Serum Level of Indolamine 2, 3-Dioxygenase as a Marker in the Evaluation of Atopic Asthma

Maged Mohamed Refaat[1], Asmaa Saber Abdel Rehim[1], Osama Mohamed Abdel Latif[1], Nesrine Aly Mohamed[2], Ahmad Talat Faheem[1]

[1]*Department of Internal Medicine, Allergy and Clinical Immunology, Faculty of Medicine, Ain Shams University, Cairo, Egypt*
[2]*Department of Clinical Pathology, Faculty of Medicine, Ain Shams University, Cairo, Egypt*

Conflict of Interest: None declared

This article has been peer reviewed.

Article Submitted on: 1st January 2016

Article Accepted on: 21st April 2016

Funding Sources: None Declared

Correspondence to: Dr Osama Mohamed Abdel Latif

Address: Department of Internal Medicine, Allergy and Clinical Immunology, Faculty of Medicine, Ain Shams University, Cairo, Egypt

Email: ozamalatif@yahoo.com

Cite this article: Refaat MM, Rehim ASA, Latif OMA, Mohamed NA, Faheem AT. Serum level of Indolamine 2, 3-Dioxygenase as a marker in the evaluation of Atopic Asthma. J Pioneer Med Sci 2016; 6(3):73-76

ABSTRACT

BACKGROUND: The changes in amino acid catabolism contribute to the regulation of immune system. The role of indoleamine 2,3-dioxygenase (IDO), and enzyme that mediates the catabolism of tryptophan (trp), remains a controversy. The aim of this study was to determine the relationship between serum level of IDO and the extent of asthma control in patients with allergic bronchial asthma.

METHODS: This was a cross-sectional study enrolling 40 atopic patients with allergic bronchial asthma; 20 patients with controlled asthma and 20 patients with uncontrolled asthma. Asthma diagnosis and the level of asthma severity and control were established according to The Global Initiative for Asthma (GINA) recommendations 2012. Atopy was defined by the presence of at least one positive skin prick test reaction to common environmental allergens. Total serum immunoglobulin E (IgE) and serum IDO concentration were measured in all patients. Twenty healthy individuals were included in this study to determine the cutoff level of serum IDO. We used unpaired t-test and Fisher's exact test for determining statistical significance.

RESULTS: Serum IDO levels were significantly different across the three study groups ($p<0.001$). Uncontrolled asthma and controlled asthma patients had significantly higher median (IQR) level of IDO [1200 (975–1950), 1000 (762.5–1650) respectively] compared to controls [300 (150–300)]. On the other hand, serum IDO level did not differ significantly between uncontrolled and controlled asthma patients.

CONCLUSION: Atopic patients with bronchial asthma have high serum IDO levels. There is no relationship between IDO levels and asthma control.

Keywords: Adult Asthma; Allergic Asthma, Allergy; Asthma; Atopy; Immunoglobulin E (IgE); Total IgE

INTRODUCTION

Disturbed immunoregulatory mechanisms within lymphocytes are the cornerstone in the pathogenesis of bronchial asthma with resultant excess production of IgE antibodies and finally an allergic inflammatory state [1]. Immune regulation is specific in fine adjustment of innate immunity and modification of adaptive immunity resulting in control of inflammation and self-tolerance. Immune responses are partly regulated by essential amino acid catabolism, such as tryptophan [2]. Tryptophan is converted to its metabolites kynurenine or quinolinic acid by indoleamine 2, 3-dioxygenase (IDO) enzyme. IDO protein is widely expressed in a variety of cells such as macrophages, dendritic cells, eosinophils, B lymphocytes, endothelial cells, and certain tumor cells [3].

Studies have examined the inhibitory role of IDO by activating the T-helper-1 lymphocytes line. However, the role of IDO in modulating the function of T-helper-2 lymphocytes has not been thoroughly investigated. It is also unclear whether IDO involves antigen-induced tolerance or allergic inflammation in the airways [4].

Therefore, the aim of this study was to determine the relationship between serum IDO levels and extent of asthma control in atopic patients with allergic bronchial asthma.

METHODS

The was a cross-sectional study performed on 40 atopic patients with allergic bronchial asthma attending the Allergy and Immunology Clinic of Ain Shams University Hospitals from December 2013 to August 2014. Asthma diagnosis was established according to The Global Initiative for Asthma (GINA) recommendations 2012 [5], based on clinical asthma symptoms and lung function tests. Atopy was defined by the presence of at least one positive skin-prick test reaction to common environmental allergens including mites, animal epithelia, pollens and molds. The skin-prick test was considered positive if the wheal diameter was 3mm larger than the negative control. The level of asthma severity and control was determined on the basis of GINA guidelines 2012 [5]. We selected convenient sample of 20 patients with controlled asthma symptoms and another 20 patients with uncontrolled asthma symptoms. Twenty healthy age- and sex-matched individuals were also included in this study to determine the cutoff value for serum IDO levels. All controls had no history of asthma or other allergic diseases, and had normal lung function tests and negative skin-prick test reactions. Exclusion criteria included current or past inflammatory or septic conditions; any associated autoimmune disease, any organ failure, history of smoking, any associated bronchopulmonary disorders or respiratory disease, concomitant nasal allergy, history of antihistamine therapy for past 15 days and glucocorticoid therapy for past 6 weeks. Informed consent was obtained from all subjects. The conduct of our study was approved by Ain Shams University; Faculty of Medicine research Ethics Committee Federal Wide Assurance no. FWA 000017585.

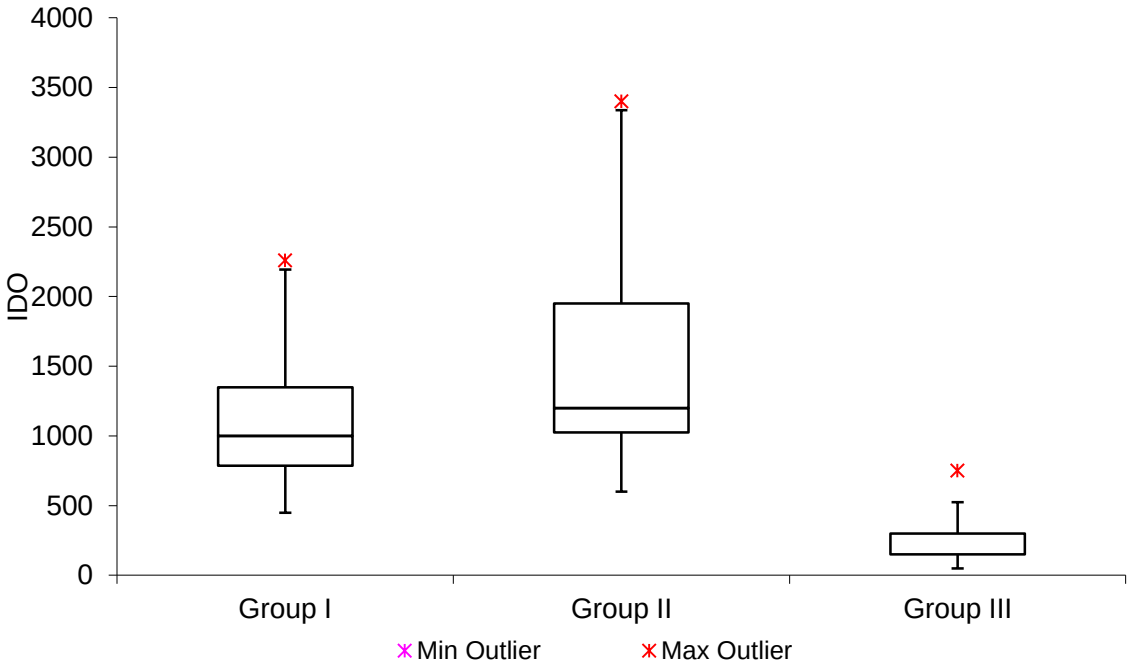
Laboratory investigations: Venous blood (8 ml) was drawn from each patient where 5 ml was placed in EDTA tube for performing complete blood count (CBC) and erythrocyte sedimentation rate (ESR) and 3 ml of blood was collected in plain vacutainers for analysis of ANA (antinuclear antibody), total serum immunoglobulin E (IgE), IDO, AST, ALT, creatinine, and blood urea nitrogen. Serum samples were stored at -20 ºC until the time of assay. They were measured in the clinical laboratory of Ain shams University Hospital. CBC was done using Coulter counter (T660). ESR was done by the Westergren method. ANA was performed by indirect immunofluorescence assay using IMMCO Diagnostics, USA, on Hep-2 substrate. Total IgE levels were measured using IgE Accubind ELISA (Monobind, Inc. Lake Forest, CA, USA) according to the manufacturer's instructions. This procedure has a sensitivity of 1 IU/ml. The normal level of total IgE in adults is less than 100 IU/ml. IDO concentration (pg/ml) was evaluated using IDO enzyme immunoassay (ELISA) kit (Glory Science, New York, and USA), according to the instructions of the manufacturer. The minimum detectable concentration was 60 pg/ml.

Statistical analysis: Analysis of data was performed using the SPSS version 15. Data were expressed as mean and standard deviation (SD) for continuous variables, and as median and interquartile range (IQR) for categorical variables. Comparison between same variables between two groups was done using unpaired t-test. Comparison of non-parametric data was done using Fisher exact test. Two-tailed p value>0.05 was considered statistically insignificant, $p<0.05$ was considered statistically significant and $p<0.001$ was considered highly significant.

RESULTS

Of the 60 patients enrolled in this study, 20 were atopic controlled asthmatic patients, 20 atopic uncontrolled asthmatic patients, and 20 apparently healthy individuals as control group. Age and gender were comparable between all three groups. Both asthma groups had comparable disease duration. There was a statistically significant difference in the serum level of IDO across the three study groups ($p<0.001$). Uncontrolled asthma patients had significantly higher median (interquartile range [IQR]) level of IDO [1200 (975–1950)] as compared to controls [300 (150–300)] and controlled asthma patients' had significantly higher median (IQR) level of IDO [1000 (762.5–1650)] as compared to controls. However, serum IDO level did not differ significantly between uncontrolled and controlled asthma patients (Figure 1). Serum IDO levels among controlled and uncontrolled asthma patients' did not correlate significantly with age, disease duration, or total IgE levels. No significant relationship was observed between the number of positivity of skin test and serum IDO levels. Upon comparing the IDO level between patients according to asthma severity the difference was

Figure 1: Box plots showing IDO level among the three patients groups



not statistically significance.

DISCUSSION

In this present study, we have shown that serum IDO levels were higher in patients with allergic bronchial asthma than in normal individuals, yet there was no relationship between level of clinical asthma severity (asthma control) and serum level IDO. Even upon comparing between IDO levels in disease-controlled or poorly-controlled asthmatics and normal persons, higher values of serum level IDO was found in the asthma groups. There was no relationship between serum IDO levels and patients with regards to age, sex, duration of disease, serum total IgE level and number of positive allergens in the skin prick test.

IDO has shown to be a negative regulator of the immune system due to its effect on dendritic cells (cells that are important in presentation of antigen to T cells). Interestingly, IDO has been investigated in vitro and in various animal models. The study of its role in vivo is limited in human [6]. Studies have demonstrated the role of IDO in acquired peripheral tolerance through modulating T-helper 1 lymphocytes [2, 4]. In agreement with our study, Xu et al's experiment on IDO deficient mice concluded that IDO plays no role in antigen-induced immune tolerance in mice airways. Instead, IDO has a positive role in initiating antigen-driven T-helper 2 lymphocytes responses through dendritic cells. IDO accumulates mature dendritic cells in the draining lymph nodes in lungs of mice in response to exposure to antigen [4].

Two important residual cells in the lung, myeloid dendritic cells and epithelial cells, express IDO protein, which in turn recruit another group of cells, the plasmacytoid dendritic cells and the eosinophil, leading to allergic inflammation. The stimulatory (in eosinophils) or inhibitory role of IDO depends on the target cell and the model under study [7, 8]. Honkanen et al. reached the same results in cases with chronic rhinosinusitis. They found that IDO expression is associated with chronic rhinosinusitis with nasal polyps and antrochoanal polyps [9].

On the other hand, Von Bubnoff et al. showed that different types of antigen-presenting cells express IDO differently, and in turn, affect the immunoregulation process. They stated that induction of IDO occurs in atopic monocytes after their attachment to the high-affinity receptor for IgE, FcεRI, leading to regulation of T lymphocyte responses in atopic patients especially in asymptomatic atopic individuals sensitized to common aeroallergens. This occurs especially in the presence of atopic family background, and highly expressed FcεRI in peripheral monocytes [10]. Other experiments established IDO as a tolerance-inducing enzyme

in allergy. Hayashi et al found that IDO induction in the lung cells results in inhibition of T-helper 2 lymphocyte-driven asthma and suppression of airway hyper-reactivity and inflammation [11]. Maneechotesuwan investigated the effect of inhaled glucocorticoid (ICSs) on IDO activity and found that IDO activity in sputum was lower in patients with mild intermittent and mild-to-moderate persistent asthma than controls. On administration of ICS or ICS/long-acting β2-agonist, IDO activity increased [12]. They concluded that ICSs led to increased IL-10 secretion in the macrophages along with enhanced IDO activity [12]. Ciprandi et al assessed levels of serum tryptophan and kynurenine in pollen-induced allergic rhinitis patients during and after pollen season, and compared the results with values in healthy subjects. Levels were higher in these patients during off-season. They considered tryptophan and its metabolites as essential biomarker in allergic rhinitis [13].

Several mechanisms can potentially explain the seemingly contradictory observations that IDO acts sometimes as pro-inflammatory while other times as tolerance-inducing enzyme. Specific local tissue micro-environment may direct IDO as a defense mechanism against immunosuppression and decrease proliferation of T cells in order to protect tissue from damage [14]. As most experiments were performed on knock-out mice, such models may react in a different way than wild-type mice [6]. In addition, most studies were performed on animal model, which in turn arouses the question if human IDO reacts differently than animal IDO. Furthermore, within humans, genetic polymorphism of IDO gene may help to explain the variability in response to IDO enzyme.

CONCLUSION

In conclusion, we postulate the possible role of IDO in bronchial asthma as a proinflammatory mediator (regardless of level of asthma control) with significant rise in IDO level, but there was no correlation between its level and severity of symptoms. Further studies may help to differentiate IDO level in different types of asthma and other obstructive lung diseases.

REFERENCES

1. Pietras T, Panek M, Tworek D, Oszajca K, Wujcik R, Górski P, et al. The Bcl I single nucleotide polymorphism of the human glucocorticoid receptor gene h-GR/NR3C1 promoter in patients with bronchial asthma: pilot study. *Mol Biol Rep*. 2011; 38:3953-3958
2. Fallarino F, Grohmann U, Puccetti P. Indoleamine 2,3-dioxygenase: from catalyst to signaling function. *Eur J Immunol*. 2012; 42 (8):1932-1937.
3. Beutelspacher SC, Tan PH, McClure MO, Larkin DF, Lechler RI, George AJ. Expression of indoleamine 2,3-dioxygenase (IDO) by endothelial cells: implications for the control of alloresponses. *Am J Transplant*. 2006; 6 (6): 1320-1330.
4. Xu H, Oriss TB, Fei M, Henry AC, Melgert BN, Chen L, et al. Indoleamine 2,3-dioxygenase in lung dendritic cells promotes Th2 responses and allergic inflammation. *Proc Natl Acad Sci USA*. 2008; 105 (18):6690-6695.
5. Global Strategy for Asthma Management and Prevention, Global Initiative for Asthma (GINA) 2012. http://www.ginasthma.org/local/uploads/files/GINA_Report_March13_1.pdf
6. Von Bubnoff D, Bieber T. The indoleamine 2,3-dioxygenase (IDO) pathway controls allergy. *Allergy*. 2012; 67 (6):718-725.
7. Odemuyiwa SO, Ghahary A, Li Y, Puttagunta L, Lee JE, Musat-Marcu S, et al. Cutting edge: human eosinophils regulate T cell subset selection through indoleamine 2,3-dioxygenase. *J Immunol*. 2004; 173 (10):5909-5913.
8. Grohmann U, Volpi C, Fallarino F, Bozza S, Bianchi R, Vacca C, et al. Reverse signaling through GITR ligand enables dexamethasone to activate IDO in allergy. *Nat Med*. 2007; 13 (5):579-586.
9. Honkanen T, Luukkainen A, Lehtonen M, Paavonen T, Karjalainen J, Hurme M,et al. Indoleamine 2,3-dioxygenase expression is associated with chronic rhinosinusitis with nasal polyps and antrochoanal polyps. *Rhinology*. 2011; 49 (3):356-363.
10. Von Bubnoff D, Fimmers R, Bogdanow M, Matz H, Koch S, Bieber T. Asymptomatic atopy is associated with increased indoleamine 2,3-dioxygenase activity and interleukin-10 production during seasonal allergen exposure. *Clin Exp Allergy*.2004; 34 (7):1056-1063.
11. Hayashi T, Beck L, Rossetto C, Gong X, Takikawa O, Takabayashi K ,et al. Inhibition of experimental asthma by indoleamine 2,3-dioxygenase. *J Clin Invest*. 2004; 114 (2):270-279.
12. Maneechotesuwan K, Supawita S, Kasetsinsombat K, Wongkajornsilp A, Barnes PJ. Sputum indoleamine-2, 3-dioxygenase activity is increased in asthmatic airways by using inhaled corticosteroids. *J Allergy Clin Immunol*. 2008; 121 (1):43-50.
13. Ciprandi G, De Amici M, Tosca M, Fuchs D. Tryptophan metabolism in allergic rhinitis: the effect of pollen allergen exposure. *Hum Immunol*. 2010; 71 (9):911-915.
14. Mellor A. Indoleamine 2,3 dioxygenase and regulation of T cell immunity. *Biochem Biophys Res Commun*. 2005; 338 (1):20-24.