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Role of Th2 type Cytokines and IgE in Asthmatic Children

Assem Metwally Abo-Shanab[1]*, Hala Elnady[2], Iman Helwa[1], Rania Fawzy Mahmoud Abdelkawy[1], Hanan Atta[2], Dina A Salah[2] and Haiam Abdel Raouf[1]

[1]Immunogenetics Department, National Research Centre, Egypt.
[2]Child Health Department, National Research Centre, Egypt.
*Corresponding Author E-mail: asem_nrc@yahoo.com

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Bronchial asthma (BA) is usually persistent through allergic sensitization, which is likely to result in bronchial hyper-responsiveness and acute bronchoconstriction due to reactions to specific and non-specific trigger stimuli. Many research focused on the role of T cells; particularly, T helper 2 (Th2) cells which linked to controlling immunoglobulin E (IgE) production due to their role in producing different cytokines, like Interleukin-13 (IL-13), in addition to influencing the function of eosinophils through the actions of IL-5. From this perspective, we decided to study the role of IgE, IL-13, and IL-5 in asthmatic children. IL-5, IL-13, and total IgE have been measured by ELISA technique in the serum of 57 children with bronchial asthma and compared to that of 20 healthy controls. Our results reported that 38/57 (66.67%) of the patient group had a family history for allergy, and parental consanguinity was found in 4/57 families (0.07%). IgE levels showed high statistical significance in asthmatic patients in comparison to controls (p = 0.00001), while IL-13 and IL-5 levels were not significantly different in patients versus control groups (P= 0.96, P = 0.81). Moreover, IgE was found significantly increased in both groups with/without family history for allergy (p = 0.009), whereas IL-13 was significantly elevated only in the group of patients with family history for allergy (0.01). This study demonstrates that asthma is strongly related to the family history of allergy, where IgE, as well as IL-13 levels, were found high in these asthmatic patients suggesting their association with underlying symptoms. Unexpectedly IL-5 was found insignificantly decreased.

Keywords: Asthma, allergy, Cytokines, T helper cells, IgE.

Asthma is defined as a heterogeneous, chronic, inflammatory airway disease where patients suffer from recurrent bouts of bronchoconstriction, airway hyper-responsiveness as well as hyper mucus secretion. Asthma affects nearly 300 million people all over the world.[1] Five to ten percent of the adult asthma populations suffer a severe form of asthma accompanied by a high incidence of hospitalizations and mortality as well as marked symptoms, and noticeable health care costs.[2]

It is quite obvious that asthma runs in families. Moreover, children born to asthmatic parents have a higher risk of being asthmatic. However, a single gene mutation does not cause asthma. Thus, disease transmission doesn't typically pursue simple Mendelian inheritance across generations as the case with other monogenic diseases, like Huntington's disease (autosomal dominant), or sickle-cell disease (autosomal recessive).[3]

On the contrary, asthma is a polygenic, multifactorial disease, where genetic, as well as environmental factors, add to its development. Hence, the joint action of these numerous interacting genes with environmental factors leads to the situation.[3]

Several studies of asthma noted that asthmatic patients' children have a higher risk of developing asthma showing a 25% recurrence risk of asthma with one affected parent. Moreover, when both parents are concerned, the risk rises to almost 50%. However, studies on twins also supported the higher incidence of asthma when one is a genetically close relative to a patient. Accordingly, asthma's recurrence risk is higher in monozygotic twins than in dizygotic twins. This clarifies that genetic risk factors play a role in asthma.[4]

While it is clear that familial background has a strong influence on the risk of developing asthma on an individual basis, other factors, both environmental and genetic, affect the phenotypic expression of asthma. It is considered that a small number of genes sets individual background risk. Nevertheless, environmental factors and other modifying genes also act upon it.[5] Many Genome-wide association (GWA) studies of asthma and allergy have recognized some candidate genes.[6] The gene list associated with asthma counts over a hundred different genes and still growing.

There are three main categories of asthma susceptibility genes; relating to 1) immune system functioning, 2) function biology of the mucosa, in addition to 3) function of the lung and expression of the disease.[7]

Inflammation in asthma is also heterogeneous. Moreover, airway inflammation determines asthma brutality and characteristics and thus is considered a treatment target. The inflammatory pathway in asthma is determined by many cell types, immune pathways, and mediators. Theses pathways differ from one patient to the other and vary with time and variable circumstances. Thus, they are far more complex than classifying them in two known groups: T (Type) 2 and non-T2 inflammation. Probably, a combination of both pathways leads to the final airway inflammation with the dominance of either pathway and thus indicating a target for therapy and better control of the disease.[2]

According to the present understanding of T2 inflammation components and its Importance in severe asthma, many pathways have drawn awareness to the presence of novel targeted interventions. Namely, IgE, in addition to the IL-4/ IL-13 pathway as well as eosinophils. Significant and effective treatments have emerged to ameliorate asthma outcomes in severe cases based on the specific components of T2 inflammation that have been identified while studying biologics for asthma.[8] So far, in severe asthma, many medications are targeting particular downstream cytokines of the type2 high pathway as IL- 4, IL-5, in addition to IL-13. Also; IgE and thymic stromal lymphopoietin (TSLP) is included in the same context.[9]

Th-2 lymphocytes, mast cells, eosinophils, and Innate lymphoid cell 2 (ILC2) cells secrete IL-5, which is an essential type2 cytokine.[10] It is an effective eosinophilic cytokine responsible for eosinophils' maturation and differentiation in the bone marrow, in addition to activation and survival at peripheral sites of allergic inflammation.[11] Handling severe forms of eosinophilic asthma has been revolutionized by using monoclonal antibodies to block the activity of IL-5.

In the same context, Interleukin-13 is also a vital factor of the Th-2 pathway and pathogenesis of asthma. It stimulates airway hyper-responsiveness, mucus production, and subepithelial fibrosis.[12] For that reason, this study aimed at evaluating IL-13, IL-5, and total IgE in asthmatic patients.

Patients and Methods

The local medical research ethics committee (approval no: 16/381) of the National Research Centre (NRC) accepted the study, and we obtained written informed consent from all patients' parents at enrolment and before any study procedure. We enrolled fifty-seven asthmatic children, twenty-seven males, and thirty females; their age ranged between 4 years and 16 years old between February 2018 to April 2019. A group of twenty healthy children, fifteen males, and five females with ages ranging from 4 to 15 years old were included as a control group.

The inclusion criteria were as follows:

• Asthmatic children 4-16 years old (they were diagnosed as asthmatics according to the clinical manifestations described in GINA (2019)

Exclusion criteria

• Mentally impaired children.
• Any child aged below 4 years or above 16 years.
• Chronic pulmonary, cardiac, renal, or neuromuscular disorders.

The following was done for every child

• Complete history: including demographic data, allergic symptoms, asthma severity, and control as well as family history.

Clinical examination

• General & chest examination
• Anthropometric measurements in the form of weight, height, and BMI using a digital scale and a stadiometer respectively.[13]
• Spirometry and Impulse Oscillometer was done to all patients according to ATS/ERS recommendations before and after inhalation of a bronchodilator (salbutamol inhaler with a spacer) using Jaeger Master screen Pulmonary Functions apparatus.[14]

Spirometry was performed in a relaxed sitting position with participants applying nose clips to occlude the nares and the head slightly extended. The procedure required the patient to breathe into a well-fitted mouthpiece, with the lips tightly sealed. The patient first breathed normally then forcefully.

The following parameters were measured: FVC, FEV1, FEV1/FVC %, MEF25-75%

Impulse oscillometer (IOS)

Impulse oscillometer (IOS) was completed according to the "European Respiratory Society/ American Thoracic Society (ERS/ATS)" guidelines by the Master Screen IOS system (Jaeger Co., Germany).

Impulse oscillometer (IOS) necessitates that the subject should breathe at his normal pace (tidal breathing) into a mouthpiece. A loudspeaker produces an impulse shaped pressure signal into the respiratory system. The IOS system was adjusted every day before the measurements by using a syringe of 3-liter capacity. IOS measurements were completed in the sitting position while subjects were applying nasal clips. Participants breathed tidally for 30 seconds into the IOS mouthpiece while the mother's hands supported the cheeks. The physician assessed the exertions and assured that each observation comprised at least three reproducible maneuvers that did not have artifacts due to swallowing or coughing or even vocalization or breath-holding. The Impulse oscillometry was done before and after intake of salbutamol from a metered-dose inhaler with a spacer to evaluate response to bronchodilator.

The IOS parameters acquired at completion of this application were resistances (R5, 20) at 5—20 Hz, R5-R20 (resistance at 5 Hz minus resistance at 20 Hz), reactance at 5 Hz (X5), resonant frequency (Fres, the frequency where the X value is zero), and area of the reactance curve (AX, integral of X values from 5 Hz to Fres).

In both; Spirometry and Impulse Oscillometer, the attending physician assessed the patient's efforts and made sure each test consisted of at least three reproducible maneuvers free of artifacts in all procedures.

In addition, laboratory tests were also done for the patients; namely, a complete blood picture was done for each case as well as serum IL-5, IL-13, and total IgE were estimated using the ELISA technique.

Cytokines and IgE measurements

Determination of Interleukin 5

Serum IL-5 levels have been studied by the well-known "commercially-available solid-phase sandwich ELISA kit" (ELISA) (NOVA, Daxing Ind. Zone, Beijing, China.) as following the company's instructions.

Determination of Interleukin 13

Serum IL-13 levels were assessed by the "commercially-available solid-phase sandwich ELISA kit (ELISA)" (NOVA, Daxing Ind. Zone, Beijing, China.) following instructions of the manufacturer.

Determination of Total IgE

The study measured Serum IL-13 using a commercially-available "solid-phase sandwich ELISA kit (ELISA)" (Chemux Bioscience Inc, 385 Oyster Point BLVD, Suite 6, South San Francisco, USA.) following steps provided by the manufacturer.

Data analysis and statistics

The data was collected, coded, and tabulated. Statistical analysis was done using a T-test for parametric data. For nonparametric data, the Mann-Whitney test was used. A P-value of less than 0.05 was reflected in statistical significance. Quantitative data were analyzed using descriptive

statistics, i.e., mean ± SD (standard deviation) or median (min-max), while for qualitative data, it was presented as number and percentage.

RESULTS

The clinical data of 57 asthmatic patients presented in Table (1). This data demonstrated that 38/57 (66.67%) of the studied asthmatic patients had a family history of allergy. Also, parental consanguinity was encountered in 4/57 families (0.07%).

The current study has demonstrated that IgE levels show highly statistical significance in asthmatic patients as compared to apparently healthy controls (P = 0.00001). In contrast, IL-13 levels were not significantly different in asthmatic patients when compared to apparently healthy controls (P= 0.96). Whereas; IL-5 was insignificantly decreased in asthmatic patients in comparison to the control group (P = 0.81). Table (2).

Table (3) summarizes the results of IL-13, IL-5, and IgE levels in two groups of asthmatic patients. A group with a family history for allergy while the other group had no family history for allergy. These findings indicated that IgE revealed a statistically significant increase in both groups where IL-13 showed a statistically significant increase in the patient's group with a family history for allergy compared to the control group.

Our laboratory findings indicated a high statistically significant increase in serum level of IgE in the mild persistent asthmatic and intermittent asthmatic patients compared to the control group (P = 0.003 and 0.001 respectively) as well as a statistically significant increase in moderate persistent asthmatic patients as compared to the control group (p= 0.016). Besides, the results of the study demonstrated that IL-13 level was significantly higher in mild persistent asthmatic children in comparison to the apparently healthy control group (P = 0.05), as presented in Table (4).

Table 1. Clinical findings among an asthmatic group

Variables	Asthmatics(n =57)
Age years, mean (SD)	10.1 (3.4)
BMI, mean (SD)	19.1 (3.9)
TLC cell/mm^6, mean (SD)	7.4 (2.3)
Sex N%	
Male (%)	27 (47.37%)
Female (%)	30 (52.63%)
Family history for allergy N%	38 (66.67%)
Consanguinity N%	4 (0.07%)
Severity N%	
Moderate persistent asthma	13 /57 (23.8%)
Mild persistent asthma	8 /57 (14.04%)
Intermittent	36/57 (63.16%)

Table 2. Laboratory parameters in study subjects

	Normal Control (n = 20)	Asthmatics (n =57)	P value
Total IgE IU/mL	6 (2-355)	105 (3-996)	0.00001*
IL13 pg/mL	295 (161.7-553.3)	297.8 (14.5-3178.3)	0.96
IL5 pg/mL	166.2 (55.9-367.4)	172.6 (17.4-818.7)	0.81

Results were expressed as Median (Min-Max).
* Significant versus controls (by Mann Whitney U Test).

Table 3. Comparing parameters according to family history

	Family history for allergy Positive (n = 38)	P-value	Family history for allergy Negative (n = 19)	P-value
Total IgEIu/mL	69 (15-405)	0.009*	61.5 (3-996)	0.0009
IL13 pg/ml	288.05 (14.5-3150.6)	0.01*	603.35 (58.7-3178.3)	0.32
IL5 pg/ml	144.35 (39.2-476.4)	0.4	155.9 (17.4-818.7)	0.9

Results were expressed as Median (Min-Max).
* Significant versus controls (by Mann Whitney U Test).

Table 4. Biomarkers comparing apparently healthy control with different asthmatic groups

	Mild persistent asthma n=13	Moderate persistent asthma n= 8	Intermittent n= 36
Total IgEIu/mL	42 (30-330)*	25 (3-120)*	120 (27-996)*
IL13 pg/ml	131.1 (17.3-328.3)*	253.3 (14.5-256.1)	383.3 (95-3178.3)
IL5 pg/ml	68.7 (39.2-180.3)	178.9 (50.8-808.5)	249.5 (17.4-818.7)

Results were expressed as Median (Min-Max).
* Significant versus controls (by Mann Whitney U Test).

DISCUSSION

BA is usually persistent through allergic sensitization, which is likely to result in bronchial hyper-responsiveness and acute bronchoconstriction due to reactions to specific and non-specific trigger stimuli.[15] Among children, asthma is considered the most common chronic disease. It varied geographically from 2% to 32% in different countries.[16]

It is considered a multifactorial condition with numerous predisposing factors, among which an individual's genetics plays a central role. Many loci related to asthma have been reported in Genome-wide studies.[17]Other environmental factors as the structure of house designs as well as dust mites, pollution, molds, pets, tobacco smoke, food, infection, and others, are considered to trigger factors in asthma.[18] Pathogenesis of asthma involves innate and adaptive immunity pathways as well as memory immunity.[19]

In severe asthma, inflammation of the airway has been broadly divided into either T (type) 2 high or T2 low. Th2 cells, release interleukins as IL-4, IL-5, and IL-13. Airway eosinophilia mediated by Th2 is not only mediated by adaptive immunity but also linked to innate immunity. This happens via an intercellular connection between immune cells as innate lymphoid cells, dendritic cells, and bronchial epithelial cells as well. As a result of the biological activity of both Th2 and ILC2 cells, airway eosinophilia occurs. This significantly participates in the pathogenic process of type-2 inflammation in both eosinophilic allergic and nonallergic asthma.[20]

ILCs are defined as immune cells having a lymphoid morphology yet lacking antigen receptors. On exposure to an allergen, ILC2s produce many type 2 cytokines like IL-4, IL-5, and IL-13.[21] Although the asthma phenomena are characterized by Th2 overactivation, Th1 response is linked to the continuation of the asthma.[22]

The current study demonstrated that IgE levels showed a high statistically significant difference in asthmatic patients compared to apparently healthy controls (P = 0.00001) *(Table 2)*. This came in agreement with *(Yu et al., 2014)*,[20,] who reported increased IgE expression in asthma, and also with *(Nakajima et al., 1992),* who proved that Th2 cells-like mast cells and basophils release IL-4. This induces immunoglobulin class switching from IgM to IgE by B lymphocytes.[23]

In our study, IL-13 levels are non-significantly higher in asthmatic patients compared to controls (P= 0.96) *(Table 2)*. Wong *et al*. reported the same findings. in *2010* who recognized that, in an asthma model, Thymic Stromal Lymphopoietin (TSLP) cause a decrease in airway hyperreactivity through upregulation of natural killer T cells that on their behalf increase production of IL-13.[24] Moreover, *Peebles et al.* .in *2019* stated that, concerning innate allergic immune response, ILC2 are stimulated by IL-33 leading to enhancement of Th2-type cytokines production, as IL-13.[25]

Differentiation of naive T cells into Th2 cells is stimulated by IL-13, which also causes induction of IgE production. Moreover, vascular cell adhesion protein 1 (VCAM-1) expression is initiated by IL-13. This causes many immune cells to migrate directly to sites *of* allergic inflammation.[26] We noticed that IL-5 is insignificantly decreased in an asthmatic patient in comparison to the control group (P = 0.81) *(Table 2).* On the contrary, *(Ngoc et al., 2005) and (Finkelman et al., 2010)* confirmed an enhanced gene expression or secretion of IL-4, IL-5, IL-13.[2, 27] Also *Dorman et al.* in *2004* reported that in asthmatic patients, the bone marrow increases eosinophil production in response to

an environmental irritant.[28] Nevertheless, in asthmatics experiencing acute and late reactions of asthma, this event is linked to increasing IL-5 mRNA compared to subjects with early bronchial reactions. Away from the effect of IL-5 on the bone marrow, it was noted that IL-5 enhances eosinophil maturation in the airways of allergic patients.

All of the above harmonized with *(Chung and Barnes, 1999)* who reported that Th2 cells release diverse proallergic inflammatory cytokines, such as IL-4, IL-5, and IL-13 in addition to "Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF)".[29] The later was found to generate basophils and eosinophils and raise mucus secretion in the patients' airway. This also came in accordance with *Bossios et al.* in *2010* who confirmed that IL-5 could stimulate the production of eosinophils and play a role in their differentiation, proliferation, and survival.[30]

Nevertheless, in patients with asthma, some studies described the high expression of IL-5 in their bronchoalveolar lavage (BAL) fluid and bronchial biopsies. Nevertheless, IL-5 mRNA was found to be regulated in bronchial mucosa, after confronting the allergen, in a manner proportionate to activity of the disease.[31]

On the other hand, investigating of mouse models has revealed that increased eosinophils are linked to type 2 inflammation and an increase of interleukin (IL)-4, -5, and -13.[32]

Our laboratory findings indicated a high statistically significant increase in serum level of IgE in the mild persistent asthmatic and intermittent asthmatic patients compared to the control group (P = 0.003 and 0.001 respectively) and a significant increase in moderate persistent asthmatic patients compared to the control group (p= 0.016) *(Table 4)*. That came matching with another study, which concluded that doubt still exists over the exact role of this relationship of IgE-mediated sensitivity to familiar aeroallergens in the development of asthma, especially in children.[33]That came unlike the findings of *Rogala et al.* in *2015* who found that in some asthma patients especially when severe; total IgE serum level was considerable low.[34]

In addition, study results demonstrated that IL-13 level was considerably higher in mild persistent asthmatic children in comparison to the control group (P = 0.05) as presented in (Table 4*)*. This is similar to what *Doran et al.* stated in *2017* as they found appreciably high results of serum IL-13 in severe asthma patients who are relatives of healthy volunteers, and these levels were strongly related to the type 2 gene signature in bronchial epithelium. However, in moderate to severe asthma patients, there was a strong positive correlation between serum IL-13 and blood eosinophil counts.[35]

Our findings indicated that IgE significantly increased in asthmatic patients with and without family history where IL-13 significantly elevated only in the group of patients with a family history for allergy in comparison to controls *(Table 3)*. That came in parallel to *Rogala et al.* in *2015*. They reported that a positive family history of allergy, in addition to a long personal history of asthma was more frequently found in patients with severe asthma. Whereas, more common positive personal history of allergy as well as concurrent rhinitis was found in mild asthma rather than in severe disease.[34]

Last but not least, in many clinical studies, the clinical value of immunotherapy has been established, yet, still some issues are to be studied. In clinical trials, numerous biologicals are being evaluated, including those involved in the inhibition of interleukins 4, 5, 9, and 13, and IgE, but most of them were tested in clinical trials, involving patients with allergic asthma.[36-38]

CONCLUSION

This study demonstrates that asthma is strongly related to a family history of allergy where IgE, as well as IL-13 levels, were found high in these asthmatic patients suggesting their association with underlying symptoms. Unexpectedly IL-5 was found insignificantly decreased.

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Conflict of Interest

The authors declare that there is no financial conflict regarding research and publication.

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