



## Estimation of serum immunoglobulin E level in asthma and its correlation with Epstein Barr Virus (EBV) infection

Alaa Younis Mahdy Al-Hamadany

Technical Institute- Mosul , Northern Technical University , Mosul , Iraq

### ARTICLE INFO.

#### Article history:

-Received: 17 / 4 / 2018

-Accepted: 3 / 6 / 2018

-Available online: / / 2018

**Keywords:** IgE level, Asthma, EBV

#### Corresponding Author:

**Name:** Alaa Younis Mahdy

**E-mail:**

[alaa.mahdy156@yahoo.com](mailto:alaa.mahdy156@yahoo.com)

**Tel:**

### Introduction

Asthma is currently defined as “a chronic inflammatory disorder of the respiratory airways. The chronic inflammation is associated with airway hyper-responsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness and coughing, particularly at night or early in the morning. These episodes are usually associated with widespread, but variable, airflow obstruction within the bronchial tree that is often reversible either spontaneously or with treatment [1]. Although epidemiological studies of childhood bronchial asthma are particularly difficult, there is evidence that the mortality and morbidity from asthma have been increasing, especially over the last 20 years [2]. Asthma is most frequently mild to moderate, but significant proportion of patients has severe asthma [3].

Asthma is two types – extrinsic and intrinsic types. Atopic asthma is a subtype of extrinsic asthma in which patients who have a hyper-responsive airway, the scene for the reaction is set in large part by initial sensitization to inhaled antigens and chemical antigens [4,5]. These stimulate induction of Th-2 Tcells, which release cytokines like IL-4 and IL-5. The released cytokines in turn promote IgE production by B cells, growth of mast cells (IL-4) and growth and activation of eosinophils. Subsequent IgE

### Abstract

This study carried out to show the association between Epstein-Barr Virus (EBV) infection and asthma. a cohort 65 serum samples were collected from asthma patients within age group of 15-62 years ( 37 females and 28 males) and 20 healthy controls. The levels of IgE and EBV IgM antibodies were measured by ELISA assay. The results showed that IgE levels increased with severity of asthma (327.95 IU/ml) and the high level of IgE in asthma patients with age group (45-55 years) was 306 IU/ml. Sero detection of EBV showed that EBV infection increased with severity of asthma and was highest in females with severe asthma. serum IgE levels were high in asthmatic as compared to healthy controls and the highest levels of it was in severity of asthma. On other hand, there was weak significant correlation between levels of IgE and EBV infections in asthma patients.

mediator reaction to inhaled allergens elicits acute and late phase reaction[6] .

Viruses are felt to be one of the most common causes of asthma in children and a major cause of asthma in adults. The studies have also demonstrated that only specific respiratory infectious agents cause asthmatic exacerbations. Respiratory syncytial virus [7], parainfluenza virus, and rhinovirus commonly cause exacerbations, adenovirus causes asthmatic exacerbations less often and cytomegalovirus, herpes simplex virus , and bacteria are not associated with asthmatic flares [8]. Viruses exacerbate asthma via complex multi-mechanistic processes that involve epithelial injury and the induction and/or alteration of local inflammation [9].

Anyway, there are no many researchers documented the investigating the effect of EBV in asthma and relationship between age and IgE . In this study, we evaluated the effects of EBV, age and specific IgE level in patients with asthma.

### Materials and methods

#### 1. Patients

Sixty five patients (28 males & 37 females) with asthma and 20 healthy controls were studied in Alzahrway Teaching hospital. All the asthma patients underwent clinical examination and were diagnosed as asthma by specialized physician. There were 28 males and 37 females in the group and their age

ranged from 15 to 62 years in period from 15 January to 31 June 2013.

## 2. Estimation of total serum IgE

The total IgE was estimated in all serum samples using kit provided by Human Gesellschaft für Biochemica und Diagnostica mbH, Germany.

### Interpretation of the Total IgE Results

< 25 IU/ml No indication of an allergic reaction

25 –100 IU/ml Allergy possible -confirmation of diagnosis recommended (specific IgE)

> 100 IU/ml Allergy very probable

## 3. Detection of EBV

EBV has been detection in all samples by using Anti-EBV-CA ELISA (IgM) kit (Euroimmune, Germany), which provides a semi-quantitative in vitro assay for human anti-bodies of IgM class against Epstein Barr Virus capsid antigen (EBV-CA) in serum or plasma. In brief, the first reaction steps, diluted patient samples are incubated for 30 minutes in wells then wash three times used 300 µl of working wash buffer and empty the wells. In the case of positive samples, specific IgM antibodies will bind to antigens. To detect the bound antibodies, a second incubation has been carried out after added conjugate enzyme (peroxidase-labelled anti-human IgM) into each well, after wash added 100 µl of substrate into microplate wells and incubated in dark for 15 minutes at room temperature then finally stopped the reaction and read the absorbance at 450nm within 30 minutes by using Microelisa (washer and reader) applied by Biotek, USA in Al-Salam Teaching hospital. The result is evaluated semi-quantitatively by calculating a ratio of the extinction value of the control or patient sample over the extinction value of calibrator by using the following formula to calculate the ratio:

$$\text{Ratio} = \frac{\text{Extinction of the control or patient sample}}{\text{Extinction of calibrator}}$$

Interpreting result as following:

Ratio < 0.8: Negative

Ratio > 0.8 <1.1: Borderline

Ratio > 1.1: Positive

## 4. Statistical Analysis:

All data were analyzed using the statistical package for social sciences SPSS version 14 software for windows 7. The results were expressed as mean. The chi square test was used to test the association of IgE with anti-EBV-CA and the statistical significance level was set at 0.01.

## Results and Discussion

The mean IgE level in healthy controls and cases were 19 IU/ml, 181 IU/ml respectively and significantly differences between the levels of cases when compared with healthy controls. In this study the asthmatics were categorized into three groups depended on levels of IgE via mild, moderate and sever group (Table 1). The analysis of the data revealed that mean IgE levels were significantly different ( $P < 0.01$ ) among the groups. its indicated

that there are tend in IgE levels normal in control group and highest in severe group. This shows the important role played by IgE in the severity of asthma; but whether this high level is a causative factor in severity of symptoms could not be ascertained.

Several studies suggest a role of sex hormones in the pathogenesis of asthma[10]. More females than males develop asthma during puberty and thereafter, so the prevalence of adult asthma becomes higher in females than in males [11], this in harmony with our study that asthma high in females more than males.

**Table 1: Total Serum IgE levels in patients with asthma**

Group	No. (male/female)	concentration (IU/ml) (Mean)
Controls	20(10/10)	19
Mild	13(6/7)	97.67
Moderate	30(13/17)	118.00
Sever	22(9/13)	327.95
Total	85(38/47)	181.21

The presnt study showed that the highest EBV infection were in severe asthma 7(53.85%) followed by moderate and mild 5(38.46%) and 1(7.69%), according to gender, the results showed that highest EBV infections in females with severe asthma 4(30.77%) followed by females of moderate and males of severe asthma 3(23.08%) while the infection not appeared in males with asthma (Table 2). In relation to age group, the results showed that high level of IgE in asthma patients with age group (45-55 years) was 306 IU/ml followed by age group (35-45 years) was 287 IU/ml while the lowest level of IgE with age group (25-35 years) was 193 IU/ml. on other hands, the results revealed that the high percent of EBV infection with age group (35-45 years) and (45-55 years) were 4 cases (30.77%) while the lowest percent of infection was 7.7 % with age group (25-35 years) (Table 3). The current study showed the EBV associated with high level of IgE in asthma patients, this results agreement with many previous studies who documented that the viral infection has been likened to the onset of atopy and increases in systemic IgE levels have been found following infection with the EBV [12], CMV and the measles virus [13] and following vaccination with whole virion influenza vaccine.

EBV has a different mechanism of protection of allergic diseases. It induces T-reg cells as an escape mechanism from the immune system, and although this mechanism has not been well elucidated, it seems to involve an elevation of IL-10 and transforming growth factor-beta (TGF-β). Wingate and collaborators [14] and Marshall [15] found in the EBV genome a well-preserved sequence homologous to IL-10, which may be influencing this immunomodulation [16].

**Table 2: Seropositive of EBV in asthmatic patients according to severity and gender**

Group	Mild No (%)	Moderate No. (%)	Severe No. (%)	Total No. (%)
Males	- (0)	2(15.38)	3(23.08)	5(38.46)
Females	1(7.69)	3(23.08)	4(30.77)	8(61.54)
Total	1(7.69)	5(38.46)	7(53.85)	13(100)

**Table 3: Total IgE levels and seropositive EBV in asthmatic patients according to age group**

Age group	Mean IgE levels (IU/ml)	Seropositive EBV No.(%)
15-25	201	2(15.38)
25-35	193	1(7.70)
35-45	287	4(30.77)
45-55	306	4(30.77)
55-65	246	2(15.38)

**Correlation between of EBV infection and IgE**

Anti-EBV-CA and characteristic markers of asthma disease (IgE) were examined (Table 4). Significant weak correlation was often observed between anti-EBV-CA and IgE, further suggesting a close association between EBV infection and the development of asthma diseases. Several studies have reported EBV associated with protection of atopy [17,18] and has been shown that RSV infection induce the production of virus-specific IgE, the magnitude of the response correlating with degree of wheezing and children with an atopic predisposition developing an IgE response more readily [19] also Specific IgE antibodies to parainfluenza virus [20]. Other studies have indicated associations between EBV infection, serum IgE levels, and diseases mediated, at least in part, by IgE. These observations include an elevation of serum IgE during acute EBV infection, [21]. increased numbers of patients with anti-EBV antibodies and concomitant high levels of IgG anti-VCA antibodies in atopic children [22] and in adults with atopic dermatitis and urticarial. [23].

**References**

- [1] Boulet, L.P. (2009). Influence of comorbid conditions on asthma. *Eur. Respir. J.*, 3(4): 897-906.
- [2] American Thoracic Society (ATS). (2000). Proceedings of the ATS workshop on refractory asthma: current understanding, recommendations, and unanswered questions. *Am. J. Respir. Crit. Care. Med.*, 162:2341-51.
- [3] Holgate, S.T; Holloway, J; Wilson, S; Howarth, P.H; Haitchi, H.M; Babu, S; & Davies, D. E. (2006). Understanding the pathophysiology of severe asthma to generate new therapeutic opportunities. *J. Allergy Clin. Immunol.*, 117:496-506.
- [4] Husain, A. N and Kumar, V. (2007). The lung. In: Kumar, V; Abbas, A. K and Fausto, N; editors. Robbins and Cotran pathologic basis of disease. 7<sup>th</sup> ed. Philadelphia W B: Saunders; pp: 711-72
- [5] Mathias, C.B; Freyschmidt, E.J; & Oettgen, H.C. (2009). Immunoglobulin E antibodies enhance pulmonary inflammation induced by inhalation of a chemical hapten. *Clin. Exp. Allergy*, 39:304-306.

According to the above studies and documents, the asthma patients' complaints of an increase in EBV-associated symptoms during their allergy season or when exposure to specific antigen. On the other side, other study reported that is no association between viral infections and the presence of specific IgE to environmental allergens and also between viral infection and asthma disease [24]. This difference in findings in different geographic regions indicates that environmental and genetic factors may be influencing the ability of this virus to immune modulate atopy and allergy or not. Also indicating that exposures to this infection may be associated with suppression of immediate hypersensitivity responses [24]. The immune response of allergic diseases is typically Th2; thus, it is possible that pathogenic or non-pathogenic organisms that suppress this response can induce protection from allergies. Among the known factors that can suppress a Th2 immune response, one can highlight Th1 and T-regulatory (T-reg) cells stimulators [24]. *In vitro* studies have shown that HSV DNA can induce tumor necrosis factor TNF- $\alpha$  and interleukin IL-6, a Th1 immune response, and release interferon IFN- $\gamma$  [25]. In Conclusion, Total serum immunoglobulin E levels were high in asthmatics patients as compared to normal subjects. On the average, the IgE levels increased as the severity of asthma increased and as within age group 45-55 years. The very fact that IgE levels significantly weak associated with EBV infection.

**Table 4: Correlation of IgE with anti-EBV**

Anti-EBV-CA		IgE
	Correlation coefficient	0.124
	P value	0.110*
	N	13

\* Significant P &lt; 0.01

- [6] Kindt, T.J; Goldsby, R.A; & Osborne, B. (2007). Kuby Immunology 6<sup>th</sup> ed. New York: WH Freeman and Company, pp. 380-385.
- [7] Weiss, K.B; Gergen, P.J; & Hodgson, T.A. (1992). An economic evaluation of asthma in the United States. *N. Engl. J. Med.*, 326:862-866.
- [8] Pattemore, P.K; Johnston, S.L; & Bardin, P.G. (1992). Viruses as precipitants of asthma symptoms. I. Epidemiology. *Clin. Exp. Allergy*, 22:325-336.
- [9] Stark, J.M. and Graziano, F.M. (1995). Lower Airway Response to Viruses. In Asthma and Rhinitis. W.W. Busse, and S.T. Holgate, editors. Blackwell Scientific Publications, Boston, MA. 1229-1243.
- [10] Arbes, S. J; Guo, X; Orelie, J. *et al.* (2004). Interaction between sex and age in the prevalence of current asthma. *J. Allergy Clin. Immuno.*, 113: 302.
- [11] Global Strategy for Asthma Management and Prevention. (2006). Global Initiative for Asthma (GINA). [www.ginasthma.org](http://www.ginasthma.org).
- [12] Frick, O. L, German, D.F; & Mills, J. (1979). Development of allergy in children: association with

virus infection. *J. Allergy Clin. Immunol.*, 64:228-241.

[13] Griffin, D. E, Cooper, S. J; Hirsch, R.C; Johnson, R.J; Lindo de Soriano, I; Roedenbeck, S. *et al.*, (1985). Changes in plasma IgE levels during complicated and uncomplicated measles virus infections. *J. Allergy clin. immunol.*, 76:206-213

[14] Wingate, P. J; McAulay, K.A; Anthony, I.C; & Crawford. D.H. (2009). Regulatory T cell activity in primary and persistent Epstein-Barr virus infection. *J. Med. Virol.*, 81(5):870-877.

[15] Marshall, N.A; Culligan, D.J; Johnston, P.W; Millar, C; Barker, R.N; Vickers, M. A. (2007). CD4 (+) T-cell responses to Epstein-Barr virus (EBV) latent membrane protein 1 in infectious mononucleosis and EBV-associated non-Hodgkin lymphoma: Th1 in active disease but Tr1 in remission. *Br. J. Haematol.*, 139(1):81-89.

[16] Raftery, M.J; Wieland, D; Gronewald, S; Kraus, A.A; Giese, T; & Schonrich, G. (2004). Shaping phenotype, function, and survival of dendritic cells by cytomegalovirus-encoded IL-10. *J. of Immunol.*, 173(5): 3383-3391

[17] Nilsson, C; Larsson Sigfrinius, A.K; Montgomery, S. M; Sverremark - Ekstrom, E; Linde, A; Lilja, G; & Blomberg, M. T. (2009). Epstein-Barr virus and cytomegalovirus are differentially associated with numbers of cytokine-producing cells and early atopy. *Clin. Exp. Allergy*, 39(4):509-517

[18] Nilsson, C; Linde, A; Montgomery, S. M; Gustafsson, L; Nasman, P; Blomberg, M.T; & Lilja, G. (2005). Does early EBV infection protect against IgE sensitization?. *J. Allergy Clin. Immunol.*, 116(2): 438-444.

[19] Welliver, R.C; Wong, D.T; Sun, M; Middleton, Jr. E; Vaughan, R.S; & Ogra, P. L. (1981). The development of respiratory syncytial virus-specific IgE and the release of histamine in nasopharyngeal secretions after infection. *N. Engl. J. Med.*, 305:841-846

[20] Welliver, R; Wong, D; Middleton, E; Sun, M; McCarthy, N; Ogra, P. (1982). Role of parainfluenza virus-specific IgE in pathogenesis of croup and wheezing subsequent to infection. *J. Pediatr.*, 101: 889-896.

[21] Bahna, S. L; Horwitz, C.A; Fiala, M; & Heiner, D.C. (1978). IgE response in heterophil-positive infectious mononucleosis. *J. Allergy. Clin. Immunol.*, 62:167

[22] Strannegard, I. L. and Strannegard, O. (1981). Epstein-Barr virus antibodies in children with atopic diseases. *Int. Arch. Allergy Appl. Immunol.*, 64: 314.

[23] Rystedt, I; Strannegard, I.L; & Strannegard, O. (1984). Increased serum levels of antibodies Co Epstein-Barr virus in adults with history of atopic dermatitis, *Int. Arch. Allergy Appl. Immunol.*, 75:179,

[24] Veiga, R. V; Sergio, S. C; Vitor, C. D; Álvaro, C. C; Phillip, J. C; Laura, C. R; Mauricio, L. B; & Neuza, M. A. (2011). Chronic virus infections suppress atopy but not asthma in a set of children from a large latin american city: a cross-section study. *BMC Pulmonary Medicine*, 11:24

[25] Lundberg, P; Welander, P; Han, X; & Cantin, E. (2003). Herpes simplex virus type 1 DNA is immunostimulatory *in vitro* and *in vivo*. *J. Virol.*, 77(20):11158-11169.

## تقييم مستوى الكلوبولين المناعي الكلي IgE في مصول مرضى الربو وعلاقته مع اصابات فايروس الحمى الغدية (EBV)

علاء يونس مهدي الحمداني

المعهد التقني - الموصل ، الجامعة التقنية الشمالية ، الموصل ، العراق

### الملخص

جرت هذه الدراسة لإظهار الارتباط بين الإصابة الاثنيتين-بار فايروس والربو. تم جمع 65 عينة من مصول مرضى الربو باعمار من 15-65 عاما (37 إناث و28 ذكور) و20 من الاصحاء كمجموعة سيطرة وتم تقدير مستويات الكلوبولين المناعي IgE والأجسام المضادة لل EBV بواسطة تقنية الامتزاز المناعي المرتبط بالانزيم ELISA. أظهرت النتائج أن زيادة مستويات IgE مترافقة مع ازدياد شدة الربو (327.95 IU/ml)، وارتفاع مستوى الكلوبولين في مرضى الربو كان ضمن الفئة العمرية (45-55 سنة) 306 IU/ml. وأظهر الكشف المصلي لل EBV زيادة الإصابات EBV مع ازدياد شدة الربو وكانت أعلى نسبة في الإناث الذين يعانون من الربو الحاد. كان IgE بمستويات عالية في المرضى المصابين بالربو بالمقارنة مع الاصحاء وإن أعلى مستويات IgE كانت في حالات الربو الشديدة. ومن جهة اخرى، كان هناك ارتباط ضعيف بين مستويات IgE واصابات EBV في مرضى الربو.