Evaluation of interleukin-35 and interleukin-27 in allergic conjunctivitis and associated allergies before and after allergen-specific immunotherapy

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Background/aim
While interleukin (IL)-35 has been identified as a novel immunosuppressive and anti-inflammatory cytokine, IL-27 has both inflammatory and anti-inflammatory functions. Allergen-specific immunotherapy is the most excellent form for custom-tailored treatment of allergic diseases. The aim of this study is to determine the role of IL-35 and IL-27 and the protective role of allergen-specific immunotherapy in allergic conjunctivitis alone or associated with other allergies.

Patients and methods
The present study enrolled 30 patients who were referred to the Allergy Lab, at the Research Institute of Ophthalmology, Giza, Cairo, Egypt. Patients were divided into two groups (15 each); patients complaining of allergic conjunctivitis alone (group 1) and patients who were suffering from allergic conjunctivitis associated with allergic rhinitis and/or allergic bronchitis (group 2). In addition, 15 healthy individuals served as a control group (group 3). Allergen SIT was prepared from natural allergenic extracts from some different crude materials causing allergy, such as pollens, animal hairs, house dust, molds, nicotine, and feathers. Group 1 was treated with local conjunctival immunotherapy as eye drops, while group 2 was treated with subcutaneous immunotherapy for 36 months. Serum levels of IL-35 and IL-27 were measured using the enzyme-linked immunoassay technique before and after immunotherapy.

Results
The present results showed significant decreases in serum levels of IL-35 and IL-27 (146.4 and 13.2 pg/ml, respectively) in all 30 allergic patients before immunotherapy than controls (235.0 and 50.4 pg/ml). However, IL-35 and IL-27 showed a significant increase (478.8 and 42.6 pg/ml, respectively) in all patients after receiving their immunotherapy either as eye drops or as subcutaneous injections when compared before starting immunotherapy. Moreover, insignificant changes were obtained between local and subcutaneous immunotherapy in the level of IL-35, while IL-27 showed a significant increase ($P<0.05$) in the subcutaneous group than the eye drops group. The present results showed a positive excellent correlation between IL-35 and IL-27 after immunotherapy ($r=0.709$, $P<0.001$), while no correlation before immunotherapy ($r=0.334$, $P=0.063$).

Conclusions
Allergic diseases are associated with significant lowered serum levels of IL-35 and IL-27. Allergen-specific immunotherapy significantly increases serum levels of IL-35 and IL-27 confirming the role of IL-35 and IL-27 in allergic diseases and proved that allergen-specific immunotherapy increases their induction.

Keywords:
allergic diseases, interleukin-35, interleukin-27, immunotherapy

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Introduction

Ocular allergy is a well-established example for studying allergic diseases as it is usually associated with other allergies. However, unfortunately severe allergic conjunctivitis may cause corneal damage, opacity, and loss of vision [1]. Allergic conjunctivitis is the only ocular disease to involve solely a type I allergic reaction (Dupuis *et al*. 2020) [2]. Allergic bronchitis and allergic rhinitis are the most ubiquitous inflammatory airway allergies. The incidence of allergic rhinitis is greater than asthma; both represent type-1 hypersensitivity reactions that involve mast cells, basophils, eosinophils, and Th2 cells. Th2 cells secrete interleukin (IL)-4, IL-5, and IL-13. IL-4 and IL-13 stimulate B cells to produce

immunoglobulin E (IgE), which binds to the high-affinity receptors (FcεRI) on mast cells and basophils in the airway subepithelium to start the known allergic response on the second exposure to the offending allergen [3]. While most published articles gave attention to pro-allergic cytokines the protective role of immunosuppressive cytokines in allergic diseases is not well clarified [1].

Recently five members of cytokines could be recognized as members of IL-12 family: IL-12, IL-23, IL-27, IL-35, and IL-39, which have been established as crucial regulators of immunity. All are heterodimeric cytokines. Each member consists of an alpha chain subunit (p19, p28, or p35) paired with a beta chain subunit (p40 or EBi3). Chain-sharing is an important key characteristic. IL-27 is composed of α chain p28 and β chain Epstein–Barr virus-induced gene 3 (EBi3), while IL-35 is composed of α chain p35 and β chain EBi3. On one side, IL-12 and IL-23 have a serious role in the pathogenesis of autoimmune diseases through the induction of Th1 and Th17 lymphocytes, but on the other side IL-27 and IL-35 suppress inflammatory responses and decrease tissue damage by increasing the proliferation of regulatory B-cell and T-cell subsets [4]. IL-35 is a relatively newly revealed cytokine that plays a role in immune suppression and was formerly thought to be produced by T regulatory cells (Tregs) only but more advanced studies proved its production from activated dendritic cells, regulatory B cells, and CD8+ Treg cells. Its immunosuppressive role is through the inhibition of Th17 while enhancing cell proliferation and differentiation of Treg cells. Moreover, every subunit of IL-35 can independently regulate the immune response as EBi3 act by downregulating the expression of retinoic acid-receptor-related orphan receptor gamma transcription factor (RORγt) in Th17 cells, thus decreasing the inflammatory process. At the same time, P35 by itself can inhibit inflammation and these functions are augmented in the IL-35 heterodimeric form [5].

While IL-35 has been recognized as a novel immunosuppressive and anti-inflammatory cytokine, IL-27 has both inflammatory and anti-inflammatory functions. Within its pro-inflammatory actions are inhibition of proliferation and function of Th17 cells and antagonizing IL-6 activity. More than one cell can release IL-27 depending on the leading cytokine profile and disease type, and this may explain its double function [6].

IL-27 has a crucial role in controlling Th2-mediated allergic diseases. IL-27 directly suppresses Th2 and Th17 differentiation. In experimental model of allergic asthma, in-vitro administration of IL-27 stimulates IFN-γ production with decreased production of IL-5 and IL-13, showing its efficient result on reducing Th2 reactions [7]. The primary target cells of IL-27 in vivo are the Tregs promoting their anti-inflammatory functions [8].

Noon and Freeman (1911) have introduced allergen-specific immunotherapy (SIT) for treating allergic diseases and since then it has been considered the earliest preventive procedure of the management of allergies through applying increasing doses of the causative allergens to decrease symptoms and signs associated with contact to these allergens and thus create tolerance [9]. Allergen-specific immunotherapy induces tolerance to selected allergens. Tolerance can be defined as the initiation and continuation of long-lasting insensitivity to allergens. Allergen SIT is the crucial curative treatment for allergic diseases with proved efficacy and safety by several studies. Allergen-specific immunotherapy is the most excellent form for custom-tailored treatment of allergic diseases. Allergen-specific immunotherapy is efficient for IgE-dependent allergies [10,11]. Different approaches of IT were applied; the first one was the conventional subcutaneous immunotherapy (SCIT), which is to date considered the most widely used approach of allergen-SIT for more than a century [11]. It requires weekly increasing building doses followed by monthly maintenance doses for 3–5 years to persuade constant decline in symptoms and signs [2].

Sublingual immunotherapy (SLIT) is receiving the selected causative allergens in the form of sublingual droplets or as sublingual dissolvable tablets. SLIT is considered a safer and an easier substitute to SCIT for at least 30 years. It is tempting for children to escape from repeated injections; however, SLIT is less efficient than SCIT [11]. Oral immunotherapy is considered a possible remedy for food allergies to peanut, milk, and eggs [11].

Local nasal immunotherapy showed efficiency on symptoms of rhinitis alone. Moreover, it needs a special technique of application that is why its use is gradually decreasing although it is still considered an attractive option to subcutaneous injections [12].

Local conjunctival immunotherapy (LCIT) as eye drops is applied for cases suffering from allergic conjunctivitis only. This route also follows the same

principles of IT by installing increasing doses of the causative allergens onto the conjunctiva. Significant improvement in clinical signs and symptoms after 1 year compared with the controls was reported by Del Prete *et al*. [13] and Nunez and Cuesta [14].

Although few publications were concerned with LCIT as eye drops for treating patients with allergic conjunctivitis, the efficacy of this route was proved by clinical and laboratory investigations. However, LCIT alone at short periods did not alleviate symptoms and signs of allergic conjunctivitis from multiple allergens [15].

Intralymphatic immunotherapy (ILIT) is a new approach for direct application of allergens into lymph nodes. The main advantage of ILIT is being of a shorter course than the conventional SCIT as the number of required low-dose allergens is only three intralymphatic injections with 1-month interval throughout the entire period of management. Even though inadequate data is available ILIT is found to be safe and the results of clinical trials are primarily promising [11].

Epicutaneous immunotherapy is another new approach for immunotherapy in which allergens are applied to the skin through patches or following abrasion. Allergens are entrapped by Langerhans cells present in the epidermis and dendritic cells in the dermis to be translocated to lymph nodes to prepare $CD4^+$ T cells [11]. Intradermal allergen immunotherapy could suppress skin late-phase responses; however, it was clinically not efficient and eventually symptoms of allergic bronchitis became worse [16].

Immunotherapy is the only therapy that can provide continued benefits after an adequate course is completed. Immunologic changes involve downregulation of the Th2 response and upregulation of regulatory T cells that produce inhibitory cytokines [2]. Allergen-specific immunotherapy increases IL-35 and decreases IL-17 in allergic rhinitis patients [17].

Also, patients with bronchial asthma had low serum levels of IL-35 and after SLIT the level of this suppressive cytokine increased. Moreover, it was associated with an improvement in clinical symptoms [18]. Recent studies on IL-35 with advanced techniques studying cytokine receptors and signal transduction suggested IL-35 to be approved as a new immunotherapy aim [5]. Evidences were provided regarding the role of IL-27 in the management of asthma, and allergic rhinitis represented IL-27 as a novel therapeutic option [3]. The aim of this study was to determine the levels of IL-35 and IL-27 before and after immunotherapy to verify the role of IL-35 and IL-27 and the protective role of natural allergic extracts in allergic diseases.

Patients and methods

Patients

The present study enrolled 30 patients who were referred to the Allergy Lab at the Research Institute of Ophthalmology, Giza, Cairo, Egypt. Patients were divided into two groups: group 1 patients complaining of allergic conjunctivitis alone (15 patients) and group 2 patients who were suffering from allergic conjunctivitis associated with allergic rhinitis and/or bronchitis (15 patients).

Inclusion criteria of patients:

(1) Patients above 12 years old.
(2) Patients diagnosed clinically as allergic conjunctivitis alone and gave positive skin prick test (SPT) more than or equal to 3 mm wheal diameter (induration).
(3) Patients diagnosed clinically as allergic conjunctivitis associated with allergic rhinitis and/or allergic bronchitis and gave positive SPT.
(4) Patients with high serum levels of IgE more than 100 IU/ml.
(5) Patients receiving antihistamines were asked to stop these medications 5 days before performing skin testing and blood sampling, while those taking corticosteroids were asked to stop them for 14 days earlier.

Exclusion criteria of patients:

(1) Children less than 12 years old.
(2) Patients with allergic dermatitis.
(3) Patients who gave negative SPT (<3 mm diameter wheal).

Ethical approval

The present study was conducted with the Code of Ethics of the World Medical Association, according to the principles expressed in the Declaration of Helsinki 2013. This study has been approved by the Local Research Ethics Committee of Research Institute of Ophthalmology, Giza, Cairo, Egypt, with approval number #1-3-17-10-21#. A written informed consent was provided by each participant before their inclusion in the study. Patients' confidentiality was preserved.

Study design

The present study enrolled 45 participants who were divided into three groups (15 each) as follows:

Group 1: patients complaining of allergic conjunctivitis alone and consequently were treated with local specific immunotherapy as eye drops for 36 months, according to the protocol recommended by Del Prete *et al.* [13] and Kasetsuwan *et al.* [15].
Group 2: patients suffering from allergic conjunctivitis associated with allergic rhinitis and/or bronchitis and consequently were treated with the conventional approach as subcutaneous injections for 36 months as recommended by Shamji and Durham [10].
Group 3: involved healthy volunteers as the control group.

Methods

Blood sampling

Blood samples were collected from all patients twice; the first time before they started immunotherapy in March 2017 and the second time when they completed their immunotherapy in March 2020, while serum from normal individuals were collected right before performing enzyme-linked immunoassay (ELISA) in April 2020. Five milliliters peripheral blood was withdrawn from each patient into a plain vacutainer tube, centrifuged, and serum kept in −20°C for measuring IL-35 and IL-27.

Preparation of natural allergenic extracts

Natural allergenic extracts were prepared in the Allergy Lab at the Research Institute of Ophthalmology, Giza, Cairo, Egypt, from crude materials according to the protocol established by Haggag [19], where weight per volume of different crude materials that cause allergy to pollens of palm tree, orange tree, Bermuda grass (El-Negeel), four types of animal hairs such as that of the cat, dog, goat, rabbit, house dust, mixed feathers, mixed molds, mites, wool, cockroaches, and nicotine were dissolved in coca's sol, which was consisted of 20 g sodium chloride and 11 g sodium bicarbonate dissolved in 1 l of sterile distilled H_2O. The crude allergen preparations were put in a shaker for few hours to be repeated for 2–3 successive days. Then primary filtration was done by the usual filter paper (Wattman No. 1), and then secondary filtration was done with 0.22 μm millipore syringe filter (CHMLAB Group ref SCA020025K-S) into sterilized sealed vaccine bottles. Checking the sterility of the prepared allergenic extracts was done by culturing on blood agar aerobically and anaerobically. By then they were ready for SPT performance to identify the causative allergens for each patient as well as for the preparation of his or her immunotherapy according to SPT results [20].

Immunotherapy

Allergen-specific immunotherapy was prepared according to the results of SPT for each patient. In the present study, allergen-specific immunotherapy was given in two forms: the first form was LCIT as eye drops to treat patients with allergic conjunctivitis alone and was prepared as diluted allergen extract with saline solution at 1/10 of the concentration required to obtain a 3-mm-diameter wheal at the prick tests. Eye drops was put in each eye at a dose of three drops a week for 4 weeks; then three drops every 15 days for 2 months; and lastly, three drops every 21 days following the schedule recommended by Del Prete *et al.* [13], for 36 months as recommended by Kasetsuwan *et al.* [15].

The second form is the conventional SCIT for treating patients suffering from allergic conjunctivitis associated with allergic rhinitis and/or allergic bronchitis following the schedule proposed by Haggag [19], which required twice SC injections per week with an insulin syringe starting with 0.1 ml of 1/1000 concentration and then increased in an ascending pattern by 0.1 ml every time to reach 1 ml of 1/1000 conc. by the end of the fifth week. An interval of 10–14 days was required before starting 0.1 ml of 1/100 concentration with the same pattern to reach 1 ml of 1/100 conc. by the end of another 5 weeks, by then a complete building dose was achieved. The maintenance dose was a monthly dose of 1 ml of 1/100 conc. for 36 months as recommended by Shamji and Durham [10]. SCIT should be continued for 3–5 years to induce sustained clinical remission [2].

Biochemical measurements

Measuring serum levels of IL-35 and IL-27 were determined according to Mansour *et al.* [21] and Luo *et al.* [22], respectively, using ELISA kit of Sunlong Biotech Co. Ltd (Hangzhou, China) as described by instructions of ELISA Kit (www.sunlongbiotech.com|sales@sunlongbiotech.com catalog No. SL1009Hu).

Statistical analysis

Data were analyzed using IBM SPSS Statistics, Version 22 (IBM Corporation, 1 New Orchard Road Armonk, New York, United States). Data were tested for normality using the Kolmogrov–Smirnov test and Shapiro–Wilk test. Quantitative data were presented as mean and SD. Mann–Whitney test was used to compare between

each group of cases versus control. Analysis of variance test was used for multiple comparisons. Spearman's correlation coefficient was used to test the correlation between IL-27 and IL-35. *P* value was set at a significant level of 0.05. All tests were two tailed.

Results

The present results indicated that before immunotherapy IL-35 was significantly lowered in all 30 allergic patients than in 15 controls. Regardless of the clinical condition of the patients whether patients in group 1 complaining of allergic conjunctivitis alone or patients in group 2 suffering from allergic conjunctivitis associated with allergic rhinitis and/or allergic bronchitis, the mean levels of IL-35 were almost the same (146.6 and 146.1 pg/ml), respectively, both were significantly lower than the IL-35 level of controls, which was 235.0 pg/ml as shown in Table 1.

After immunotherapy IL-35 was significantly increased in all 30 patients whether whom were receiving local IT as eye drops in group 1 (431.2 pg/ml) or those were receiving SCIT in group 2 (526.3 pg/ml) comparing to controls (235.0 pg/ml) as shown in Table 1.

Also, the mean levels of IL-27 before immunotherapy were significantly lowered in both groups of patients (10.4 and 16.1 pg/ml) than in the control group (50.4 pg/ml) as shown in Table 1.

However, after IT there was significant increase of IL-27 in patients of group 2 who were receiving subcutaneous treatment (54.5 pg/ml), while no significant increase was observed in patients of group 1, who were receiving eye drops (30.7 pg/ml) compared with controls (50.4 pg/ml) as shown in Table 1.

In the present study, measurements of serum levels of IL-35 and IL-27 showed a significant increase in all 30 patients after receiving their IT whether in its local form as eye drops in group 1 (431.2 and 30.7 pg/ml, respectively) or in its conventional systemic form as SC injections in group 2 (526.3 and 54.5 pg/ml, respectively) when compared with their levels before starting IT (146.6 and 10.4 pg/ml for group 1 and 146.1 and 16.1 pg/ml for group 2) as shown in Tables 2 and 3.

The present results showed a positive excellent correlation between IL-35 and IL-27 after immunotherapy for all 30 patients (r=0.709, P<0.001), while no correlation was found before immunotherapy (r=0.334, P=0.063) as shown in Table 4 and Fig. 1.

Discussion

In this study, allergen-specific immunotherapy was given in two forms: LCIT as eye drops for treating patients with allergic conjunctivitis alone and SCIT for treating patients suffering from associated allergic rhinitis and/or allergic bronchitis.

Table 1 Serum levels of interleukin-35 and interleukin-27 before and after immunotherapy in the studied groups of patients with allergic conjunctivitis alone (group 1) or associated with allergic rhinitis and/or bronchitis (group 2) compared with the control group (group 3)

ILS	Allergic conjunctivitis (n=15)	Allergic conjunctivitis with associated rhinitis and/or bronchitis (n=15)	All cases (n=30)	Control (n=15)
Before immunotherapy				
IL-35	146.6±42.6[a]	146.1±44.0[a]	146.4±42.6[a]	235.0±86.4[b]
IL-27	10.4±4.7 [a]	16.1±7.8[a]	13.2±7.0 [a]	50.4±20.3[b]
After immunotherapy				
IL-35	431.2±166.6[a]	526.3±222.5 [a]	478.8±199.1[a]	235.0±86.4[b]
IL-27	30.7±15.5 [a]	54.5±32.7[b]	42.6±27.9[a]	50.4±20.3[ab]

All data are expressed as mean±SD. IL, interleukin. All data with different letters (a, b, c) in the same row are significant at *P* value less than 0.05, using analysis of variance test.

Table 2 Comparison of interleukin-35 and interleukin-27 before and after local specific immunotherapy as eye drops for allergic conjunctivitis cases alone (group 1)

Variables	Before immunotherapy	After immunotherapy	Test	*P* value
IL-35	146.6±42.6	431.2±166.6	–3.408	0.001*
IL-27	10.4±4.7	30.7±15.5	–3.408	0.001*

IL, interleukin. *Significant difference at *P* value less than 0.05 using Mann–Whitney test.

The clinical efficiency of SCIT is established in treating allergic rhinitis and allergic bronchitis by many studies. Nelson [23] reported the long-lasting valuable benefits of SCIT after completion of the immunotherapy course which was lasting and continued afterward. Mohapatra *et al.* [9] also proved the effectiveness of SCIT to an extent much more than SLIT.

Passalacqua and Canonica [12] reported the efficacy of local nasal immunotherapy in improving symptoms of rhinitis alone. Both SLIT and LINIT were effective as long as they were administered, but once they had been stopped symptoms were back again as observed by our two successive studies [24,25].

Table 3 Comparison of interleukin-35 and interleukin-27 before and after subcutaneous immunotherapy for allergic conjunctivitis cases associated with allergic rhinitis and/or allergic bronchitis (group 2)

Variables	Before immunotherapy	After immunotherapy	Test	*P* value
IL-35	146.1±44.0	526.3±222.5	–3.408	0.001*
IL-27	16.1±7.8	54.5±32.7	–3.408	0.001*

IL, interleukin. *Significant difference at *P* value less than 0.05 using Mann–Whitney test.

Table 4 Correlation between interleukin-35 and interleukin-27 before and after immunotherapy for all 30 patients

ILS	*r**	*P* value
IL-35 and IL-27 before IT	0.334	0.063
IL-35 and IL-27 after IT	0.709	<0.001

IL, interleukin; IT, immunotherapy. *Spearman correlation coefficient, *P* value is set significant at 0.05 levels.

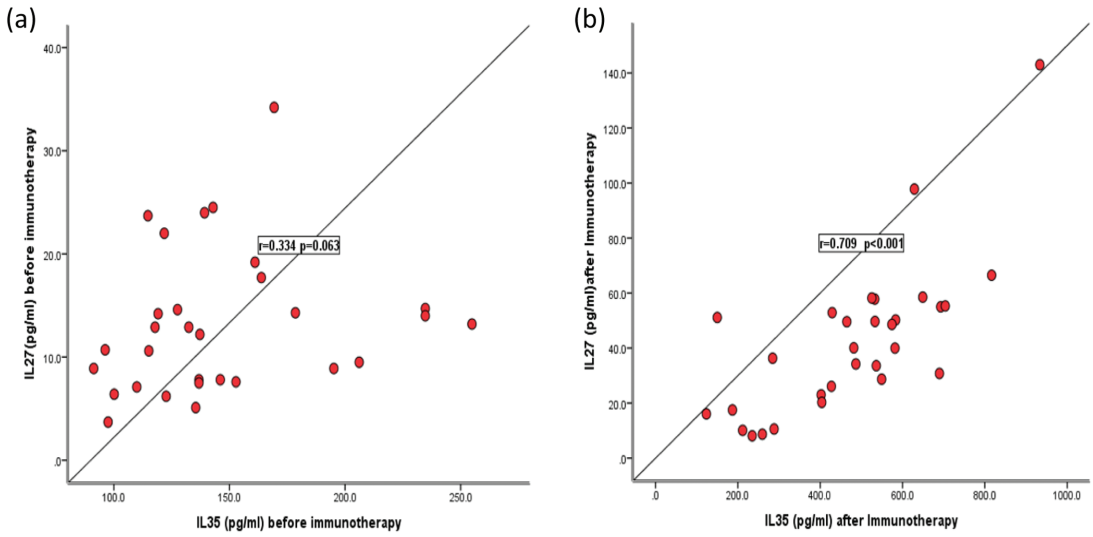
In the present study, LCIT as eye drops was chosen for treating allergic conjunctivitis as it has been used in the Research Institute of Ophthalmology since 2000 for the first time in Egypt with complete relief of symptoms and reduced using other medications, and good results were reported in our previous study [24]. Moreover, eye drops are more convenient to the patients than SCIT and safer. Efficacy of LCIT in allergic conjunctivitis was also proved in a study by Kasetsuwan *et al.* [15], who reported clinical improvement and better outcome in laboratory findings.

In the present study, IL-35 before immunotherapy is significantly lowered in all allergic cases (146.4 pg/ml) than in controls (235.0 pg/ml), while after immunotherapy IL-35 is significantly increased in all cases (478.8 pg/ml) than in controls, while insignificant changes were obtained between local eye drops and subcutaneous groups of patients.

In a study by Elazab and Hessam [17] in 20 allergic rhinitis patients his results regarding serum IL-35 levels showed lower values in patients before immunotherapy (229±64 pg/ml) than in 10 controls and showed a highly significant increase to 378 ±114 pg/ml after immunotherapy. Their results after immunotherapy showed no significant difference in the serum level of IL-35 between the patients and controls.

Wang *et al.* [26] reported a serum level of IL-35 in 25 asthmatics as 240±120 pg/ml, which was lower than that in 12 controls (450±190 pg/ml) and suggested that IL-35 can efficiently decrease IL-4 production of activated $CD4^{+}CD25^{-}$ T cells in allergic asthma,

Figure 1



Scatter diagrams show correlation between interleukin (IL)-35 and IL-27 before (a) and after (b) immunotherapy in all patients.

and that IL-35 might be a novel immunotherapy for asthmatic patients.

Mansour *et al*. [21] also reported a significantly lower level of serum IL-35 in asthmatic patients than in controls, while the result of Khoshkhui *et al*. [27] revealed no significant difference found between the serum level of IL-35 (30.9 pg/ml) in 44 asthmatic children and that (30.2 pg/ml) of 44 healthy children.

Interestingly, Gao *et al*. [28] reported the involvement of IL-35 in the physiopathology of quite variable diseases and concluded that the reported highly changeable normal levels of IL-35 in different studies might be due to the different ELISA kits and standards used.

In the present study, the serum levels of IL-27 before immunotherapy was significantly lower in all cases (13.2±7.0 pg/ml) than in controls (50.4±20.3 pg/ml), while after immunotherapy IL-27 showed insignificant increases in all cases (42.6±27.9 pg/ml) than in controls (50.4±20.3 pg/ml). Moreover, there was significant increase of IL-27 in patients of group 2 who were receiving subcutaneous treatment (54.5 pg/ml) than group 1 who were receiving eye drops (30.7 pg/ml).

Our results more or less agree with Huang *et al*. [29] where serum levels of IL-27 in 18 cases of allergic rhinitis were compared with that of 10 healthy volunteers, and the results were significantly lower in cases (21.69 ±12.6 pg/ml) than in controls (53.10±12.5 pg/ml). Gan *et al*. [30] reported lower levels of serum mRNA and IL-27 in allergic rhinitis than in controls. Luo *et al*. [22] also proved that serum IL-27 protein expression in allergic rhinitis patients was significantly lower compared with controls. Suzuki *et al*. [31] in their experimental study revealed for the first time a successful suppression of nasal allergic symptoms as a result of intranasal administration of IL-27.

In this study, serum levels of IL-35 and IL-27 showed a significant increase in allergic cases (478.8 and 42.6 pg/ml, respectively) after immunotherapy when compared with their levels before starting immunotherapy (146.4 and 13.2 pg/ml, respectively). Our results showed positive excellent correlation between IL-35 and IL-27 after immunotherapy while no correlation between them before immunotherapy. To the best of our knowledge, no study has revealed correlation between IL-35 and IL-27 for allergic diseases before and after immunotherapy.

Conclusions

Allergic diseases are associated with significant lowered serum levels of IL-35 and IL-27 than healthy controls. Allergen-specific immunotherapy significantly increases serum levels of IL-35 and IL-27 in cases more than their levels before starting immunotherapy. The present study revealed positive excellent correlation between IL-35 and IL-27 after immunotherapy while no correlation between IL-35 and IL-27 before immunotherapy. Our study confirmed the protective role of IL-35 and IL-27 in allergic diseases and also proved that allergen-specific immunotherapy increases their induction.

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Conflicts of interest

There are no conflicts of interest.

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