The Role of IL-37 in allergic Rhinitis, Asthma and Urticaria Diseases in Samples of Iraqi patients

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Abstract

Interleukin 37 (IL37) is an anti-inflammatory cytokine, secreted by monocytes, macrophages, and epithelial cells, which suppress the immune system through binding to one of the receptors and co-receptors of the IL-1R family. Different allergic disease phenotypes may be related to different biomarkers, and many mechanisms involve in the pathogenesis of allergic inflammations, so it is wealthy to study different cytokines to understand their effect on allergic diseases. Blood specimens were obtained from patients attending the Allergic Specialized Center/Baghdad-Resafa   74 patients with allergic diseases (rhinitis patients 24, asthma patients 26, urticaria patients 24 (37 men and 37 women) and 14 healthy control subjects (7 men and 7 women). Total and differential counts of WBC were determined, and total immunoglobulin E (IgE), and IL-37 concentration in serum were measured by the enzyme-linked immunosorbent assay ELISA. IL-37 levels were decreased in all types of patients whether ~~in~~ male or female in comparison with the control group with significant differences ($p<0.05$), that's may due to the inactive signaling of IL-37 which leads to inflammatory diseases, through Th2 activation and secrete allergic pro-inflammatory cytokines that contribute to exacerbation pathogenesis of allergic diseases.

Keywords: IL-37, allergic Rhinitis, Asthma, Urticaria, diseases

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دور انترليوكين 37 في أمراض حساسية التهاب الأنف والربو والشرى لدى النساء والرجال

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الخلاصة :

يعد انترليوكين 37 هو سيتوكين مضاد للالتهابات ، تفرزه الخلايا الوحيدة ، الضامة ، الخلايا الظهارية ،له قابلية قمع الجهاز المناعي من خلال الارتباط بأحد المستقبلات والمستقبلات المشتركة لعائلة IL-1R. أمراض الحساسية المختلفة قد تكون مرتبطة بمؤشرات حيوية مختلفة ، والتي تشارك في العديد من الآليات التهابات الحساسية ، و لهذا يجب دراسة السيتوكينات المختلفة لفهم تأثيرها في أمراض الحساسية.

تم الحصول على عينات الدم من مرضى يحضرون الى مركز امراض الحساسية التخصصي في بغداد/ الرصافة . 74 مريضا يعانون من أمراض الحساسية (24مريض لديهم التهاب الأنف و 26 مريض لديه الربو و 24 مريض يعاني من الشرى او الأرتكاريا (37 رجلا و 37 امرأة) و 14 شخصا من الأصحاء (7

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رجال و 7 نساء). تم تحديد العدد الإجمالي والتفاضلي لكريات الدم البيضاء ، و التركيز الكلي لـ IgE و تم
قياس تركيز IL-37 في مصول المجاميع الأربعة بواسطة مقايسة الممتز المناعي المرتبط بالإنزيم.
انخفضت مستويات IL-37 في جميع أنواع المرضى سواء في الذكور أو الإناث مقارنة بالمجموعة الضابطة
مع وجود اختلافات كبيرة (p<0.05)   وذلك قد يكون نتيجة الإشارات غير الفعالة لـIL-37 التي تسبب
أمراض التهابية من خلال تنشيط Th2 وإفراز السيتوكينات المسببة للالتهابات التي تساهم في تفاقم   إمراضية
امراض الحساسية.

Introduction

Many research focused on proinflammatory cytokines, while anti-inflammatory cytokines have less attention, interleukin 37 (IL37) is an antagonist (an anti-inflammatory cytokine) a member of IL-1 family and is secreted by many types of body normal cells; circulating monocytes, tissue macrophages, dendritic cells (DCs), tonsil B cells, plasma cells, natural killer (NK) cells, stimulated B cells, skin keratinocytes, epithelial cells, lymph node, thymus, lung, colon, uterus and bone marrow (1,2,3) and have a role in innate and adaptive immunity, by suppressing the immune system during chronic inflammatory, autoimmune diseases and cancer, through binding to one of the receptors and coreceptors of the IL-1R family (3), especially by binding to IL-18Rα and IL-1R8 (4,5).

Different allergic disease phenotypes and endotypes can be characterized by the presence of different biomarkers and the involvement of many mechanisms that participate in the pathogenesis of allergic inflammations, so different cytokine studies will be required to understand their effect on allergic diseases.

Researchers (2, 6) indicated in their reviews that IL-37 plays a role in many diseases, such as cardiovascular diseases, autoimmune diseases, and ischemia-reperfusion injury; to protect the body against endotoxic shock. The function of IL-37 inhibits the effects of proinflammatory effects during Toll-like receptor (TLR) activation (7). Kim et al., 2017 demonstrate that intranasal IL-37 can suppress Th2 and Th17 responses in an allergic rhinitis murine model (8). This was further confirmed by Meng et al., 2019 who indicate that IL-37 down-regulate Th2 in induced allergic asthmatic mice that were treated with intranasally IL-37 (9).

Charrad et al., 2016 and Elfeky et al., 2018 found that the level of IL-37 in asthmatic children patients is lower than the controls and concluded that there was a negative relationship between IL-37 level and asthma (10, 11) which support the hypothesis that IL-37 has a protective role in the immune pathogenesis of asthma, by increasing expression of IL-37 under severe inflammatory condition to inhibit excessive immune response (12, 13).

The current research aimed to estimate hematological profile of some allergic patients and to evaluate the serum level of IgE and IL-37 in allergic patients, and to determine the differences in their levels in two sexes, to contribute to other studies for more understanding of the vital role of IL37 that may lead to use of this cytokine as anti-inflammatory agent to reduce inflammatory action of asthma, rhinitis and urticarial, and in cytokine therapy which could be promising in treating of many inflammatory diseases like cancer and autoimmune diseases in the near future.

1. Materials and Methods
1.1. Patients and Controls

Peripheral blood samples were collected from the control group (7 men and 7 women) and patients groups were comprised of Rhinitis patients (12 men and 12 women), asthma patients (13 men and 13 women) and urticaria patients (12 men and 12 women) who were diagnosed by the physician through clinical profiles and by lab investigation through total IgE and specific IgE, who were referred to the Allergic Specialized Center/Baghdad-Resafa, and the

sera were separated and stored at –70 °C. All patients and control groups gave their written informed agreement before being admitted to this study.

1.2. Immune assays

The serum IL-37 concentrations were determined according to the manufacturer's instruction of commercial enzyme-linked immunosorbent assay (ELISA) kits (Shanghai Yehua Biological Technology /China), with the assay range：0.5pg/ml→150pg/ml.

Total IgE levels were estimated by Immunoenzymetic Assay using the total IgE ELISA kit according to the manufacturer's instruction (Euroimmum/German). The total and differential count of WBC was done by Cell-Dyn Rub Autoanalyzer.

1.3. Statistical analysis

Results were expressed as mean ± standard error. Data were analyzed by one-way analysis of variance followed by Fisher's test for multiple comparisons, using Statview version 5.0. Differences were considered significant when $p < 0.05$

2. Result

This study analyzed the total, differential count of WBC, total IgE, and the levels of IL-37, in three patient groups with the control subjects as shown in Table (1).

Table 1: Comparison of the mean± standard error of total, differential count of the white blood cells, IgE level and IL37 concentration in three patient groups including male and female; Asthma (A), Rhinitis (R), Urticaria (U), as compared with control (C) group

Parameters	Asthma patients (A)	Rhinitis patients (R)	Urticarial patients (U)	Healthy control (C)	comparison between four groups
WBC count Cell/ml	9.93±0.63	7.99±0.41	7.95±0.4	7.48±0.49	A vs C p<0.05 A vs R p<0.05 A vs Up<0.05
Lymphocyte count cell/ml	26.24±1.7	32.51±1.19	29.55±1.77	29.26±2.88	R vs A p<0.05
Monocyte count Cell/ml	6.75±0.54	7.32±0.36	7.74±0.61	6.72±0.65	NO significant differences
Basophil count Cell/ml	0.16±0.02	0.14±0.02	0.21±0.01	0.15±0.01	U vs C p<0.05 U vs A p<0.05 U vs R p<0.05
Eosinophil count Cell/ml	6.4±0.90	6.69±0.83	3.34±0.48	3.07±0.64	A vs C p<0.05 R vs C p<0.05 U vs R p<0.05 U vs A p<0.05
Neutrophil count Cell/ml	60.44±2.62	53.38±1.48	59.17±2.19	60.80±3.66	A vs R
Total IgE concentration IU/ml	380.08±28.08	342.46±30.96	321.76±27.49	36.59±6.78	A vs C p<0.05 R vs C p<0.05 U vs C p<0.05
IL-37 pg/ml concentration	2.69±0.78	3.26±1.25	2.68±0.56	124.61±22.07	A vs C p<0.05 R vs C p<0.05 U vs C p<0.05

Table 2: show the comparison of the mean± standard error between male and female in each patients groups

Parameters	Gender	Asthma Patients (A)	Rhinitis patients (R)	Urticarial Patients (U)	Healthy control (C)
WBC count Cell/ml	Female	10.9±0.91	8.3±0.68	9.1±0.59	7.27±0.65
	Male	8.9±0.81	7.68±0.46	6.8±0.29	7.7±0.76
P value		0.11	0.45	0.002**	0.68
Lymphocyte count Cell/ml	Female	23.32±2.83	30.8±1.67	25.4±1.6	31.5±2.08
	Male	29.17±1.63	34.2±1.6	33.8±2.7	27±5.47
P value		0.09	0.15	0.014*	0.46
Monocyte count Cell/ml	Female	5.4±0.68	6.9±0.48	6.9±0.56	6.9±0.91
	Male	8.1±0.67	7.7±0.54	8.58±1.07	6.52±0.99
P value		0.010**	0.29	0.2	0.77
Basophil count Cell/ml	Female	0.13±0.02	0.15±0.012	0.2±0.02	0.17±0.02
	Male	0.19±0.02	0.91±0.02	0.21±0.02	0.13±0.02
P value		0.035*	0.12	0.84	0.12
Eosinophil count Cell/ml	Female	5.78±1.28	4.52±0.7	3.576±0.64	3.9±0.88
	Male	7.02±1.3	8.86±1.2	3.1±0.73	2.3±0.89
P value		0.50	0.006**	0.63	0.23
Neutrophil count Cell/ml	**Female**	65.36±4.52	57.5±1.88	63.9±7.38	57.5±2.44
	Male	55.5±2.06	49.2±1.58	54.4±3.39	64.1±6.98
P value		0.059	0.003**	0.027*	0.39
Total IgE concentration IU/ml	Female	416.6±30.28	323±41.23	345±39.56	31.86±8.33
	Male	343.6±46.33	361.9±47.34	298.5±38.67	41.3±11.06
P value		0.200	0.54	0.41	0.51
IL-37 pg/ml concentration	Female	0.93±1.18	1.42±0.4	1.84±0.51	132±40.97
	Male	4.46±1.41	5.11±2.39	3.53±0.96	117.2±20.34
P value		0.020*	0.14	0.13	0.75

The comparison of total and differential counts of the white blood cells was done for healthy control and three types of allergic patients. Results show a significant increase in total WBC count in patients with asthmatic allergy as compared with control, with a slight increase in total WBC count in rhinitis and urticarial patient groups, but without significant differences, as shown in Table (1) and Figure(1).
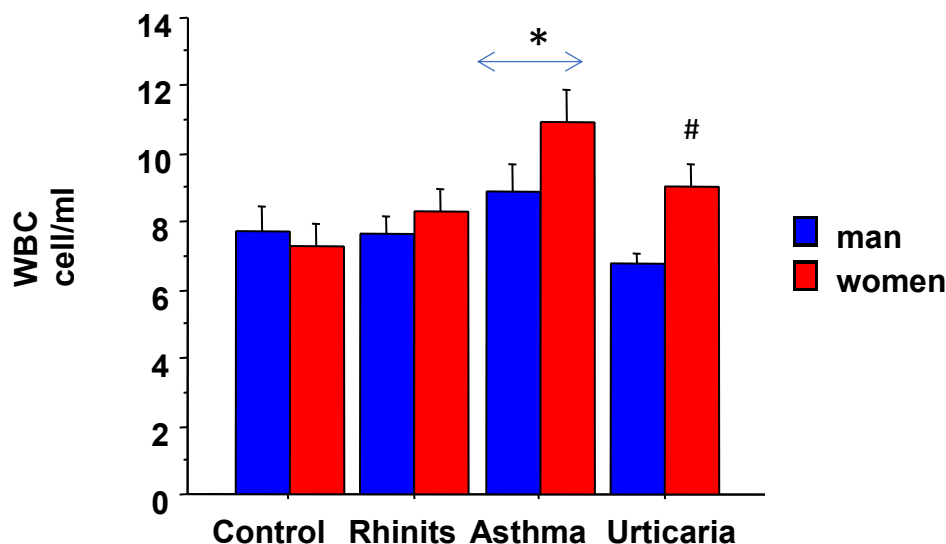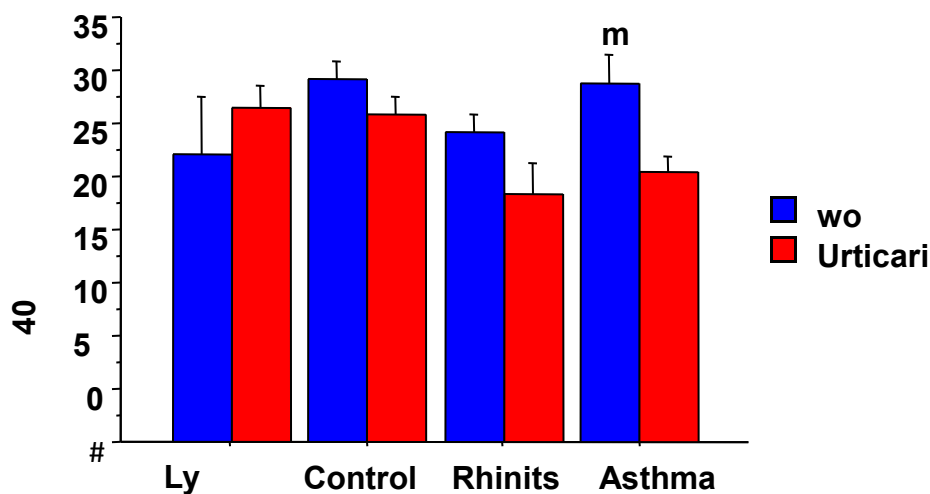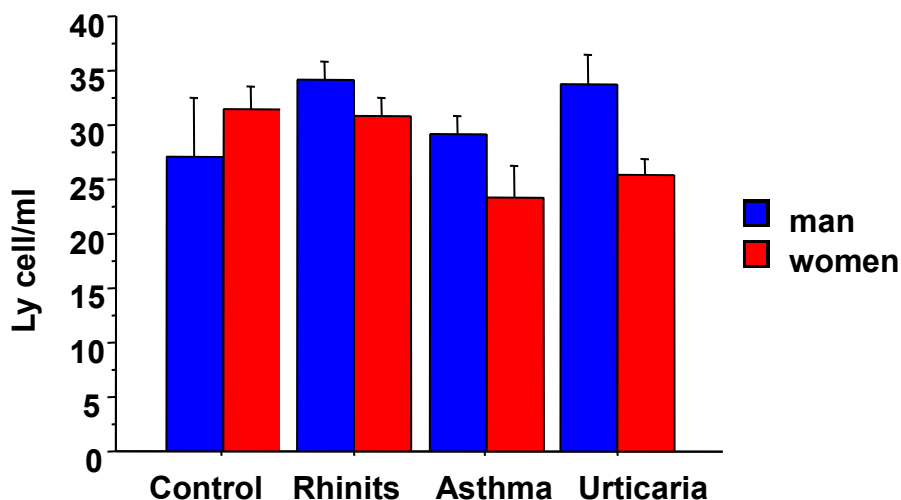
Figure 1: The total WBC count in Asthma, Rhinitis, Urticaria and Control groups
*represent the significant difference in WBC in Asthma patient as compared with the control
represents the significant difference in WBC in Urticarial female patient as compared with
the male

And when total WBC count for three groups was compared with each other, significant increase in total count in patients with asthma was seen as compared with both rhinitis and urticaria patient groups as shown in Table (1). Significant increase in WBC in females with urticaria was detected as compared with male patients, Fig (1) and Table (2). While no significant differences were observed between males and females of other groups.

Lymphocytes exhibited no significant differences in their numbers in all patient groups as compared with the control, and significant increase in lymphocyte numbers was detected in patients with asthma as compared with rhinitis patient group. Fig (2) and Table (1).

When males and females were compared, significant differences in lymphocytes were found between males and females of the urticarial group, and no differences were detected in comparison of males and females of other groups. Figure (2), Table (2).

Figure 2: The lymphocyte count in Asthma, Rhinitis, Urticaria and Control groups
#represents the significant difference between urticarial males and females.

The monocyte count did not show any significant difference between the patients groups as compared with the control, Table (1) and Figure (3), as well as between the two sexes Table (2), except for the asthma group, which showed a highly significant difference between male and female P=0.010.
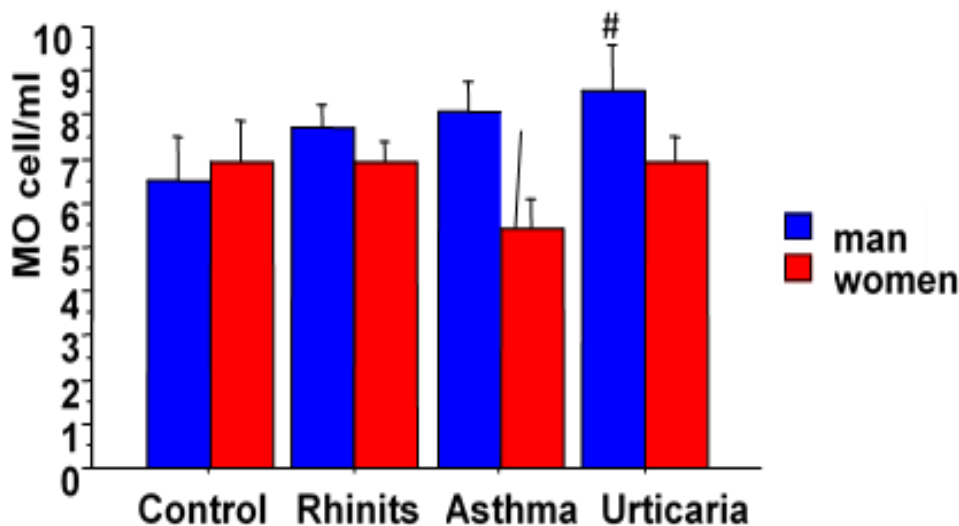
Figure 3: Monocyte count in Asthma, Rhinitis, Urticaria and control groups
represents the significant difference between asthmatic male and female

Basophil recorded a significant increase only in Urticarial group as compared with the control, table (1) and Figure (4), also significant increase was recorded in Urticarial group as compared with Rhinitis and Asthma groups. Fig (4)

Comparison of basophile numbers between males and females indicates a significant increase in their numbers in asthmatic males as compared with the female (p= 0.035), and no significant differences were recorded between the two sexes in the other groups, Table (2).
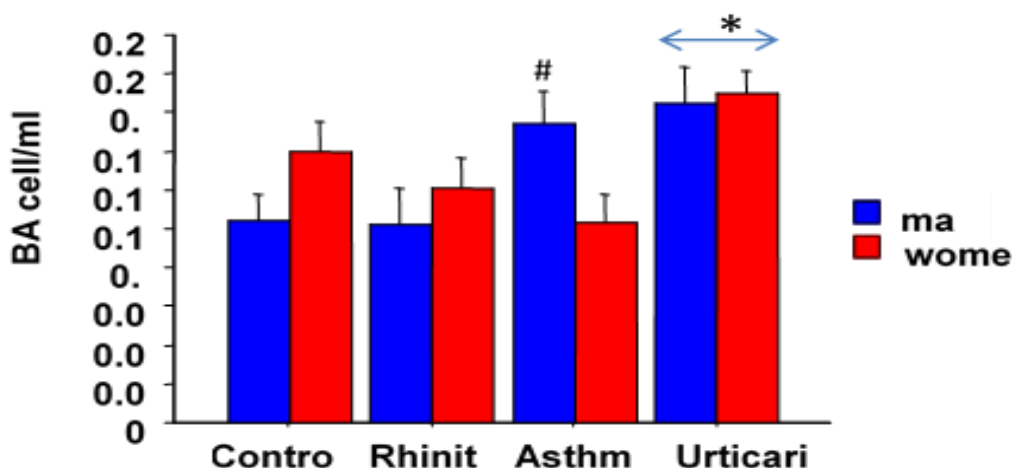
Figure 4: Basophil count in Asthma, Rhinitis, Urticaria and Control groups
                    *show significant difference between urticaria and the control
show significant difference in asthmatic males as compared with the control

Eosinophil count increased in all groups of patients but this increase was significant only in asthma and rhinitis groups as compared with the control, table (1) and Figure (5), and as compared with urticarial group, and there were no significant differences between males and females in the studied groups except for the rhinitis group, P= 0.006 (Figure 5) and table (2).
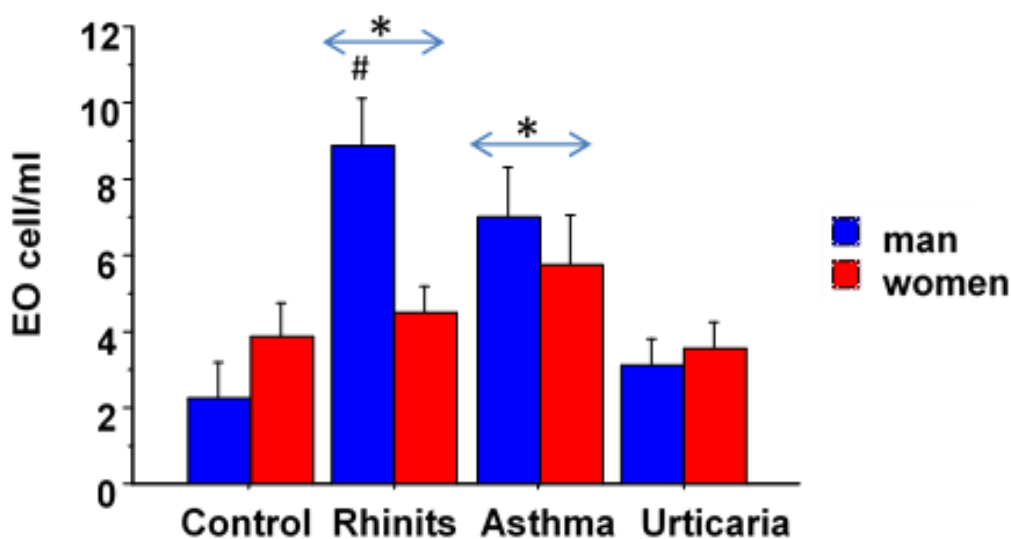
Figure 5: Eosinophil count in Asthma, Rhinitis, Urticaria and Control groups
*show significant increase in eosinophil numbers in asthma and rhinitis groups as compared with the control
#show significant increase in eosinophil numbers in rhinitis diagnosed females compared to males in the same group

Neutrophil count showed a significant decrease in rhinitis group as compared with the control, as well, as with the asthma and urticarial groups. Table (1) and Figure (6). When males and females were compared, neutrophil numbers in females showed an increase in their numbers as compared with the male, in contrast to their numbers in control group, but it was significant only in rhinitis and urticarial males. P=0.003 and P= 0.027 respectively, in Table (2) and Figure (6).
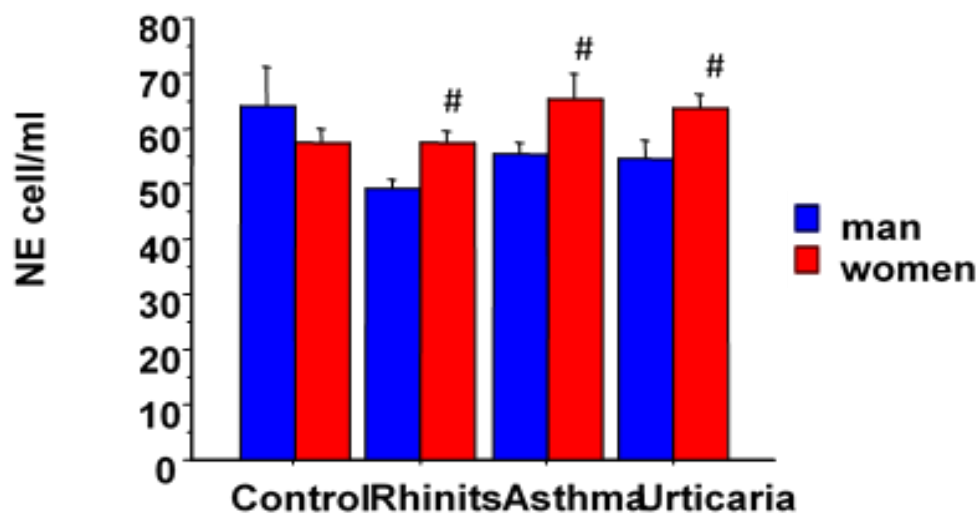
Figure 6: The neutrophil count in Asthma, Rhinitis, Urticaria and Control groups
#show significant increase in female neutrophils as compared with the male.

Immunoglobulin E (IgE) levels were determined using IgE ELISA test, table (1) and Fig (7), and it revealed a significant increase in concentration in all tested groups of patients as compared with the control and no significant differences were found when the three groups compared with each other or in male and female as well. Table (2) and Figure (7)
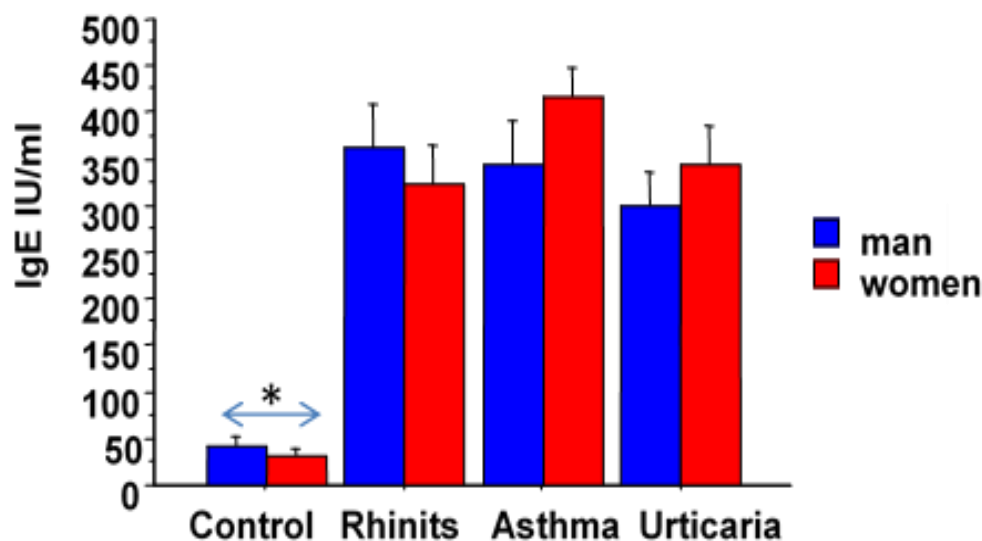
Figure 7: The IgE levels in Asthma, Rhinitis, Urticaria and Control groups

IL37 cytokine has been measured, and its level showed a significant decrease in all patients groups as compared with the control [table (1) and Figure (8)], and no significant differences observed in IL37 levels were shown when the three groups of patients compared with each other [table(1)], as well male and female comparison was done and showed a significant decrease of IL37 in female with asthma, but it was not significant in other patients groups, Table (2).
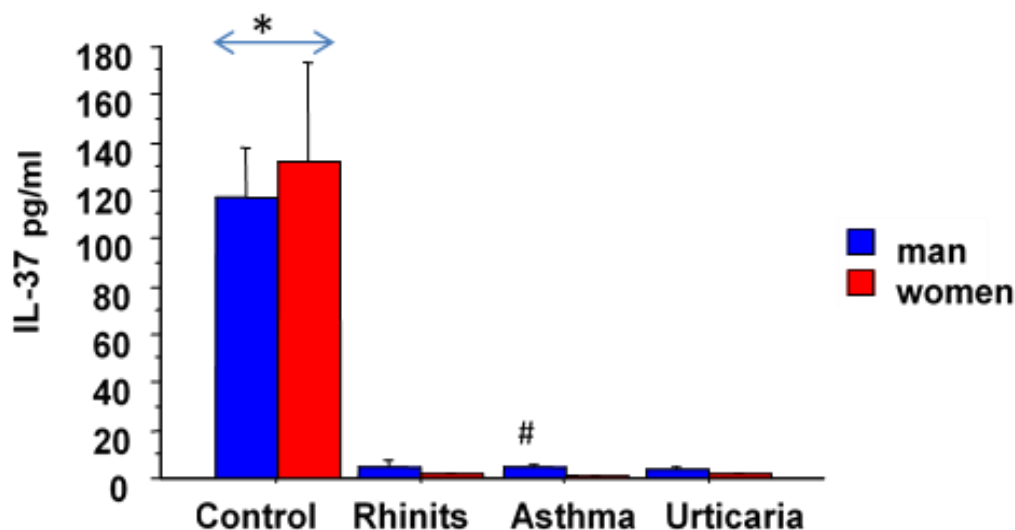
Figure 8: The IL37 level in Asthma, Rhinitis, Urticaria and Control groups
showed significant decrease of IL37 in female with asthma

3. Discussion

Asthma, rhinitis, and urticaria diseases consider type I hypersensitivity (8). They developed as a response to foreign allergens that are presented by Antigen-presenting cells (APCs) to T-cells which stimulate B-cells to produce IgE antibodies (12). That bind by the Fc region on its receptors on mast cells and basophils through the sensitization phase, subsequently, after a second exposure to the same allergens, crosslinking of these mast cell and basophil, that bound with IgE antibodies, lead to degranulation of these cells and the release different types of mediators such as histamine, cytokines, leukotrienes, proteolytic enzymes, platelet-activating factors, macrophage inflammatory proteins, prostaglandin, tryptase, etc. (13). Which leads to the emergence of different symptoms, according to the affected organ of the human, as a result of peripheral vasodilation, contraction of smooth muscle, bronchospasm, potentially hypovolemia or hypoxia, increased permeability of blood vascular, rhinitis, abdominal cramping, and pruritis, could be seen (12, 13).

From Table (1), we notice that there are some significant differences in the total and differential count of white blood cells in the three pathological groups as compared with the control and within the groups themselves, as well as significant differences between the two sexes in the three pathological groups, while there was no significant difference between the two sexes in the control group, which indicates that immune response to these diseases can be affected by sex hormones; estrogens or androgens; produced by each sex, which are known to modulate inflammation, (14,15,16), and this topic needs more studies.

This research found an increase in total WBC count in the asthma patients group in comparison with other patients groups and the control group which come in agreement with the previously well-documented study by Lewis *et al*. (17). The most affected part of the body by hypersensitivity is the respiratory system (nose, sinuses, lung), therefore the immune response, which represented by elevation in peripheral total WBC count, is higher than the other diagnostic parameters, especially in Asthma patients group. As well, the total WBC count in the rest patients groups was higher than the control group, and this matches previous studies (16, 18).

Many previous studies documented that there are significant differences between females and males in the incidence of different diseases and in the immune responses attributed to the

effects of sex hormones such as estrogen, progesterone, and androgens (18,19, 20), and leukocytes having various roles in innate and adaptive immunity and inflammation which also affected by gender.

Immunoglobulin E (IgE) levels were determined and were found to record a significant increase in all groups tested as compared with the control group because asthma, rhinitis and urticaria were considered Th2-dominant responses with increasing levels of IgE (21), and there were no significant differences found in IgE levels in the comparison between patients groups and this result is self-evident because all these diseases are of hypersensitivity type I, this was previously validated in a study in China by Xu-De *et. al.* 2021 (22) and other studies (23), therefore, this test is one of the diagnostic tests that confirm the presence of allergies(24).

No significant differences were found in the comparison between males and females that is agreed with lately published studies that indicate no influences of the age or gender on the level of the IgE (24).   In contrast to a previous study, which demonstrates an elevated IgE level in males that may be due to sex hormones like estrogen (22).

The decrease of IL-37 in female groups was not significant in urticaria and rhinitis but significant in asthma patients in comparison with the male groups in all patients groups while the opposite happens in the control group, where the concentration of IL-37 increases in females compared to males, but without significant differences, similar studies reported the low concentration of IL-37 in allergic patients compared to healthy controls in asthma patients (25, 10), and in rhinitis (26).

In allergic diseases, there is an induction of unfavorable immune response, due to exposure to different allergens, and cause type I hypersensitivity reaction and late-phase responses could occur, sensing and developing of memory cells that accelerate the production of IgE and other immune reaction agents such as immune cells, cytokines, and enzymes that remoulade the functions of these cells, especially through increasing of T helper type 2 (Th2) lymphocytes originated from inflammation area and secrete allergic pro-inflammatory cytokines by draining lymph nodes, that contribute to exacerbation pathogenesis of allergic diseases, many studies find that IL-37 suppress innate and adaptive immunity (6, 27, 28), as well as many in-vitro and animal in-vivo studies indicate that IL-37 reduces allergic symptoms through suppression of Th2 cells and their regarding cytokines (27, 8, 25).

Wang and his colleagues (2) explained the low concentration of IL-37, attributed to its redistribution of IL-37 protein in the cell between intracellular (especially nucleus) and extracellular positions (1), which impacts ~~on~~ cellular responses depending on the mediators and cytokines, and they added, that the trigging factors for the production of IL-37 protein were toll-like receptor (TLR) stimulus, interferon (IFN)γ, IL-18, IL-1b, tumor necrosis factor (TNF) and transforming growth factor β1 (29), in the same study, the addition of IL-4 plus granulocyte-macrophage colony-stimulating factor (GM-CSF) suppress IL-37 expression (2). It is obvious that IL-4 consider one of the cytokines that are responsible for allergic responses (23).

Different mechanisms associated with the production of IL37 in different cell species are attributed to different signal molecules that affect gene transcription, through a transduction mechanism especially in allergic diseases (1, 2, 3). Eisenmesser *et. al.*, (30) showed that there are missing cofactors at the molecular level for signaling and activating IL-37 and inactive signaling of IL-37 can to inflammatory diseases (31, 32, 5). Additional studies will

be needed for more understanding of IL37 role as anti-inflammatory cytokine to be used in the therapy of autoimmune diseases and cancer (33).

4. Conclusion

Our results may contribute to the clinical therapeutic application of IL-37 in the treatment of allergic diseases, since the low concentration of IL-37 in allergic diseases lead to acute inflammatory reactions in all patient groups studied, which confirm previous studies that explain the regulatory effects of IL37 in different diseases.

Ethical Statements for Human/animal experiments

The study was approved by institutional ethics committee of "University of Mustansiriyah" and informed consent was obtained in writing from each individual participant. Each participant was previously informed about the study follow-up before enrolling for the study.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that may appear to influence the work reported in this paper.

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