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Association of Serum Complement Components (C3 and C4) with Disease Activity in Yemeni Bronchial Asthmatic Patients

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ABSTRACT

Objective: To assess the role of the complement components (C3 and C4) in the pathogenesis and severity of bronchial asthma (BA) among Yemeni patients.

Methods: This cross-sectional, clinic-based study was conducted in Sana'a city, Yemen in the period from March to June 2012. It included 100 BA patients, where demographic and clinical data were collected using a pre-designed, structured questionnaire. Blood samples then collected by venipuncture, and sera were then separated and tested for the levels of C3 and C4 by immunoturbidimetry assay. Data were analyzed using suitable statistical tests using IBM SPSS Statistics, version

Results: Of 100 patients, 53% were males and 29% were older than 40 years. The mean age of patients was 34.7± 17.5 years (range: 6–80). The majority of patients had active asthma (81.0%) and experienced asthma for more than two years (66.0%). Dyspnea (81.0%) and cough (65.0%) were the most frequent clinical features, while edema (11.0%) was the least frequent clinical feature among asthmatic patients. The mean level of serum C3 among BA patients was elevated (127.9 ± 21.3 mg/dL) and was significantly higher among females, patients aged 40 years or older and those with active BA, but there was no statistically significant difference with respect to the duration of asthma. In contrast, the mean level of serum C4 was normal (27.6 ± 8.8 mg/dL) but was significantly higher among patients aged 40 years or older and those having asthma for more than two years. Wheezing was significantly associated with the mean level of serum C3 among BA patients. Similarly, the mean level of serum C4 was significantly higher among patients having cough.

Conclusions: The majority of Yemeni patients with BA have elevated levels of serum C3, which can be significantly higher in females, patients older than 40 years and those with active asthma. On the other hand, the levels of serum C4 are normal among the majority of Yemeni patients with BA, but these can be elevated among those with severe clinical features or long-term asthma.

Keywords: Active asthma, Bronchial asthma, Complement, C3, C4, Yemen

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1. Introduction

Bronchial asthma (BA) is a chronic inflammatory disease characterized by attacks of wheezing, dyspnea, chest tightness, and coughing. It could be attributed to atopic (environmental or extrinsic) and non-atopic or genetic (intrinsic) factors. It usually results from an inappropriate immunological reaction to common environmental antigens in genetically susceptible people. Although the pathways that generate and express the T helper cell type 2 (Th2)-biased immune response in asthma are still unclear, there is evidence on the role of Th2 in triggering adverse immune responses in BA characterized by serum immunoglobulin E (IgE) elevation. Moreover, inflammation with eosinophilia, airway hyperresponsiveness (AHR) and increased production of mucus were attributed to the action of Th2 cells.[1]

The complement system constitutes a major part of the innate immune system that opsonizes pathogen particles or infected cells to enhance phagocytosis.[2] The pathways for complement activation include the classical, alternative and lectin pathways. Over 30 proteins and protein fragments build the complement system, including serum proteins and cell membrane receptors. The complement pathways and their components represent a key link between the innate and specific immune responses. In addition, they have an essential role as lytic effector components against microbial infections by the regulation and activation of T cells, B cells and antigen-presenting cells.

The complement pathways may also be involved in the pathogenesis of BA by influencing the adaptive immune reaction towards inhaled allergens and promoting the Th2 immune response at initiation.[3] Recently, the serum complement factor 1 is associated with systemic lupus erythematosus activity.[4] In addition, mediators of the complement system are implicated in airway tissue injury and tissue remodeling in BA patients.[5] The pivotal role for the anaphylatoxins (C3a and C5a) in asthma pathogenesis has been shown.[6–10] The levels of C3a in induced sputum could, therefore, be used as a clinical biomarker for clinical management of asthma.[7] On the other hand, targeting the complement pathway and its mediators may have a potential therapeutic role in controlling the severe attacks of asthmatic seizures.[8] Furthermore, C3a is used by IgE to mediate the increase of interleukin (IL)-17 and cluster of differentiation (CD4+) cells in the lungs of BA patients associated with neutrophilic inflammation and AHR. This is clinically called the IgE-mediated late-phase asthmatic response.[9] The synergic action of C3a and C5a with Th2 immune response triggers allergic inflammation during the acute phase of BA. This indicates the importance of targeting the complement pathway during BA management.[10] IL-17A, IL-13 and IL-23 are regulated by complement-mediated immune reactions in severe asthma.[11] Additionally, human beta-tryptase can exacerbate the asthmatic attack by generating C3a, C4a and C5a anaphylatoxins.[12] Therefore, a further explanation for the role of complement system components in BA pathogenesis is critically needed, particularly their association with clinical features.

The present study assessed the association of serum C3 and C4 complement components with disease activity among BA patients among Yemeni patients. Furthermore, the levels of these components were compared in relation to clinical features, intake of drugs and the presence of chronic co-morbidities.

2. Methods

2.1. Study design and setting

This cross-sectional, clinic-based study was conducted at the Thoracic Diseases Clinic of Dr. Mohammed Al Shaikh and University of Science and Technology Hospital in Sana'a city, Yemen in the period from March to June 2012.

2.2. Sample size calculation and recruitment of BA patients

Based on an expected frequency of 10.0% and an acceptable value of 5.0% for the frequency of C3 and C4, a minimum of 54 BA patients were calculated for an estimated population of 50,000 patients attending the study settings at a confidence level of 95% using Epi Info™, version 6 (Centers for Disease Control and Prevention, Atlanta, GA, USA). However, 100 patients were enrolled in this study irrespective of gender and age. Patients were selected after having been diagnosed with BA according to the Global Initiative for Asthma Guidelines (2007). Clinically, signs of infection were not observed in all patients.

2.3. Data collection

A pre-designed, structured questionnaire was used to collect demographic and clinical data from the patients after obtaining written informed consent.

2.4. Blood collection

Approximately 3-5 ml of whole blood were collected aseptically by venipuncture into pre-labeled plain tubes. Sera were separated from clotted blood specimens by centrifugation at 4500 rpm for 5 minutes. Hemolyzed sera were excluded from laboratory investigations

2.5. Immunoturbidimetric measurements

The complement components C3 and C4 were measured with immunoturbidimetry using cobas® c311 fully automated clinical chemistry analyzer (Roche Diagnostics, GmbH, Mannheim, Germany) according to the manufacturer's instructions.

2.6. Statistical analysis

Data were analyzed by independent t-test using IBM SPSS Statistics, version 22.0 (IBM Corp., Armonk, NY, USA). Differences and associations were considered statistically significant at *P*-value <0.05.

3. Results

3.1. Characteristics of BA patients

Table (1) shows that 53.0% of BA patients were males and 29.0% were older than 40 years. The mean age of patients was 34.7± 17.5 years (range: 6–80). The majority of patients (81.0%) had active asthma and experienced asthma for over two years (66.0%). Dyspnea (81.0%) and cough (65.0%) were the most frequent clinical features. However, edema was the least frequent symptom, being observed among 11.0% of patients. Hypertension was observed in 14.0% of patients compared to 4.0% for those with cardiac diseases. The majority of patients (63.0%) reported the intake of bronchodilators.

Table 1. Characteristics of BA patients attending thoracic clinics in Sana'a city, Yemen (March to June 2012)*

Item	*n* (%)
Gender	
Male	53 (**53.0**)
Female	47 (**47.0**)
Age (years)	
Mean ± SD (range)	34.7± 17.5 (6–80)
≤10	5 (**5.0**)
11-20	18 (**18.0**)
21-30	24 (**24.0**)
31-40	24 (**24.0**)
>40	29 (**29.0**)
Status of asthma	
Active	81 (**81.0**)
Inactive	19 (**19.0**)
Duration of asthma (years)	
≤ 2	34 (**34.0**)
>2	66 (**66.0**)
Clinical features	
Cough	65 (**65.0**)
Dyspnea	81 (**81.0**)
Wheezing	55 (**55.0**)
Tachypnea	47 (**47.0**)
Sweating	42 (**42.0**)
Nausea	44 (**44.0**)
Edema	11 (**11.0**)
Presence of chronic co-morbidities	
Hypertension	14 (**14.0**)
Cardiac diseases	4 (**4.0**)
History of drug intake	
Analgesics	20 (**20.0**)
Antihypertensives	13 (**13.0**)
Bronchodilators	63 (**63.0**)

* Total number of patients was 100.

3.2. Serum C3 and C4 complement components among BA patients

Table (2) shows that the mean levels of serum C3 and C4 among BA patients were 127.9 ± 21.3 mg/dL and 27.6 ± 8.8 mg/dL, respectively.

Table 2. Mean levels of serum complement components C3 and C4 mong BA patients attending thoracic clinics in Sana'a city, Yemen (March – June 2012)*

Complement component	Mean level (mg/dL) ± SD
C3	127.9 ± 21.3
C4	27.6 ± 8.8

* Total number of patients was 100; SD, standard deviation; C3 normal range is 55-120 mg/dL; C4 normal range is 20-50 mg/dL

3.3. Serum C3 and C4 complement components among BA patients in relation to demographic and clinical characteristics

The mean levels of serum C3 were significantly higher among females, patients aged 40 years or older and those with active BA, but there was no statistically significant difference with respect to the duration of asthma. In contrast, serum C4 was significantly higher among patients aged 40 years or older and those having asthma for more than two years (Table 3).

Wheezing was significantly associated with the mean level of serum C3 among BA patients. The mean level of serum C4 was significantly higher among patients having cough and wheezing. Serum C4, but not C3, was significantly higher among hypertensive patients. However, no statistically significant association was observed between the use of bronchodilators and the mean levels of either C3 or C4. Moreover, the mean level of C3 was significantly higher in patients on analgesics (Table 3).

4. Discussion

BA is a chronic inflammatory disease characterized by increased Th2 cytokine-producing T-cells and mast cells. Studies revealed the significance of complement pathways and their mediators in the immunopathogenesis of BA.[4, 6, 8] Indeed, complements system has a critical role in airway inflammatory, AHR and remodeling.[5, 9, 13] Most importantly, C3 is the main key protein in the cascade of the three complement pathways.

Table 3. Serum complement components C3 and C4 among BA patients attending thoracic clinics in Sana'a city, Yemen (March – June 2012)

Variable		C3		C4	
		Mean ± SD	*P*-value	Mean ± SD	*P*-value
Gender					
	Male	121.9 ± 17.8	0.003	26.9 ± 8.5	0.333
	Female	134.6 ± 23.2		28.1 ± 7.6	
Age (years)					
	< 40	122.3 ± 19.9	0.010	25.5 ± 8.4	0.051
	≥ 40	134.6 ± 19.6		39.2 ± 7.4	
Status of Asthma					
	Active	130.3 ± 21.8	0.018	27.9 ± 8.4	0.133
	Inactive	117.5 ± 15.8		24.8 ± 5.8	
Duration of asthma (years)					
	≤2	123.8 ± 22.5	0.168	25.2 ± 7.8	0.064
	>2	130.0 ± 20.5		28.4 ± 8.0	
Clinical features					
Cough					
	Yes	130.2 ± 21.4	0.137	28.5 ± 8.4	0.045
	No	123.5 ± 20.8		25.1 ± 7.0	
Dyspnea					
	Yes	129.6 ± 21.8	0.088	27.8 ± 8.4	0.153
	No	120.4 ± 17.9		24.9 ± 5.7	
Wheezing					
	Yes	132.2 ± 22.3	0.023	28.7 ± 8.3	0.051
	No	122.5 ± 18.9		25.6 ± 7.4	
Tachypnea					
	Yes	131.6 ± 23.5	0.097	28.5 ± 8.7	0.148
	No	124.5 ± 18.7		26.2 ± 7.3	
Sweating					
	Yes	129.9 ± 22.5	0.426	27.5 ± 8.2	0.812
	No	126.4 ± 20.5		27.1 ± 8.0	
Nausea					
	Yes	127.2 ± 23.8	0.078	27.2 ± 8.9	0.892
	No	128.4 ± 19.3		27.4 ± 7.4	
Edema					
	Yes	137.4 ± 21.9	0.115	28.6 ± 7.6	0.565
	No	126.7 ± 21.1		27.1 ± 8.1	
Presence of chronic co-morbidities					
Hypertension					
	Yes	129.4 ± 15.3	0.779	32.1 ± 7.4	0.016
	No	127.6 ± 22.2		26.5 ± 8.0	
Heart diseases					
	Yes	136.2 ± 6.7	0.430	30.4 ± 7.7	0.437
	No	127.5 ± 21.6		27.2 ± 8.1	
Intake of bronchodilators					
	Yes	128.3 ± 20.3	0.775	27.1 ± 8.2	0.743
	No	127.0 ± 23.1		27.6 ± 7.9	
Intake of analgesics					
	Yes	143.7 ± 27.4	0.005	31.7 ± 11.8	0.084
	No	123.9 ± 17.6		26.7 ± 7.6	

C3, complement component 3; C4, complement component 4; SD, standard deviation.

Increased serum C3 levels have been associated with childhood asthma and are considered as a diagnostic biomarker. [14, 15] In line with this finding, the present study showed significantly elevated serum C3 levels in the majority of Yemeni BA patients in association with clinical features. The mean serum C3 level was significantly higher among patients older than 40 years in the present study. Najam et al.[14] also observed that the mean

serum C3 was significantly higher in Indian children with active asthma. Serum C3 is an inflammatory marker and is positively correlated with the severity of asthma.[16] Therefore, complement activation could contribute to bronchospasm and inflammation in asthma. It was also demonstrated that IL-4 and IL-13 are involved in asthma by inducing RNA expression for C3 in human epithelial cells.[17, 18] The significantly higher levels of serum C3 among females in the present study could be partly attributed to the effects of female hormones on asthma severity. It has been suggested that women show worsening asthma during the premenstrual and menstrual times.[19, 20]

The significant association of sweating with elevated levels of serum C3 in the present study is in line with the fact that excessive sweating may be affected by the immunologic effects of C3.[21] We also observed elevated C3 levels in our patients that taking analgesics. In line with this, the cyclooxygenase (COX) inhibitors such as indomethacin and aspirin cause activation of C3, C4, and C5 by the classical pathway,[22] independently from antigen-antibody reactions.[23] However, the lack of association between aspirin-sensitive asthma and complement activation was also reported.[24]

The significantly elevated serum C3 levels in active BA patients compared with those with inactive BA is consistent with that reported among Indian asthmatic children.[15] On the other hand, Hasegawa et al.[25] found an association between proteins of the complement system and asthma development among Japanese asthmatic patients. They showed that C3, C5, C3aR and C5aR gene polymorphisms are related to elevated IgE levels and asthma in children and adults.[25] It is noteworthy that the balance between the function of mesenchymal cells and the components of innate and adaptive immune responses affects asthma sensitization and progression.[26] Furthermore, the pathogenesis of asthma is strongly related to the innate immune response, particularly that controlled by the complement system.[27] The present study also revealed significantly higher mean levels of serum C3 and C4 in patients experiencing asthma for more than two years, which could be attributed to bronchodilator tolerance that may induce inflammatory processes.[28]

In contrast to previous studies that showed a significant increase in the mean levels of serum C4 in patients with BA,[14, 15] the present study showed normal mean levels of serum C4 in the majority of BA patients. However, a significant increase was observed in patients with severe clinical features.

5. Conclusions

The majority of Yemeni patients with BA have elevated serum levels of C3, which can be significantly higher in females, patients older than 40 years and those with active asthma. Therefore, targeting the complement pathways could be a suitable therapeutic strategy in BA management. The utility of C3 as a biomarker for monitoring BA severity among Yemeni patients needs to be further investigated. On the other hand, serum C4 levels are within normal range among the majority of Yemeni patients with BA, but these can be elevated among those with severe clinical features or long-term asthma.

Ethical considerations

The protocol of this study was approved by the Ethics Committee of the Faculty of Medicine and Health Sciences, Sana'a University, Sana'a, Yemen. Informed consent was obtained from the patients or their parents.

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Authors' contributions

MA designed the study, performed the laboratory investigations, analyzed the data and wrote the manuscript. EI, MS, MSH, AR, SA and MAW contributed to data analysis and interpreted the study results. KM supervised and revised the manuscript. All authors read and approved the final version of the manuscript submitted for publication.

Competing interests

The authors declare that they have no competing interests associated with this article.

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