Role of IL-33, TSLP, and their receptor in the pathogenicity of asthmatic patients in Basrah province

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Abstract
Case /control study were started from March 2021 to January 2022 in Basrah province, Southern Iraq. Eighty-eight cases were divided into two groups 58 (66%) patients and 30 (34%) healthy control individuals their ages ranged between 30 and 60 years old. they were divided by sex into 44 (50%) males and 44 (50%) females. According to the age, the patients and healthy control were divided into three age groups (30–40 years old, 41–50 years old, and 51–60 years old), and their numbers were 29 (33%), 30 (34 %), and 29 (33%), respectively. The results revealed that all markers which used in this study (IL-33, ST2, TSLP and TSLP-R) are elevated significantly in asthmatic patients when compared with healthy control, P. value (0.001, 0.003, 0.001 and 0.001), respectively.
Keywords: Asthma, IL-33, ST2, TSLP, TSLP-R, ELISA.

1. Introduction

Asthma is a term used to describe a variety of chronic airway inflammation phenotypes, including allergic asthma (extrinsic), which is caused by inhaling environmental allergens such as house dust mites, and non-allergic asthma (intrinsic), which often develops later in life and has no family history of allergy (1).
Interleukin 33 (IL-33) was identified as a new member of the IL-1 cytokine family to generate Th2 and intensify Th1 and Th2 reaction forms (2).The atopic dermatitis and asthma is associated with mutations in the IL1RL1 (ST2) gene. IL-33/ST2 known as important initiators of the immune response of type 2 and are suggested contributing to asthma pathogenesis. IL-33/ST2 considered to be essential early initiators of type 2 immune responses and are proposed to contribute to the pathogenesis of asthma (Shakerian et al., 2022).
ST2 polymorphisms are significantly associated to allergic problems and asthma, according to the findings of a genome-wide association study (GWAS) (3).
Thymic stromal lymphopoietin (TSLP) is an upstream cytokine that or- chest rates inflammatory responses in asthma and overexpressed in the airways of severe asthmatics. TSLP works by binding to a heteromeric receptor complex made up of TSLPR and IL-7R with a high affinity. Polymorphisms in the TSLP gene have been linked to airway hyperresponsiveness, IgE, eosinophilia, and asthma (4).
Thymic stromal lymphopoietin (TSLP) appears to be a special promoter of atopic inflammation, and it frequently functions in a positive feedback loop, enhancing Th2 inflammatory responses and contributing to their chronicity. Many genetic variations that affect its expression have been discovered. As a result, a variety of non-specific stimuli, such as infectious agents, may cause it to be re- leased and establish a persistent Th2 inflammation in those who are prone to it. Because of its critical involvement in chronic atopic disorders (5).
Thymic stromal lymphopoietin protein receptor (TSLP-R) also named Delta and CRLM-2 (cytokine receptor-like module-2), was originally cloned as a novel type 1 cytokine receptor with similarity to the common gamma chain. It was subsequently identified to be a subunit of the cellular receptor for the IL-7- like cytokine TSLP and termed TSLP R, also known as Delta and CRLM-2, is expressed on regulatory T cells (Treg), some subsets of helper T cells, innate lymphoid cells (ILC), monocytes, and dendritic cells. It associates with IL-7R alpha to form a high affinity receptor complex for epithelium-derived TSLP (thymic stromal lymphopoeitin) (6).
Thymic stromal lymphopoietin protein receptor (TSLP-R) expression is ubiquitous in the immune and hematopoietic cells but is up regulated in Th2-skewed cells. Cells expressing TSLP R alone bind TSLP with low affinity. Co- expression of TSLP R and IL-7 R alpha is required for high-affinity TSLP binding and signal transduction. The TSLP R and IL-7 R alpha are expressed primarily on monocytes and dendritic cells and at lower levels in lymphoid cells. TSLP has been shown to induce the release of T cell-attracting chemokines from monocytes and enhance the maturation of CD11c+ dendritic cells (7).
Present study aims to investigate the role of IL-33, ST2, TSLP and TSLP-R in asthma pathogenicity of asthmatic patients in Basrah province.

2. Materials and Methods

Blood samples were collected from patients from March 2021 to January 2022, at the Specialized Center for Asthma and Allergy Diseases in Basra, southern Iraq.

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All enrolled patients diagnosed by the specialist physician according to the Iraqi national guidelines for the diagnosis and treatment of asthma, common symptoms included shortness of breath.

The study was approved by the Ethics Committee, which is a specialized committee in the Ministries of Health, Higher Education and Scientific Research in Iraq, and prior consent was obtained from participating patients before collecting data and samples. Informed consent is waived for patients who are unable to obtain informed consent .For medical examinations, blood samples were drawn from patients as follows:

Three ml of venous blood were drawn from patients through a 5 ml syringe. 3 mL of whole blood was in a gel tube to obtain serum.

Whole blood were taken with oriented consents, from 58 patients with age about (30-60) years old who were visiting the Allergy and Asthma center of Basrah province therapy in the period of March 2021 to January 2022. Each patient gave written informed consent before participate in the study that was approved by the local medical ethical. At the institute of training and development of the Basrah health 58 patients enrolled in this study with history of Asthma disease who were selected from those attended to the Allergy and Asthma center of Basrah province.

Criteria of patients

Inclusion criteria

A total of (58) patients of three age groups attended in asthma and allergy Basrah center. Were included in this study and diagnose as asthma disease. The patients were examined under supervision of the physician.

Exclusion criteria:

The study excluded any patients had COVID-19 and allergic patients instead of bronchial asthma patients with immunocompromised infectious dis-eases. The study also excluded any patients under 30 years of old and over 60 years of old.

The controls 30 persons no attending the allergic and asthma disease Clinic and have no any syndrome of allergy and did not have any history of allergic disease. There was range from 30 to 60 years (Male & Female). All control and patients having all the exclusion criteria of the study.

Blood samples (3 ml) were collected from asthmatic patients and healthy subjects.

Serum was used for determining (IL-33, TSLP, TSLPR and ST2). The sera were kept frozen during the period of sampling. All sample was transported at deep-freezing during sample collection.

Three ml of blood were collected in GEL tube. The serum was separated by centrifuge for 5 minutes at 1500 rpm to determine the levels of IL-33, TSLP, ST2, and TSLPR by sandwich ELISA.

3. Statistical Analysis

Data analysis were performed using statistical software (SPSS) version 26.0, several statistical criteria were applied to the given data. Independent student, t- test & one-way analysis of variance were used to test the significant different between treatments, while ANOVA test was applied as multiple comparison test & P. value ≤ 0.05 was set as significant level & P. value ≤ 0.01 as highly significant. Pearson correlation coefficient was performed for finding out the degree of correlation between variables. For non-parametric data used chi- square, all data were presented mean ± standard deviation (mean ± S.D.) Or Percent values.

4. Results

Characterization of Asthmatic patients

Demographical pictures

The sample population consisted of eighty-eight cases, consisting of 58 patients (66%) and 30 (34%) control samples, they were divided by sex into 44 (50%) males and 44 (50%) females. According to the age, the patients and healthy control were divided into three age groups (30–40 years old, 41–50 years old, and 51–60 years old), and their numbers were 29 (33%), 30 (34 %), and 29 (33%), respectively. Their ages ranged between 30 and 60 years old.

Distribution of patients according to age.

The results of the current study documented that the mean age of asthmatic groups were 44.72 ±8.94 with not significant (P. value = 0.7).

The results of this study also observed the most cases of asthma were founded among the third to fourth decade (30-40) years about 20 (69.0 %) out of 58 cases, while the age groups included (41-50) and (51-60) were founded to had equal frequencies and percentages with 19 (65.5%) respectively, statistically these differences was not significant (P. value = 0.9) as arranged in Table and Figure (1).

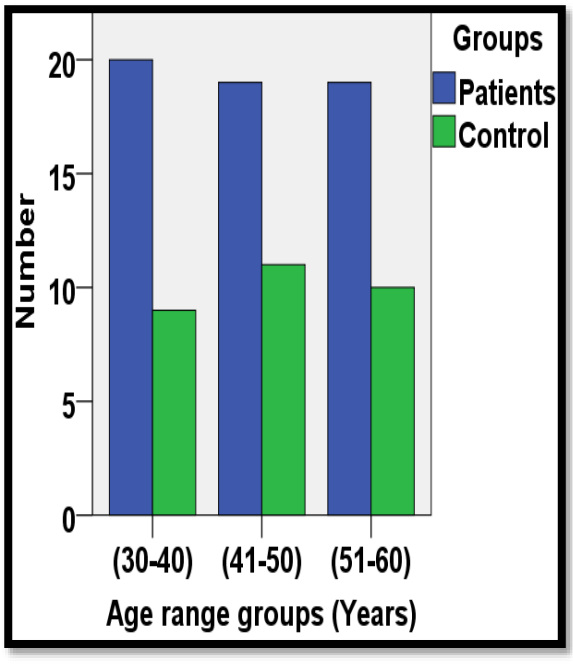

Figure 1: Distribution of patients according to age.

Table 1: Distribution of patients according to age				
Age range group	Studied groups		P-value for total	P-value for each age range group
	Patients (N=58)	Control (N=30)		
(30-40) years	20 (69.0 %)	9 (31.0%)	0.9 (N.S)	0.001
(41-50) years	19 (63.3 %)	11 (36.7%)		0.001
(51-60) years	19 (65.5 %)	10 (34.5%)		0.001

Distribution of patients according to gender

Regarding to the gender the number of asthmatic cases were equally recorded in frequencies and percentage among both male and female groups with 29 (50.0%), 29 (50.0%) from total study cases 58, respectively, statistically there was no differences were founded with (p. value = 1 ≤ 0.05) as arranged in Figure (2).

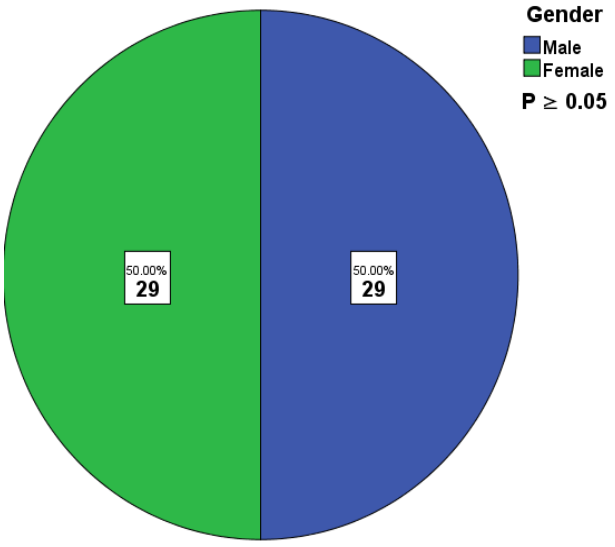

Figure 2 Distribution of patients according to gender.

Determination of IL-33 concentration

Regarding to the levels of IL33 (pg/ml) there were highly increasing in the levels of IL-33 among asthmatic patients than control group (50.93 ±17.49, 9.69±2.48), statistically these differences in the levels between the two groups were highly significant (p. value ≤ 0.001) as arranged in Figure (3).

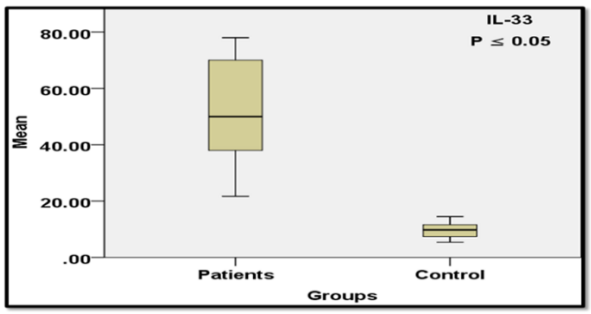

Figure (3): Levels of IL-33 among asthmatic patients and control groups.

Determination of ST2 concentration

The results of the current study observed that there were an elevation in the levels of ST2 (5.11±2.04, 1.48 ±0.36pg/ml) receptor among asthmatic patients than control group respectively, these differences in the levels statistically were highly significant (p. value ≤ 0.003) Figure (4)

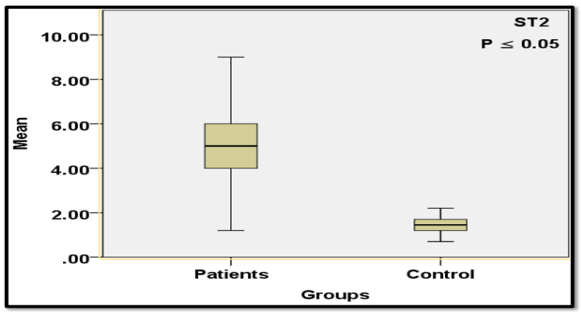

Figure (4): Levels of ST2 among asthmatic patients and control groups.

Determination of TSLP and TSLPR concentrations

Related with the levels of the TSLP (65.19±30.97, 12.63±4.41pg/ml) cytokines and its receptor TSLPR (197.88±100.22, 12.72±4.85pg/ml) there were an elevation in the levels among asthmatic patients than control groups with highly significant differences (P. value ≤ 0.001, 0.001) respectively Figure (5) and (6).

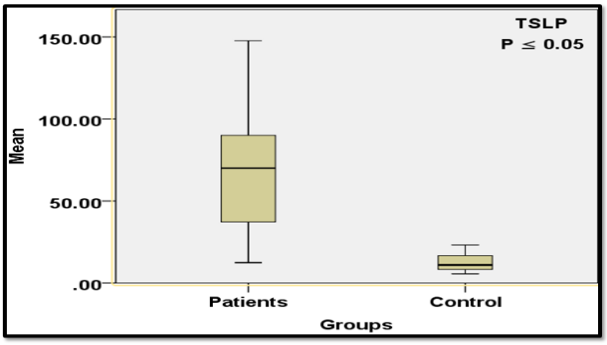

Figure 5: Levels of TSLP among asthmatic patients and control groups.

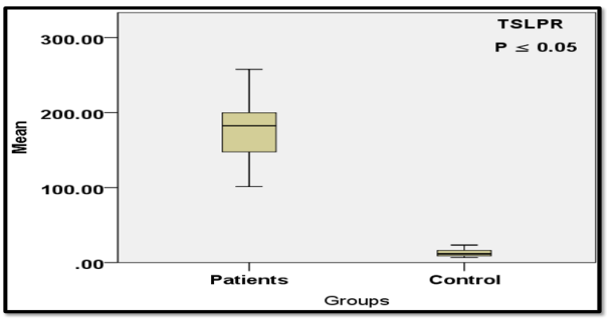

Figure 6: Levels of TSLPR among asthmatic patients and control groups.

Levels of immunological parameters (IL-33, ST2, TSLP and TSLP-R) according to patients age range groups.

The results of the current study documented that there were a significant difference in the levels of the TSLP among age range group (p. value = 0.01).

The results of this study also revealed there were not significant differences in the levels of IL-33, ST2, TSLP and TSLPR among age range group (p. value = 0.06, p. value = 0.6, p. value = 0.01, p. value = 0.8) respectively as illustrated in Figure (7,8, 9 and 10).

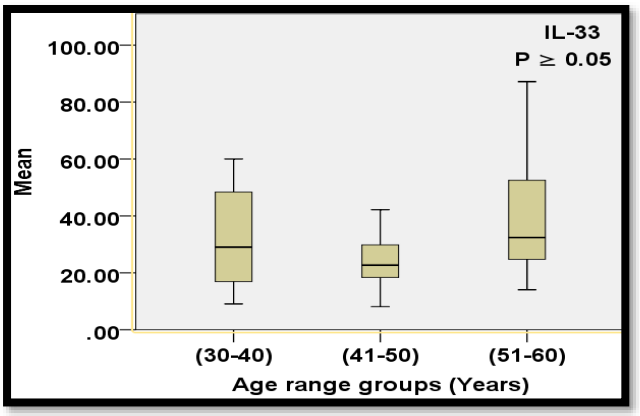

Figure 7: Levels of IL-33 among age range groups (Years).

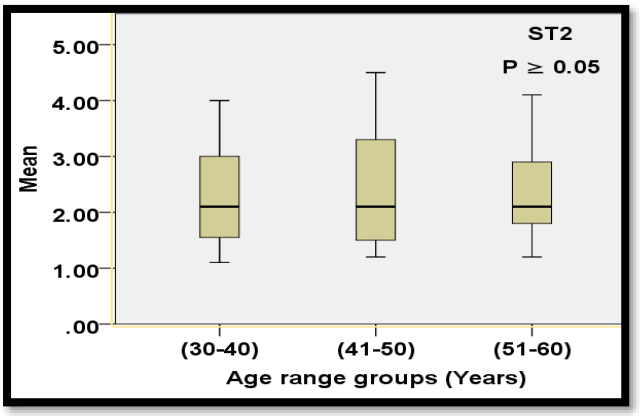

Figure 8: Levels of ST2 among age range groups (Years).

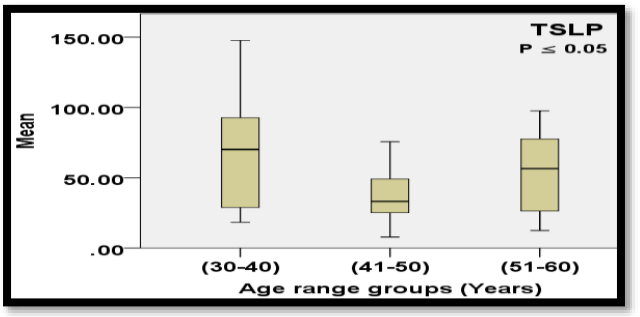

Figure 9: Levels of TSLP among age range groups (Years).

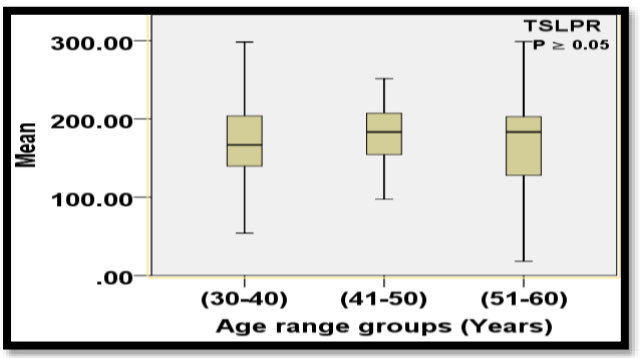

Figure 10: Levels of TSLPR among age range groups (Years).

5. Discussion

The hallmark signs and symptoms of asthma include airway hypersensitivity, allergic inflammation, high blood IgE levels, and enhanced Th2 cytokine production. Since IL-33 strongly induces Th2 immune responses, its function in asthma has been thoroughly researched (8).

Because of prior research, showing that TSLPR and ST2 signaling encourages allergic inflammation mediated by adaptive immunity; TSLP and IL-33 have been identified as therapeutic targets for allergic diseases (9).

Characterization of patients

Distribution of patients according to the Age groups

All subjects of the current study were male and female divided into three groups

1. (40-30) year [patients 20(69.0%) and control 9 (31.0%)]
2. (50-41) year [patients 19(63.3%) and control 11(36.7%)]
3. (60-51) year [patients 19(65.5%) and control 10 (34.5%)]

In this study, Asthma were confirmed by symptoms, all asthma patients are diagnosed through clinicians. In the Asthmatic and control classes, there was no substantial difference between the groups in terms of ages. The patient's age range was (30-60) years

Regarding to the age group, the highest percentage (69.0 %) was (30-40) years old and the lowest percentage (63.3 %) was (41-50) years old, this outcome could have shown higher prevalence of bronchial asthma in individuals from (30-40) years old. Considerably may be because this age mostly smokes a cigarette or may be due to this group is a worker with high chance to contact with allergens.

Distribution of patients according to the gender

All subjects of the current study were male and female divided into two groups

1. Male [patients 29(65.9%) and control 15(34.1%)].
2. Female [patients 29(65.9%) and control 15(34.1%)].

Male and females were equal.The percentage of females was(50.0%), whereas male was (50.0%).Throughout data analysis. these results may be due to a number the samples selected for the study between males and females are equal.

Mean concentration of IL-33 in serum of Asthmatic patients and control

Current data documented highly significant elevation (P. value = 0.001) of IL-33 in asthmatic patients in compare with control

Increased IL-33 levels in asthmatic patients may be related to IL-33's role in allergic reactions that are

accompanied by neutrophil and monocyte activity! There has been an increase in cytokine production in macrophages. The fact that IL-33 can directly act on both eosinophils to increase their survival is also highlighted (10).

IL-33 is the functional ligand for ST2 and ST2/IL-33 signaling regulating inflammation and immunity. IL-33 and its receptor are part of IL-1 family, and their interactions promote a variety of actions from a number of different cell types. The IL-33/ST2 axis is thought to be intimately involved in the promotion and maintenance of allergic inflammation via a number of cell types that include Th2 cells, mast cells and basophils, and structural cells such as airway epithelium and smooth muscle cells. IL-33/ST2 signaling pathway activates air way eosinophils that exacerbate air way inflammation (11).

IL-33 is now considered to be crucial for the development of allergic disorders through the activation of not only acquired immunity but also innate immunity. In particular, IL-33 is a key cytokine for inducing and activating ILCs in allergic disorders. In addition to allergic disorders, IL-33 plays key roles in the development of non-Th2-type immune responses such as host defense against a variety of pathogens and the development of autoimmune disorders. Thus, IL-33 neutralization may provide a potential target for novel therapeutics for such disorders (12).

Mean concentration of ST2 in serum of Asthmatic patients and control

The present result showed highly significant elevation (P. value = 0.003) of ST2 in asthmatic patients in compare with control, and because the IL-33 is the functional ligand for ST2 and ST2/IL-33 signaling regulating inflammation and immunity so increased ST2 level indicated the inflamma-tory process in patients within study, and their interactions encourage several distinct cell types to take a range of activities. Th2 cells, mast cells, basophils, and structural cells such the airway epithelium and smooth muscle cells are hypothesized to play key roles in the development and maintenance of allergic inflammation via the IL-33/ST2 axis. Eosinophils in the airways are activated by the IL-33/ST2 signaling pathway, which increases airway inflammation. Additionally, there was a substantial increase in blood levels of both Interleukin-33 and its receptor ST2, which provided additional evidence for the connection between eosinophil and IL-33/ST2.

The fact that IL-33 is one of the cytokines that regulates crucial biological processes like immune response or hematopoiesis and is involved in the pathophysiology of many illnesses may also be used to explain why IL-33 levels were elevated in the current research. Their quantities in biological fluids and tissues are either undetectable or very low in the physiological state.

Any rise in their concentrations therefore denotes the activation of pathways involved in the inflammatory response or the progression of illness. Because of this, cytokines may be utilized as possible biomarkers of different illnesses, and variations in their quantities may be monitored. Additionally, the cytokine profile in the acute versus chronic phases of the illness frequently varies. The diagnosis of an illness may be made with the use of measurements of cytokine concentrations, and these concentrations are related to the disease's stage.

Interlukin-33 and its receptor, sST2, both considerably increased in blood levels in the current study's asthmatic patients, and this increase was much greater in patients than in control subjects.

Mean concentration of TSLP in serum of asthmatic patients and control

Highly significant elevation (P. value = 0.001) of TSLP in was recorded asthmatic patients in compare with control.

This increasing of TSLP may help to understand the role of TSLP and IL-33 in a positive feedback loop between the airway epithelium and inflammatory cells infiltrating the airways in obstructive lung diseases (Hong., et al 2020).

whereas overexpression of TSLP in the lung, results in the development of severe airway inflammation so, their increasing in serum also indicated that there was an inflammatory responses because TSLP play a role in pro-allergic responses by acting on maturation and increased costimulatory expression including upregulation of ox-40 ligand thus allow DC to prime naïve CD4 + T cells to differentiate into proinflammatory Th2 cells expressing IL-4, IL-5, IL-13, and TNF-α, so increasing of serum TSLP in studied asthmatic patients indicated the inflammation in there patients (14).

Mean concentration of TSLPR in serum of Asthmatic patients and control

Documented data showed highly significant increasing (P. value = 0.001) in TSLPR in asthmatic patients in compare with control, this can be that studied patients with in the inflammatory stage of disease because TSLPR and ST2 signaling promotes adaptive immunity-driven allergic inflammation, and as a result, TSLP and IL-33 have been identified as therapeutic targets for allergic disorders (15).

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