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ORIGINAL ARTICLE

Efficacy of Sublingual Immunotherapy versus Subcutaneous Immunotherapy in Treatment of Children with Grass Pollen Induced Vernal Keratoconjunctivitis by Eosinophilic Cationic Protien

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ABSTRACT

Objective: *The aim of this study was to compare efficacy of sublingual allergen immunotherapy versus subcutaneous allergen immunotherapy for treatment of children with grass pollen induced VKC.* ***Methodology:*** *This study involved 46 cases with grass pollen induced vernal keratoconjunctivitis as proved by specific IgE test. According to the route of administration of immunotherapy, the cases were randomly distributed into 2 groups; group (A) included cases received sublingual immunotherapy (23 cases) and group (B) included cases received subcutaneous immunotherapy (23 cases). The response to the treatment was evaluated in the two groups using a clinical scoring system which comprises the total subjective symptom scores (TSSS) and the total ocular sign score (TOSS) every 3 months and also by measurement of the level of specific IgE and ECP every 6 month for one year.* ***Results:*** *There was statistically significant improvement in VKC cases treated with either sublingual immunotherapy (group A) or subcutaneous immunotherapy (group B) (p<0.001) as proved by specific IgE test, ECP test, total subjective symptom scores (TSSS) and total ocular signs score (TOSS). Our data indicate that there was no statistically significant difference between a long-term treatment with grass pollen SLIT and SCIT in children with VKC as regard specific IgE neither at 6 months nor 12 months of treatment (P1= 0.315 and P1=1.01). There was also no statistically significant difference between both methods as regard ECP (P1= 0.61 and P1=0.61). Our study indicates also that there was no statistically significant difference between SLIT and SCIT as regard TSSS score at 3 month (p=0.187), at 6 month (p=0.88), at 9 month (p=0.47), and at 12 month (p=0.43) of treatment. Our study shows also that there was no statistically significant difference between SLIT and SCIT as regard TOSS score at 3 month (p=0.34), at 6 month (p=0.38), at 9 month (p=0.79), and at 12 month (p=0.83) of treatment.* ***Conclusions:*** *Sublingual immunotherapy is considered a viable alternative to subcutaneous Immunotherapy as there is better adherence to sublingual Immunotherapy. Protocols of SLIT have a more convenient and shorter schedules compared with that of SCIT, they have less anaphylactic reactions and preferred to children. Moreover SLIT is preferred in children. In addition, SCIT has more anaphylactic reaction.*

INTRODUCTION

Vernal keratoconjunctivitis (VKC) is one of the most common eye diseases affecting adolescents and children. It is a chronic aggressive inflammatory eye disease that may seriously affect vision[1] .

Corneal lesions in VKC is not uncommon and may occur in most patients with VKC as erosion of the cornea, superficial keratopathy, corneal plaque , persistent epithelial defects of the cornea and corneal ulcers[2,3]. Severe impairment of vision can occur[4]. Treatment with topical steroids has many drawbacks as glaucoma, eye infections and cataract. Glaucoma induced from topical steroids is a serious sight-threatening complication [5].

Desensitization (allergen immunotherapy) implicates the administration of allergen extracts in gradually increasing doses at a regular way for a relatively long time (months to years). Subcutaneous injections, sublingual drops, tablets or sprays are all different forms for immunotherapy administration [6].

Desensitization means that the body would not experience again the violent symptoms when exposed to a certain allergen (itching, watery eyes, redness and burning sensation) but instead it will begin to develop a normal immune response to the allergen[7]. Desensitization is a valuable treatment approach for many allergic patients as it leads to long-term release of allergy symptoms even after treatment is stopped [8].

Immunotherapy has the disadvantage that it may take long time (several months or even years) to

produce satisfactory effect, but one the other hand it has the advantage that it can avoid many serious ocular complications of other allergy medications [7].

The practice of allergen immunotherapy was firstly described by Noon in 1911 in treatment of allergic diseases[9]. As subcutaneous allergen-specific immunotherapy is considered a safe method for desensitization, Werfel et al encouraged the use of this technique [10]. Nevertheless other authors as Burastero et al [11] and Malling et al [12] favored the use of sublingual form of immunotherapy in treatment of allergic diseases.

The present study was planned to compare sublingual immunotherapy with subcutaneous immunotherapy in treatment of pollen induced vernal keratoconjunctivitis in children who suffered much more from the refractory symptoms and the serious ocular side effects of medication.

METHODOLOGY

Study Population

This study was a prospective randomized study that was conducted during the period from September 2014 to August 2016.

It included 46 patients with vernal keratoconjunctivitis which were resistant to topical corticosteroids (for more than one month), and antihistamines drugs and/or mast cell stabilizers (for more than 3 months) of treatment. These cases were proven to have an allergy to grass pollen by specific IgE test.

Exclusion criteria include:

- Coexisting of other ocular diseases such as uveitis, glaucoma, and ocular infection.
- Systemic diseases other than accompanying allergic rhinitis, atopic dermatitis and asthma.
- Allergy to any allergen except pollen.
- History of previous immunotherapy treatment.
- The instability of environmental conditions (we excluded from the study patients changing their environmental conditions)

Diagnostic Procedures: Cases were subjected to:

Ophthalmic History: including age, sex, occupation, present history of allergy and family history.

Full ophthalmological examination*:* with stress on conjunctival signs of vernal keratoconjunctivitis

Clinical Scoring System

There are two scoring systems to assess cases of vernal keratoconjunctivitis. The first one is the total subjective symptom scores (TSSS) which depends on patient complaints and the second is the total ocular sign score (TOSS) which is objective depending on the observed ocular signs [13,14]. TSSS and TOSS scores were used for comparison between the two groups. These scores were recorded at first visit then every 3 months. Maximal values of both were 15[14].

The cases were divided according to the route of immunotherapy treatment administration into two groups:

Group A: included 23 children with grass pollen induced VKC treated by sublingual immunotherapy.

Group B: included 23 children with grass pollen induced VKC treated by subcutaneous immunotherapy.

Measurement of serum specific IgE.

Serum was separated from the blood samples (5 mL) at the time of diagnosis and stored at -20. Immune blot assay was used for the quantitative determination of specific IgE in samples against aero-allergens and use anti-goat IgG as positive control with Allergy Screen Panel 2A EGY (MEDIWISS analytic GmbH, Underinger, Germany) according to the manufacturer's instructions. Briefly, serum pipetted into a trough of nitrocellulose membrane coated with particular allergens, followed by addition of biotin coated anti-human IgE antibody, streptavidin conjugated with alkaline phosphatase and substrate; in same order. The colour reaction of each precipitates line on the test trough specified specific antibody content. Serum specific IgE was analyzed by Rapid Reader (Improvio, Germany) using the densitometer curve of the membrane and concentration data for each intensity. The result was expressed in IU/ml with a detection limit of 0.35 kAU/L. The test was valid if positive control Ig E > 3.5 IU/ml.

All our patients were allergic to grass pollen and we measure serum specific IgE level of it at first visit and after 6 and 12 months of treatment.

Measurement of serum eosinophil cationic protein (ECP)

Blood samples were collected from patients and serum was separated and stored at -20 C for quantitative measurement of serum ECP by Human Eosinophil Cationic Protein (ECP) ELISA Kit (AVISCERA BIOSCIENCE, INC. USA) with a sensitivity of 0.05 ng/ml. The micro titer plate included in the kit is coated with antibody specific to ECP. Then samples were added to the plate with polyclonal antibody preparation specific for ECP. We added Avidin conjugated to Horseradish Peroxidase (HRP) to each microplate well and incubated. TMB substrate was then added to the well. Only wells that have ECP, biotin-conjugated antibody and enzyme-conjugated Avidin will display a change in color. The enzyme-substrate reaction was accomplished by the addition of sulphuric acid solution and the change in color is measured spectro-photometrically at a wave length of 450 nm..

ECP test was done to all patients at the start of treatment and at after 6 and 12 months of treatment.

Treatment Protocol

Subcutaneous immunotherapy (SCIT)

SCIT includes two phases the initial build-up phase, when the concentration and dose of allergen immunotherapy extract are increased, and the

maintenance phase, when the patient obtains an effective therapeutic dose over a period of time.

Preparation of vials

We prepare the first 3 vials (1/10000, 1/1000, 1/100) at one time. The Extracts were purchased as an aqueous solution (OMEGA LABORATORIES LTD, Montreal, Quebec Canada) as 5000 PNU. We began preparation with the third vial (1/100) by adding 0.05 ml of extract to 4.95 ml of saline. We utilized 10-fold dilutions in preparing the subsequent allergy vials by adding 0.5 ml of vial 3 to 4.5 ml of saline to prepare 5 ml total volume of vial 2 (1/1000), 1 ml of vial 2 to 4.5 ml of saline to prepare vial 1(1/10000). Maintenance vial is the same concentration as vial 3 (1/100)

Dose protocol

During the initial build-up phase, volumes had increased as follow 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml and finally 1ml of each prepared vial were injected twice weekly subcutaneously. After the initial build-up phase, one ml of maintenance vial (diluation number 3) was administered every week as maintenance treatment. The intervals between maintenance immunotherapy injections were 4 weeks[15].

Sublingual Immunotherapy (SLIT)

SLIT includes 2 phases escalation and maintenance phases. The escalation phase continued for twelve weeks and included 4 vials with increasing concentrations.

Preparation of vials

We prepared the first 4 droppers at the same time. The fourth escalation vial was prepared by making 1: 25 dilution of the grass pollen concentrate; 10,000 BAU/mL (Jubilant HollisterStier LLC 14110 Collections Drive, Chicago USA) (adding 0.2 ml of concentrate to 4.8 ml of 50% glycerine). The panel utilized fivefold dilutions in formulating the subsequent vials by adding 1 ml of vial 4 to 4 ml of 50% glycerin to create five ml total volume of vial 3, one ml of vial 3 to 4 ml 50% glycerine to prepare vial 2, and 1 ml of vial 2 to 4 ml of 50% glycerin to prepare vial 1.

Maintenance vial (10ml vial) was prepared by mixing 0.4 ml concentrate allergen to the dropper and add 50% glycerin to prepare ten mL volume the dropper vial[16,17].

Dose protocol[16,17]

For the escalation phase, from each dropper the patient began with 1 drop every day for 7 days, and then increase the dose to 2 drops daily for 7 days, and finally the patient took 3 drops daily for 7 days. (One drop was about 0.03 to 0.07 mL). The patient repeated the same protocol until the fourth vial was reached (maintenance dose). The patient continued to consume 3drops every day until vial number 4 was depleted.

The following droppers would be at maintenance concentration, and the patient took 3 drops daily for one year[16,17].

RESULTS

This study involved forty six cases with grass pollen induced vernal keratoconjunctivitis as proved by specific IgE test.

All the cases were resistant to medical treatment as topical steroid, sodium chromoglycate, topical and systemic antihistamines for at least one month of treatment.

According to the route of administration of immunotherapy, the cases were divided into 2 groups; group (A) included cases received sublingual immunotherapy (23 cases) and group (B) included cases received subcutaneous immunotherapy (23 cases).

The male-to-female ratio in group (A) was 1.55 (60.68% male and 39.13% females) and in group (B) was 2.29 (69.56% male and 30.43% females), the mean age was 10.9±2.8 years (range 6.1-13.9 years) in group (A) and 8.9±2.3 years (range 5.8-14.5years) in group (B).

There was no statistically significant difference between the two groups as regard to age, sex, type and duration of VKC (table1).

Table 1: Demographic characteristics of the studied groups

	Group (A) (N=23)		***Group (B) N=23)(***		***P value***
Sex	**No**	**%**	**No**	**%**	
Male	**14**	**60.68**	**16**	**69.56**	**0.54**
Female	**9**	**39.13**	**7**	**30.43**	
Age					
Mean ±SD	**10.9±2.8**		**8.9±2.3**		**0.32**
Range	**(6.1-13.9)**		**(5.8-14.5)**		
Age group					
6-10 years	**14(60.86)**		**10(43.47)**		**0.23**
10-15 years	**9(39.13)**		**13(56.52)**		
Duration of disease before ttt	**2.84±2.14 (0.08- 8)**		**4.21±3.41 (2-9)**		**0.53**
Type of VKC					
Limbal	**6 (26.08)**		**6(26.08)**		**0.44**
Tarsal	**12(52.17)**		**15(65.21)**		
Mixed	**5(21.73)**		**2(8.69)**		

There was statistically significant improvement in VKC cases treated with sublingual immunotherapy (group A) as proved by specific IgE, ECP as shown in table 2.

Table 2: Difference in response of sublingual treatment (group A) as regard specific IgE and ECP

Variable	*Before treatment*	*At 6 months*	*At 12 months*	*P value*
Specific IgE **Mean ±SD** **Range**	**17.77± 3.44** **(11- 24)**	**8.99±1.1** **(8-11)**	**5.67±1.05** **(3-7)**	**P1<0.001** **P2<0.001** **P3<0.001** **P4<0.001**
ECP **Mean ±SD** **Range**	**1420.0± 277.19** **(1050- 1950)**	**701.35±141.46** **(570-980)**	**244.60±67** **(130-310)**	**P1<0.001** **P2<0.001** **P3<0.001** **P4<0.001**

P1: represent the difference in response between all the visits of follow up.
P2, 3 and 4: represent this difference between any two individual visits.

There was statistically significant improvement in VKC cases treated with sublingual immunotherapy (group A) as proved by total subjective symptom scores (TSSS) and total ocular signs score (TOSS) as shown in table 3.

Table 3: Difference in response of sublingual treatment (group A) as regard total subjective symptom scores (TSSS) and total ocular signs score (TOSS)

	Before treatment	**At 3months**	**At 6 months**	**At 9months**	**At 12 months**	**P1<0.001** **P2<0.001**
(TSSS) **Mean ±SD** **Range**	**11.89± 1.56** **(9-11)**	**7.99±1.74** **(5-9)**	**3.66±1.07** **(3-7)**	**2.92±0.79** **(2-4)**	**1.38±0.5** **(1-2)**	**P3<0.001** **P4<0.001** **P5<0.001** **P6<0.001** **P7<0.001** **P8<0.001** **P9<0.001** **P10<0.001** **0.001<p11**
(TOSS) **Mean ±SD** **Range**	**12.69±1.39** **(11-15)**	**10.22±1.07** **(9-12)**	**7.2±1.22** **(5-9)**	**4.1±0.76** **(3-5)**	**1. 69±0.73** **(1-3)**	**P1<0.001** **P2<0.001** **P3<0.001** **P4<0.001** **P5<0.001** **P6<0.001** **P7<0.001** **P8<0.001** **P9<0.001** **P10<0.001** **0.001<p11**

P1: represents the difference in response between all the visits of follow up.
P2,3,4,5,…: represent differences between any two individual visits.

There was statistically significant improvement in VKC cases treated with subcutaneous immunotherapy (group B) as proved by specific IgE and ECP as shown in table 4.

Table 4: Difference in response of subcutaneous treatment (group B) as regard specific IgE and ECP

Variable	Before treatment	At 6 months	At 12 months	P value
Specific IgE **Mean ±SD** **Range**	 **19.7± 4.27** **(11-25)**	 **9.69 ±1.81** **(7-12)**	 **5.55 ±1.05** **(4-7)**	**P1<0.001** **P2<0.001** **P3<0.001** **P4<0.001**
ECP **Mean ±SD** **Range**	 **1366.15 ±337.88** **(900- 1900)**	 **667.93 ±156.13** **(450-990)**	 **241.51 ± 55.01** **(150-300)**	**P1<0.001** **P2<0.001** **P3<0.001** **P4<0.001**

P1: represent the difference in response between all the visits of follow up.
P2, 3 and 4: represent this difference between any two individual visits.

There was statistically significant improvement in VKC cases treated with subcutaneous immunotherapy (group B) as proved by total subjective symptom scores (TSSS) and total ocular signs score (TOSS) as shown in table 5.

Table 5: Difference in response of subcutaneous treatment (group B) as regard total subjective symptom scores (TSSS) and total ocular signs score (TOSS)

	Before treatment	At 3months	At 6 months	At 9months	At 12 months	P1<0.001 P2<0.001
(TSSS) **Mean ±SD** **Range**	**10.90±1.33** **(9-14)**	**6.76±1.65** **(5-8)**	**3.72±0.77** **(3-5)**	**2.76±0.76** **(2-4)**	**1.42± 0.07** **(1-2)**	**P3<0.001** **P4<0.001** **P5<0.001** **P6<0.001** **P7<0.001** **P8<0.001** **P9<0.001** **P10<0.001** **0.001<p11**
(TOSS) **Mean ±SD** **Range**	**13.27 ± 0.81** **(11-14)**	**10±0.91** **(8-11)**	**7.34±1.2** **(5-9)**	**3.92± 1.41** **(2-6)**	**1.71± 0.80** **(1-3)**	**P1<0.001** **P2<0.001** **P3<0.001** **P4<0.001** **P5<0.001** **P6<0.001** **P7<0.001** **P8<0.001** **P9<0.001** **P10<0.001** **0.001<p11**

P1: represent the difference in response between all the visits of follow up.
P2, 3, 4, 5, …: represent this difference between any two individual visits.

There was no statistically significant difference between sublingual treatment (group A) and subcutaneous treatment (group B) as regard specific IgE and ECP as shown in table 6 , figure 1 and figure 2.

Table 6: Difference between sublingual treatment (group A) and subcutaneous treatment (group B) as regard specific IgE and ECP

	Sublingual treatment (Group A) (n=23)	Subcutaneous treatment (Group B) (n=23)	P value
Specific IgE			**0.288**
Before treatment Mean ±SD Range	**17.77± 3.44 (11- 24)**	**19.7± 4.27 (11-25)**	**0.315**
At 6months Mean ±SD Range	**8.99±1.1 (8-11)**	**9.69 ±1.81 (7-12)**	**1.01**
At 12 months Mean ±SD Range	**5.67±1.05 (3-7)**	**5.55 ±1.05 (4-7)**	
ECP			
Before treatment Mean ±SD Range	**1420.0± 277.19 (1050- 1950)**	**1366.15 ±337.88 (900- 1900)**	**0.60**
At 6months Mean ±SD Range	**701.35±141.46 (570-980)**	**667.93 ±156.13 (450-990)**	**0.61**
At 12 months Mean ±SD Range	**244.60±67 (130-310)**	**241.51 ± 55.01 (150-300)**	**0.61**

***test of significance : Independent t test**

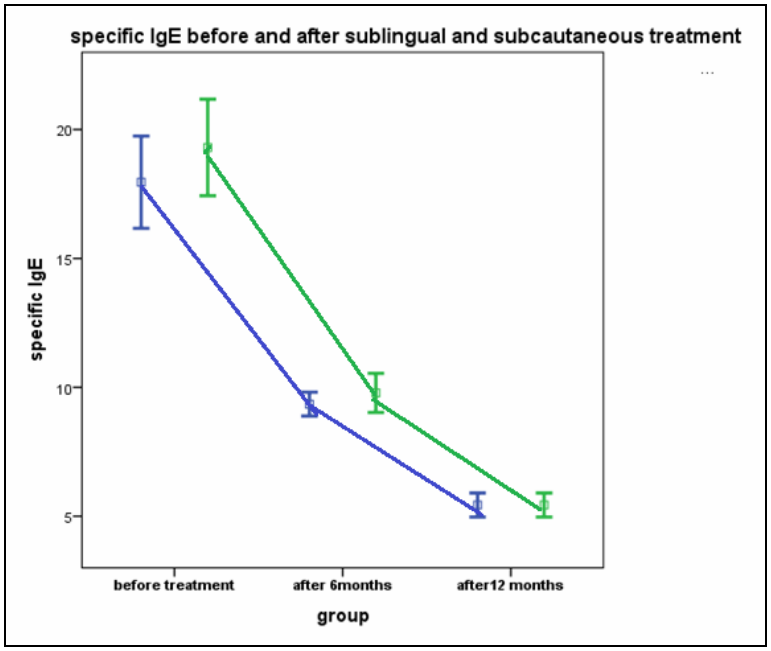

Fig. 1: Difference between sublingual treatment (group A) and subcutaneous treatment (group B) as regard specific IgE.

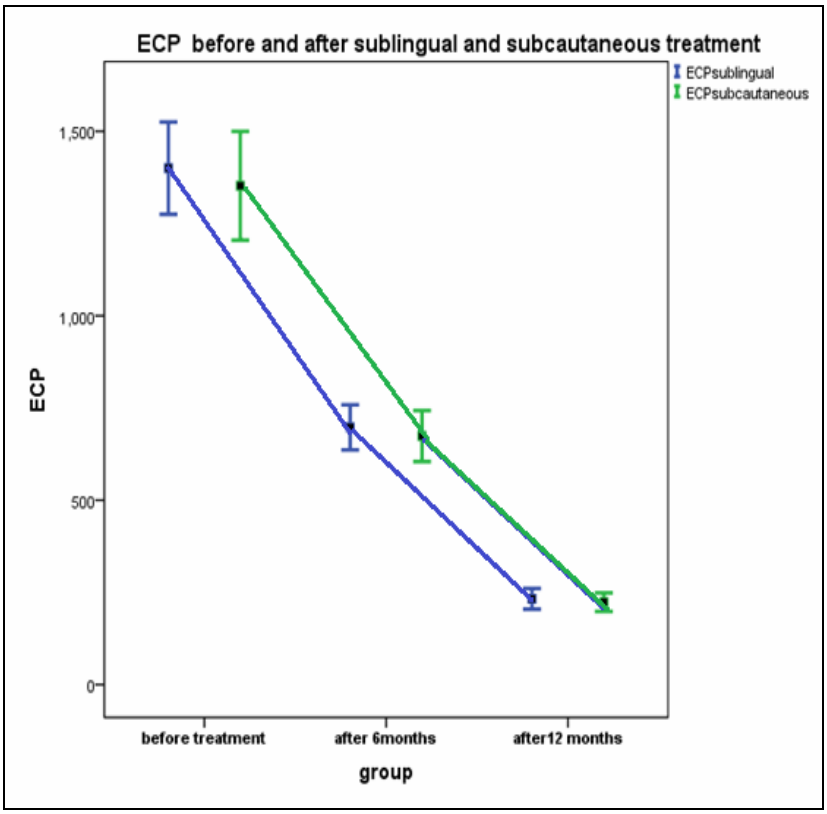

Fig. 2: Difference between sublingual treatment (group A) and subcutaneous treatment (group B) as regard ECP.

There was no statistically significant difference between sublingual treatment (group A) and subcutaneous treatment (group B) as regard total subjective symptom scores (TSSS) as shown in table 7 and figure 3.

Table 7: Difference between sublingual and subcutaneous treatment (group B) as regard total subjective symptom scores (TSSS)

	Sublingual treatment (group A) (n=23)	Subcutaneous treatment (Group B) (n=23)	P value
Before treatment			
Mean ±SD	**11.89± 1.56**	**10.90±1.33**	**0.55**
Range	**(9-11)**	**(9-14)**	
At 3 months			
Mean ±SD	**7.99±1.74**	**6.76±1.65**	**0.187**
Range	**(5-9)**	**(5-8)**	
At 6months			
Mean ±SD	**3.66±1.07**	**3.72±0.77**	**0.88**
Range	**(3-7)**	**(3-5)**	
At 9 months			
Mean ±SD	**2.92±0.79**	**2.76±0.76**	**0.47**
Range	**(2-4)**	**(2-4)**	
At 12 months			
Mean ±SD	**1.38±0.5**	**1.42± 0.07**	**0.43**
Range	**(1-2)**	**(1-2)**	

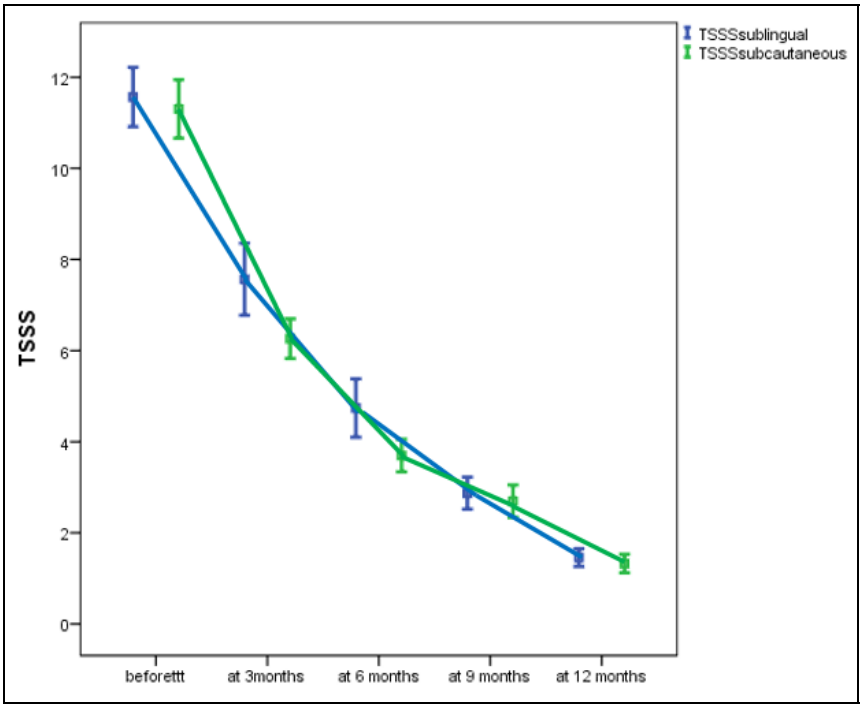

Fig. 3: Difference between sublingual treatment (group A) and subcutaneous treatment (group B) as regard total subjective symptom scores (TSSS)

There was no statistically significant difference between sublingual treatment (group A) and subcutaneous treatment (group B) as regard total ocular signs scores (TOSS) as shown in table 8 and figure 4.

Table 8: Difference between sublingual and subcutaneous treatment (group B) as regard total ocular signs score (TOSS).

	Sublingual treatment (Group A) (n=23)	Subcutaneous treatment (Group B) (n=23)	P value
Before treatment			
Mean ±SD	**12.69±1.39**	**13.27 ± 0.81**	**0.129**
Range	**(11-15)**	**(11-14)**	
At 3 months			
Mean ±SD	**10.22±1.07**	**10±0.91**	**0.34**
Range	**(9-12)**	**(8-11)**	
At 6months			
Mean ±SD	**7.2±1.22**	**7.34±1.2**	**0.38**
Range	**(5-9)**	**(5-9)**	
At 9 months			**0.79**
Mean ±SD	**4.1±0.76**	**3.92± 1.41**	
Range	**(3-5)**	**(2-6)**	
At 12 months			**0.83**
Mean ±SD	**1. 69±0.73**	**1.71± 0.80**	
Range	**(1-3)**	**(1-3)**	

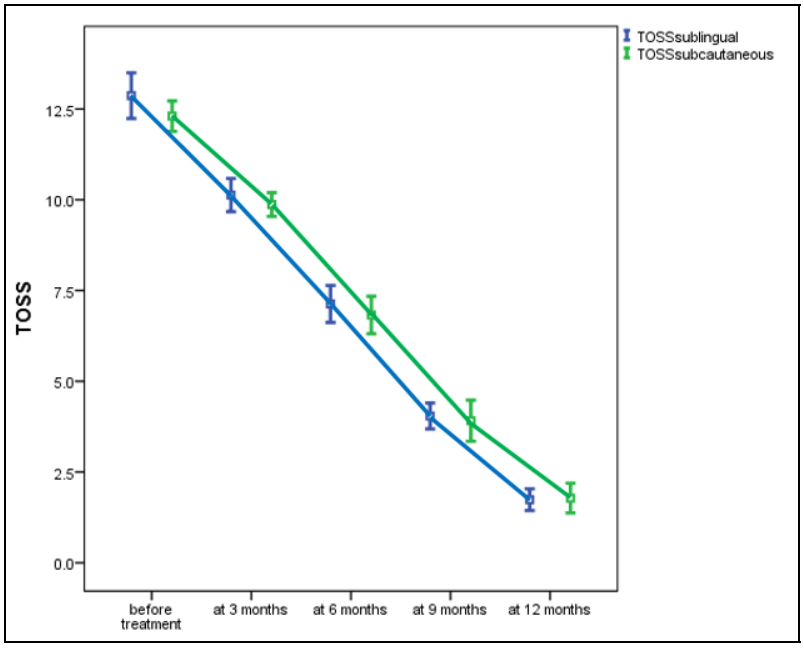

Fig. 4: Difference between sublingual treatment (group A) and subcutaneous treatment (group B) as regard total ocular signs score (TOSS)

DISCUSSION

Vernal keratoconjunctivitis (VKC) is a chronic inflammatory eye disease usually occur in adolescents and children. VKC frequently appears before ten. The disease lasts 2 to 10 years and usually resolves by puberty[18].

Limbal, tarsal and mixed VKC are common types of VKC. Many patients developed corneal lesions as corneal plaque, superficial keratopathy, corneal ulcers and persistent epithelial defects of the cornea[19].

Cases of VKC are regularly treated by steroid eye drops which usually induce serious sequlae as cataracts, infections of the eye and glaucoma. Glaucoma induced from steroid frequantly lead to blindness[20].

VCK is considered one of immunological complications that facing ophthalmologists especially in warm seasons in Egypt, Use corticosteroids may have many complications. Immunotherapy of patients against the causal allergens may have the future strategy in treatment of cases of VKC[7].

Subcutaneous immunotherapy is curative method by modifying immune responses[21] The first research of subcutaneous immunization of allergic patients with grass pollen allergy with allergen extract was published 100 years ago[22]. After that many researches evidenced that SCIT is a useful therapy for VKC[23].

Many researches show that SCIT has good effect in decreasing the development of new allergic diseases. Moreover, it is well recognized that SICT can decrease the risk of development of asthma in children with allergic conjunctivitis[24].

Many years ago, many researches were done to overcome the fallacies of SCIT by instituting alternative methods of immunotherapy. SLIT has many advantages including compliance as injections could be escaped, and the risk of allergic reactions is reduced, making it especially attractive alternative to SIT especially in children[25].

The present study was planned to compare sublingual allergen immunotherapy with subcutaneous allergen immunotherapy in treatment of grass pollen induced vernal keratoconjunctivitis in children who suffered much more from the refractory symptoms and the serious ocular side effects of medication. In our study, we use specific IgE, ECP, TOSS and TSSS scores for comparison between the two groups.

In our study, our patients were allergic to grass pollen as most studies found great correlation between VKC and grass pollen allergy[26]. Clinical scoring of ocular allergy (TOSS and TSSS) was used in evaluation and follow up in treatment of ocular allergy. Also quantitative specific IgE level is a good tool of evaluation[26].

ECP is an ocular marker accurately reflect the clinical status of VKC patients. Measurement of ECP levels may prove useful not only in the diagnosis and monitoring of allergic disease, but also as an objective factor for the evaluation of new anti-allergic therapies including immunotherapy[27].

In our study, there was statistically highly significant difference between results of specific igE and ECP before the start of sublingual immunotherapy (SLIT) treatment, at 6 month and at 12 month of treatment ($p<0.001$). Our data indicate also that there

was statistically highly significant difference between TSSS score and TOSS scores before the start of SLIT treatment, at 3 month, at 6 month and at 12 month of treatment ($p<0.001$). This was in accordance with Volkmar et al. who found that SLIT had reduced medication score.[26] Many studies had identified a significant decrease in medication scores after SLIT particularly for seasonal allergens [28,29].

In contrast to our results, other studies found that there was no significant decrease in medication scores or symptom by SLIT, but numbers of children in these previous studies were small. Moreover, different allergens (Molive pollen, Parietaria and Dermatophagoides pteronyssinus) and dose had been used [30].

Subcutaneous immunotherapy (SCIT) was introduced a century ago. It represents the third most important mainstay treatment accessible to allergic patients and become the only means of changing the abnormal immune response that causes allergic disease [31]. In our study, there was statistically highly significant difference between results of specific IgE and ECP before the start of subcutaneous immunotherapy (SCIT) treatment, at 6 month and at 12 month of treatment ($p<0.001$). Our data indicate also that there was statistically highly significant difference between TSSS score and TOSS scores before the start of SCIT treatment, at 3 months, at 6 months and at 12 months of treatment ($p<0.001$). This was in accordance with Radtke et al who found that subcutaneous immunotherapy (SCIT) researches resulted in decline of 70% of symptoms [32].

In a prospective study, Mahdy et al. comared the effectiveness of SCIT versus medical treatment in 64 Egyptian patients with VKC. The study publicized that 72% of patients treated with SCIT had shown symptom reduction, while 59% in medical treatment ($P<0.05$) reported symptom reduction[33].

Sublingual immunotherapy (SLIT) is a matter of the last 15 years only, but it counterparts that of subcutaneous immunotherapy (SCIT), since the initial studies were only aimed at validating the clinical efficacy. Then, SLIT is now documented as a viable alternative to SCIT [34].

Many studies compare between SLIT and SCIT treatment. Mauro et al. conducted a one-year study on allergic patients with birch-apple syndrome to estimate the effect of SLIT compared with SCIT. It was reported that there was no statistically significant difference between SLIT treatment and SCIT treatment in decrease of symptom scores (4.67 versus 3.93 SLIT versus SCIT) after one year. However, systemic reactions were estimated in SCIT (16%) compared with SLIT (0%) [35].

Our data indicate that there was no statistically significant difference between a long-term immunotherapy treatment with grass pollen SLIT and SCIT in children with VKC as regard specific IgE neither at 6 months nor 12 months of treatment ($P1= 0.315$ and $P1=1.01$). There was also no statistically significant difference between both methods as regard ECP ($P1= 0.61$ and $P1=0.61$). Our study indicate also that there was no statistically significant difference between SLIT and SCIT as regard TSSS score at 3 month ($p=0.187$), at 6 month ($p=0.88$), at 9 month ($p=0.47$), and at 12 month ($p=0.43$) of treatment.

Our study shows also that there was no statistically significant difference between SLIT and SCIT as regard TOSS scores score at 3 month ($p=0.34$), at 6 month ($p=0.38$), at 9 month ($p=0.79$), and at 12 month ($p=0.83$) of treatment. This was in accordance with Antúnez et al. who found that children with respiratory allergic diseases receiving SLIT or SCIT had a similar improvement of clinical scoring [36].

Immunotherapy is a must in any of the following conditions: coexisting allergic rhinitis and asthma; poor response to pharmacotherapy; wish to reduce or avoid long-term pharmacotherapy, unacceptable adverse effects of medications; and the cost of medication; and possible prevention of asthma in patients with allergic rhinitis[33].

Although SCIT is used worldwide, sublingual immunotherapy (SLIT) has been conducted with single allergen extracts more recently. SLIT is considered a viable alternative to SCIT[30].

As SCIT had many disadvantages include, patients receiving beta blockers and severe or unstable asthma. SCIT with food extracts may be effective for severe allergies to fish and peaches, but may be complemented by anaphylactic side effects[32].

In conclusion, sublingual immunotherapy treatment is considered a viable alternative to subcutaneous Immunotherapy treatment as there is better adherence to sublingual Immunotherapy compared to the subcutaneous Immunotherapy treatment. Protocols of SLIT have a more convenient and shorter schedules compared with that of SCIT. Moreover SLIT is preferred in children. In addition, SCIT has more anaphylactic reaction.

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