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Correlation of serum total immunoglobulin E levels, eosinophilia and sensitization to common aero- allergens among asthmatics in Sudan.

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Abstract

Asthma is a chronic inflammatory disorder of pulmonary airways in which many cells and cellular elements play a role. Chronic inflammation is responsible for increased serum total Immunoglobulin E (IgE) levels, specific IgE, and blood eosinophils (B-Eos) count.

We aimed in the present study to determine quantitative physiological traits: serum total IgE, and B-Eos count and assess their correlation with skin prick test (SPT) among Sudanese asthmatics, and evaluate these findings with the persistence of developing asthma. 281 subjects; 146 asthmatics and 135 controls were recruited. Pulmonary function tests (PFTs), and SPT for commonly encountered allergens were performed using the standard methods. Serum total IgE was measured using the ELISA technique, and blood smears for B-Eos count were measured using a colter counter. An interview questionnaire was filled out for each individual to determine the duration of the disease, allergic status, and environmental factors. In the majority of asthmatics; Positive SPT response to seven allergens ($P < 0.01$), the highest exposure allergen was found to be mixed molds (85.3%), and the strongest sensitized allergen was house dust mite (HDM) ($P < 0.001$). Serum total IgE and B-Eos count were highest ($P<0.001$) having a significant positive SPT response ($P<0.001$).

Conclusion: There is a strong association between sensitization to some allergens and asthma.

High serum total IgE levels, and high eosinophil count has been found to be correlated with asthma

Keywords: Asthma; Immunoglobulin E; Blood Eosinophil; Skin Prick Test; Aero Allergens

1 Introduction

Asthma is one of the common chronic lung diseases. The chronic inflammation is responsible for increased levels of serum total IgE, elevated levels of IgE specific aero allergens, and elevated B-Eos count, and is also responsible for increased airway hyperresponsiveness (AH) to a variety of stimuli, such histamine [1, 2]. The definition of asthma remains limited to the description of its key clinical features, with broad reference to the underlying inflammatory characteristics and heterogeneity of asthma phenotypes, this definition provided by many studies. The Severe Asthma Research Program (SARP) clustered on clinical, demographic, and natural history [3], which were distinguished primarily by lung function and the age of asthma onset [4]. Some studies classified asthma phenotypes according to

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onset age and persistence of wheezing, despite diverse factors affecting the development and progression of asthma [5]. Other studies have suggested that the decline in force expiratory volume1 ($FEV_{1)}$ may be related to the persistence of airway inflammation [6]. However, while some studies have included many variables to identify asthma subtypes, most have continued to be based on clinical/demographic characteristics or pathological characteristics [7]. Although the World Asthma Phenotypes study (WASP) redefine asthma phenotypes in children, adolescents, and young adults using a combination of clinical, demographic, and pathological data in five centres; in the UK, New Zealand, Brazil, Ecuador and Uganda [8]. There have been the number of studies that try to identify asthma phenotypes based on non-invasive type 2-markers, such as B-Eos count, serum total IgE [9,10]**.** Allergic asthma represents the most frequent endotype of asthma representing over the 60% where as non atopic eosinophilic phenotype represents about 25–30% of the cases [11]. Previous studies have used a range of clinical variables in combination with simple, easy to apply, and measures of airways inflammation assessed in induced sputum and Type-2 inflammation [12, 13]. Asthma phenotypes are all generally categorized under the broad umbrella of asthma because they meet the simple criteria for clinical diagnosis of this disease, however the actual clinical application of phenotyping asthma was based on symptom severity [14]. Although persistent troublesome wheeze is known to be associated with atopy, bronchial hyper-responsiveness (BHR), and decreased pulmonary function compared with controlled asthma [15]. Asthma is a multi-factorial condition; the strongest risk factor in the etiology of asthma is atopy, and may be defined as the production of abnormal amounts of IgE antibodies in response to common aero allergens. IL-4 produced by Type 2 T-helper cell (Th2) stimulates IgE production in B-cells [16]. Although asthma is characterized by the presence of increased numbers of eosinophils, neutrophils, lymphocytes, and plasma cells in the bronchial tissues, bronchial secretions, and mucus [17] involving allergen exposure. IgE- mediated sensitization with a Th2 lymphocyte response and subsequent IL-5 mediated eosinophilic airways inflammation, resulting in reversible airflow obstruction and clinical symptoms [6]. IgE has important functions in the development of allergic disorders and asthma, the pathophysiological mechanism of asthma is associated with atopy and eosinophilic inflammation, however it has been shown that asthma is a heterogeneous disease that involves IgE production and eosinophilia [18]. IgE is involved early in the inflammatory cascade and can be considered as a cause of allergic asthma, eosinophilia can be considered a consequence of the whole process [19]. Three primary environmental exposures that lead to airway inflammation and allergic symptoms have been identified in many studies. Some studies have shown dust mite-specific IgE in the bronchial secretions of intrinsic asthmatics as well as the production of IgE specific to *S. aureus*enterotoxin [20]. Epidemiologic studies have considered the instability of asthma phenotypes over time and identified associated risk factors for each asthma phenotype, however such studies considering environmental factors for the classification of asthma phenotypes are limited, although environmental factors play an important role as causative and provocative factors for asthma [21]. Our study aimed to estimate environmental allergens triggers for IgE and esinophils production and to analyse correlation of these findings on asthma development by using these phenotypes as diagnostic tools in asthma disease.

2 Material and methods

2.1 Subjects and Data collection

281 subjects aged between 7-65 were included, females predominated represent 52%. Asthmatic subjects (n=146) were selected from respiratory outpatient clinics located in Khartoum state. The apparently normal control subjects (n=135) were randomly selected from Khartoum state residents. Data were collected using the Portuguese versions of International Study of Asthma and Allergies in Children (ISAAC) questionnaires for children and adults, regarding asthma, rhinitis, and allergic diseases [22]. An interview questionnaire was filled by each adult participant or the parents in the case of children. From the questionnaire, personal and family history of respiratory symptoms, illnesses, allergies to food or animals, and exposures to different aero allergens were assessed. Subjects with a positive family history of asthma and/or any other allergic condition like atopic dermatitis, and allergic rhinitis were selected and undergone PFTs, SPTs and blood tests for IgE assays and B-Eos count.

2.2 Pulmonary Function Testing

Pulmonary function testing is a valuable tool for evaluating the respiratory system, representing an important adjunct to patient history. PFT was conducted according to American Thoracic Society (ATS) criteria, [23] using a hand-held spirometer (Micromedical Limited, UK). Spirometry was performed in the standing position, using bronchial reversibility that included spirometry before and after the administration of inhaled salbutamol as a bronchodilator. FEV1 and peak expiratory flow rate (PEF) was measured and recorded as initial (before bronchodilator) records. FEV1 and PEF were then re-measured after 15 minutes following administration of inhaled bronchodilator (salbutamol 400 µg) via a spacer and recorded as post-bronchodilator records. An increment of 12% in FEV1 or 20% or more in PEF was considered significant reversibility and confirms the diagnosis of asthma [24]. All parameters are expressed as a percentage of the predicted value; based on age, gender, and height (according to GINA

guidelines). We calculated the percentage change of FEV1 from baseline as follows: ([Post-FEV1] − [Pre-FEV1])/(Pre-FEV1 × 100%) and defined a change of 12% or greater as evidence of bronchodilator reversibility (BDR).

2.3 Skin Prick Testing

A panel of thirteen inhalant allergens that commonly found in asthmatic patients in Sudan were tested. The commercial extracts (NELCO laboratories, USA) included; HDM; (D. pteronyssinus and D. farinae), cockroaches (Blattella germanica and Periplanetaamericana), mixed molds (Alternaria alternata, Cladosporiumherbarum, and Aspergillusfumigatus), mixed ragweed (Ambrosia artemisifolia, Artemisia vulgaris), mixed standardized grass pollens (Loliumperenne, Quercus alba, and Betulaverrucosa), goat dander, animal dander (Felisdomesticus and Canisfamiliaris), mixed trees, mixed weeds, mixed feathers, dog epithelium, standardized cat hair, and mosquito. After explaining the test procedure and assurance of its safety to the selected subjects. Lancets were used to prick the epidermis through the allergen extract drops on the flexor surface of their forearm to the thirteen allergen extracts as well as a positive control (histamine solution) and a negative control (0.9% saline). The tests responses were read after 15 minutes. A positive response was defined as a mean wheal diameter of greater than 3 mm with an absent response to the negative control solution, an atopy was defined as a positive response to 1 or more of house dust mite, cat, or grass pollen [25].

2.4 Measurement of Serum Total 1gE

Serum total IgE levels were measured using ELISA technique with a commercially available kit (Monobind, INC, Costa Mesa, and CA92627 reagents, USA). Total serum IgE levels were expressed in international units per milliliter (IU/ml), =100 U/ml considered up normal value in adult, for children (6-9 years) =155U/ml, (10-15 years) =199U/ml. 1gE calibrator, patient specimen, or control was first added to streptavidin (capture antibody) coated well. Biotinylated monoclonal antibody (specific for 1gE) was added and the reactants mixed. The reaction between 1gE antibodies and the native IgE forms the complex that bound with the streptavidin-coated to the well. Another enzyme-labeled monoclonal antibody specific to 1gE was added to the wells. The enzyme-labelled antibody bound to the IgE was immobilized on the well through its binding with the biotinylated monoclonal antibody, the absorbent was read using a micro plate reader (Human Geselleschat for Biochemical and Dignostica 65205 Wiesbaden/Germany).

2.5 Eosinophil counts

Blood smears from peripheral samples were stained (Wright-Giemsa stain) and evaluated under a microscope. A 200-count white blood cell differential was performed, the percentages and absolute eosinophil counts were calculated accordingly. A B -Eos count ≥300/mm3 was used to define a blood eosinophilia asthma phenotype [26]. B-Eos count ≥5%. Peripheral blood eosinophil levels above 450 cells/mL are generally considered to be abnormal [27]

2.6 Ethical Approval

The study was approved by the Research Ethics Committee of the Institute of Endemic Diseases, University of Khartoum, Sudan. All participants were informed about the objectives and the need for this study; self-confidentiality was assured and their written consent to participate was taken before being involved in the study.

2.7 Statistical Analysis

The obtained data were analysed using the SPSS program (Version 18; Chicago, IL, USA). Chi-square tests were used for the association of variables between asthma cases and controls and logistic regression analysis was performed to obtain the adjusted odds ratios. A value of $p < 0.05$ was considered significant.

3 Results

281 subjects were included. The majority of cases had multiple allergic symptoms, especially those of long duration. The duration of disease between 2-20 years, (the majority were 5 years).

3.1 SPT

Out of the tested 13 allergens, positive SPT response for seven allergens in the majority of cases (P <0.5), (Table 1). House dust sensitization was the strongest among cases (P = 0.001), the highest exposure allergen among cases was found to be mixed molds 85.3%. The majority of subjects 64.2% were sensitized to three or more allergens. The exposure to overall allergens was found to be significant among asthmatics in comparison with non-asthmatics ($P < 0.001$).

3.2 Serum total IgE level and B-Eos count

Serum total IgE level was significantly higher among cases (72.7%) compared to controls (*P*< 0.001) (Table 2). Median serum total IgE level in cases and controls (fig.1). Eosinophilia was significantly more observed among cases (59%) compared to controls (P < 0.001), (Table 2). Serum total IgE and B-Eos count were highest among cases, having significant positive skin. The asthma-related phenotypes outcome regarding gender, the analysis reveals the following; the prevalence of single and multiple phenotypes among females cases, was found to be higher; (SPT=80%, IgE=71%, B-Eos count=60%), both high IgE level & eosinophilia (62.5%) (Table 3). Although, having along duration of disease>20 had higher prevalence of phenotypes (Table3).

Table 1 The sensitization rate on inhalant allergens in cases and controls subjects

	Allergens	**Cases (n=146)**	**Controls (n=135)**	**P -value**
1	House dust	70% (103)	45% (61)	0.001
2	D. Peterenyssinus	66% (97)	32.5% (44)	0.01
3	Mixed molds	82%(120)	57.8%(78)	0.01
4	Grass pollen	44.4% (60)	26.6% (36)	0.01
5	Mixed rag weeds	17.1% (25)	6.6% (9)	0.01
6	Cat hair	35.6% (52)	21.4% (29)	0.02
7	Mixed feathers	22% (31)	12.4% (16)	0.05

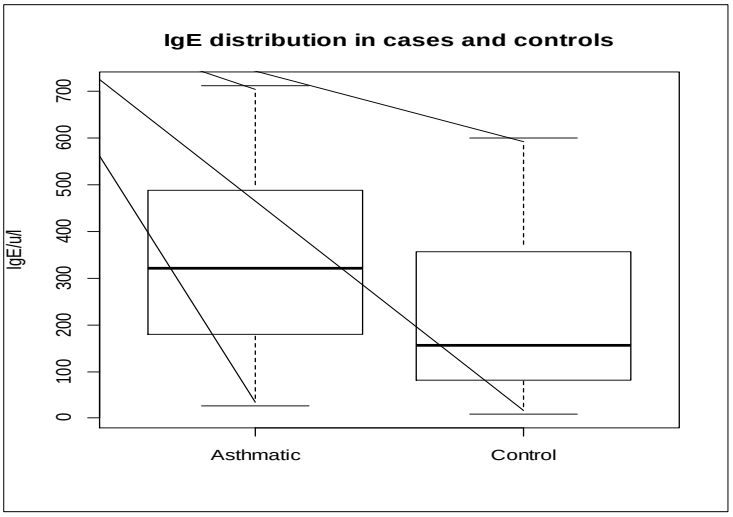

Figure 1 Median serum total IgE level in cases and controls

Median (IQR) 321 (180, 488) 157 (81, 357)

Table 2 Comparison of serum IgE and B-Eos count between cases and controls

Phenotype	**Cases (n=146)**	**Controls (n=135)**	**P-value**
High IgE level	72.7% (106)	26% (35)	0.001
Normal IgE level	27.3%(40)	74% (100)	
High B-Eos count	59%(86)	23.7% (32)	0.001
Normal B-Eos count	41.0%(60)	76.3%(103)	

Table 3 Comparison of high serum IgE level, eosinophilia, and +ve SPTs in relation to gender and duration of disease among cases

Phenotype	Female(n= 80)	Males(n= 66)	Duration of disease
SPT (**+ve** to>3 allergens)	81% (65)	60% (40)	8-20
High IgE level	71% (57)	57.5% (38)	8-20
Eosinophilia	60% (48)	50% (33)	8-20
High IgE level & Eosinophilia	62.5% (50)	%60 (40)	**>**20

4 Discussion

Asthma and allergic diseases witnessed an increased over the world in a short time period. A possible explanation for difficulties in identifying causes of asthma is that phenotypic heterogeneity, which underlies differences in the pathophysiology of asthma, each having different genetic and environmental influences [28]. However there have been few novel insights into how environmental factors might influence the onset of specific asthma, which can help in the prevention of disease [28]. In this study, on the analysis of the asthma-related phenotypes [SPT, IgE, B-Eos count], our results showed a high prevalence of positive SPT, elevated serum total IgE level, as well as eosinophilia with significant values. An explanation may be due to a major increase in exposure to allergens [29], our results seem to be in line with the view of many findings; the presence of asthma or atopy may be confirmed by high levels of specific IgE or total IgE in the serum or by positive responses to skin sensitization [29,30,31]. However, other findings focused on allergic asthma phenotypes based on IgE levels [32]. This study highlights the importance of environmental exposures in the development of asthma and atopy in Sudanese. We investigate common environmental factors that might influence asthma which are specific to Sudanese. We found that subjects having a specific combination of asthma phenotypes gave concordant results. The more prevalent combinations of phenotypes observed were; positive SPT (75.2 %) together with high serum total IgE level (75.2 %), which in agree with study reveal serum total IgE level was higher in the individuals sensitized with food allergy [33]. Other studies considering environmental factors for the classification of asthma phenotypes are limited, although environmental factors play an important role as causative and provocative factors for asthma [21]. This study supports the suggestion that sensitization to various indoor allergens has a differential effect on allergy and asthma, and the highest risk was found to be among individuals sensitized to house dust. Studies conducted among Sudanese found that most triggering allergens are; molds, house dust mite, grass pollen, and cat [34]. In this study 61.4% of subjects were atopic sensitized with three or more positive SPT, a graded effect was observed with the number of positive SPT sensitivity, the risk of asthma in our subjects increasing with the increased number of positive SPT reactions (3+ve to11+ve range). Nearly 75% of the individuals with three or more SPT reactions had chronic asthma, furthermore, there was relationship between allergen exposure, sensitization, and the development of allergic asthma. This supports the view that atopy-related asthma in-line with previous studies reported that, atopy is an important risk factor for allergic diseases such as asthma [29]. In the USA and Europe, some sensitizations may be actual risk factors for the development and severity of allergic diseases [35, 36]. Studies conducted in Australia, allergies and hay fever are very common among adolescents with asthma [37]. Although as reported in Zimbabwe, poly sensitizations may increase the risk of allergic symptoms [38]. However our findings were not agree with a study conducted in Angola, showed no relationship was detected between SPT response and asthma [39]. Furthermore, studies have shown that factors such as skin reactivity to allergens, allergic disease history, and age can all influence eosinophilia [40]. Evidence supporting the role of IgE in asthma includes the correlation of elevated serum IgE levels with asthma severity and allergic diseases [19]. Importantly, study reported the atopic individuals to have significantly greater probability of developing asthma, and individual with a family history of atopic diseases are at greatest risk of asthma as confirmed [41]. Study reported in Australia, allergies and hay fever are very common among adolescents with asthma [39]. A recent study reported, atopy, as indicated by a positive SPT to common aero allergens, higher total serum IgE or allergic rhinitis were associated with elevated blood eosinophil counts [1]. Eosinophilic inflammation of the airways characterises disease severity in subsets of individuals with severe asthma and there is a direct relationship between eosinophil count and the frequency of asthma exacerbations [6, 41]. Although it is clear documentation of increased eosinophil numbers and increased eosinophil activation in the blood, lungs, and sputum of asthmatics was closely associated with asthma severity [42]. The study showed that sex is a risk factor, there was a female predominance among phenotypes, particularly in the serum IgE level and skin sensitization, which seem to be in line with the study showed that sex is a risk factor for different asthma phenotypes [43]. Finally, this is the first study concerning these parameters; serum total IgE level, peripheral B-Eos count, and aero allergen sensitization conducted in Sudan, although few studies conducted in Africa. The knowledge of common triggering aero-allergens, may be useful for controlling the reactivity of these allergens and development of asthma disease.

Abbreviations

- IgE: Immunoglobulin E.
- B-Eos: Blood Eosinophils.
- AH: Airway Hyperresponsiveness.
- SPT: Skin Prick Test
- ELISA: Enzymatic Link Immuno Sorbent Assay
- Th2: T-Helper Cell 2
- HDM: House Dust Mites
- GINA: Global Initiative of Asthma Guideline
- SARP: Severe Asthma Research Program
- $FEV_{1:}$ Force Expiratory Volume1
- WASP: World Asthma Phenotypes Study
- BHR: Bronchial Hyperresponsiveness
- ISAAC: International Study of Asthma And Allergies In Children
- PFTs: Pulmonary Function Tests.
- ATS: American Thoracic Society
- PEF: Peak Expiratory Flow Rate
- BDR: Bronchodilator Reversibility

5 Conclusion

High serum total IgE levels, eosinophilia have been found to be correlated with asthma. There is a significant association between hypersensitivity SPT, eosinophilia, and high serum total IgE levels among asthmatics in Sudan. Females had a significantly higher prevalence of asthma related phenotypes. The overall findings were that subjects having multiple phenotypes & single phenotype with long duration of disease had a significantly higher proportion of developing asthma. Characterization of asthma phenotypes might help clarify the underlying mechanisms through which asthma occurs and improve the power to detect causal factors and proper treatment.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no conflicts of interest.

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