three children with pneumonia. He found two children had low C3 levels and one had both low C3 and C4 levels.

The present study indicated that, the normal value of complement (inactive complement) of C3 and C4 were recorded in 57.1 % of Pneumonia patients.

The obtained results were in agreement with those reported by many authors including Zhu *et al.* (2013), Meng *et al.* (2013) and Coonrod and Rylko-Bauer (1977) who found that complement levels were normal in the pneumonia patients.

Complement C3 and C4 in Hemoptysis:

The present work revealed that the normal value of complement (inactive complement) was recorded in 14.3% of the hemoptysis patients as for C3 but it was found in 42.8 % of the patients as concerns C4.

Results were in agreement with the findings of Dienstl *et al.* (1998), Dogukan *et al.* (2000) and Lee *et al.* (2014) who found that the serum complement levels (C3 and C4) were normal.

The results revealed activation of the complement system in 85.7% of hemoptysis patients as concerns C3 (71.4% high and 14.3% low) and in 57.1% of patients (14.3% high and 42.8% low) as regards C4. The abnormal levels of C3 and C4 indicate the classical and alternatives pathway activation of the complement system. However, there are not any authors who agreed with this result.

Complement C3 and C4 in Pulmonary Fibrosis:

The present work revealed that the normal value of complement (inactive complement) was recorded in 100 % of the pulmonary fibrosis patients as for C3 but it was found in 42.9 % of the patients as concerns C4.

The present study was in agreement with the findings of Wulffraat *et al.* (1994) who found that serum complement levels were normal in pulmonary fibrosis patients.

The results revealed that the levels of C4 were lower than normal in 57.1% of pulmonary fibrosis patients. The abnormal levels of C4 indicate the classical pathway activation of the complement system.

The present studies were in agreement with many Götz and Lubec (1978) and Strunk *et al.* (1977) who found that levels of C3 and C4 were lower than normal in pulmonary fibrosis patients.

The obtained result was in disagreement with those reported by Schiøtz *et al.* (1979), Church *et al.* (1981) and Meliconi *et al.* (1990) who found that serum C3 and C4 concentrations were higher than normal. Also, Holzhauser *et al.* (1976) recorded significant elevations of mean C3 levels in cystic fibrosis (CF) patients.

Conclusion

The present study comes to a conclusion that: The analysis of activity of C3 together with C4 in lung diseases indicated that Chronic Obstructive Pulmonary Disease (COPD) has shown low activity of complement. Lung cancer; pulmonary fibrosis; Asthma and Pneumonia have shown moderate activity of complement. Bronchiectasis and Hemoptysis have shown high activity of complement. All lung diseases that have been studied in this work showed classical pathway activation except Hemoptysis which showed both classical and alternative pathways of activation of complement. The current work draws attention that complement proteins C3 and C4 can be used as important biomarker in the differential diagnosis of lung diseases.

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