



## The Effect of Immunotherapy on Some Immunological Parameters of Airway Allergic Patients in Province of Babylon/Iraq

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### Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

### Article Information

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### ABSTRACT

Twenty-five blood samples had been collected from patients who attend the center of the allergy and asthma in the province of Babylon, 25 samples from patient who have received subcutaneous immunotherapy, 10 samples from non-allergic persons as a control. These samples were categorized into three groups (before treatment group, after treatment group and control group), the level of immune parameters, which includes histamine, IL-10 and IL-12 have been measured by using ELISA technique. The level of histamine for the first group (before treatment) has reached  $34.8292 \pm 6.47680$  ng/ml as compared with the second group (after treatment) which was  $9.3238 \pm 3.55296$  ng/ml and control is  $6.9844 \pm 3.05477$  ng/ml, while the level of IL-10 for the first group is  $14.9604 \pm 6.48170$  pg/ml as compared with second group which was  $43.0476 \pm 21.26668$  pg/ml and control is  $24.4969 \pm 10.21066$  pg/ml, finally the level of IL-12 for the first group was  $17.753 \pm 8.39343$  pg/ml as compared with second group which was  $39.1929 \pm 17.73975$  pg/ml and control  $26.7731 \pm 12.93092$  pg/ml. The group of immunotherapy treated patients was divided into four subgroups, according to the period of immunotherapy treatment which were two months, six months, twelve months and more than twenty-four months, It had been found that there was a gradual decrease in the level of histamine with increasing the period of immunotherapy treatment that are  $11.1811 \pm 5.33203$  ng/ml,  $9.8948 \pm 2.91382$  ng/ml,  $8.8024 \pm 2.57390$  ng/ml and

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8.1191±5.76472 ng/ml respectively, on the contrary, for IL-10 its level increased with increasing the period of immunotherapy treatment that were 13.9605±2.36278 pg/ml, 39.5547±16.60696 pg/ml, 56.8740±11.15175 pg/ml and 65.6659±2.80207 pg/ml respectively. The same thing for the level of IL-12 which increased with increasing the period of immunotherapy treatment that were 16.1298±4.70059 pg/ml, 30.5796±5.40110 pg/ml, 54.0523±5.63154 pg/ml and 56.4601±1.24127 respectively.

**Keywords:** Allergic diseases; immunotherapy; histamine; IL-10; IL-12; type 1 regulatory T cells.

## 1. INTRODUCTION

Allergic diseases including asthma and rhinitis are group of common disorders which are regarded to be mediated by immunoglobulin E (IgE). Cross linking of IgE bound to high affinity receptors on mast cells result in the production of allergic mediators as well as other cytokines, chemokines and growth factors [1]. Allergen immunotherapy was first used by Leonard Noon 1911, since then it has been widely used in the treatment of respiratory allergic diseases [2]. Allergen immunotherapy is an effective treatment for allergic asthma and rhinitis, in addition to reducing symptoms it can change the course of allergic disease and induce allergen specific immune tolerance [3]. Allergen immunotherapy is the only available treatment able to affect the natural course of allergy [4] and confers long-term benefit for at least 3 years after discontinuation [5], Its continues to be used worldwide in the management of allergic rhinitis and asthma [6].

Number of immunological parameters has different roles in allergic diseases; from these parameters are histamine, IL-12 and IL-10. Histamine is a major allergic mediator, it is a primary amine synthesized from histidine in the Golgi apparatus from where it is transported to the cytoplasmic granules of mast cells and basophils for storage, it is a potent vasoactive agent, bronchial smooth muscle constrictor and stimulant of nociceptive itch nerve [7].

Interleukin-12, a heterodimeric cytokine composed of two chains, p35 and p40, produced mainly by monocyte/macrophage, neutrophils and dendritic cells [8,9]. It is a key cytokine involved in regulating the balance between Th1 and Th2 cells by promoting Th1 response, reduced capacity to produce this cytokine could lead to aberrant Th2 cells development [10], which considered the main characteristic feature of allergic response [11].

Interleukin-10 is a unique cytokine with a wide spectrum of anti-inflammatory effects and a profile of activity that suggest that it may be a powerful inhibitor of allergic response [12]. It was first discovered as an inhibitor cytokine secreted from Th2 cells that suppress Th1 cells [13,14]. but it is now found that a subtype of CD4 T cells termed type 1 regulatory T cells (Tr1) are a major source of IL-10 and play a major role in suppressing both Th1 and Th2 cells [15]. Because of the worldwide distribution of allergic disease, this studied was aimed to find the differences in the level of histamine, IL-10 and IL-12 among airway allergic patients.

## 2. MATERIALS AND METHODS

Fifty blood samples had been collected from airway allergic patients, (25 asthma and 25 allergic rhinitis). Twenty five samples before immunotherapy treatment which were (14 asthma and 11 allergic rhinitis) and 25 after immunotherapy treatment were (11 asthma and 14 allergic rhinitis), their ages range between 17-58 in addition to 10 blood samples from healthy persons as a control whom their ages ranged between 20-55. Patients were enrolled from November 2013 to February 2014. They were from both sexes. All the patients have been diagnosed under the supervision of consultant physician in allergy and asthma center in Babylon province, the patients were sensitized to the following allergens : pollen (1 patient), mite (3 patients), DP *Dermatophagoid espteronysinus* (1 patient), DPDF *Dermatophagoi despteronysinus* and *Dermatophagoi desfarinae* (5 patients), M3+M4 *Aspergillus fumigatus* + *Muccor racemosus* (2 patients), pollen + DPDF (1 patient), DP+M4 (1 patient), pollen + mite (6 patients), date pollen + DP (1 patient), DP + pollen (3 patients) and DPDF + chenopodium (1 patient). Immunotherapy treated patients are given the following allergens extracts: pollen (2 patients), mite (5 patients), DP (2 patients), DPDF (2 patients), pollen+ mite (12 patients), DP+

pollen (1 patient) and DF+5 grasses mix (1 patient).

## 2.1 Enzyme-Linked Immunosorbent Assay (ELISA)

Blood is allowed to clot, then centrifuged and sera were collected. Sera divided into a number of eppendorf tubes stored at  $-20^{\circ}\text{C}$  the level of histamine concentrations were determined according (Creative Diagnostics, USA) company. The concentrations of both IL-10 and IL-12 were estimated according (Boster, USA).

## 2.2 Statistical Analysis

Statistical analysis was done by using computer software Statistical Package for Social Science (SPSS) version 20. Results were expressed as mean  $\pm$  standard deviation (SD), and analyzed by using of one-way ANOVA. The level of statistical significance was  $P < 0.05$  and  $P < 0.01$ .

## 3. RESULTS

This study was included 50 patients with asthma (37 female and 23 male), and 10 healthy person as control (5 male and 5 female). The mean age of study groups was (17-58) years.

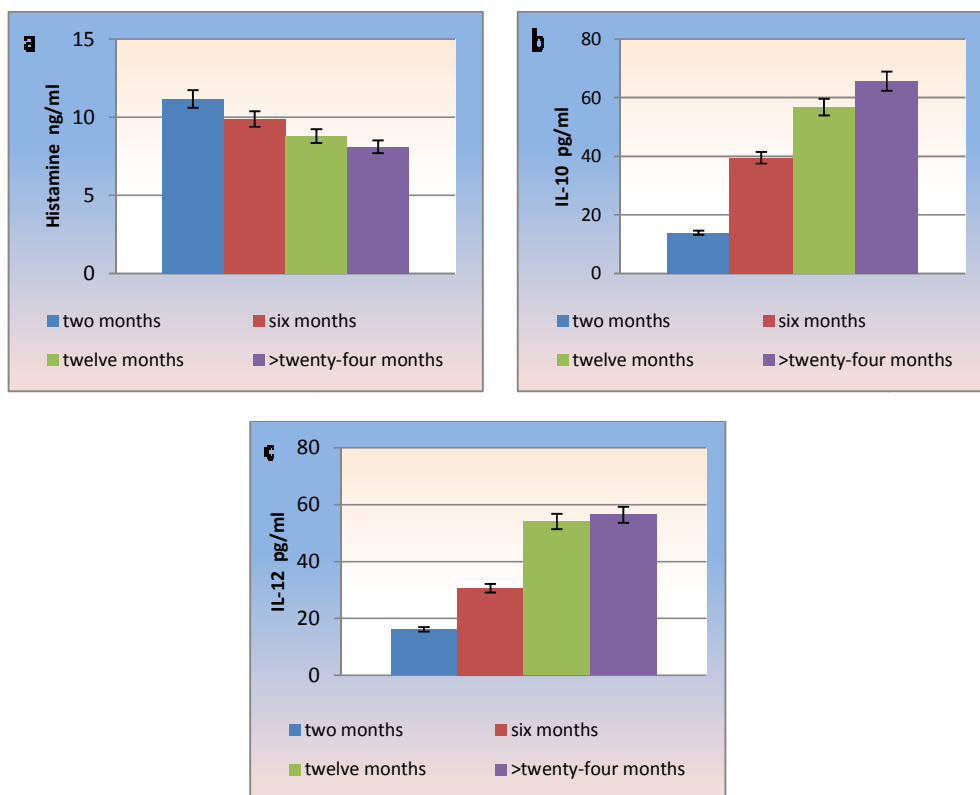
The results appeared the level of histamine in allergic patients was increased before treatment  $34.8292 \pm 6.47680$  ng / ml as compared with the patients after treatment  $9.3238 \pm 3.55296$  ng/ml and the control  $6.9844 \pm 3.05477$  ng / ml. The presence of significant differences between the first group before treatment and both after treatment and the control at the level of probability  $< 0.05$  and  $P < 0.01$ , on the contrary. The level of IL-10 and IL-12 were studied, IL-10 it reached in allergic patients before treatment were  $14.9604 \pm 6.48170$  pg/ml while in allergic patients after treatment were  $43.0476 \pm 21.26668$  pg/ml and the control  $24.4969 \pm 10.21066$  pg/ml with the presence of significant differences between the first group before treatment and the second group after treatment at the level of probability  $P < 0.05$  as well as between the second group and control at the level of probability  $P < 0.05$  and  $P < 0.01$ , while the level of IL-12 for allergic patients before treatment  $17.753 \pm 8.39343$  pg / ml as compared with allergic patients after treatment  $39.1929 \pm 17.73975$  pg/ml and the control  $26.7731 \pm 12.93092$  pg / ml with the presence of significant differences between the first group

before treatment and the second group after treatment at the level of probability  $P < 0.05$  as well as between the second group and the control at the level of probability  $P < 0.05$  and  $P < 0.01$ .

The group of allergic patients who received immunotherapy treatment through this study categorized into four groups according to the period of treatment, they are two months (6 patients), six months (6 patients), twelve months (6 patients) and more than twenty-four months (7 patients). The level of histamine according to these groups decrease gradually as follow  $11.1811 \pm 5.33203$  ng/ml,  $9.8948 \pm 2.91382$  ng/ml,  $8.8024 \pm 2.57390$  ng/ml and  $8.1191 \pm 5.76472$  ng/ml respectively. While the level of both IL-10 and IL-12 increases gradually by increasing the period of immunotherapy treatment, the level of IL-10 for these groups reaches  $13.9605 \pm 2.36278$  pg/ml,  $39.5547 \pm 16.60696$  pg/ml,  $56.8740 \pm 11.15175$  pg/ml and  $65.6659 \pm 2.80207$  pg/ml respectively, and the level of IL-12 for these groups is  $16.1298 \pm 4.70059$  pg/ml,  $30.5796 \pm 5.40110$  pg/ml,  $54.0523 \pm 5.63154$  pg/ml and  $56.4601 \pm 1.24127$  respectively Fig. 1.

## 4. DISCUSSION

Histamine is a biogenic amine synthesized and stored mainly in mast cells and basophils and plays a prominent role in the pathophysiology of allergic diseases such as asthma and rhinitis [16]. During this study it has been found that, there was a rise in the level of histamine for allergic patients before immunotherapy treatment, the interpretation of this rise back to the liberation of histamine from inflammatory cells, when allergic patients exposed to allergens causing their symptoms, these allergens will be cross-linked to IgE molecules found on the surfaces of both mast cells and basophiles and stimulate these cells to the liberalization of the allergic mediators, including histamine [17]. These results are similar to a study by Lin *et al* on allergy patients (food, respiratory, drug and skin allergy) of both sexes noticed a significant increase in the level of histamine [18] the level of histamine decreases after immunotherapy treatment this is due to the fact that mast cells and basophiles suffer from a local decrease affected by immunotherapy [19,20], also found that the first step in the immunotherapy is the desensitization of inflammatory cells bearing high affinity receptors Cori which are mast cells and basophiles, and the inhibition of its ability to



**Fig. 1. The mean of immune parameters according to the period of immunotherapy, (a) Histamine, (b) IL-10 and (c) IL-12**

produce histamine and other inflammatory mediators [21]. Histamine considered as an important modular of immune function [22]. It has an important role in the pathogenesis of allergy differential regulation of T cells, histamine enhance the secretion of Th2 cytokines and inhibit the production of Th1 cytokines, it has been found that histamine receptor H4R is expressed on dendrite cells and T cells and therefore it is possible that this receptor directly mediate dendritic cells activation of T cells in vivo , the effect of H4R on T cells response is clearly illustrated by the defect in the production of Th2 cells cytokines IL-4, IL-5 and IL-13 in the absence of H4R signaling [23], while the inhibitory effect of histamine is due to its receptor H2R which found on Th1 cells [22].

Interleukin-10 is important to achieve a normal immune response Through the balance among cytokines produced by immune cells, But in the case of loss of balance between these cytokines, it leads to a shift of the immune response from protective to harmful immune response (as is the case of hypersensitivity) [24]. Allergic responses

characterized by a decreased in the level of IL-10 the immunoregulatory cytokine, it had been found through this study, that there was a significant decreased in the level of IL-10, because there was an inverse correlation between Th2 cells and IL-10 producing Tr1 cells, and because there is a dominance of Th2 cells and their inflammatory cytokines in allergic response, the level of IL-10 will negatively affected [25,26]. These results were similar to the study of Hawrylowicz who noticed a decrease in the level of IL-10 of allergic patients [27], but it had been found that the level of IL-10 increased in patients having immunotherapy treatment, because immunotherapy can stimulate the generation of immature dendritic cells (iDC) it also known as a tolerogenic DC , these cells play an important role in the generation of IL-10 producing Tr1 cells, iDC characterized by possessing a few levels of surface molecules CD40 and the absence of proinflammatory cytokines such as IL-6 and TNF- $\alpha$  [28,29,30].

Interleukin-12 considered as main regulator of cellular immunity (Th1 cells) [31]. The dominance

of Th2 cells in allergic response which lead to lack of balance between T cells Th1 / Th2 cells response [32], therefore, it had been found that there was a decrease in the level of IL-12 for allergy patients through this study. These results were similar to Rahi through his study on children with asthma [33]. While Al-Quraishi found that the level of IL-12 did not change between asthma patients and healthy controls in a study conducted on the children with asthma [19]. Immunotherapy can act by returning the balance between T cells [34] through the regulatory role played by immunoregulatory cytokine IL-10 produced by Tr1 cells which inhibit inflammatory cytokines produced by Th2 cells [32,33] this lead to the activation of Th1 cells and increase the production of IFN- $\gamma$ , which is positively correlate with IL-12, this lead us to that the effect of IL-12 depends on the presence of IFN- $\gamma$  [9].

## 5. CONCLUSION

In conclusion, according to this study there was a significant increase in the histamine level for allergic patients as compared with control, but there was a decrease in the level of both IL-10 and IL-12 in allergic patients as compared with control. After treatment with subcutaneous immunotherapy the level of histamine decrease gradually by increasing the period of immunotherapy treatment, on the contrary for both IL-10 and IL-12 which increased gradually by increasing the period of treatment.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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