

Assessment of Serum Level of Interleukin-37 in Asthmatic Children at Benha University Hospital

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Asthma is a complex inflammatory disease, characterized by airway hyperresponsiveness, inflammation, and reversible airway obstruction. Interleukin-37 (IL-37), functions as a fundamental inhibitor of innate inflammatory and immune responses, and it is an important cytokine in the control of asthma by suppressing the production of inflammatory cytokines. This study aimed to reveal the possible role of IL-37 in asthma through assessment of its serum level in controlled and uncontrolled asthmatic children as compared to controls. Serum IL-37 level was measured by ELISA. The serum level of IL-37 was significantly lower in patients than controls and in uncontrolled than in controlled asthma ($P<0.001$). It is concluded that there is negative relation between serum level of IL-37 and asthma, which is more evident in uncontrolled asthmatic group, this observation may support the protective role of IL-37 in immune pathogenesis of asthma.

Asthma is a complex inflammatory disease, characterized by airway hyperresponsiveness (AHR), airway inflammation, and reversible airway obstruction, affecting up to 300 million people worldwide [1]. The development of asthma is a multifactorial process which is associated with a variety of risk factors, including environmental factors and genetic factors [2]. In asthmatic patients, allergic episodes trigger the bronchoalveolar infiltration of various immune cell populations, mostly eosinophils, mast cells, and activated CD₄⁺ T-cells. Effector T-helper 2 (Th2) cells play a central role in distributing the immune response to allergens by releasing cytokines that trigger the predominant features of asthma. The secretion of IL-4 and IL-13 contributes to B-cell production of IgE, the release of IL-5 drives eosinophilic inflammation, and IL-9 stimulates mast cell proliferation [3]. In 2016, the prevalence of asthma in rural and urban schools was 5.34 and 6.58%,

respectively, with a total prevalence of 6.09% among primary school children in Menoufiya governorate, Egypt. A low socioeconomic level, a positive family history of similar disease, and exposure to smoke showed a significant effect as risk factors for asthma. [4]. Bronchial asthma had a significant effect on delayed weight gain, disturbed sleep, missed school days, limited activity, and emergency room visits and had a significant effect on delayed growth [4]. Early diagnosis of asthma among children can facilitate clinical control and result in reduction in morbidity. Delayed diagnosis causes lung dysfunction while proper treatment at the right time can inhibit it [5].

Interleukin-37 (IL-37) is an anti-inflammatory cytokine that can bind to IL-18-receptor and IL-18-binding protein (BP). IL-37 can be secreted by monocytes, macrophages and epithelial cells [6]. IL-37 has five different isoforms, of which IL-37b is the largest isoform. IL-37 is a reported

molecular of IL-1 family with anti-inflammatory effect. IL-37 has potent anti-inflammatory properties and many studies have elucidated its precise role in autoimmune disease such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), inflammatory bowel disease (IBD), guillain-Barré syndrome, and atopic dermatitis (AP) [7]. IL-37, functions as a fundamental inhibitor of innate inflammatory and immune responses [7], [8], and it is an important cytokine in the control of asthma by suppressing the production of inflammatory cytokines [9,10]. IL-37 plays different roles in the pathogenesis of the different phenotypes of asthma. IL-17, which can be suppressed by IL-37 is involved in both neutrophilic and eosinophilic asthma. IL-17 produced by Th17 cells or macrophages not only promotes the infiltration of neutrophils into the lung but a recent evidence also suggests that it can contribute to the development of allergic eosinophilic asthma [11,12] where it acts synergistically with Th2 cytokines to promote airway hyperresponsiveness (AHR) [11]. IL-37 can suppress Th1, Th2, and Th17 effector responses to modulate inflammatory responses [13,14]. Defective IL-37 signaling can lead to both Th2- and Th1-mediated inflammatory diseases [15], including asthma [13].

This study aimed to shed light on the role of IL-37 in asthma by assessing its serum level in controlled and uncontrolled asthmatic children versus controls.

Patients and Methods

Study populations

This case control study was conducted on sixty asthmatic children at the Allergy and Asthma Clinic of the Pediatric Department, Benha university hospital and the Microbiology and Immunology Department, Benha Faculty of Medicine, during the

period November 2016 till May 2017. Thirty apparently healthy children age and sex matched served as controls. This study was reviewed and approved by the Ethical Committee of Benha University. Study children and controls were enrolled after obtaining an informed consent from their parents.

Inclusion criteria

Children aged from 4 to 12 years diagnosed as bronchial asthma according to the Global Initiative for Asthma (GINA) guidelines (2011) [16], stable asthma with different disease control levels.

Exclusion criteria

Any chronic diseases other than bronchial asthma.

The asthmatic children were subdivided into two subgroups according to. Asthma control test, the children and their parents answered questions to assess the frequency of shortness of breath, general asthma symptoms, use of rescue medications, the effect of asthma on daily functioning, and overall self-assessment of asthma control during the past 4 weeks and, then according to asthma score, the level of asthma control was calculated, Score ≥ 20 indicates controlled asthma, while ≤ 19 indicates uncontrolled asthma according to Nithan *et al.*, [17]

- Group A

Thirty asthmatic children with uncontrolled bronchial asthma (UBA), score ≤ 19

- Group B

Thirty asthmatic children with controlled bronchial asthma, Score ≥ 20 (CBA)

- Control Group

It included 30 apparently healthy children age and sex matched.

Each child was subjected to:

1-Full history taking laying stress on asthma history and positive family history of atopy.

2-Thorough clinical examination including general and systemic examination, Body Mass Index (BMI) was calculated as weight (Kg)/ height (m^2) [18].

3- Peak flow meter: To assess degree of asthma. Peak flow meter is a small handheld device that measures the fastest rate of air that a person can blow out of his lungs. It shows how well the airways are open. It measures peak expiratory flow rate (PEFR) % [19].

4- Serum Interleukin 37 level assay

It was measured using human Interleukin 37 ELISA Kit (CUSABIO TECHNOLOGY, China), based on standard sandwich ELISA. Monoclonal antibodies specific for IL-37 have been precoated onto 96-well plates. It was done according to the manufacturer's instructions.

All candidates had undergone this assay, 2 milliliters of blood were withdrawn under complete aseptic precautions, put in glass tube and left to clot for 20 minutes. Serum was separated by centrifugation for 20 minutes at 800xg and stored at -20°C in Eppendorf tubes until further processing.

Standard and samples were added to the wells and combined to the specific antibody. The wells were washed then a horseradish peroxidase (HRP) conjugate mixed with biotinylated anti-human antibody was added, and incubated, unbound conjugates were washed away. TMB substrate solution was added to each well, produced blue color which turned yellow after addition of the acidic stop solution. The optical density (OD) was measured spectrophotometrically by a full-automated Microplate Reader MB-580 (Shenzhen Huisong Technology Development Co., Ltd, China) at a wave length of 450nm. The OD value is proportional to the concentration of IL37.

Statistical Analysis

The collected data were summarized in terms of mean \pm Standard Deviation (SD), range for quantitative data and frequency and percentage for qualitative data. Comparisons between the different study groups were carried out using the Chi-square test and Fisher's Exact Test (FET) to compare differences between proportions as appropriate. The Student's t-test (t) and the Mann-Whitney test (z) were used to test differences between two groups regarding parametric and non-parametric data respectively. While, the One-way Analysis Of Variance (ANOVA; F) was used to test differences between more than two groups followed by post-hoc test using the Bonferroni method to detect differences in pairs. The Pearson correlation coefficient and the Spearman correlation coefficient were used to test for the correlation between IL-37 levels and quantitative variables. Statistical significance was accepted at $P < 0.05$ (S). A P value > 0.05 was considered non-significant. All statistical analyses were carried out in STATA/SE version 11.2 for

Windows (STATA Corporation, College Station, Texas).

Results

There was no statistically significant difference between the studied groups regarding to age, sex and body mass index while there was statistically significant difference between them as regarding history of other atopic conditions and regarding positive family history of atopy $P < 0.001$, $P = 0.001$, respectively. These data are shown in Table 1.

The mean \pm SD of the PEFR of group A [uncontrolled bronchial asthma (UBA)], B [controlled bronchial asthma (CBA)] and the control group was 117.33 ± 7.04 , 137.33 ± 11.63 and 332.67 ± 52.02 liters per minute, respectively and so there was statistically significant decrease in PEFR in patients groups than the control group $P < 0.001$ and in uncontrolled asthma than controlled asthma. These data are summarized in Table 2.

The Mean \pm SD of the serum level of interleukin 37 in group A [uncontrolled bronchial asthma (UBA)], B [controlled bronchial asthma (CBA)] and the control group was 1.78 ± 0.45 , 2.27 ± 0.71 , 3.81 ± 0.84 pg/ml, respectively, i.e. a statistically significant decrease in serum level of interleukin 37 in the patients group than the control group $P < 0.001$, and in the uncontrolled asthma group than in the controlled asthma group ($P < 0.001$). These data are shown in Table 3 & Figure 1.

There was a positive correlation between serum level of interleukin 37 and PEFR. These data are shown in Figure 2.

Table 1. Demographic data of the studied groups

Variable		Group A(UBA), (No.=30)		Group B(CBA) (No.=30)		Controls (No.=30)		P value
		No.	%	No.	%	No.	%	
Sex	Male	12	40.0	18	60.0	14	46.67	NS
	Female	18	60.0	12	40.0	16	53.33	
History of atopic condition other than asthma	Yes	14	46.67	16	53.33	0	0.0	<0.001
	No	16	53.33	14	46.67	30	100.0	
Positive FH of atopy	Yes	22	73.33	18	60.0	8	26.67	0.001
	No	8	26.67	12	40.0	22	73.33	
		Mean \pm SD	Range	Mean \pm SD	Range	Mean \pm SD	Range	
Age (years)		9.07 \pm 1.41	7-11	8.17 \pm 2.54	4-12	8.53 \pm 1.81	5-11	NS
BMI (kg/m ²)		21.5 \pm 1.43	17-24	22.3 \pm 2.58	18-25	21.4 \pm 1.84	19-24	NS

FH=Family history, $P>0.05$ is not significant (NS)

Table 2. Comparison between group A, group B and control regarding peak expiratory flow rate (PEFR) (Litres per minute).

Variable	Group A(UBA) (No.=30)		Group B(CBA) (No.=30)		Control (No.=30)		P value
	Mean \pm SD	Range	Mean \pm SD	Range	Mean \pm SD	Range	
PEFR	117.33 \pm 7.04	110-130	^a 137.33 \pm 11.63	120-150	^{ab} 332.67 \pm 52.02	250-390	<0.001

a: significant difference compared to Group A

b: significant difference compared to Group B

$P<0.05$ is significant.

Table 3. Comparison between group A, group B and control regarding serum level of Interleukin 37(pg/ml).

Variable	Group A(UBA) (No.=30)		Group B(CBA) (No.=30)		Control (No.=30)		P value
	Mean \pm SD	Range	Mean \pm SD	Range	Mean \pm SD	Range	
IL-37	1.78 \pm 0.45	1.14-2.41	^a 2.27 \pm 0.71	1.17-3.59	^{ab} 3.81 \pm 0.84	1.19-4.60	<0.001

a: significant difference compared to Group A

b: significant difference compared to Group B

$P<0.05$ is significant.

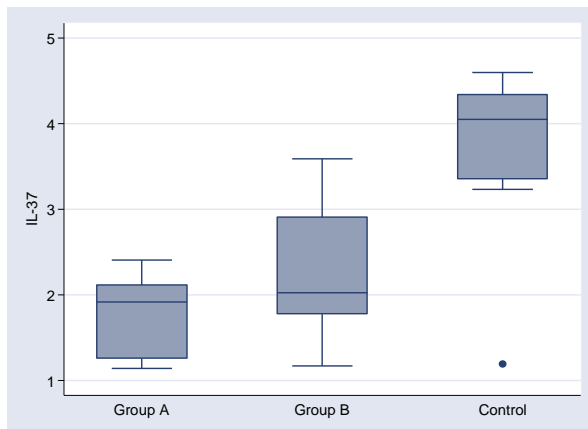


Figure 1. Comparison between group A, group B and control regarding serum level of Interleukin 37(pg/ml).

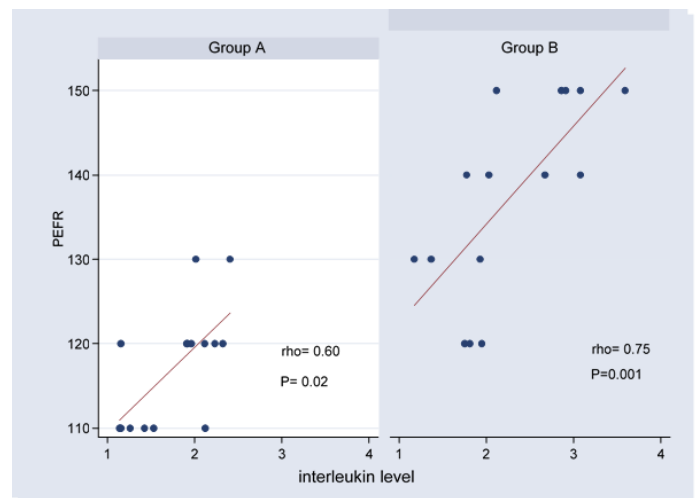


Figure 2. Correlation between serum level of Interleukin 37 and PEFR

Discussion

This study aimed to reveal the possible role of IL-37 in asthma through assessment of its serum level in controlled and uncontrolled asthmatic children as compared to controls.

The present study showed that, there was no significant difference between the studied groups regarding to age and sex, which agrees with previous studies [7,8]. However other studies reported that asthma has a higher prevalence in boys than in girls before puberty and a higher prevalence in women than in men in adulthood. It is likely that hormonal changes and genetic susceptibility both contribute to the change in prevalence that occurs about the time of puberty [20,21].

This study showed that there was no significant difference between the studied groups regarding BMI which is in line with that reported by Charrad *et al.* [8], while most studies supported a link between obesity and asthma [22,23]. There are many possible mechanisms for the relationship between asthma and obesity including

airway inflammation, mechanical changes in airway hyper responsiveness and changes in physical activity and diet [24].

This study showed a significant increase of positive family history of atopy among the patients group than the control group ($p = 0.01$). This agrees with studies reported by Liu *et al.* [7], Jain & Bhat [25] and Al-Mazamand & Mohamed [7,25,26]. Parental asthma has been shown in several studies to be a strong predictor of asthma in the children [7, 25, 26, 27].

The current study showed a significant decrease in PEFR in the patients group than the control group and also a significant decrease in PEFR in the uncontrolled asthma group than in the group with controlled asthma. This agrees with Srinivasa & Ushakiran and Lin *et al.* [28,29]. It was considered that the decrease in PEFR reading is a criterion of severity of asthmatic exacerbation [30].

This study showed a significant decrease in serum level of interleukin 37 in the patients group than the control group and in

the uncontrolled asthma group than in the controlled asthma group. This is in line with Charrad *et al.* [8] who assessed IL-37 production in asthmatic children and reported that the expression of IL-37 mRNA in asthmatic patients was significantly lower than that observed in healthy controls and its expression correlated with asthma severity. Such findings suggested that IL-37 could be an important cytokine in the control of asthma by suppressing the production of inflammatory cytokines.

This also agrees with Lunding *et al.* [13] who found that IL-37 production of human peripheral blood mononuclear cells was significantly lower in allergic asthmatics vs healthy children. In wild-type mice, intranasal administration of IL-37 ablated allergic airway inflammation as well as cytokine production and subsequently diminished the hallmarks of experimental asthma including mucus hyperproduction and AHR. In contrast, local application of IL-37 produced none of these effects in mice lacking either IL18R α or SIGIRR/IL-1R8.

Another study by Hu [31] highlighted the anti-inflammatory role of IL-37 in asthma and found decreased IL-37 in the patients group than in the control groups [31]. Other studies reported that the expression of IL-37 mRNA in asthmatic patients was significantly lower than that observed in healthy controls. Defective IL-37 signaling can lead to both Th2- and Th1- mediated inflammatory diseases including asthma [32,15]

This study showed a positive correlation between IL-37 and PEFr. This agrees with Liu *et al.* [7] who observed a significant correlation between IL-37, PEFr and the degree of severity of asthma.

In the current study, it was found that IL-37 significantly decreased in uncontrolled asthma than controlled asthma, this in

agreement with Smolnikova *et al.*, [33], who reported that there are clinical, immunological and genetic changes present in uncontrolled asthmatic children.

In conclusion, a negative relation between serum level of interleukin 37 and asthma was observed, and it is more evident in uncontrolled asthma. Future studies are needed to better understand the role of IL-37 in asthma and its possible usage in asthma therapy.

References

1. Masoli M, Fabian D, Holt S. The global burden of asthma: executive summary of the GINA Dissemination Committee report. *Allergy* 2004; 59: 469-78.
2. Zhu S, Chan-Yeung M, Becker AB, Dimich-Ward H, Ferguson AC, Manfreda J, Watson WT, Paré PD, Sandford AJ. Polymorphisms of the IL-4, TNF-alpha, and Fcepsilon R1beta genes and the risk of allergic disorders in at-risk infants. *Am J Respir Crit Care Med* 2000; 161: 1655-9.
3. Rosenberg HF, Phipps S, Foster PS. Eosinophil trafficking in allergy and asthma. *J Allergy Clin Immunol.* 2007; 119(6):1303-10
4. El-Mashad GM, Mahmoud AA, Abdel Hafez AA. The prevalence of bronchial asthma among primary school children in Menoufiya Governorate (El-Bagour Center). *Menoufia Med J* 2016; 29:89-94
5. (5). Portnoy JM, Jones EM. Diagnosing asthma in young children. *Curr Allergy Asthma Rep.* 2002; 2:447-52.
6. Boraschi D, Lucchesi D, Hainzl S, Leitner M, Maier E, Mangelberger D. IL-37: a new anti-inflammatory cytokine of the IL-1 family. *Eur Cytokine Netw.* 2011; 22:127-147.
7. Liu W, Deng L, Chen Y, Sun C, Wang J, Zhou L, Li H, and Luo R. Anti-Inflammatory Effect of IL-37b in Children with Allergic Rhinitis, *Mediators of Inflammation*, 2014; 746846, 1-13
8. Charrad R, Berraies A, Hamdi B, Ammar J, Hamzaoui K, Hamzaoui A. Anti-inflammatory activity of IL-37 in asthmatic children: correlation with inflammatory cytokines TNF-alpha, IL-beta,

- IL-6 and IL-17A. *Immunobiology*. 2016; 221:182–187.
9. Wang M, Wang B, Ma Z, Sun X, Tang Y, Li X. Detection of the novel IL-1 family cytokines by QAH-IL1F-1 assay in rheumatoid arthritis. *Cell Mol Biol*, 2016;62:31–34.
 10. Nold-Petry CA, Lo CY, Rudloff I, Elgass KD, Li S, Gantier MP, Lotz-Havla AS, Gersting SW, Cho SX, Lao JC, Ellisdon AM, Rotter B, Azam T, Mangan NE, Rossello FJ, Whisstock JC, Bufler P, Garlanda C, Mantovani A, Dinarello CA, Nold MF.
 11. IL-37 requires the receptors IL-18R α and IL-1R8 (SIGIRR) to carry out its multifaceted anti-inflammatory program upon innate signal transduction. *Nat Immunol*. 2015; 16:354–365.
 12. Wilson RH, Whitehead GS, Nakano H, Free ME, Kolls JK, Cook DN. Allergic sensitization through the airway primes Th17-dependent neutrophilia and airway hyperresponsiveness. *Am J Respir Crit Care Med*. 2009; 180:720–730.
 13. Barczyk A, Pierzchala W, Sozanska E. Interleukin-17 in sputum correlates with airway hyperresponsiveness to methacholine. *Respir Med*. 2003; 97:726–733
 14. Lunding L, Webering S, Vock C, Schröder A, Raedler D, Schaub B, Fehrenbach H, Wegmann M. IL-37 Requires IL-18R α and SIGIRR/IL-1R8 to Diminish Allergic Airway Inflammation in Mice. *ALLERGY*. 2015;70:366–373
 15. Ye L, Jiang B, Deng J, Du J, Xiong W, Guan Y. IL-37 alleviates rheumatoid arthritis by suppressing IL-17 and IL-17-triggering cytokine production and limiting Th17 cell proliferation. *J Immunol*. 2015; 194:5110–5119.
 16. Xia T, Zheng XF, Qian BH, Fang H, Wang JJ, Zhang LL. Plasma Interleukin-37 is elevated in patients with rheumatoid arthritis: its correlation with disease activity and Th1/Th2/Th17-related cytokines. *Dis Markers*. 2015; 2015:795043.
 17. Global initiative for asthma GINA. Global strategy for the Diagnosis and management of asthma 2011; :<http://www.ginasthma.org>.
 18. Nathan RA, Sorkness CA, Kosinski M, Schatz M, Li JT, Marcus P, Murray JJ, Pendergraft TB. Development of the asthma control test: a survey for assessing asthma control. *J Allergy Clin Immunol* 2004; 113(1):59–65.
 19. Must A, Anderson SE. Effects of obesity on morbidity in children and adolescents. *Nutr Clin Care*. 2003; 6(1):4–12.
 20. Adeniyi BO, Erhabor GE. The PFM and its use in clinical practice. *Afr J Resp Med* 2011; 6: 5–8.
 21. Subbarao P, Mandhane PJ, Sears MR Asthma: epidemiology, etiology and risk factors. *CMAJ : Canad Med Asso J*, 2009; 181(9), E181–E190.
 22. Postma DS. Gender differences in asthma development and progression. *Gend Med*. 2007; 4:133-46
 23. Chinn S. Obesity and asthma. *Paediatr Respir Rev*. 2006; 7:223–8.
 24. Flaherman V, Rutherford GW. A meta-analysis of the effect of high weight on asthma. *Arch Dis Child*. 2006; 91(4):334-9.
 25. von Mutius, E., Schwartz, J., Neas, L.M., Dockery, D., Weiss, S.T. Relation of body mass index to asthma and atopy in children: the National Health and Nutrition Examination Study III. *Thorax*. 2001; 56: 835–838
 26. Jain A, Bhat HV. Prevalence of bronchial asthma in rural Indian children: a cross sectional study from South India. *The Indian J Pediat*, 2009; 77 (1): 31 – 35.
 27. Al-Mazam A, Mohamed AG. Risk factors of bronchial asthma in Bahrah, Saudi Arabia. *J Family & Comm Med*, (2001): 8(1), 33–39.
 28. Sherman CB, Tosteson TD, Tager IB, Speizer FE, Weiss ST Early childhood predictors of asthma. *Am. J. Epidemiol*. 1990; 132:1990-83-95
 29. Srinivasa K, Ushakiran CB. Clinical study of bronchial asthma in children aged 5 to 12 years with special reference to peak expiratory flow rate. *Inter J Contemp Pediat*, 2015; 2 (4): 297 – 302.
 30. Lin R, Guan R, Liu X. Significant rise of the prevalence and clinical features of childhood asthma in Qingdao China: cluster sampling investigation of 10,082 children *BMC Public Health*. 2014; 14: 1002
 31. Reddel HK, Taylor DR, Bateman ED, Boulet L, Boushey HA, Busse WW. An official American Thoracic Society/European Respiratory Society statement: Asthma control and exacerbations: Standardizing endpoints for clinical trials and

- clinical practice. *Amer J Resp Criti Care Med.* 2009; 80:59–99.
32. Hu D. Role of Anti-inflammatory Cytokines IL-35 and IL-37 in Asthma 2017; 40: 697.
33. Raedler D, N. Ballenberger, E. Klucker, A. Böck, R. Otto, O. Prazeres Da Costa, O. Holst, T. Illig, T. Buch, E. von Mutius, B. Schaub Identification of novel immune phenotypes for allergic and nonallergic childhood asthma. *J Allerg & Clin Immunol* 2015; 135: 81–91
34. Smolnikova MV, Smirnova SV, Freidin MB, Tyutina OS Immunological parameters and gene polymorphisms (C-590T IL4, C-597A IL10) in severe bronchial asthma in children from the Krasnoyarsk region. West Siberia. *Int J Circumpolar Health*, 2013; 72:21159.