EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 2
Issue: 7
425-429
Year: 2015

INTERLEUKIN-6 AND INTERLEUKIN-17 LEVELS OF ASTHMATIC PATIENTS IN THI-QAR PROVINCE

***Ali Naeem Salman and Hind Hussein Salih**

Biology Dept., Collage of Education for Pure Science – Thi-Qar Univ.

***Author for Correspondence: Ali Naeem Salman**
Biology Dept., Collage of Education for Pure Science – Thi-Qar Univ.

Article Received on 23/09/2015 **Article Revised on 12/10/2015** **Article Accepted on 02/11/2015**

ABSTRACT
The current study was conducted at the Al-Hussein Teaching Hospital in Thi- Qar province, during the period from October 2014 to May 2015. The study aimed to evaluate immune status of asthmatic patients by measuring the levels of interleukins (IL-6, IL-17) in the serum using a technique enzyme-linked immunosorbent assay (ELISA), the study included a total of 60 patients with bronchial asthma they were (22 males and 38 females) and they were aged between 17-62 years and compared with 20 apparently healthy people as control. The results showed a significant increase ($P \leq 0.001$) in the levels of interleukins (IL-6, IL-17) in the serum of all patients with bronchial asthma compared to the control group.

KEYWORDS: IL-6, IL-17, Asthma, Thi-Qar.

INTRODUCTION
Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. causes recurrent episodes of wheezing, breathlessness, chest tightness, and coughing. These episodes cause airflow obstruction, often reversible either spontaneously or with treatment.[1] it is a complex disease that is caused by a combination of genetic and environmental factors.[2] These factors influence its severity and its responsiveness to treatment.

asthma is a significant public health problem. affecting approximately 300 million individuals worldwide.[3] Currently, the prevalence of allergic asthma is increasing globally due to air pollution and other environmental irritants. These environmental exposures are especially evident in developing countries. where industrialization is progressing rapidly.[4]

Chronic airway inflammatory processes result in intense recruitment of activated eosinophils and T-helper (Th)2 lymphocytes at the site of injury and an inappropriate immune response to common allergens.[5] Recurrent inflammation and subsequent abnormalities in the tissue repair mechanisms lead to structural changes in the airway wall that manifest the clinically detectable features of epithelial injury, goblet cell hyperplasia, subepithelial thickening, airway hyperplasia and angiogenesis.[6] Thus, allergic asthma is characterized as a complex airway remodelling disease.[7] While there is currently no cure for asthma, the standard of care for asthma is limited to symptomatic control of disease mediators with potent inhaled corticosteroids (ICS), long-acting β-adrenergic agonists and leukotriene modifiers.[8][9]

Cytokines
Cytokines are low-molecular weight regulatory proteins or glycoproteins secreted by white blood cells and various other cells in the body in response to a number of stimuli. Cytokines include chemokines, interferons, interleukins, lymphokines, Cytokines are produced by a broad range of cells, including immune cells like macrophages, B lymphocytes, T lymphocytes and mast cells, as well as endothelial cells, fibroblasts, and various stromal cells; a given cytokine may be produced by more than one type of cell.[10] The main biological activities of a number of cytokines include both cellular and humoral immune responses, induction of inflammatory responses, regulation of hematopoiesis, control of cellular proliferation and differentiation, and induction of wound healing. They are important in health and disease, specifically in host responses to infection, immune responses, inflammation, trauma, sepsis, cancer, and reproduction.[11]

Interleukin 6 (IL-6)
IL-6 is a proinflammatory cytokine, affects various processes including, the immune response reproduction, bone metabolism and aging. IL-6 is synthesized by mononuclear phagocytes, vascular endothelial cells, fibroblasts and other cells in response to trauma, burns, tissue damage and inflammation.[12] However, recent studies suggest that IL-6 plays an important role in determining the type of adaptive immune response, primarily in the differentiation of effector CD4+ T

cells.[13] IL-6 has been shown to promote Th2 differentiation of CD4+ T cells while suppressing Th1 differentiation through independent pathways that IL-6 promotes allergic airway inflammation.[14][15] and could also influence lung physiology by promoting an increase in airway wall thickness, subepithelial fibrosis and smooth muscle hypertrophy and proliferation, as supported by different studies.[16]

Interleukin 17 (IL- 17)

IL-17 is a proinflammatory Th1 cytokine produced by activated T helper cells.[17] IL-17 is an early trigger of the T lymphocyte-induced inflammatory response and has potent chemotactic activity for inflammatory cells. It can remarkably increase recruitment of inflammatory cells in the airways. IL-17 can be involved in airway inflammation through a variety of ways: (1) it can induce bronchial epithelial cells, bronchial fibroblasts, and venous endothelial cells to release proinflammatory cytokines such as IL-6, IL-8, GM-CSF and TNF-γ, which can stimulate the inflammatory tissues and thus indirectly causes tissue invasion and tissue damage.[18] (II) it can promote dendritic cell maturation and stimulate the relevant cells to produce a series of inflammatory mediators and cytokines, causing inflammatory response[19] and (III) the Th17 cells can produce IL-17 and thus mediate the endothelial cells, fibroblasts, and macrophages to secret a series of inflammatory chemokines, which promotes the aggression of inflammatory cells and the chronic inflammation, resulting in immune-inflammatory damage.[20]

MATERIALS AND METHODS

study desigen

This study was performed on (60) Iraqi patients with bronchial asthma, who attended the Al-Hussein Teaching Hospital in in Al-Nasiriya city in the period from beginning of October 2014 to the end of May 2015. Also, this study included (20) person apparently healthy individuals as a control group., who have no history or clinical evidence of asthma or any other chronic disease and no obvious abnormalities.

Blood Samples Collection

Blood samples were collected by venipuncture from 60 patients and 20 controls (five milliliters of venous blood) were drawn by disposable syringe under aseptic technique. The blood samples were placed in a sterile plane tube and allowed to clot, then serum was separated by centrifugation at 4000 rpm for 15 minutes. The serum was stored at -10C˚. These sera (60 asthmatic patients and 20 controls) were used for estimating the concentration of interleukin (IL- 6 and IL-17).

METHODS

Kit of (IL-6&IL-17) provided by (Elabscience Company, China). The sera of patients and controls were assessed for the level of tow cytokines, which were IL-6, IL-17, by means of ELISA that were based on similar principles.

A - Principles of Assay

This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for IL-6, IL-17 has been pre-coated onto a micro plate. Standards and samples were pipetted into the wells and any IL-6, IL-17 present was bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for IL-6, IL-17 was added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) was added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution was added to the wells and color develops in proportion to the amount of IL-6, IL-17 bound in the initial step. The color development was stopped and the intensity of the color is measured.

B – Assay procedure

All reagents were bring and samples to room temperature before use. The samples were centrifuged again. It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents, working standards and samples as directed in the previous sections.
2. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 4°C.
3. Add 100µl of standard and sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at 37°C. A plate layout is provided to record standards and samples assayed.
4. Remove the liquid of each well, don't wash.
5. Add 100µl of Biotin-antibody (1x) to each well. Cover with a new adhesive strip. Incubate for 1 hour at 37°C. (Biotin-antibody (1x) may appear cloudy. Warm up to room temperature and mix gently until solution appears uniform.).
6. Aspirate each well and wash, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (200µl) using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher and let it stand for 2 minutes, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
7. Add 100µl of HRP-avidin (1x) to each well. Cover the microtiter plate with a new adhesive strip. Incubate for 1 hour at 37°C.
8. Repeat the aspiration/wash process for five times as in step 6.
9. Add 90µl of TMB Substrate to each well. Incubate for 15-30 minutes at 37°C. Protect from light.
10. Add 50µl of Stop Solution to each well, gently tap the plate to ensure thorough mixing.

11. Determine the optical density of each well within 5 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. Subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

Statistical analysis

The analysis of data was expressed as mean ± SD. The comparisons between each asthmatic group with matched healthy control were performed with T-test by using computerized Minitab 14 program. P<0.001 was considered to be the least limit of significance, the statistical analysis was done by using Pentium-4 computer through the (SPSS program) Statistical Package For Social Sciences (version-20).

RESULTS

The present study showed the presence of a significant increase ($P \leq 0.001$) in the rate of concentrations of IL-6, IL-17 in sera of patients with asthma, compared with the average concentration in the sera of healthy control group, as was the rate of concentration of IL-6 in patients (4.770pg/dl) compared to the control group (2.051 pg/dl) with significant difference (0.001), while IL-17 concentration (28.214 pg/dl) for patients compared to the healthy control (12.722pg/dl) with a significant difference (0.001).

Table (1) Comparison of serum (IL-6, IL-17) concentrations (pg/dL) of the patient groups with healthy controls group.

Parameter	Subject	No of cases	Mean ± SD	T-value	Df	P-value
IL – 6	Pateints	60	4.770± 3.086	3.659	78	0.001
	Control	20	2.051 ± 1.573			
IL – 17	Pateints	60	28.214 ± 15.810	3.877	78	0.001
	Control	20	12.722 ± 6.948			

DISCUSSION

In this study, the level of **IL-6** in serum from patients with asthma was significantly higher than that from controls. The increased level of IL-6 in patients with asthma suggests that local inflammatory processes may play a role in increasing level of IL-6 in asthmatic patients.

Many studies showed that circulating IL-6 levels were higher in asthma patients compared with control patients[21][22] increased release of IL-6 from alveolar macrophages from asthmatic patients after allergen challenge[23] as well as increased IL-6 secretion from lung epithelial cells collected from asthmatics.[24][25]

IL-6, a cytokine produced by inflammatory cells is also produced by primary lung epithelial cells in response to a variety of different stimuli including allergens, respiratory virus and exercise[26][27] IL-6 has emerged as an important regulator of effector CD4 T cell fate[28], promoting IL-4 production during Th2 differentiation, inhibiting Th1 differentiation and together with TGFβ, promoting Th17 cell differentiation. The pleotropic nature of these immunoregulatory roles suggests that IL-6 could be a potential wide-ranging contributor to asthma as well as other pulmonary diseases where the lung epithelium is damaged.

The results showed that there was a significant increase of **IL-17** in the asthmatics compared with healthy controls and Many studies showed that circulating IL-17 level was significantly higher in patients with allergic asthma than those in the control group.[29][30]

IL-17 is a proinflammatory cytokine playing an important role in the induction and propagation of inflammation in asthma.[31] IL-17 may be one of the major cytokines involved in exacerbation of bronchial asthma.[32]

IL-17 can enhance the development and maturation of neutrophils, recruit neutrophils, promote the maturation and differentiation of multiple cells and drives the allergic TH2 response, which produces the cytokines IL-4, IL-5, and IL-13 and thereby promotes IgE production, eosinophilia and mucus secretion into the airway.[33][34]

IL-17 can cause the remodeling of airway and lung tissue, which may because IL-17 can activate the neutrophils to produce neutrophil elastase, which can degrade the elastin, promote the secretion of gland cells and thus affect the structures of airway and lung tissues.[35] The activated neutrophils release matrix metalloproteinase-8 and -9, resulting in the massive degradation of the components of the extracellular matrix and the changes in airway structures.[36]

REFERENCE

1. M. Fitz Gerald, Chair; P. Barnes, N. Barnes, E. Bateman, A. Becker, J. De Jongste, J. R. Lemanske, P. O' Byrne, K. Ohta, S. Pedersen, E. Pizzichini, H. Reddel, S. Sullivan, S. Wenzel. Global Initiative for Asthma GINA updated 2010 Global strategy for asthma management and prevention.
2. Martinez FD "Genes, environments, development and asthma: a reappraisal". European Respiratory Journal, 2007; 29(1): 179–84.

3. Masoli, M. et al. The global burden of asthma: executive summary of the GINA Dissemination Committee report. Allergy, 2004; 59: 469-478.
4. Lee SY, Chang YS, Cho SH. Allergic diseases and air pollution. Asia Pac Allergy, 2013; 3: 145–54.
5. Paul WE, Zhu J. How are T(H)2-type immune responses initiated and amplified? Nat. Rev. Immunol, 2010; 10: 225–35.
6. Busse WW, Lemanske RF Jr. Asthma. N. Engl. J. Med., 2001; 344: 350–62.
7. Xu KF, Vlahos R, Messina A, Bamford TL, Bertram JF, Stewart AG. Antigen-induced airway inflammation in the Brown Norway rat results in airway smooth muscle hyperplasia. J. Appl. Physiol., 2002; 93: 1833–40.
8. Holgate ST, Polosa R. Treatment strategies for allergy and asthma. Nat. Rev. Immunol, 2008; 8: 218–30.
9. National Institutes of Health, National Heart, Lung, and Blood Institute. Full report of the expert panel: guidelines for the diagnosis and management of asthma (EPR-3) 2007 [Internet]. National Asthma Education and Prevention Program.
10. Cytokine in Stedman's Medical Dictionary, 28th ed. Wolters Kluwer Health, Lippincott, Williams & Wilkins, 2006.
11. Dinarello CA "Proinflammatory cytokines". Chest, August 2000; 118(2): 503–8.
12. Davidsons, Sir Satnly; Davidsons principle and practice of medicine". Churchill living stone Elsever, 2010; 21: 70-71-72 -977-1233-1239.
13. Dienz O, Rincon M: The effects of IL-6 on CD4 T cell responses. Clinical immunology (Orlando, Fla), 2009; 130(1): 27-33.
14. Wang J, Homer RJ, Chen Q, Elias JA: Endogenous and exogenous IL-6 inhibit aeroallergen-induced Th2 inflammation. J Immunol, 2000; 165(7): 4051-4061.
15. Doganci A, Eigenbrod T, Krug N, De Sanctis GT, Hausding M, Erpenbeck VJ, Haddad el B, Lehr HA, Schmitt E, Bopp T, Kallen KJ, Herz U, Schmitt S, Luft C, Hecht O, Hohlfeld JM, Ito N, Nishimoto N, Yoshizaki K, Kishimoto T Rose-John S, Renz H, Neurath MF, Galle PR, Finotto S: The IL-6R alpha chain controls lung CD4+CD25+ Treg development and function during allergic airway inflammation in vivo. The Journal of clinical investigation, 2005; 115(2): 313-325.
16. Ammit AJ, Moir LM, Oliver BG, Hughes JM, Alkhouri H, Ge Q, Burgess JK, Black JL, Roth M: Effect of IL-6 trans-signaling on the pro-remodeling phenotype of airway smooth muscle. American journal of physiology, 2007; 292(1): L199-206.
17. Aarvak T, Chabaud M, Miossec P, Natvig JB. IL-17 is produced by some proinflammatory Th1/Th0 cells but not by Th2 cells. J Immunol, 1999; 162: 1246±51.
18. Tanaka S, Yoshimoto T, Naka T, et al. Natural occurring IL-17 producing T cells regulate the initial phase of neutrophil mediated airway responses. J Immunol, 2009; 183: 7523-30.
19. Besnard AG, Togbe D, Guillou N, et al. IL-33-activated dendritic cells are critical for allergic airway inflammation. Eur J Immunol, 2011; 41: 1675-86.
20. Bettelli E, Korn T, Oukka M, et al. Induction and effector functions of T(H)17 cells. Nature, 2008; 453: 1051-7.
21. Yokoyama A, Kohno N, Fujino S, et al. Circulating interleukin-6 levels in patients with bronchial asthma. Am J Respir Crit Care Med., 1995; 151: 1354–8.
22. Canöz M, Erdenen F, Uzun H, Müderrisolu C, Aydin S. The relationship of inflammatory cytokines with asthma and obesity. Clin Invest Med., 2008; 31(6): E373-E379.
23. Gosset P, Tsicopoulos A, Wallaert B, et al. Increased secretion by tumor necrosis factor and interleukin 6 by alveolar macrophages consecutive to the development of the late asthmatic reaction. J Allergy Clin Immunol., 1991; 88: 561–71.
24. Marini M, Vittori E, Hollemborg J, Mattoli S: Expression of the potent inflammatory cytokines, granulocyte-macrophage-colony-stimulating factor and interleukin-6 and interleukin-8, in bronchial epithelial cells of patients with asthma. The Journal of allergy and clinical immunology, 1992; 89(5): 1001-1009.
25. Kicic A, Sutanto EN, Stevens PT, Knight DA, Stick SM: Intrinsic biochemical and functional differences in bronchial epithelial cells of children with asthma. American journal of respiratory and critical care medicine, 2006; 174(10): 1110-1118.
26. Broide DH, Lotz M, Cuomo AJ, Coburn DA, Federman EC, Wasserman SI. Cytokines in symptomatic asthma airways. J Allergy Clin Immunol, 1992; 89: 958–67.
27. Dienz O, Rincon M. The effects of IL-6 on CD4 T cell responses. Clin Immunol, 2009; 130: 27–33.
28. Wang X, Ma CY, Zhang YJ, et al. Expressions of Serum Interleukin -17 and Leukotriene B4 in Childhood Asthma and Their Clinical Significance. Journal of Applied Clinical Pediatrics, 2012; 27: 257-9.
29. Agache I , Giobanu C, Agache C, Anghel M. Increased serum IL-17 is an independent risk factor for severe asthma. Respir Med, 2010; 104: 1131-37.
30. Albano GD, Disano C, Bonnano A, Ricobanno L, Gagliardo R. TH17 immunity in children with Allergic Asthma and Rhinitis: A pharmacological approach. PloS ONE, 2013; 8(4): e58892.
31. Gong YX, Yu HP, Guo XH, Deng HJ, Chen X. Expression of interleukin-17A in asthmatic mice and its significance, 2009; 29(2): 256-58.
32. Miyamoto M, Prause O, Sjostrand M, et al. Endogenous IL-17 as a mediator of neutrophil recruitment caused by endotoxin exposure in mouse airways. J Immunol, 2003; 170(9): 4665–72.

33. Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stockinger B. TGF beta in the context of an inflam - matory cytokine milieu supports de novo differentiation of IL-17 producing T cells. Immunity, 2006; 24(2): 179–89.
34. Wilson RH, Whitehead GS, Nakano H, et al. Allergic sensitization through the airway primes Th17-dependent neutrophilia and airway hyperresponsiveness. Am J Respir Crit Care Med, 2009; 180: 720-30.
35. Lindén A. Interleukin-17 and airway remodelling. Pulm Pharmacol Ther, 2006; 19: 47-50.