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## The effect of TSLP, IgE and Eosinophil on Atopic Dermatitis Patients

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### Abstract

Atopic dermatitis in another word Atopic Eczema is a chronic inflammatory skin disease that effecting all ages, described by lesions of eczematous and acute itch. As the objective of this study for determine the effect of variation in TSLP, IgE, and Eosinophil levels in patients with atopic dermatitis compared with a healthy control group. The level of TSLP was almost the same between the patients and the control group, and the reason was that most of the patients were treated with anti-allergic drugs. In this study, there is a significant ( $P = 0.004$ ), serum level of TSLP in atopic dermatitis patient's pre-treatment with immunotherapy (corticosteroids and antihistamines) is compared with atopic dermatitis patients' post-treatment with immunotherapy and controls (23.06 vs. 29.48 and 23.99 pg/ml). The median level of overall T-IgE of the Atopic Dermatitis group was substantially greater than controls, (91.64 vs. 49.72 IU/ml), ( $P < 0.023$ ). Eosinophils count of Atopic Dermatitis patients was approximately equal to the control group, ( $3.1 \pm 2.43$  vs.  $2.9 \pm 1.05$  %), ( $P$ -value 0.07). This study was conducted on a case series of 60 patients that have Atopic Dermatitis, who volunteered due to their review of the Allergy Center in Al-Rusafa, Baghdad, during the fall of 2020. Thirty healthy and non-Atopic Dermatitis volunteers of the same type, the center volunteered as a control group.

**Keywords:** TSLP, TSLPR, AD, Eosinophil, Immunoglobulin E, DCs.

## تأثير الساييتوكين نوع TSLP ومجموعة الغلوبولين المناعي نوع IgE وكذلك الحمضات الدموية على مرضى التهاب الجلد التأتبي

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### الخلاصة

لتحديد تأثير الاختلاف في مستويات TSLP و IgE و Eosinophil في المرضى الذين يعانون من التهاب الجلد التأتبي مع مجموعة اشخاص اصحاء لغرض المقارنة. كهدف من هذه الدراسة لتحديد تأثير التباين في مستويات TSLP و IgE و Eosinophil في المرضى الذين يعانون من التهاب الجلد التأتبي مقارنة مع مجموعة الاصحاء. كان مستوى TSLP متساوياً تقريباً بين المرضى ومجموعة الاصحاء ، والسبب هو أن معظم المرضى قد تمت معالجتهم بأدوية مضادة للحساسية. في هذه الدراسة ، هناك مستوى ذو دلالة احصائية واضحة ( $P = 0.004$ )، حيث كان مستوى مصل TSLP في التهاب الجلد التأتبي (AD) ، هو

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أعلى عند المرضى الذين لم يعالجوا مسبقاً بالعلاج المناعي مقارنة بالمرضى المعالجين ومجموعة الاصحاء. كان المستوى الوسيط العام لـ T-IgE لمجموعة التهاب الجلد التحسسي أكبر بكثير من مجموعة الاصحاء. كان عدد الحمضات لمرضى التهاب الجلد التحسسي مساوياً تقريباً لمجموعة الاصحاء. أجريت هذه الدراسة على سلسلة حالات مكونة من 60 مريضاً مصاباً بالتهاب الجلد التحسسي (AD)، الذين تطوعوا من خلال مراجعتهم لمركز الحساسية في الرصافة بغداد ، خلال فصل الخريف من عام 2020. ثلاثون شخصاً يتمتعون بصحة جيدة وغير مصابين بالتهاب الجلد التحسسي من نفس النوع. تطوعوا من خلال نفس المركز كمجموعة اصحاء.

## Introduction

Atopic dermatitis is a chronic inflammatory skin disease that usually begins in childhood and manifests itself in the first few years of life [1]. Recurrent eczematous lesions and extreme itching define atopic dermatitis, a common inflammatory skin condition. Multiple comorbidities, such as food allergies, asthma, allergic rhinitis, and mental health disorders, are linked to atopic dermatitis. The pathophysiology is complicated, with a strong hereditary susceptibility, epidermal malfunction, and T-cell-driven inflammation all playing a role. Although type-2 mechanisms are the most common, there is growing evidence that the illness is caused by a combination of immunological pathways, [1]. Thymic stromal Lymphopoietin (TSLP) is a cytokine that is most closely related to interleukin 7 (IL-7). It was first discovered in the cultured supernatant of a mouse thymic stromal cell line, and it was known for its potential to activate B lymphocytes and myeloid DCs [2] and [3]. TSLP is produced by epithelial cells on the skin, lungs, and gut barrier surfaces [4]. The epidermis of lesioned skin in individuals with allergic dermatitis, such as AD, expresses more TSLP than the epidermis of unaffected skin or skin from patients with non-allergic dermatitis or cutaneous lupus erythematosus. DCs in the afflicted skin develop an activated phenotype, exit the epidermis, and move to the draining lymph node, indicating that TSLP plays a role in tissue-resident DC responses [5]. Selective ablation of the transcription regulator COUP-TF-interacting protein 2 (CTIP2; also known as BCL11b) in epidermal keratinocytes recently triggered a human-AD-like skin inflammatory phenotype characterized by high levels of Th2-type cytokines/chemokines (IL-4, IL-13, CCL17, and TSLP) and elevated plasma IgE levels [6]. Degranulation occurs often in eosinophils transported to inflammatory regions, releasing a variety of cationic cytotoxic compounds such as major basic protein (MBP) and eosinophil peroxidase (EPX), as well as various cytokines (IL-4, IL-13, CCL17, IL-9, IL-7, and IL-2), chemokines, and growth factors. These have a big impact on how immunological and inflammatory responses progress [7].

It is necessary to mention the aim of this study is to find new ways to diagnose atopic dermatitis by determining the effect of the cytokine responsible for the emergence of symptoms of the disease. This discovery leads us to find new and alternative ways to treat the disease. This is done by targeting the immune component responsible for the symptoms rather than administering traditional allergy medications that suppress almost all of the immune components of the human immune system. For achieving this goal, the below steps were used:

- Evaluation of TSLP cytokine, and total IgE and specific IgE levels in blood serum of Iraqi patients suffering from Atopic dermatitis.
- Determination of the relationship between the level of TSLP and Atopic dermatitis diseases under study.
- The investigation of the relation between Eosinophil and Atopic dermatitis.

## Materials and Methods

A descriptive study was carried out on atopic dermatitis patients visiting the Allergy Center in Al-Rusafa, Baghdad from September 2020 to December 2020. This study was conducted on a case series of 60 patients with Atopic Dermatitis ,who volunteered through their review of the Allergy Center in Al-Rusafa, Baghdad, during the fall of 2020. Thirty healthy and non-Atopic Dermatitis was volunteered as a control group. People with atopic disorders were asked to participate in this study. They matched patients based on their age and gender.

### Materials that used in this study and their origins

**Table 1:** Kits for immunology Diagnosis

#	Chemicals and Diagnostic Kits	Company name	Origin
1	Total IgE ELISA kit	Euroimmun	German
2	Human TSLP ELISA Kit	MYBIOSOURCE	USA
3	Beckman coulter kit (Solution)	Beckman	German

The total serum IgE antibody was determined using the small VIDAS Enzyme Linked Immune Fluorescent Assay method (ELFA). Bio Mérieux, a French company, offered the kit. A quantitative in vitro test for human total IgE antibodies was included in the kit. Quantitative Detection of TSLP (Thymic stromal Lymphopoietin) by Enzyme-Linked Immune-sorbent Assay technology (ELISA) (Human TSLP ELISA Kit), MYBIOSOURCE, USA company. The Hematological examination (eosinophils count) was carried out by a Beckman Coulter analyzer, German all these materials and origins showed in Table 1.

## Methods

The total serum IgE antibody was estimated using five milliliters of blood from both the patients and the healthy control group. After letting the blood coagulate at room temperature, it was centrifuged at 3000 rpm. Thymic Stromal Lymphopoietin TSLP serum level was measured by the same principle of the IgE test, the reading calculation, (the relative O.D 450) = (the O.D 450 of each well) – (the O.D 450 of the blank well). The standard curve is plotted as the relative O.D 450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The target concentration of the samples was interpolated from the standard curve using the equation ( $Y=5E-07X^2+0.0018X+0.1085$ ). Eosinophil count test after 2ml as blood sample collected from the patients and control group then put into CBC device (Beckman Coulter analyzer) and the results from the analyzer were used for the purpose of this study.

## Experiments

- Sample collection
- Determination of serum level of TSLP
- Determination of serum level of total IgE
- Eosinophil count test

## Statistical methods

To summarize the data in this investigation, different descriptive statistical methods were applied. The mean differences in eosinophil counts between patients and controls were compared using an independent two-sample student one-way ANOVA and T-test. The TSLP and total serum IgE levels were compared using the Mann Whitney test, which employed the

Median  $\pm$  Standard Error to distinguish between different levels of AD severity. A significant p-value of less than 0.05 was used. SPSS ver. 13 was used for statistical processing (SPSS inc, Chicago, Ill).

## Results and Discussion

Atopic dermatitis is a distressing inflammatory skin disease that affects a large number of children worldwide, and it is a syndrome made up of an identifiable group of signs and symptoms that represents the dermatological manifestation of the atopic diathesis [8]. The number of atopic dermatitis patients selected for the current study was 60 in total. The healthy individuals (N = 30) represented the control group. The age range of atopic dermatitis patients was between 6 to 65 years. It has a variety of clinical presentations and courses, and it is a syndrome made up of an identifiable group of signs and symptoms that represents the dermatological manifestation of atopic diathesis. The study comprised 60 instances in all, with the majority of the patients being female (32, 53 percent). This contradicts Johansson et al's findings, who found that 95.0 percent of AD patients were females and 5.0 % were males [9]. Both sexes (Male and female) are affected, however in adults, females are more likely to get the condition, but in children, boys are more likely to have atopic dermatitis [10]. In a study performed in Iraq, the IgE levels were no significant differences between both males and females of patients or controls [11]. In the first six months of life, male gender and atopy in the family were linked to an elevated risk of AD. These data show that genetic and prenatal effects play a role in both genders' differences [12]. There is no clear reason for this disparity, other than the fact that it is more prevalent in boys during childhood. In 84 cases (84%) the family history was positive, while 16 cases (16%) had a negative family history. This is consistent with Blumenthal's findings, which indicated that the majority of AD cases had a positive family history [13]. This could be because atopic dermatitis disease is a hereditary condition that runs in families, but there is no obvious line of inheritance, which explains why clinically normal parents could have affected children, ruling out simple dominant inheritance. Other families, on the other hand, may have both parents sick but the children are unaffected, barring a simple recessive trait [14].

## TSLP level and AD

Thymic Stromal Lymphopoietin was targeted as serum level in atopic dermatitis patients and the results were non-significant in this stud. As shown in Table 2 the Atopic Dermatitis group, the median level of TSLP was equal in the controls group, (27.7 vs. 27.8), (p-value = 0.58).

**Table 2:** Levels of TSLP in AD Patients and Control Group.

TSLP in Group	NO.	TSLP (pg/ml) Median $\pm$ S.E
Patients	60	27.7 $\pm$ 10.96107
Controls	30	27.8 $\pm$ 13.76096
<b>p- value = 0.58</b>		

## No.: Number, P: P-value significant $\leq$ 0.05, S.E: Standard Error, pg: pictogram

The level of TSLP in this study was almost equal between the patients and the control group, and the reason was that most of the patients were treated with anti-allergic drugs. Where this was done by asking patients through a questionnaire or by looking at the patient registry at the Allergy Center in Al-Rusafa, Baghdad. Yeo, et al.2021 [15], and Nettis, et al.,2020 [16] supported the result of this study.

### TSLP level in treated and non-treated patients

The serum level of TSLP in Atopic Dermatitis patient's post and pre-treated. The correlation between the serum levels of TSLP in pre-treatment and post-treatment patients who were treated with corticosteroids and anti-histamines (immunotherapy) is illustrated in Table 3. The results showed a significant increase in TSLP levels in atopic dermatitis patients at post-treatment with immunotherapy in comparison with those at pre-treatment (29.48 vs. 23.06 ng/ml, p-value <0.004).

**Table 3:** TSLP Levels Among Studied Groups.

Treatment	No.	TSLP (pg/ml) Median ± S.E
TREAT	39	23.06 ± 0.87592
NON TREAT	21	29.48 ± 8.89097
CONTROLS	30	23.99 ± 39.25424
a, b P-value = 0.04		c P-value = 0.4

(a). Serum level of TSLP pg/ml in patient's pre-immunotherapy compared with controls. (b). Serum level of TSLP pg/ml in patient's pre-immunotherapy compared with post-immunotherapy. (c). Serum level of TSLP pg/ml in patients post-immunotherapy compared with controls.

The content of TSLP in blood serum in both children and adults with AD has been shown to be considerably higher than in healthy people [17] and [18]. Furthermore, greater cytokine expression was seen in keratinocytes of AD patients, which correlates with the severity of the disease's course and reduced epidermal barrier function as determined by stratum corneum hydration and transdermal water loss. TSLP levels are reduced when moisturizing drugs are used, as are the symptoms of AD and the severity of the disease course [18].

### Total IgE serum level in atopic dermatitis patients

One of the essential parameters in atopic dermatitis diagnosis is the serum level of total IgE. Table 4 shows the concentrations of total IgE in the serum of allergic asthma patients in comparison with that of the control. The median level of overall T-IgE of the Atopic Dermatitis group was substantially greater than controls, (91.64 vs. 49.72 IU/ml), (P<0.023).

**Table 4:** Total IgE levels in AD patient compare controls in Group

Total IgE in Group	No.	Total IgE (IU/ml) Median ± S.E
Patients	60	91.64 IU/ml* ± 43.34059
Controls	30	49.72 IU/ml ± 31.79012
P-value = 0.023		

**No.:** Number, **P:** Probability, **IU:** International unit, **S.E:** Standard Error.

Several research [19] and [20], including the current one have demonstrated the central involvement of IgE in the etiopathogenesis of AD. The researchers discovered that the mean total serum IgE level in atopic dermatitis patients was six times greater than in non-atopic participants. According to the manufacturer's reference figure, a cutoff value of 100 IU/ml is used. In this study, 65 percent of patients had total serum IgE levels greater than 100 IU/ml. The increased IgE level was consistent with some research' findings while being slightly lower than that reported by others. In the literature, percentages ranging from 70% to 88 percent [21] of atopic patients have been reported. These disparities could be explained by the different cutoff values used, the methodologies used to estimate IgE, the participants' age, and the severity of the disease. This result confirms the results of each Tokura et al., 2010 [22]; Krawiec et al., 2004 [23]; Uekert et al., 2006 [24]; Barrenas et al., 2008 [25] results of their

studies found that a highly significant increase in the mean serum total IgE in patients with atopic dermatitis (compared with healthy controls). All of these studies support the primary role of IgE in immune response and maintaining symptoms severity in AD diseases. IgE is an immunoglobulin that plays a significant role in chronic inflammatory allergic diseases and acute allergic reactions [26]

### Total IgE level in treated and non-treated patients

The serum level of total IgE in Atopic Dermatitis patient's post and pre-treated. The correlation between the serum levels of total IgE in pre-treatment and post-treatment patients who were treated with corticosteroids and anti-histamines (immunotherapy) is illustrated in Table 5. The results showed a non-significant increase in total IgE level in atopic dermatitis patients at post-treatment with immunotherapy in comparison with those at pre-treatment (92.629 vs. 91.112 ng/ml, p-value <0.07).

**Table 5:** Total IgE levels in AD patient pre-treating and post-treating in group

Total IgE in Group	No.	Total IgE (IU/ml) Median ± S.E
Post-treatment Patients	13	92.629 IU/ml* ± 92.70594
Pre-treating Patients	47	91.112 IU/ml ± 49.55294
<b>P-value = 0.071</b>		

**No.:** Number, **P:** Probability, **IU:** International unit, **S.E:** Standard Error.

### Eosinophil Count

Eosinophils count of Atopic Dermatitis patients was approximately equal to the control group, ( $3.1 \pm 2.43$  vs.  $2.9 \pm 1.05$  %), (P-value 0.07) as shown in Table 6.

**Table 6:** Eosinophil Count levels in studied subjects

Eosinophil % in group	N	Mean ± Std. Deviation
Patients	60	$3.10 \pm 2.43$
Controls	30	$2.9 \pm 1.05$
<b>P-value= 0.07 Non-significant</b>		

**No.:** Number, **P:** P significant  $\leq 0.05$ , **%:** Percent.

Despite the small sample studied, the study proved that there is no association between eosinophils with atopic dermatitis patients due to there is non-significant increase in eosinophil count percentage in patient groups compared with controls ( $3.1 \pm 2.43$  vs.  $2.9 \pm 1.05$  %), (P-value 0.07).

### Eosinophil Count level in treated and non-treated patients

The count level of Eosinophil in Atopic Dermatitis patient's post and pre-treated. The correlation between the count levels of Eosinophil in pre-treatment and post-treatment patients who were treated with corticosteroids and anti-histamines (immunotherapy) is illustrated in Table 7. The results showed a non-significant increase in Eosinophil levels in atopic dermatitis patients at post-treatment with immunotherapy in comparison with those at pre-treatment ( $3.7685$  vs.  $2.917$  %, p-value <0.2).

**Table 7:** Eosinophil Count levels in AD patient pre-treating and post-treating in group

Eosinophil % in group	N	Mean ± Std. Deviation
Post-treatment Patients	13	$3.7685 \% \pm 2.97032$
Pre-treating Patients	47	$2.917 \% \pm 2.26568$
<b>P-value = 0.2</b>		

**No.:** Number, **P:** Probability, **IU:** International unit, **Std.:** Standard.

## Conclusion

The level of TSLP in patients with atopic dermatitis who were treated with anti-allergic drugs was equal to the control group, when comparing the untreated patients with the control group, the result showed a higher level of TSLP in the untreated patients compared to the control group, and this indicates the effect of TSLP with immunotherapy (corticosteroids and antihistamines). In this study, there was a significant increase in the mean serum level of total IgE for patients with atopic dermatitis compared to the control group, and this is also evidence that the level of total IgE was not affected by allergy drugs. Despite the smallness of the studied sample, the study proved that there is no association between eosinophils and atopic dermatitis patients due to the presence of a non-significant rising in the percentage of eosinophil counts in the atopic dermatitis patient groups compared to the contro

## ETHICAL CLEARANCE

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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