EVALUATION OF SERUM LEVELS OF C3 AND C4 COMPLEMENT SYSTEM COMPONENTS IN ASTHMATIC CHILDREN

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ABSTRACT

Background. Asthma is one of the most common respiratory disorders in children and is charecterized by distal airway inflammation and hyperresponsiveness. This disease challenges human health because of its increasing prevalence, severity, morbidity, and the lack of a proper and complete cure. Complement proteins are responsible for many pathophysiological features of asthma, including inflammatory cell infiltration, mucus secretion, increase in vascular permeability, and smooth muscle cell contraction.

Objective. To assess the serum levels of complement factors C3 and C4 in Egyptian asthmatic children.

Patients and methods. This case controlled study comprised of 60 Egyptian children with the diagnosis of bronchial asthma and 30 age-and sex-matched healthy controls.It was conducted in Al-Azhar University Hospitals, from January 2016 to Septemper 2016. All candidates were subjected to athorough clinical study, complete blood counts, absolute eosinophilic count and serum complements (C3, C4).

Results. Serum C3 levels were significantly higher in asthmatics when compared to controls (179.08 ± 44.04 mg/dl *vs* 123.70 ± 22.17 mg/dl respectively, $p < 0.01$). Also, C4 levels were higher compared to controls (42.7±15.58 mg/dl *vs* 27.3 ± 6.89 mg/dl respectively, $p < 0.001$). There was a significant positive correlation between severity of asthma and serum C3 and serum C4.

Conclusions. Serum levels of C3 and C4 are elevated in children with bronchial asthma, with a positive correlation between their levels and severity of asthma.

Key words: *Asthma; Children; Complement 3,4.*

INTRODUCTION

Allergic disorders are one of the most common diseases of man. About 25- 30 % of the total population is suffering from allergic diseases including bronchial asthma, allergic rhinitis, allergic conjunctivitis, atopic dermatitis, food allergy and drug Allergy ***(WHO, 2009*)**.

Asthma is a common chronic respiratory disease affecting 1-18% of the population in different countries. ***(GINA, 2015*)**.

As regard asthma, it is a very common chronic disease in respiratory system in which the constricted airways become inflamed and are lined with excessive amount of mucus ***(Harold and Nelson, 2008).***

In susceptible individuals air-ways inflammation may cause recurrent or persistent broncho-spasm which causes symptoms including wheezing, breathless-ness, chest tightness, particularly at night or after exercise ***(Girish and Payel, 2009).*** Asthma is caused by a complex interaction of environmental and genetic factors ***(Martinez, 2007).***

These episodes may be triggered by such things as exposure to an environmental stimulant such as an allergen, environmental tobacco smoke, cold or warm air, perfume, moist air, exercise or emotional stress. In children the most common triggers are viral illness such as those that cause the common cold ***(Zhao et al., 2002).***

In the past three decades it was witnessed dramatic increase in the prevalence of asthma and allergic diseases worldwide most notably in a western lifestyle ***(Balicer et al., 2007).***

As regard the complement system, it was discovered a century ago as a potent defense cascade of innate immunity. After its first description, continuous experimental and clinical research has been performed and three pathways of activation were established. Upon activation by traumatic or surgical tissue damage, complement reveals beneficial functions of pathogen and danger defense by sensing and clearance of injured cells. However, latest research efforts have provided a more distinct insight into the complement system and its clinical subsequences. Complement has been shown to play a significant role in the pathogenesis of various

inflammatory processes such as sepsis, multi-organ dysfunction, ischemia/reperfusion, cardiovascular diseases and many others **.** ***(Christian et al., 2004)***

The role of the complement system in asthma was suggested by ***(Roitt et al., 1993)***. possibly through initiation and or amplification of the inflammatory response of the complement cascade. If this system is involved in the pathogenesis of asthma, serum levels of the complement components are expected to be altered. Complements in asthma have been studied to a great extent, although reports were conflicting. Some studies have reported significantly increased serum C3 levels where as others have demonstrated no change ***(Najam et al., 2005).***

The aim of this study was to evaluate serum levels of C3 and C4 in asthmatic children and to detect if there is a correlation between severity of bronchial asthma and their serum levels.

PATIENTS AND METHODS

This case-controlled study was conducted in Al-Azhar University Hospitals, from January 2016 to Septemper 2016. It comprised sixty Egyptian asthmatic children and 30 age-and sex-matched healthy controls. Patients aged between 1 to 12 yr and diagnosed according to **Global *Initiative for Asthma Guidelines 2015***, were included. Patients who had acute chest infection or other acute inflammations and those who refused to participate in the study, were excluded.

An informed written consent was obtained before enrollment. All candidates were subjected to a complete clinical study. The following investigations were performed: Chest X ray, Complete blood counts (CBC), absolute eosinophilic count and serum complement factors (C3, C4).

Statistical Design: Data collected were reviewed. Coding for the collected data was done manually. These numerical codes were fed to the computer where statistical analysis was done using the statistic package for social science version 22(SPSS 22) for windows.

Chi-square-test was applied for comparison of qualitative data. The data were considered signifi-cant if p value was equal to or less than 0.05.

RESULTS

Our results were tabulated in the following tables (1-5).

Table (1): Demographic and Clinical characteristics of studied cases.

Characteristics	**Cases (60)**
Age (years)	6.88 ± 3.01
Sex (male/female)	33/27
Residence (urban/rural)	45/15
Severity of asthma	
Mild	5 (7.5%)
Moderate	31 (52.5%)
Severe	24 (40%)
Level of Control	
Uncontrolled	15 (25%)
Controlled	45 (75%)

Among the asthmatic group, 55% were male and 45% were female. Their age ranged from 1-12 years. Patients from rural areas constituted 25% and those from urban side constituted 75% of our cases.Using GINA guidelines (2010) for grading patient's asthma we had 7.5% of the cases with mild persistent asthma, 52.5% with moderate persistent asthma and 40% with severe persistent asthma. Also we classified our cases into 2 groups, controlled group (25% of cases) while 75% were uncontrolled asthmatic children.

Table (2): Comparison between the 2 studied groups according to history of atopy.

groups / Parameters	**Cases (n = 60)**		**Control (n = 30)**		**Test of sig.**
	No.	**%**	**No.**	**%**	
Family history of atopy					
-ve	25	41.5	30	100.0	p <0.001
+ve	35	58.5	0	0.0	
Patient Associated atopy					
+ve atopy	**9**	**15.0**	**0**	**0.0**	p = 0.165
Skin allergy	3	5.0	0	0.0	
Allergic rhinitis	3	5.0	0	0.0	-
Skin allergy + rhinitis	3	5.0	0	0.0	

There was positive family history of atopy in 58.5% of the asthmatic children and positive personal history of atopy in 15% of asthmatic children in the form of skin allergy and/or allergic rhinitis.

Table (3): Comparison between the two studied groups according to serum C3 and C4 levels.

groups / Parameters	Cases (n = 60)		Control (n = 30)		Test of sig.
	No.	%	No.	%	P value
C3					
Normal	15	25.0	30	100.0	
Increased	45	75.0	0	0.0	<0.001
Range (mg/dl)	92.0 – 225.0		97.0 – 175.0		
Mean ± SD	179.08 ± 44.04		123.70 ± 22.17		Z = 3.866
Median	197.50		115.50		<0.001
C4					
Normal	27	45.0	30	100.0	
Increased	33	55.0	0	0.0	<0.001
Range (mg/dl)	23.10 – 69.30		16.20 – 39.20		
Mean ± SD	42.70 ± 15.58		27.31 ± 6.89		Z = 3.584
Median	42.95		26.75		<0.001

There was positive family history of atopy in 58.5% of the asthmatic children and positive personal history of atopy in 15% of asthmatic children in the form of skin allergy and/or allergic rhinitis.

Table (4): Levels of C3 and C4 in relation to asthma grade in patients group.

Asthma grade / parameters	Mild persistent (n = 5)	Moderate persistent (n = 31)	Severe persistent (n = 24)	Test of sig. P value
C3				
Range(mg/dl)	92.0 – 98.0	94.0 – 206.0	201.0 – 225.0	**P**1 0.010
Mean ± SD	95.33 ± 3.06	166.67 ± 41.34	211.06 ± 7.36	**P**2 <0.001
Median	96.0	189.0	210.50	**P**3 <0.001
C4				
Range(mg/dl)	24.90 – 30.20	23.10 – 60.20	45.20 – 69.30	**P**1 0.007
Mean ± SD	27.30 ± 2.69	32.97 ± 9.87	58.35 ± 7.69	**P**2 <0.001
Median	26.80	30.20	58.25	**P**3 <0.001

p1 : p value for Mann Whitney test between mild persistent and moderate persistent asthma.
p2 : p value for Mann Whitney test between moderate persistent and severe persistent asthma.
p3 : p value for Mann Whitney test between mild persistent and severe persistent asthma.

This table shows that there was significant relation between asthma grade and the levels of C3, C4, as asthma severity increases, the level of C3 and C4 is increased.

Table (5): Serum levels of C3 and C4 in relation to level of asthma control in patients group.

Level of control / Parameters	Uncontrolled (n = 45)	Controlled (n = 15)
C3		
Range(mg/dl)	185.0 – 225.0	92.0 – 122.0
Mean ± SD	203.40 ± 10.84	106.10 ± 11.72
Median	204.0	103.0
C4		
Range(mg/dl)	23.20 – 69.30	23.10 – 30.20
Mean ± SD	48.21 ± 14.09	26.15 ± 2.45
Median	48.90	25.25

There were significant relations between levels of C3 ,C4 and the level of asthma control in our cases.

DISCUSSION

In the present study, the results showed that there was significant elevation in mean C3 level in the asthmatic children in comparison to normal healthy children as the mean level of C3 in asthmatic children was 179.08 ± 44.04 in comparison to 123.7±22.17 for control group (with $p< 0.001$).This is in agreement with other studies. **Abdel Fattah et al. (2010**), reported that serum levels of C3 were elevated in children with stable asthma, with a positive correlation between serum C3 and severity of asthma.

Another study done by **Najam et al. 2005**, reported higher serum C3 level in asthmatic patients in comparison to control children. Regarding C4, in the present study, there was significant elevation of C4 in asthmatic children. The mean level of C4 was 42.7±15.58 in asthmatic children and 27.31 ± 6.89 in the control group with ($p<0.001$). This is in consistent with **Kay et al. 2011**; who stated that C4 levels were significantly higher than normal in asthmatic children. On the other hand, ***Abdel Fattah et al. 2010 and Najamet al 2005,***

reported that C4 level was not affected in asthmatic children. Some old studies reported results similar to ours. ***Bour et al. (1980),*** who studied 16 asthmatic patients, reported significant increase in C3 and C4 in 5 patients during immediate reaction and in 7 patients during late phase of asthma**.**

Also, ***Lin et al. 1992*** stated that C3 level in patients with acute attack of asthma was significantly higher than normal, also serum C4 tend to be higher but the difference did not reach significance. All investigators agreed that the apparent discrepancy is due to the fact that the level of serum complement components reflects a balance between its rate of production and consumption. Increased levels resulting from over-production might be masked later on by the normal or increased catabolism of the components depending on the disease state ***(Michel et al., 1996)***. That is why the level of individual complement components might vary from one study to another .Also, measurement of individual complement componenetsC3 or C4 determines only the amount of the protein without regard to its biological activity ***(Schaller, 1992).***

Nagata and Glovesky, 2000, noted that the increased complement activation in some asthmatic patients might be attributed to several factors as allergen induced complement activation, with several types of antigens capable of exerting this effect such as house dust, mites and aspergillus.

In the present study, we found a significant relation between the levels of C3, C4 and the level of asthma control. We observed that none of the controlled asthmatics had elevated levels of C3 or C4 while all the uncontrolled asthmatics had increased levels of C3 and 73.3% of them had increased levels of C4 as the mean C3 level was 203.4 ±10.8 and mean C4 level was 48.2±14.09 in uncontrolled cases in comparison to 106.1±11.72 and 26.15±2.45 for C3 and C4 respectively in controlled cases with $p<0.001$. This could be explained by the chronic ongoing inflammatory process that occurring in uncontrolled asthma leading to activation of either entire complement cascade or the specific cleavage of C3 and/ or C5 producing C3a,C5a which are pro-allergic and play an important role in the recruitment and activation of inflammatory cells (i.e., eosinophils, mast cells) known to be important in the induction of the cardinal features of asthma, including airway hyperresponsive-

ness, mucus metaplasia, and airway remodeling ***(Wills-Karp, 2007).***

In our study, there were significant relations between asthma grade and the levels of C3, C4 as the means for C3 were 95.33±3.06, 166.67±41.34 and 211.06±7.36 for mild persistent, moderate and severe grade of asthma respectively.

On the other hand,the mean for C4 was 27.3±2.69, 32.97±9.87 and 58.35±7.69 for mild persistent, moderate and severe grade of asthma respectively. This is in agreement with ***Abdel Fattah et al. (2010)***, who stated that the patients with increasing severity of asthma had higher C3 levels. Also, this is in agreement with the results of ***Solomon et al. 2007***, who reported a positive correlation between plasmaC3 and severity of asthma, *and stated that,* with increasing severity of the disease, systemic inflammatory mediators of asthma like tumor necrosis factor-α and interleukin-1 might be exerting a stimulatory effect on the liver to produce larger amounts of C3, which in turn might have augmented the severity of asthma. On the other hand, ***Wills-karp and Koehl, 2005;*** did not find a significant difference between serum C4 levels in asthmatic children and the control group. And there was no correlation between C4 levels and severity of asthma**.**

CONCLUSIONS

From the present study, we concluded that:

- Serum levels of C3 and C4 were elevated in children with asthma in comparison to control group.
- There was a significant positive correlation between serum C3 and C4 and severity of asthma grade.
- Serum levels of C3, C4 were significantly higher in uncontrolled asthmatics than controlled patients.

Recommendations

We recommend further multicenter studies on larger number of asthmatic children to evaluate:

- Complement profile in asthmatic patients during asthma exacerbations and in between asthma attacks.
- Complement profile during the early and late phase of asthma, to detect possible sequential changes in complement during various stages of the attack as well as the relation between various patient´s asthma characteristics and changes in complement activity.

- Complement profile after bronchial challenge with different allergens to find out the relation (if any) of the type of allergen and complement activation.
- Effect of complement components activation on type, duration and clinical severity of asthma. Use of serum C3 level as an indicator for efficacy of management of bronchial asthma.
- Possibility of using serum levels of complement in the classification of asthma severity.
- Use of serum levels of complement as a prognostic parameter for asthma control.

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تقييم مستويات المكون المتمم الثالث والرابع في مصل الدم في الاطفال المصابين بالربو الشعبي

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خلفية البحث: الربو الشعبي هو واحد من أكثر أمراض الجهاز التنفسي شيوعا في الأطفال وهو عبارة عن التهاب مزمن بالشعب الهوائية. هذا المرض يمثل تحديا لصحة الإنسان وذلك بسبب زيادة معدل انتشاره وشدته وقدرته علي احداث مضاعفات وعدم وجود العلاج التام له. ويشارك النظام المتمم بصفة أساسية في ميكانيكية الربو الشعبي من خلال زيادة خلايا الالتهاب وإفراز المخاط وزيادة نفاذية الأوعية الدموية وانقباض الخلايا العضلية في ممرات التنفس.

الهدف من البحث: إلقاء الضوء علي إمكانية حدوث تغيرات في مستوي المكون المتمم الثالث والرابع عند الأطفال المصابين بالربو الشعبي عن طريق قياس مستوي هذه المكونات في مصل الدم .

المرضى وأساليب الدراسة: تم إجراء الدراسة على ستين طفلا مصابين بالربو الشعبي الذين ترددوا علي مستشفيات جامعة الأزهر في الفترة من يناير 2016 حتى سبتمبر 2016 وكذلك علي ثلاثين طفلا سليما من نفس العمر والجنس. وقد خضعت كل الأطفال الي: التاريخ المرضي كاملا والفحص السريري الشامل وفحوصات مثل أشعة عادية علي الصدر وصورة دم كاملة وقياس مستوي المكونات المتممة سي 3 وسي 4 في مصل الدم.

النتائج: مستوى سي3 كان أعلى بكثير في مرضى الربو بالمقارنة مع الضوابط (179,08± 44,04 ملجم/ ديسيليتر مقابل 123 ± 22,17 ملجم/ ديسيليتر على التوالي ، المعدل الاحصائي p اقل من من 0.01) وكانت مستويات سي4 اعلى ايضا مقارنة مع الضوابط (42,7 ±15,58 ملجم/ ديسيليتر مقابل 27,3± 6,8 ملجم/ديسيليتر على التوالي ،المعدل الاحصائي p اقل من0,001) مما يدل علي ان هناك علاقة ايجابية ذات دلالة احصائية بين شدة الربو ومستوى سي3 وسي4 في مصل الدم. **الاستنتاج:** ارتفاع مستويات المكون المتمم سي3 وسي4 في مصل الدم في الاطفال المصابين بالربو الشعبي مع وجود علاقة ايجابية بين مستوياتهم وحدة الربو.

التوصيات: نحتاج الي العديد من الدراسات في المستقبل علي عدد اكبر من الاطفال المصابين بالربو الشعبي ودلك من اجل:

- تقييم مستوي المكونات المتممة بعد التعرض لمحفز لنوبة الربو الشعبي لإيجاد علاقة بين هدا المحفز ومستوي المكونات المتممة.
- امكانية استخدام المكون الثالث والرابع في تقييم نوبة الربو الشعبي الحادة.
- استخدام المكون الثالث والرابع كمؤشر لكفاءة علاج نوبة الربو الشعبي.
- استخدام المكون الثالث والرابع كهدف لعلاج نوبة الربو الشعبي.