

Levels of five biochemical parameters in asthmatic patient's blood as markers for bronchial asthma induced by the house-dust mite allergy

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ABSTRACT

The study examines the validity of five biochemical parameters as indicators for the activity and intensity of asthma induced by the house-dust mite allergy. Blood samples of forty children (28 males and 12 females of 0.5 - 15 years old) suffering from moderate (31) and severe (9) attack of asthma and of 10 non asthmatic children (6 males and 4 females of similar age range) were examined. Levels of histamine, total serum IgE, SIgE, ECP and AEC were significantly higher in the asthmatic- than in the normal cases. Furthermore, severe asthmatic cases had significantly higher levels as compared to the moderate ones, i.e., mean values for [Moderate < Severe] > Check cases. As a conclusion, the elevated levels of such biochemical parameters are associated with HDM asthmatics and are correlated with the severity of asthma attacks. Thus these five parameters may be considered as good markers for HDM asthma activity and severity.

Keywords: HDM allergy, Histamine, Immunoglobulin E, Eosinophil cationic protein, Absolute eosinophil count, Asthmatic patients. Cairo, Egypt.

INTRODUCTION

The house-dust mites (HDM's) are present in most of the indoor environments and live in association with man and have been shown to be the important source of indoor allergens associated with asthma and other allergic conditions (Milian and Diaz, 2004; O'Neil *et al.*, 2006 and Yassin, 2011).

Asthma induced by allergy to HDM's is a world wide problem (Platts-Mills and de Weck, 1989) and is among the most common diseases in the world (Ghaffari *et al.*, 2010) that affects millions of people. Numerous studies have shown that the prevalence of asthma is increasing (Milian and Diaz, 2004) due to the increase in HDM populations as a direct consequence of the improved housing conditions. Under the Egyptian environmental conditions, mite induced

allergy especially bronchial asthma forms a problem (Frankland and El Hefny, 1971). It was suggested (Gamal-Eddin *et al.*, 1985) that HDM allergy occurs more common than to any other allergen in the Egyptian asthmatic patients.

Baldacci *et al.* (2001) reported that studies of allergy and respiratory disease have traditionally used allergic markers such as serum total and specific IgE, skin reactivity and peripheral blood eosinophilia to identify atopic subjects. The measurement of serum IgE antibodies and skin-prick testing may give complimentary information and can be applied in clinical and epidemiological settings. Peripheral blood eosinophilia is less used, but is important in clinical practice to demonstrate the allergic aetiology of disease, to monitor its

clinical course and to address the choice of therapy.

This work was planned for and objected at examining the validity of five biochemical parameters (histamine release, total and specific serum immunoglobulin E (IgE and SIgE), eosinophil cationic protein (ECP) and absolute eosinophil count (AEC)) as markers for the activity and intensity of bronchial asthma induced by HDM allergy.

MATERIALS AND METHODS

Allergic Cases

The present study included 40 children from Cairo suffering from moderate and severe attack of asthma caused by the house-dust mites (31 moderate cases and 9 severe cases). The cases were chosen from those attending the Allergy and Immunology Pediatric Clinic, El-Husseini Hospital, Al-Azhar University in Cairo. The criteria for differentiation between moderate and severe asthmatic cases were those of Mostafa (1996). They were 28 males and 12 females, with ages ranged from 6 months to 15 years. In addition, 10 children, 6 males and 4 females of similar age range with no history of any atopic disease were chosen as normal controls from the pediatric outpatients' clinic of the same hospital.

Estimation of Biochemical Parameter Levels in Blood

Venous blood samples were taken from each asthmatic case and check individuals and used to estimate the followings: (1) histamine release by Radio Immuno Assay (RIA) using Biomerica histamine RIA reagents (Biomerica 1533 Montovia AVE., Newport beach, CA 92663, USA), (2) total serum immunoglobulin E (IgE) using pathozyne-IgE enzyme immunoassay (EIA) [Omega Diagnostics Limited, Scotland, United Kingdom]. The pathozyne IgE tests is a solid phase enzyme-linked immunoserbent assay

(ELISA) based on the sandwich principle (Engvall, 1980), (3) specific immunoglobulin E (SIgE) using an EIA [Ridascreen® Spezifisches IgE EIA (R-Biopharm Diagnostic Technologies Limited, Darmstadt, Germany)], (4) eosinophil cationic protein (ECP) by pharmacia ECP double antibody RIA [Pharmacia Diagnostic AB, SE-751 82 Uppsala, Sweden] and (5) absolute eosinophil count (AEC) or the total number of eosinophils per mm³ of blood (Brown, 1993) based on counting of the white blood cells and of the differential white blood cells (Dacie and Lewis, 2001)

Statistical Analysis

Means and standard deviations (SD) of the estimated biochemical parameters were computed and compared by the one-way Analysis Of Variance (ANOVA). Significantly different means were further exposed to multiple pairwise comparisons by Bonferroni method. All analysis were carried out using GPIS (Graph Pad In Stat, version 1.0 by H. J. Motulsky) computerized software.

RESULTS

The present study included 40 children suffering from moderate (31) and severe (9) attack of asthma due to house-dust mites. Ten normal children with no history of atopy were also included as check cases. For all cases, levels of histamine release, total IgE, SIgE, ECP and AEC were determined and compared.

Each of the five investigated biochemical attributes (Table1) showed significantly different levels (ANOVA, $P < 0.01$) among the asthmatic and non-athmatic cases. Multiple comparisons (Table 2) indicated that in comparison to the normal cases, significantly higher levels ($P < 0.05$ - $P < 0.001$) of histamine, IgE, SIgE, ECP and AEC were detected in the moderate (*ca.* 9, 9,16,2 and 3 folds for the 5 attributes, respectively), severe (*ca.* 14, 20,26,3, and 5 folds for the 5

attributes, respectively) and the total of asthmatic cases (*ca.* 10, 11,19,2, and 4 folds for the 5 attributes, respectively). Within the asthmatic cases, patients with severe attacks have higher levels of the 5 attributes than those with moderate attacks ($P < 0.05$ - $P < 0.001$).

Table 1: Histamine release, Total serum immunoglobulin E (IgE), Specific immunoglobulin E (SIgE), Eosinophil cationic protein (ECP) and Absolute eosinophil count (AEC) of the asthmatic and normal (check) cases.

Attributes	Mean (SD) ¹			Check cases	ANOVA $F_{(3,86)}^2$
	Asthmatic cases				
	Moderate	Severe	Total		
Histamine (ng/ml.)	20.84 (9.96) ^A	32.83 (14.62) ^B	23.54 (12.08) ^{AB}	2.28 (0.92) ^C	13.86
IgE (IU/ml.)	272.26 (135.39) ^A	629.11 (87.69) ^B	352.55 (209.90) ^A	31.30 (11.76) ^C	20.28
SIgE (IU/ml.)	8.28 (3.88) ^A	13.92 (4.40) ^B	9.55 (4.61) ^A	0.53 (0.25) ^C	18.90
ECP (µg/l)	17.31 (6.43) ^A	28.61 (8.71) ^B	19.86 (8.38) ^A	8.34 (1.34) ^C	12.89
AEC (cell/mm ³)	659.00 (308.07) ^A	1037.00 (251.85) ^B	744.05 (334.02) ^A	200.70 (34.22) ^C	13.45

1. Horizontally, means with different letters are significantly different (Bonferroni test, $P < 0.05$ - $P < 0.001$).
2. All are significant, $P < 0.001$.

Table 2: Pairwise multiple comparisons (Bonferroni test) of the five biochemical parameters estimated for the asthmatic and non asthmatic (check) cases

Parameter	Comparison of cases / P value [‡]					
	Moderate x			Severe x		Total x
	Severe	Total	Check	Total	Check	Check
Histamine	< 0.05	NS	< 0.001	NS	< 0.001	< 0.001
IgE	< 0.001	NS	< 0.01	< 0.001	< 0.001	< 0.01
SIgE	< 0.01	NS	< 0.001	< 0.05	< 0.001	< 0.001
ECP	< 0.01	NS	< 0.05	< 0.05	< 0.001	< 0.01
AEC	< 0.05	NS	< 0.001	< 0.05	< 0.001	< 0.01

[‡] NS = Not significant, $P > 0.05$.

DISCUSSION

In the present study, released histamine, total serum IgE, specific SIgE, ECP and AEC were estimated for 40 asthmatic children (0.5-15 years old) suffering from moderate (31 children) and severe (9 children) attacks of HDM asthma and compared with those for a normal (check) 10 children with no history of atopy.

Histamine is a basic amine that is stored in mast cells and basophils which is released due degranulation of these cells as a result of the interaction between allergen and IgE. According to Spiegelberg (1989), histamine produces many of the effects of inflammation and hypersensitivity. Present results indicated

significantly higher histamine level in HDM asthmatic individuals than in normal (check) ones ($P < 0.001$) and in severe asthmatics than in moderate ones ($P < 0.05$). No comparable results are available however, the observed elevation in histamine level in HDM asthmatic cases, may add another marker (to those reported by Baldacci *et al.*, 2001) for disease activity and severity.

In comparison to the normal individuals, significantly higher levels of total IgE was detected in sera of the moderate ($P < 0.01$) or severe ($P < 0.001$) asthmatic cases. Similarly, patients with asthma are shown to exhibit increased IgE levels in blood (Zetterstorm and Johansson, 1981; Ishigaki *et al.*, 2000;

Ghaffari *et al.*, 2010 and Mouthuy *et al.*, 2011). Olmedo *et al.* (2011) observed that increasing allergen exposure was associated with increased probability of sensitization (IgE) to cockroach ($P=0.001$), dust mite ($P=0.009$) and cat ($P=0.001$). The observed increase in IgE levels for severe ($P<0.001$) than for moderate asthmatics coincide with the observation of Di-Gioacchino *et al.* (2000) who reported that the degree of airways hyperreactivity in asthmatic patients was directly related to the increase of the total IgE levels in serum. Thus the levels of total IgE can be used as a biomarker for the risk of developing prolonged asthma symptoms and for the effective monitoring of anti-inflammatory treatment and allergen-specific immunotherapy.

It was reported (O'Neil *et al.*, 2006) that HDM cause allergic disease and elicits SIgE responses in humans. Comparing to the normal cases, the moderate or severe asthmatic cases showed significantly higher ($P<0.001$) SIgE levels in agreement with the observation of Rizzo *et al.* (1997) on the Brazilian children with asthma caused by HDM. Mouthuy *et al.* (2011) reported that both total IgE and Derp-specific IgE levels are increased in intrinsic asthma patients compared to healthy nonatopics. Taketomi *et al.* (2006) indicated that several studies have shown that the presence of IgE antibodies to HDM is an important risk factor for asthma. Thus mite SIgE should be considered as a screening test in asthmatic children (Santoso, 1998) and for diagnostic purposes to identify patients with specific sensitivity (Tsai *et al.*, 1998).

The ECP is a higher basic protein released from activated eosinophils during the inflammation process. Present results indicate that (1) asthmatic patients have significantly higher ($P<0.01$) levels than the normal (check) individuals, (2) patients with severe asthma attacks have significantly higher ($P<0.01$) levels than

those with moderate attacks. These results coincide with those of Fujitaka, *et al.* (2001) and Pohunek *et al.* (2001) who pointed out that serum ECP is a good and best marker of disease activity, intensity of inflammation in patients and of treatment efficacy in bronchial asthma.

According to several authors (*e.g.* Tang and Chen, 2001) there is convincing evidence that biologically, eosinophils play a key role in bronchial asthma by damaging respiratory epithelium and by generating inflammatory mediators which give the features of HDM allergic rhinitis. Eosinophils are found in increased number in the circulation and sputum usually in relation to the severity of asthma. Present observation revealed a significantly higher ($P<0.001$) eosinophil count in the asthmatic cases than in the normal (check) individuals. Such increased number of eosinophils in the asthmatics as compared to control has also been demonstrated by Lonkvist *et al.* (2001) and others.

Among the asthmatic individuals those with severe attacks had significantly ($P<0.05$) higher AEC than those with moderate attacks. Similarly, Fujitaka *et al.* (2001) observed that the peripheral blood eosinophil counts in patients with severe asthma were significantly higher than in those with mild asthma. Also Ghaffari *et al.* (2010) reported that eosinophil count was elevated in 40.5% of asthmatic patients. Such findings indicating that the changes in blood eosinophils reflect the disease activity (Youroukova *et al.*, 1998) and that eosinophil count play an important role in determining the severity of asthma in young individuals (Obase *et al.*, 2001).

In conclusion, based on the estimated mean values in the blood of the studied cases, the overall relations can be represented by: [(Moderate < Severe) = Total] > Check cases for histamine and by: [(Moderate = Total) < Severe] > Check cases for IgE, SIgE, ECP and

AEC. This means that: (1) higher levels of such parameters are associated with HDM asthmatics and (2) the severity of asthma attacks and elevated levels of such biochemical parameters are correlated. Thus these five parameters may be considered as good markers for HDM asthma activity and severity.

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ARABIC SUMMARY

مستويات خمس قياسات كيموحيوية في دم مرضى حساسية الصدر كمدلولات للأزمة الربوية الناتجة عن الحساسية لحلم غبار المنازل

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تتناول الدراسة تقديرات المستويات لخمس قياسات كيموحيوية في دم مرضى الحساسية كمدلولات لنشاط وشدة الأزمات الربوية بسبب حلم غبار المنازل بمصر. تم إجراء دراسة على أربعين حالة أطفال (28 ذكور و 12 اناث) تتراوح اعمارهم ما بين 6 شهور الى 15 عام) مصابين بحساسية بسبب حلم غبار المنازل مقسمين إلى حالات متوسطة (31) وشديده (9)، بالإضافة إلى عشرة أطفال أصحاء (6 ذكور و 4 اناث) شهور الى 15 عام). بتحليل عينات الدم، وجد أن مستويات انطلاق الهيستامين، الأجسام المضادة الكلية (هـ)، الأجسام المضادة النوعية (هـ) و البروتين الكاتيونى، والعد الكلى للخلايا الأكلة لحالات مرضى الحساسية أعلى إحصائياً مقارنة بمجموعة الأطفال الطبيعيين وفي الحالات الشديده اعلى إحصائياً عنها في الحالات المتوسطة. لذا يمكن ان يكون الترتيب طبقاً لمتوسطات القياسات كما يلي: (الحالات الشديده < الحالات المتوسطة) < مجموعة الأطفال الطبيعيين. خلصت الدراسة الى ان المستويات العاليه لهذه المدلولات الكيموحيوية يرتبط بشدة الأصابه بالأزمات الربوية لذا يمكن اعتبار هذه المدلولات كمحددات لنشاط وشدة مثل هذه الحالة من الحساسية.