The Role of IL-5, IL-33 and Total IgE in a Sample of Workers Suffering from Paints and Mills Asthmatic Patients

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Abstract

A case-control retrospective study was conducted during November 2020 on 88 asthmatic patients (38 male mill and 50 male paints workers)with age ranged between (15-25),and 44 control with age ranged between (15-25)years to assess the role of total IgE ,interleukine5(IL-5),and interleukin33(IL-33)serum levels in pathophysiology of disease using ELISA and multiplex immunoblot kits. Some hematological changes such as(Eosinophil and Basophil%)by Beckman coulter. The patients' samples were obtained from Nature Dyes Company Ltd in the industrial region of Aweerij Industrial, as well as Al-Dora flour mill in Baghdad, which is part of the general company for grain processing. The tests were carried out at the Specialized Center of Allergy in Baghdad/Al-Rusafa from October 2020 to April 2021. Specialized respiratory disorders and Asthma diagnose these cases, which are clinically diagnosed according to international guide lines. The present study confirms the highly significant role of total IgE and IL-5,IL-33 levels compared with control in pathophysiology of bronchial asthma and it correlation with disease severity and allergen type in adults. Results showed that there were highly significant increase in Eosinophil and basophil % in cases asthma of paints and mills patients with control group. In addition, there were a positive correlation between Eosinophil and Basophil in asthma of paints, and in asthma of mills. There was positive correlation between total IgE with IL-5 and IL-33 in asthmatic group.

Keywords:*Asthma,Eosinophil,Basophil,TotalIgE,IL-5,IL-33*

Introduction

Hypersensitivity Reactions(allergy)are immune responses that are amplified or inappropriate in response to an antigen or allergen. Asthma is a heterogeneous condition characterized by persistent airway inflammation and marked by repeated symptoms of wheezing, shortness of breath, chest tightness, and coughing that differ in duration and severity and are associated with variable expiratory flow limitation[1]. Work-related asthma is the term used to define asthma worsened by the workplace and encompasses both Occupational Asthma (OA) and work-exacerbated asthma [2,3]. OA is de novo asthma induced by either sensitization to a specific substance or a chemical at work,which is termed Sensitizer-Induced Occupational Asthma (SI-OA) or by exposure to high concentrations of an inhaled irritant found in the workplace, which is termed Irritant-Induced Occupational Asthma(II-OA)[4]. The diagnosis of OA is difficult, requiring confirmation for the diagnosis of asthma, plus evidence that the asthma was caused

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by workplace conditions. An accurate diagnosis of OA is a very important viewing of the significant health consequences for affected workers, but also the substantial socio-economic impact[5]. The level of exposure to a sensitizing agent is the most recognized environmental risk factor for OA but evidences suggest that occupational exposure to vapors, dust, gas and fumes increases prevalence of asthma[6]. Several host factors have been associated with OA. Atopy is a strong risk factor for OA due to High Molecular Weight HMW agents(e.g., bakers/pastry makers, laboratory animals workers)[3]. Abundant eosinophilia and elevated levels of immunoglobulin E(IgE)are inflammation[7]. It has addressed that IgE is a key immunoglobulin in triggering the inflammatory response in asthmatic patients, as well as asthma evolution and chronicity[8]. IgE plays a role in immediate hypersensitivity. cytokine with pro-inflammatory properties, and development of inflammation[9].

Interleukin 5 is a 13-amino acid protein that forms a 52-kDa homodimer related to both Granulocyte-Macrophage Colony- Stimulating Factor (GM-CSF) and IL-3[10]. The early response is characterized by marked synthesis of IL-3,IL-4,IL-5and IL-13, as well as the chemokine ligand-5and (GM/CSF).These mediators recruit neutrophils, eosinophils, basophils, macrophages and T lymphocytes to the site of inflammation in the late allergic response[11]. IL-5 is synthesized and secreted by eosinophils, TH2 cells, mast cells, CD34+ progenitor cells, Natural Killer (NK) T cells, and type 2 innate lymphoid cells as [12].

Interleukin 33 is produced as a 30 kDa pro-peptide, like IL-1b and IL-18, and is cleaved by caspase-1 to generate mature 18 k D IL-33. The IL33 is a regulatory cytokine from IL-1 cytokine family and it consider as an alarmin that alerts the immune system, its produce by many types of cells like epithelial cells of (skin, lungs, and gastrointestinal tract that exposure to the environmental allergens), endothelial cells, osteoblast, fibroblasts, adipocytes, smooth muscle cells, macrophages and dendritic cells[13].

Materials and Methods

A case-control retrospective study was conducted during November 2020 on 88 asthmatic patients (38 male mill and 50 male paints workers) with age ranged between (15-25) year, and 44 control with age ranged between (15-25) years to assess role of total IgE, interleukine5(IL-5), and interleukin33(IL-33) serum levels in pathophysiology of disease using ELISA and multiplex immunoblot kits. Some hematological changes such as (Eosinophil and Basophil) by Beckman coulter. The patients' samples were obtained from Nature Dyes Company Ltd in the industrial region of Aweerij Industrial, as well as Al-Dora flour mill in Baghdad, which is part of the general company for grain processing. The tests were carried out at the Specialized Center of Allergy in Baghdad/Al-Rusafa from October 2020 to April 2021.

Eight ml of blood was drawn from each subject using 10 ml disposable syringes in sterile conditions. (2ml) of blood was collected in a sterile EDTA tube for hematological assay (Basophil and Eosinophil%) by Beckman analyzer counter.

In the gel tube, 6 ml of blood was obtained and allowed to clot at room temperature. After blood clotting, the serum was centrifuged at 3000 rpm for 5 minutes before being separated into equal parts and stored at(-20°C) for immunological testing of total-IgE, IL-5, and IL-33 levels[14].

Data Analysis

The data of this study were analyzed for normality, homogeneity and normal distribution firstly to determine the suitable statistical tests for calculating the mean, standard error, standard deviation, and the probability by using student T-test, ANOVA test,

Pearson's chi- square test and Pearson's correlation via using the Graphpad Prism version 4.0[15]. The probability considered significant when it (P<0.01), (P<0.05).

Results

Asthma patients were further characterized according to severity of disease, family history and allergen type. The (mean±SD) of eosinophil, basophile, levels of T IgE, IL-5, and IL-33

Eosinophil Cell Count (%)

The results in studied groups were compared to the Eosinophil Cell (%); asthma of paints was(4.806±1.289),asthma of mills was(5.083±1.519), and control(2.164±0.9421).There were highly significantly in asthmatic groups(p=<0.0001) (P<0.01).

Basophil Cell Count (%)

The results in studied groups were compared to the Basophil Cells (%), asthma of paints was (0.4068±0.1944), and asthma of mills was (0.3816±0.2137). While control was (0.2734±0.1479). There were highly significantly in asthma of paints with control (P=0.0021) (P<0.01), and there were significantly in asthma of mill with control (P=0.0264) (p<0.05). But there were no significant in asthmatic groups (0.8046) (P>0.05). As shown in table(1) .

Table (1)The percentage of• Eosinophils and Basophils in asthmatic groups

Hematology assay	A.Paints(n=50)	A.Mills(n=38)	Controls(n=44)	P-Value
	Mean ±S.D	Mean ±S.D	Mean ±S.D	ANOVA
Eosinophil count (%)	4.806±1.289	5.083±1.519	2.164±0.9421	<0.0001****HS (p<0.01)
Basophil count (%)	0.4068±0.1944	0.3816±0.2137	0.2734±0.1479	0.0020**HS (P<0.01)

The Serum level of Total Immunoglobulin IgE(T-IgE) in studied groups.

The results total IgE in patients with asthma of paints, asthma of mills and control were (Mean±SD) (373.9±252.2), (219.6±158.4), (46.02±25.32). There were highly significantly in asthmatic groups(p=<0.0001) (P<0.01) with control, as shown in Table (2).

Table (2) Distribution of total IgE level in studied groups

Immunological assay		No.	Mean	Std. Deviation	Std. Error	P- value
Total IgE (IU/ml)	A .paints	50	373.9	252.2	35.66	<0.0001** HS
	A .Mills	38	219.6	158.4	25.70	
	Controls	44	46.02	25.32	3.817	
	Total	132				
(**HS) Highly significant , (P<0.01) .						

Interleukin -5 levels

The results of the level of IL-5 increased in the serum of asthmatic group. The level of IL-5 (mean±SD)in asthma of paints were(129.9±119.2) pg/ml, and asthma of mills were(47.19±26.89),While in control were(54.05±20.27)pg/ml. There were highly significantly in asthmatic groups with control, but there were no significantly between asthmatic groups.

Interleukin-33 level

The results of the level of IL-33 increased in the serum of asthma group patients. The level of IL-33 (mean±SD) in asthma of paints were (24.91±22.01) pg/ml, and asthma of mills were (20.19±14.86) pg/ml. While (13.57±6.039) pg/ml in the control. Statistical analysis showed that there were highly significantly in asthmatic groups with control group (P=0.0038) (P<0.01).

Table (3) comparison the serum levels of interleukins(IL-5,IL-33)between asthmatic groups.

Immunology assay	A.paints(n=50)	A.mills(n=38)	Controls(n=44)	P-value
	Mean ±S.D	Mean ±S.D	Mean ±S.D	ANOVA
IL-5 (Pg/ml)	129.9±119.2	47.19± 26.89	54.05± 20.27	< 0.0001** HS(P<0.01)
IL-33(Pg/ml)	24.91± 22.01	20.19± 14.86	13.57± 6.039	0.0038**HS (P<0.01)

The correlation between the total IgE and interleukins (IL5-IL33) in asthmatic group with age group

The present study that there was a relationship between the total IgE with IL-5and IL-33, where it was found a positive correlation with significantly in age group in asthma of paints (r=-0.3141)(p=0.0263*) (p<0.05) ,and no significantly in asthma of mills(r=-0.1492) (p=0.3712)(p>0.05) .

Table (6) The correlation between total IgE and IL-5, IL-33 in asthmatic group with age groups

Total IgE		Age	IL-5	IL-33
Asthma Paints	r	-0.3141	-0.06075	0.05557
	p	0.0263*	0.6751	0.7015
Asthma mills	r	-0.1492	-0.1234	-0.2693
	p	0.3712	0.4605	0.1020

Discussion

The results showed increased Eosinophils. There were highly significantly in asthmatic groups with control. Eosinophilic gastrointestinal disorders have increased levels of eosinophils in portions of the gastrointestinal tract (esophagus, stomach, small intestine, large intestine, or multiple segments)[16]. Eosinophils release cytokines, growth factors, and leukotrienes, which induce additional inflammation and generate the disorder's characteristic and frequent symptoms, such as hyperactivity in response to various stimulating events[17]. Eosinophils are linked with the development of allergic asthma in the respiratory tract and are recruited to the lungs by cytokines produced by activated Th2 cells as part of the inflammatory response. The authors of this new research into the general population want to identify the factors linked to high blood eosinophil levels. interpret the blood eosinophil count with a more nuanced approach, suggesting that age, sex, comorbidities, and lifestyle should all be considered[18].

Also, the results showed increased of Basophils. There were highly significantly in asthmatic group with control. Basophils, which make up less than 1% of all leukocytes in the blood, have been found to play an important role in the immune system and to produce high amounts of Th2 cytokines like IL-4[19]. This study agrees with certain Iraqi studies, such as[20]. Basophils have several characteristics of tissue-resident mast cells, such as the presence of basophilic granules in the cytoplasm, the display of the IgE receptor(FcεRI) on the cell surface, and the release of chemical mediators in response to various stimuli[21].

The result appears increased total IgE there were highly significantly in asthma group with control. Both innate and adaptive immune responses play a role in the severity and pathogenicity of allergic asthma. Immunoglobulin E is a well-known component of allergic responses, as it is generated during the sensitization process, which begins with the first exposure to the allergen[22,23]. High IgE levels and a high eosinophil count were seen in 33% of their English patients with severe allergic asthma. Similarly,[24] the found that allergic asthmatic patients produced more total and specific IgE than non-allergic and healthy subjects, and they also confirmed that total and specific IgE were produced locally in both allergic and non-allergic asthmatic patients. The found that individuals with allergic asthma had higher IgE levels than healthy people, suggesting that increased IgE was a key risk factor for chronic childhood asthma[25].

The results appear increasing of IL-5. There were highly significant differences between asthma groups with control. T-helper 2 cells generate and release IL-5 after a complex activation process involving allergens inhaled and dendritic cells [26]. Interleukin 5 The reason for the reduction in IL-5 levels is that it can control the Th1/Th2 balance and improve the airway inflammation produced by acute Bronchial Asthma (BA), as well as the clinical symptoms and lung function of individuals with acute BA [27]. The results are consistent with a previous study [28], which found a strong association between blood eosinophilia and IL-5. Furthermore, found that IL-5 is the main cytokine responsible for eosinophil maturation, activation, proliferation, and survival [29]. T cells are the primary generator of IL-5 in the lungs, and IL-5 is required for eosinophil recruitment, proliferation, survival, and activation. Furthermore, in severe asthma, neutrophils play a role in eosinophil stimulation to cause airway inflammation by forming extracellular traps[30].

The results of this study disclosed that the level of IL-33 increased in the serum of asthma group patients. there were highly significant difference between asthma of paints compared with control, whereas no significant differences between asthma of mills compared with control[30] in vivo studies

discovered that IL-33 plays critical roles in allergic inflammation, type-2 immunity, and eosinophil homeostasis, and added that IL33 is released after cell damage and necrosis and activates allergic inflammation by increasing the synthesis of inflammatory and chemotactic proteins that increase allergic inflammation, such as IL-4, IL-5, and IL-13[23].

Conclusions

There are highly significantly in total IgE and IL-5,IL-33 between the asthma group and control, and there were significant positive correlation between total IgE and IL-5, IL-33 in patients' group with age groups(P>0.01).There were highly significantly in eosinophils and Basophiles asthma groups with control.

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