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Evaluation of Serum Levels of Calprotectin in Asthmatic Children

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Abstract

Background

Calprotectin is a cytosolic protein that belongs to the S-100 protein group with immunomodulatory and antiproliferative actions. The level of this protein increases in infection, inflammation, and malignancy. The aim of this study was to evaluate the level of calprotectin in asthmatic children as indicator of asthma severity.

Materials and Methods

This is a prospective study that included forty-five children with bronchial asthma and admitted to pediatric department, Minia University Children's Hospital, Egypt, they were (15 intermittent asthma, 15 mild persistent asthma and 15 moderate persistent asthma), and 20 apparently healthy children were included in the study and subjected to thorough history taking, full clinical examination, lung function test, CBC, Immunoglobulin E and measurement of serum levels of calprotectin using ELISA.

Results

The study revealed a statistically significant increase in the level of calprotectin in asthmatic children compared to that of the control group ($p<0.000$). Also, there was a significant increased level of calprotectin in children with persistent asthma compared to those with mild intermittent asthma and control group ($p<0.001$). In addition, there was a positive correlation between serum level of calprotectin and eosinophil count ($r=0.83$, $p=0.001$), and negative correlation with lung functions (FEV1 and PEEF) ($r=-0.89$ and $r=-0.88$, respectively, $p<0.05$).

Conclusion

Increased levels of calprotectin in asthmatic children suggest that it may play a role in asthma, also it was associated with poor lung functions suggesting that there is a close relation between calprotectin level and the severity of childhood asthma.

Key Words: Asthma, Calprotectin, Children, Immunoglobulin E, Pulmonary functions tests.

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1- INTRODUCTION

Asthma is the most common chronic inflammatory disease of the airways associated with airway hyper-responsiveness that leads to recurrent episodic airflow obstruction within the lungs (1). Although the cause of childhood asthma has not been definitely determined, current research includes a combination of environmental respiratory exposures, such as inhaled allergens, respiratory viral infections, air pollutants, and genetic predisposition (2). Calprotectin is a calcium binding protein which is a member of the S100 family of proteins that play important roles in inflammation, barrier function, cancer and innate immunity (3). Calprotectin promotes phagocyte migration to the site of inflammation by promoting the polymerization and stabilization of tubulin microfilaments (4).

Also, calprotectin has proinflammatory functions, such as antimicrobial activity against many bacteria and fungi (5). Calprotectin is released by neutrophils and monocytes immediately after host pathogen interaction and can be measured in plasma, synovial fluid, cerebrospinal fluid, oral fluids and urine by ELISA (6). Calprotectin has been proposed for the diagnosis and follow-up of many inflammatory conditions such as inflammatory bowel disease, Necrotizing enterocolitis (NEC), celiac disease, intestinal cystic fibrosis, acute appendicitis, juvenile idiopathic arthritis, Kawasaki disease, and glomerulonephritis (7-9). It is also possible that calprotectin, at a high concentration, might have a dangerous effect on fibroblasts and influence the recovery of inflammatory tissue (10). The gene responsible for calprotectin is located on chromosome 1q21 (11). There are several advantages to the use of cerebral palsy (CP) as an inflammatory marker in pediatric diseases (12). Studies to detect the role of calprotectin in pediatric asthma are scarce, so this study aimed to detect the level of calprotectin in children with bronchial asthma and correlate its levels with the severity of asthma.

2- MATERIALS AND METHODS

2-1. Study Design

This prospective study was conducted at department of pediatrics, Minia University Children's Hospital, Egypt, during the period from January 2017 till January 2019. Forty-five children were diagnosed with bronchial asthma according to Global Strategy for Asthma Management and Prevention Classification (13); in addition 20 apparently healthy children were taken as control group. Also, informed consent from parents was obtained for every child before the study. Children with severe asthma or suffering from other diseases or on long term steroid therapy were excluded from the study; the protocol was approved by the local Ethics Committee of the faculty. Children with asthma were divided into 3 groups according to severity of asthma;

Group (1): included 15 children (5 males and 10 females) who were diagnosed as mild intermittent asthma, their age ranged from 6 to 11.5 years.

Group (2): included 15 children (9 males and 6 females) who were diagnosed as mild persistent asthma, their age ranged from 7 to 12 years.

Group (3): included 15 children (8 males and 7 females) who were diagnosed as moderate persistent asthma, their age ranged from 6 to 11.5 years.

Group (4): control group included 20 apparently healthy children (11 males and 9 females), their age ranged from 6 to 12.5 years.

2-2. Method

Both asthmatic children and control groups were subjected to the following:

- Thorough history taking and detailed clinical examination was performed. Anthropometric measurements including the height, weight and body mass index (BMI) was assessed.
- Lung function test was done using spirometer, it was done three times and the highest value of the three tests was accepted. Values of Forced Expiratory Volume in the first second (FEV1), peak expiratory flow (PEF) were compared to normal values according to sex, height and age, and were expressed as a percentage of the predicted value.

2-3. Laboratory investigations

CBC was done for all asthmatic patients who attended the clinic and control group; 3 ml of venous blood was withdrawn from each subject; 0.5 ml on EDTA tube for CBC (Sysmex KX-21N, Japan), and the other 2.5 ml were allowed to clot and then were centrifuged at 2,500 g for 15 min and the serum obtained was stored at –70 ℃ for calprotectin and Immunoglobulin E (IgE) assessment. Serum calprotectin (ng/ml), and serum IgE were measured by ELISA (Biotech, China), and (Sigma Diagnostics, USA), respectively. The measurement technique was done according to the manufacturer instructions.

2-4. Statistical Analysis

Data were analyzed using SPSS software version 13.0 (SPSS Inc., Chicago, Illinois, USA), using descriptive statistics, Chi-Square, Fisher Exact Test, Independent-samples t-test, and Pearson's correlation coefficient (r) test was used for correlating data.

3- RESULTS

Table.1 summarized the clinical and laboratory data of both asthmatics and controls, also there was a significant increase in calprotectin level in children with asthma compared to controls ($p<0.000$). **Table.2** shows the serum calprotectin levels were significantly higher in children with mild and moderate persistent asthma compared to children with mild intermittent asthma ($p=0.01$, $p=0.001$), and controls ($p=0.001$) **(Table.3).** There was a significant positive correlation between calprotectin level and eosinophil count ($r=0.83$). As regards lung functions, there was a significant negative correlation between calprotectin level and FEV1 and PEF ($r=-0.88$, -0.89, respectively).

Table.1: Clinical and laboratory data of the patients and control.

Variables	Asthmatic children Number= 45	Control Number= 20	P- value
Age (year); Range Mean ±SD	7-13.5 9.4 ± 2.5	7-13. 9.2 ± 2.4	0.32
Gender; Male Female	22 23	12 8	0.12
Weight (kg) Weight/age percentile	26.8±9 56.6± 30.4	25.8±8.2 56.6± 30.4	0,12
Height (cm) height/age percentile	126.5±10 40.6 ±24.5	129.5±8 44.5 ±22.5	0.26
BMI value	16.1±2.5	17.2±1.9	0.42

Family history of asthma	24(40%)	0	
Family history of atopy	12 (20%)	0	
Eosinophil's count (cell/µl)	525±120	130±68	0.001
Serum Ig E level (IU/ml)	320±110	110±58	0.001
PEFR	82.2 ±5.4	98.2 ±0.9	0.001
FEV1	83.8±6.7	99.1±0.58	0.001
Serum calprotectin level (ng/ml)	18.5±1.62	3.5±1.2	0.001

SD: Standard deviation; BMI=body mass index; PEFR=peaked expiratory flow rate; FEV1= forced expiratory volume in 1st second.

Table-2: Laboratory data and lung functions among patients and controls.

Parameter	Group I n=24	Group II n=21	Group III n=15	Group IV n=40	P- value			
					I vs. IV	II vs. IV	III vs. IV	III vs. I
Eosinophil's count (cell/µl)	366±58	433±102	525±29	154±68	0.05	0.001	0.001	0.01
IgE level (IU/ml)	202±100.8	229.6±107.8	223±110	119±58.4	0.05	0.001	0.001	0.01
PEFR	86.7±3.7 1	85.9±3.8	72.8±9.7	98.2 ±0.9	0.05	0.001	0,001	0.01
FEV1	88.9±2.7	82.5±1.7	70.8±6.7	99.1±0.58	0.05	0.001	0.001	0.01
Serum calprotectin level (ng/ml)	7.5±0.8	12.2 ±1.3	20.5±0.9	3.5±1.2	0.05	0.001	0.001	0.01

Group (1): mild intermittent asthma, Group (2): mild persistent asthma, Group (3): moderate persistent asthma, Group (4): control group included 20 apparently healthy children. IgE: Immunoglobulin E; PEFR=peaked expiratory flow rate; FEV1= forced expiratory volume in 1st second.

Table-3: Correlation between level of calprotectin and laboratory data among asthmatic children.

Parameter	Serum calprotectin level	
	Pearson correlation coefficient	P-value
Eosinophils count (cell/µl)	0.83	0.00 1
IgE level (IU/ml)	0.22	0.1
FEV1	-0.88	0.001
PEFR	-0.89	0.001

IgE: Immunoglobulin E; FEV1: Forced Expiratory Volume in the first second; PEFR: peak xpiratory flow rate.

4- DISCUSSION

Calprotectin (CP) is a 36.5-kDa heterodimer composed of one light (MRP8), and two heavy (MRP14), calcium- and zinc-binding proteins of the S100 family (14), CP is expressed mainly in the neutrophils with important extracellular activity (about 5% of their total protein contents and 30–60% of their cytosolic protein (15, 16). CP has many functions including antibacterial, apoptosis-inducing (17), and chemotactic functions (18). CP can be used as an inflammatory marker in pediatric diseases, faecal calprotectin (FCP) is an objective and non-invasive test reflecting different pathological processes occurring in the intestine of patients (19, 20). While

serum calprotectin (SCP) could be a sensitive nonspecific inflammatory marker in various pediatric conditions (21). It also participates in leukocyte interactions with the endothelium, cellular adhesions leading to the recruitment of leukocytes to inflamed intestinal tissue (22, 23), and with the inflammatory and thrombogenic response of endothelial cells (24). Studies to measure the level of calprotectin in children with asthma are scarce so the aim of this study was to evaluate the level of calprotectin in children with asthma and correlate its level with asthma severity in the present study; serum level of calprotectin was significantly increased in children with asthma compared with the control group ($p=0.001$). There was insignificant increase in the level of calprotectin in asthmatic children with mild intermittent asthma compared with that of the control ($p>0.05$); while there were significant higher levels of serum calprotectin in children with mild persistent and moderate persistent asthma than the level in the control group ($p<0.001$).

The increased serum level of calprotectin may result from the effect of inflammatory mediators that are released during acute asthma attack and that cause lung damage. These findings are in agreement with Orivuori et al. (25), they found high level of fecal calprotectin in asthmatic children; they are also in agreement with Aoki et al. (26) who found high level of calprotectin in human cultured bronchial cells after activation by allergen. In contrast, Cobanoglu et al. (27) found that there was no difference in serum level of calprotectin in asthmatic children and healthy controls. The study showed that the level of calprotectin was significantly higher in children with moderate persistent asthma compared to those with mild asthma and controls, this is in agreement with Kato et al. (28), they found that, calprotectin levels were only elevated in uncontrolled asthma patients compared to controls and newly diagnosed subgroup ($p<0.05$). In addition, as bronchial asthma is associated with recurrent exacerbations, changes observed during the course of each episode, including the levels of calprotectin have been shown to be of potential value in the exacerbation pattern prediction (29, 30). Also, the pulmonary circulation in such patients has been found to be characterized by hyperpermeability, remodeling that involves the whole bronchial tree, increase in subepithelial blood flow and endothelial dysfunction (31, 32). In addition, upregulation of Calprotectin in both settings of endothelial dysfunction and in the inflammatory processes of similar molecular patterns. This demonstrates that there are adequate grounds for future investigation of its role in asthma as well as in the follow-up of affected patients (33, 34).

4-1. Study Limitations

The present study has some limitations. First, the number of patients recruited was relatively small, it would be useful to repeat the study on a larger sample of patients in future. Second, the concentration of calprotectin was measured only initially, in the first 24 h after the inclusion in the study, and the dynamics of concentration during the asthmatic evolution has not been evaluated. Our study is one of the few studies to investigate the predictive value of calprotectin for asthma. We have demonstrated that calprotectin can predict the severity of asthmatic attack. In addition, combined clinical variables with biological biomarkers such as calprotectin may play an important role in early therapeutics or preventative approaches for asthma.

5- CONCLUSION

The study concluded that serum calprotectin level increased in asthmatic children with increasing severity. Also, its level positively correlated with peripheral blood eosinophil (PBE) counts and reversely correlated with lung functions. These establish that calprotectin may play a role in systemic inflammatory cascade in asthmatic children.

6- CONFLICT OF INTEREST

None of the authors has a financial relationship with any commercial entity that has an interest in the subject of this paper. We have no conflict of interest to declare.

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