THE EGYPTIAN JOURNAL OF IMMUNOLOGY Vol. 24 (2), 2017
Page: 127-140

Validity of Specific Immunoglobulin E Assay in Tear Film for Detection of Allergens Inducing Different Types of Allergic Conjunctivitis

Randa S. Abdel-Latif[1], Nissreen E. ELBadawy[1], Basem M. Ibrahim[2]

Departments of [1]Medical Microbiology & Immunology and [2]Ophthalmology, Faculty of Medicine, Zagazig University, Zagazig, Egypt.

This is a prospective interventional comparative study which aimed to investigate correlation between tear film allergen specific IgE levels and the skin prick test in diagnosing different types of allergic conjunctivitis. One hundred twenty patients with allergic conjunctivitis were included in this study and were classified into 4 groups based on the type of allergic conjunctivitis. Patients were subjected to skin prick test (SPT). Micro capillary method was used to collect tear samples for the quantitative assessment of specific IgE by Immune blot assay. The most common allergens were mixed mould, mixed pollen, and mixed mite. The results of tear film specific IgE in detection of allergens were evaluated against the SPT. The Receiving Operating Characteristic Curve (ROCs) revealed that tear film allergen-specific IgE specificity was 100% and sensitivity was 75%-100% to the three common allergens in the 4 studied groups. The correlation between tear's specific IgE and skin prick test was statistically significant for pollen, mite, and mould allergens in patient with SAK (r = 0.821, *P* < 0.001 for pollen, r = 0.964, *P* < 0.001 for mite, and r= 0.811, *P* < 0.02 for mould), PAC (r = 0.851, *P* < 0.001 for pollen, r = 0.826, *P* < 0.001 for mite, and r= 0.861, *P* < 0.001 for mould) and VKC (r = 0.802, *P* < 0.001 for pollen, r = 0.894, *P* < 0.001 for mite, and r= 0.861, *P* < 0.061 for mould). In patient with AKC, the correlation was statistically significant for only mite allergen (r = 1, *P* < 0.001). We concluded that Tear film specific IgE test can be considered as a good alternative to skin prick test in diagnosis of the causative allergens in allergic conjunctivitis.

Allergies are thought to affect about 20% of the general population andallergic eye diseases represent 20% of these allergies [1]. The term allergic conjunctivitis refers to conjunctival inflammation caused by an allergic reaction. This is most commonly type 1 hypersensitivity reaction.Allergic conjunctivitis represents about 15% of all eye problems [2]. Allergic conjunctivitis could be categorized as seasonal allergic conjunctivitis (SAC), perennial allergic conjunctivitis (PAC), vernal kerato-conjunctivitis (VKC) and atopic keratoconjunctivitis (AKC) [3]. History and clinical examination are the basis of diagnosis of an allergic disease and this can be confirmed by means of skin prick tests or measurement of antigen-specific IgE levels so an appropriate diagnosis of allergic conjunctivitis will necessitate assistance between ophthalmologists and allergists [4].

Tear film allergin specific IgE-mediated allergic reactivity can simply be tested for by an in vivo skin prick testor by an in vitro enzyme or fluorescence-based immuno-assay, frequently called a radio-allergosorbent test [5].

Many studies have confirmed tough relationship between serum IgE levels and skin prick test to different allergens, yet the particulars of these relations is still not obvious [6]. Allergen-specific IgE testing is frequently used in the diagnosis of IgE-mediated atopic diseases, but there is still a question about the comparative values of the in vitro IgE assay in comparison to in vivo skin tests [7].

This study was planned to investigate the validity of specific immunoglobulin E assay in tear film for detection of allergens inducing different types of allergic conjunctivitis.

Patients and Methods

This study was carried out in the allergy and immunology unit and ophthalmology department, Zagazig university hospitals between January 2016 and December 2016.

One hundred and seventy six patients with suspected allergic conjunctivitis were examined with a slit lamp for verification of the diagnosis and classification of allergic conjunctivitis. The exclusion criteria were infective conjunctivitis, giant papillary conjunctivitis and toxic conjunctivitis.

Cases of allergic conjunctivitis were classified as follow:

Seasonal allergic conjunctivitis (SAC)

Airborne pollens are usually the causative allergens in SAC. Manifestations usually occur in summer and spring, and decrease in the winter months [8].

Perennial allergic conjunctivitis (PAC)

Diagnostic features of SAC and PAC are similar; but the allergens to which the patient is allergic are different. In PAC the allergens are present throughout the year and the allergy is perennial with exposure to these allergens [9].

Vernal keratoconjunctivitis (VKC)

VKC has three clinical forms: palpebral, limbal, and mixed, with an overall majority in males. Young people are usually affected; the most characteristic sign is giant papillae (cobblestone-like swellings) on the upper tarsal conjunctiva usually 10–20 in number. The cornea may be affected in VKC [10].

Atopic keratoconjunctivitis (AKC)

Eczematous skin lesions (red and elevated) are characteristic and may be found on any place on the body including the eyelids. Ocular findings have a wide range of manifestations [11].

One hundred twenty patients were confirmed to have allergic conjunctivitis and agreed the consent; these patients were classified into four groups according to the type of allergy as follow:

Group 1: includes patients with perennial allergic conjunctivitis (PAC).

Group 2: includes patients with seasonal allergic conjunctivitis (SAC).

Group 3: includes patients with vernal keratoconjunctivitis (VKC).

Group 4: includes patients with atopic keratoconjunctivitis (AKC).

All patient demographic data, clinical data and history were recorded.

The patients were instructed to avoid antihistamines (first generation 3 days, second generation 5 days), systemic corticosteroids (6 days), and mast cell stabilizers (2 days)

All patients were subjected to the following:

Skin prick test (SPT)

A routine skin prick test was performed by aeroallergen panel using kits containing different inhalant allergens, negative control (saline 0.9%) and positive control (histamine 1 mg/ml). It included the following inhalant allergens: mixed pollen (grass, birch, ragweed), mixed mould (Aspergillusfumigatus, Aspergillusniger, Alternaria species), mixed mite (D. pteronyssinus and D. farina), Candida albicans, dog epithelium, cat epithelium, feather mix, cockroach, hay dust, house dust, and smoke, supplied in 5 ml vials (Omega Laboratory-Montreal, Canada).

A drop of solution of each test allergen, and controls was placed on the flexor surface of the forearm, and skin was pricked at drop midpoint.

The reactions were recorded in accordance with the recommendations of the Standardization Committee of the Northern Society of Allergology as shown in table 1 [12].

Table 1.Grading of skin prick test

Grades	Wheal size
Grade 0	No reaction
Grade 1	1/3 of positive control
Grade 2	1/2 to 2/3 of positive control
Grade 3	Same as positive control
Grade 4	Larger than positive control
Grade 5	Pseudopod (irregular wheal)

Tear sampling

A micro capillary method was used to collect tear samples from the temporal side of tear meniscus without local anesthesia, the patient was asked to look in the opposite direction during sampling and not to blink [13].

Measurement of tear specific IgE

Immune blot assay was used for the quantitative determination of specific IgE in tear samples against aeroallergen (same allergens used in the skin prick tests) and anti-goat IgG as positive control with Allergy Screen Panel 2A EGY (MEDIWISS analytic GmbH, Underinger, Germany) according to the manufacturer's instructions. Briefly, tear was pipetted into a trough of nitrocellulose membrane coated with specific allergens, followed by addition of biotin coupled anti-human IgE antibody, streptavidin conjugated with alkaline phosphatase and substrate; in order. The colour reaction of each precipitates line on the test trough indicated specific antibody content. Tear 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 and classified into 6 classes reflecting allergen specific Ig E content of tear according to the manufacture instructions. The test was valid if positive control Ig E> 3.5 IU/ml, (Table 2).

Statistical Analysis

The collected data were coded and analyzed by computer using a data base software program, Statistical Package for Social Science version 19 (SPSS). For quantitative variables mean, standard deviation, and range (minimum and maximum) were computed. Independent t- test was used for quantitative normally distributed data for detection difference between two different groups. Chi squareand Fisher's exact tests were used to detect relation between different qualitative variable. Receiving Operating Characteristic Curve (ROCs) analysis was done to determine the cut off areas under the curve, sensitivity and specificity of specific igE test. Correlation estimates the amount of dependency of one factor on the other, the closeness of the association is measured by the correlation coefficient r. The value of r range from +1 to -1. A test of significance (t- test for correlation) is used to test the level of significance of the association (to measure *P* value).

Table 2. Relationship between the classes found and tear film allergen specific Ig E content

Classes	Ig E level (IU/ml)	Allergen specific IgE
Class I	0.35-0.69	Low
Class II	0.7-3.4	Increased
Class III	3.5-17.4	Significantly increased
Class IV	17.5-49.9	High
Class V	50-100	Very high
Class VI	>100	Extremely high

Results

This study included 120 patients with allergic conjunctivitis. The female to male ratio was 0.79 (44% females&56% male) and the mean age was 21.3 years (range 6 to 46 years); the exclusion for this was VKC where there was a significant male dominance (73%) and younger age with a mean of 11.5 years old. Students represent forty five percent of all cases and 68 % were from rural areas. The majority (40%) of the patients had PAC, (29%) had SAC, (25%) were VKC, and (6%) were AKC.

Sixty percent of patients had a positive family history of allergy of which 32% had allergic conjunctivitis, 36% had allergic rhinitis, 26% had bronchial asthma, and 6% had atopic dermatitis.

There is a statistically significant difference in age and sex distribution in VKC group as regard to other groups as shown in table 3.

Some allergic diseases can be associated with allergic conjunctivitis as shown in table 4.

Table 3. Comparison of demographic data between different studied groups

	PAC (n=48)		SAC (n=35)		VKC (n=30)		AKC (n=7)		*P* value
	N	%	N	%	N	%	N	%	
Sex									
Male	30	62.5	12	34.3	22	73.3	3	42.9	0.007*
Female	18	37.5	23	65.71	8	26.7	4	57.1	
Family history	29	60.4	16	45.7	23	76.7	4	57.1	NS
Age mean±SD	25.4± 12.4		22.3±5		11.5±3		25.9±13.2		<0.001*

* Fishers Exact test. *P* value > 0.05 is not significant(NS) *

Table 4. Allergic diseases associated with allergic conjunctivitis.

	PAC (n=48)		SAC (n=35)		VKC (n=30)		AKC (n=7)		*P* value
	N	%	N	%	N	%	N	%	
No accompanying disease	23	47.9	15	42.9	22	73.3	0	0.0	0.002*
Allergic rhinitis	16	33.3	13	37.1	6	20.0	0	0.0	NS
Bronchial asthma	4	8.3	5	14.3	0	0.0	0	0.0	NS
Eczema	0	0.0	0	0.0	0	0.0	6	85.7	<0.001*
Allergic rhinitis with asthma	1	2.1	0	0.0	1	3.3	0	0.0	NS
Allergic rhinitis with Eczema	0	0.0	0	0.0	0	0.0	1	14.3	NS
Allergic rhinitis with Eczema and asthma	4	8.3	2	5.7	1	3.3	0	0.0	NS

**P* value > 0.5 is not significant (NS). Test of significance is fisher exact test.

In this study skin prick test and tear film specific IgE were done to eleven different allergens and the results revealed that the most common allergens were mixed pollen, mixed mould, and mixed mite, as shown in figure 1.

There was a statistically significant difference in grading results of skin prick test where few cases were grade1 and no cases exceed grade 4 as shown in table 4. No cases were positive to mixed mould in the AKC group neither for skin prick test nor for IgE specific test (Table 5).

There was no statistically significant difference between IgE level to the most common allergens in the studied groups except for the SAC group as shown in table 6.

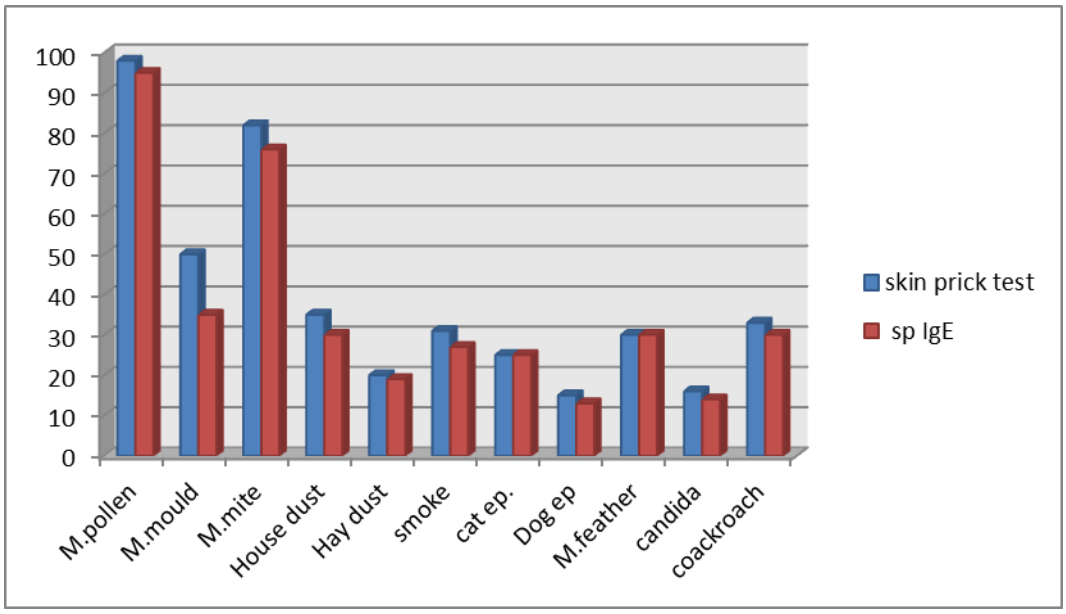

Figure 1. Bar chart showing numbers of positive cases of skin prick test and Specific IgE to different allergens. The most common allergens were mixed pollen, mixed mould, and mixed mite.

Table 5. Grading of skin prick test results for the most common allergens in the studied groups.

	Grade 1		Grade2		Grade3		Grade4		Grade5		*P*- value
* PAC	N	%	N	%	N	%	N	%	N	%	
Mixed pollen(n=35)	5	14.3	11	31.4	18	51.4	1	2.9	0	0.0	
Mixed mite(n=40)	2	5.0	13	32.5	21	52.5	4	10.0	0	0.0	<0.001*
Mixed mould(n=27)	0	0.0	13	48.1	13	48.1	1	3.7	0	0.0	
*SAC											
Mixed pollen(n=31)	2	6.5	10	32.2	18	58.1	1	3.2	0	0.0	
Mixed mite(n=15)	3	20.0	3	20.0	7	46.7	2	13.3	0	0.0	<0.001*
Mixed mould(n=13)	2	15.4	4	30.8	5	38.5	2	15.4	0	0.0	
*VKC											
Mixed pollen(n=25)	2	8.0	10	40.0	12	48.0	1	4.0	0	0.0	
Mixed mite(n=23)	5	21.7	7	30.4	9	39.1	2	8.7	0	0.0	<0.001*
Mixed mould(n=10)	3	30.0	1	10.0	5	50.0	1	10.0	0	0.0	
* AKC											
Mixed pollen(n=7)	0	0.0	1	14.3	5	71.4	1	14.3	0	0.0	
Mixed mite(n=4)	0	0.0	1	25.0	3	75.0	0	0.0	0	0.0	<0.001*
Mixed mould	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	

**P*< 0.5 is significant. PAC: perennial allergic conjunctivitis. SAC: Seasonal allergic conjunctivitis.

VKC: Vernal keratoconjunctivitis. AKC: Atopic keratoconjunctivitis.

Table 6. Classes of tear film specific IgE level to the most common allergens in the studied groups.

	classI		classII		classIII		classIV		classV		*P*- value
	N	%	N	%	N	%	N	%	N	%	
*PAC											
Mixed pollen(n=32)	0	0.0	10	31.2	16	50.0	5	15.6	1	3.1	NS
Mixed mite(n=36)	0	0.0	12	33.3	20	55.6	3	8.3	1	2.8	
Mixed mould(n=20)	0	0.0	11	55.0	7	35.0	2	10.0	0	0.0	
*SAC											
Mixed pollen(n=30)	1	3.3	6	20.0	16	53.3	7	23.3	0	0.0	0.04*
Mixed mite(n=13)	0	0.0	1	7.7	7	53.8	3	23.1	2	15.4	
Mixed mould(n=10)	3	30.0	2	20.0	4	40.0	0	0.0	1	10.0	
*VKC											
Mixed pollen(n=22)	2	9.1	9	40.9	10	45.5	1	4.5	0	0.0	NS
Mixed mite(n=23)	1	4.3	4	17.4	10	43.5	7	30.4	1	4.3	
Mixed mould(n=5)	0	0.0	1	20.0	2	40.0	2	40.0	0	0.0	
*AKC											
Mixed pollen(n=6)	-		1	16.7	4	66.7	1	16.7	0	0.0	NS
Mixed mite(n=4)	-		1	25.0	3	75.0	0	0.0	0	0.0	
Mixed mould(n=0)	-		0	0.0	0	0.0	0	0.0	0	0.0	

**P*> 0.5 is not significant (NS). PAC= perennial allergic conjunctivitis. SAC= Seasonal allergic conjunctivitis. VKC= Vernal keratoconjunctivitis. AKC= Atopic keratoconjunctivitis.

No cases were positive for mixed mould in the AKC group neither for skin prick test nor for IgE specific test.

Validity of tear film specific immunoglobulin E in detection of allergens inducing different types of allergic conjunctivitis were assessed against the skin prick test which is used as a gold standard in this field as shown in tables 7, 8, 9, 10.

There was IgE specificity of 100% and sensitivity ranged from 74% to 100% to the 3 conmen allergens in PAC group (table7).

There was IgE specificity of 100% and sensitivity ranged from 77% to 97% to the 3 conmen allergens in SAC group (table 8).

There was IgE specificity of 100% and sensitivity ranged from 50% to 100% to the 3 conmen allergens in VKC group (table 9).

There was IgE specificity of 100% to mite and sensitivity ranged from 86% for pollen to 100% for mite in patients with AKC (table 10).

There was a statistically significant correlation between specific IgE and skin prick test in patients with PAK (table 11 and figure 2).

There was a statistically significant correlation between tear film specific IgE and skin prick test in patients with SAK (table 12 & figure 3).

There was a statistically significant correlation between tear film specific IgE and skin prick test in patients with VKC except for mould allergen (table 13 and figure 4).

There was a statistically significant correlation between tear film specific IgE and skin prick test for mite allergen in patients with AKC (table 14 and figure 5).

There was statistically significant correlation between tear film specific IgE and skin prick test in all studied groups (table 15).

Table 7. Validity of detection of tear film specific IgE test in diagnosis of patients with PAC

	Cut off	AUC	95%CI	*P Value	sensitivity	specificity	PPV	NPV	Accuracy
Pollen	0.35>	0.969	0.872 - 0.998	<0.0001	91.42	100.0	100.0	81.25	93.75
mite	0.35>	0.961	0.861 - 0.996	<0.0001	90.0	100.0	100.0	66.67	91.66
mould	0.3>	94.4	0.837- 0.990	<0.0001	74.07	100.0	100.0	75.0	85.41

**P*< 0.5 is significant.

Table 8. Validity of detection of tear film specific IgE test in diagnosis of patients with SAC

	Cut off	AUC	95%CI	*P Value	sensitivity	specificity	PPV	NPV	Accuracy
Pollen	0.35>	0.969	0.872 - 0.998	<0.0001	91.42	100.0	100.0	81.25	93.75
mite	0.35>	0.961	0.861 - 0.996	<0.0001	90.0	100.0	100.0	66.67	91.66
mould	0.3>	94.4	0.837- 0.990	<0.0001	74.07	100.0	100.0	75.0	85.41

**P* value < 0.5 is significant.

Table 9. Validity of detection of tear film specific IgE test in diagnosis of patients with VKC

	Cut off	AUC	95%CI	*P Value	sensitivity	specificity	PPV	NPV	Accuracy
Pollen	0.35>	0.969	0.872 - 0.998	<0.0001	91.42	100.0	100.0	81.25	93.75
mite	0.35>	0.961	0.861 - 0.996	<0.0001	90.0	100.0	100.0	66.67	91.66
mould	0.3>	94.4	0.837- 0.990	<0.0001	74.07	100.0	100.0	75.0	85.41

**P*< 0.5 is significant.

Table 10. Validity of detection of tear film specific IgE test in diagnosis of patients with AKC

	Cut off	AUC	95%CI	*P Value	sensitivity	specificity	PPV	NPV	Accuracy
Pollen	0.35>	0.969	0.872 - 0.998	<0.0001	91.42	100.0	100.0	81.25	93.75
mite	0.35>	0.961	0.861 - 0.996	<0.0001	90.0	100.0	100.0	66.67	91.66
mould	0.3>	94.4	0.837- 0.990	<0.0001	74.07	100.0	100.0	75.0	85.41

**P*< 0.5 is significant.

Table 11. Correlation between tear film specific IgE test and skin prick test in patients with PAK

Variable	Tear's specific IgE	
	r	**P* value
Skin prick test		
Pollen	0.851	<0.001
Mite	0.826	<0.001
Mould	0.861	<0.001

***P* value **< 0.5 is significant**

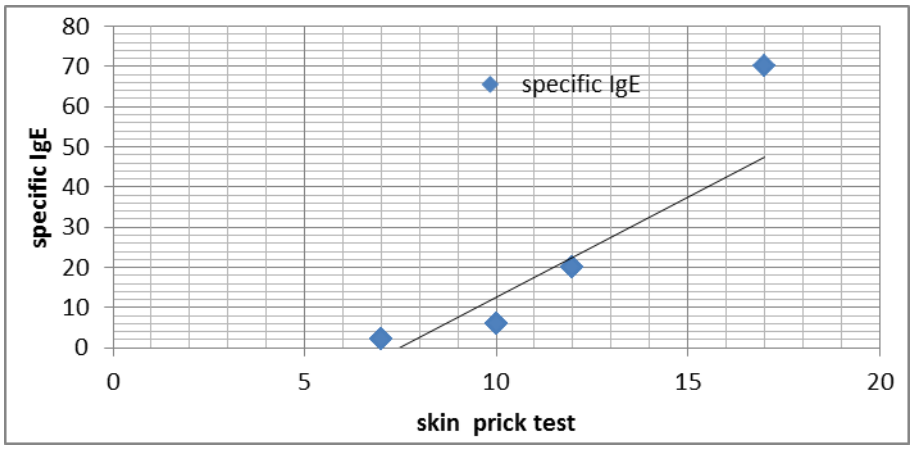

Figure 2. Correlation between tear film specific IgE and skin prick test in patients with PAC.

Table 12. Correlation between tear film specific IgE test and skin prick test in patients with SAC

Variable	Tear's specific IgE	
	r	**P* value
Skin prick test		
Pollen	0.821	<0.001*
Mite	0.964	<0.001*
Mould	0.811	0.02*

**P*> 0.5 is not significant (NS).

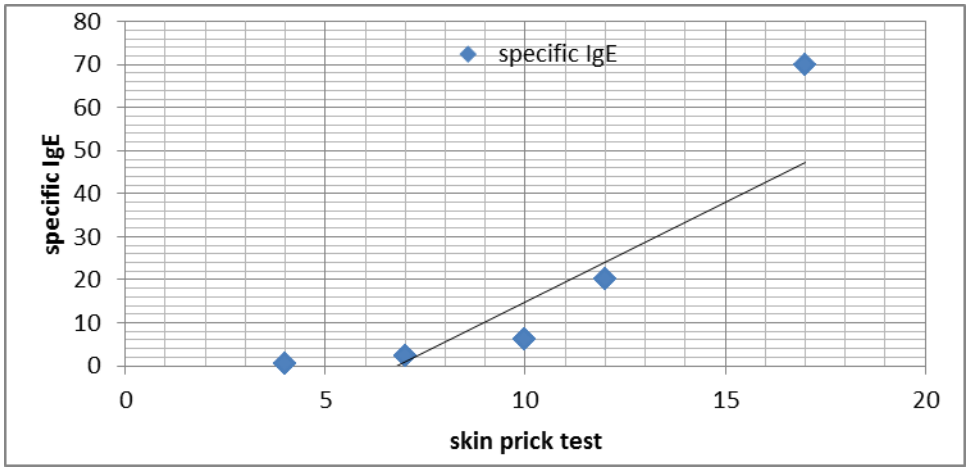

Figure 3. Correlation between tear film specific IgE and skin prick test in patients with SAC.

Table 13. Correlation between specific IgE test and skin prick test in patient with VKC.

Variable	Tear's specific IgE	
	r	*P* value
Skin prick test		
Pollen	0.802	<0.001*
Mite	0.894	<0.001*
Mould	0.861	NS

**P*> 0.5 is not significant (NS).

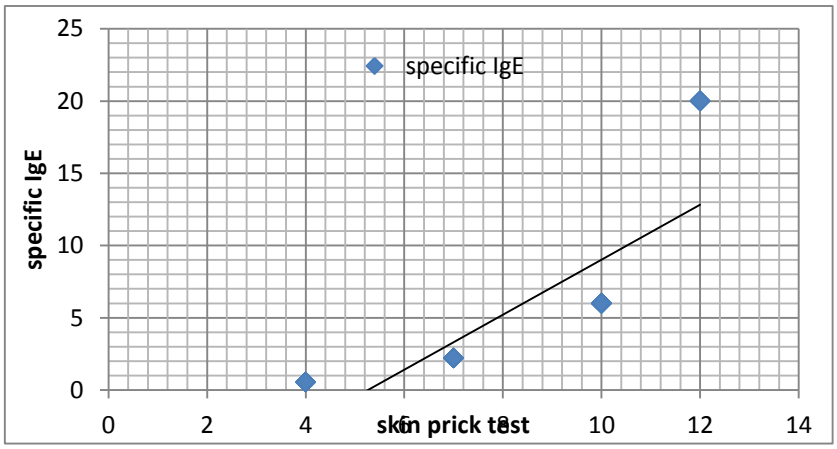

Figure 4. Correlation between tear film specific IgE and skin prick test in patients with VKC.

Table 14. Correlation between tear film specific IgE test and skin prick test in patients with AKC .

Variable	Tear's specific IgE	
	r	*P value
Skin prick test		
Pollen	0.826	0.043
Mite	1.000	<0.001
Mould	-	-

**P*> 0.5 is not significant (NS).

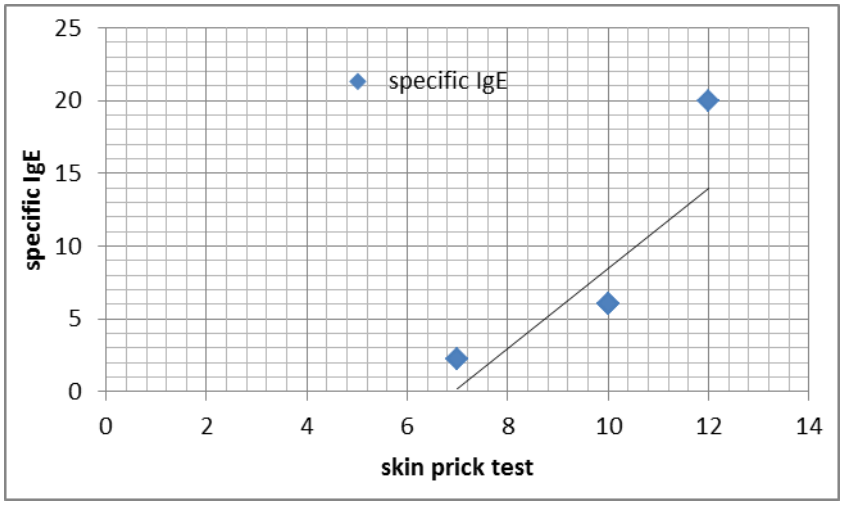

Figure 5. Correlation between tear film specific IgE and skin prick test in patients with AKC.

Table 15. Correlation between tear film specific IgE test and skin prick test in all studied groups.

Variable	Tear's specific IgE	
	r	*P value
Skin prick test		
PAC	0.871	< 0.001*
SAC	0.839	< 0.001*
VKC	0.790	< 0.001*
AKC	0.829	0.02*

**P*< 0.5 is significant.

Discussion

Allergy has significantly increased in the last few decades. Allergic conjunctivitis is a very common condition encountered in clinical practice [14]. Allergic conjunctivitis is mainly caused by type I hypersensitivity reaction. Allergic conjunctivitis includes four types: perineal conjunctivitis (PAC), seasonal conjunctivitis (SAC), vernal keratoconjunctivitis (VKC) and atopic keratoconjunctivitis (AKC) [15]. Seasonal allergic conjunctivitis and perennial allergic conjunctivitis affects about 15–20% of the

population and are the most common forms of ocular allergies [15]. In this study 40% of patients were PAC, (29%) were SAC, (25%) had VKC, and (6%) had AKC.

In this study, allergic rhinitis represents the most common accompanying allergic disease followed by bronchial asthma then eczema. As regard AKC, czema was the most common accompanying allergic disease. Tolga *et al.* found that associated allergic rhinitis was present in 73.7% of his patients with allergic conjunctivitis [16].

Hesselmar *et al.* and Kosrirukvongs *et al.* found a higher percentages of associated allergic rhinitis in their series 88% and 92% respectively [17,18]. Hay fever or allergic rhino-conjunctivitis are non specific terms used to describe such allergies [19]. Approximately 50% of the patients have a self or family history of other allergy [16]. In this study 60% of patients had a positive family history of allergy, genetic factors may play a role in development of allergic rhino-conjunctivitis [20].

Ocular allergy diagnosis is mainly clinical, but some laboratory tests can help the diagnosis. Skin prick tests or intra-dermal injections of allergen can be performed by allergists for specific allergens [15]. Skin testing is considered the gold standard in allergy diagnosis [21]. *in-vitro* tests for IgE antibodies to specific allergens are widely used [15]. As regards ocular allergy IgE has been considered to play an important role; thus it can be useful in diagnosis of such conditions [22].

This study was designed to evaluate correlation and validity of tear film specific IgE in relation to the skin prick test (gold standard) in different types of allergic conjunctivitis. Measurement of serum IgE concentration which is usually used for diagnosis of allergic diseases does not differentiate between local and systemic allergy [13].

In allergic conjunctivitis the regulation of IgE production at local levels is largely dependent on inflammatory cells. IgE combines with the antigen on the surface of local mast cells. Afterward, these cells transfer to the systemic circulation and at the same time as IgE local concentrations is high, a very low systemic concentrations of IgE is present [23]. Friedlaender *et al*. found that measurement of IgE in tear samples is useful for both the diagnosis of allergic conjunctivitis and determinations of its severity [24].

In this study there were statistically significant correlations ($P< 0.05$ in all the studied groups) between specific IgE of tear samples and the skin prick test ($r = 0.871$, 0.839, 0.790 and 0.829) in the four studied groups respectively. This was in agreement with Wohrl *et al.* who found 85% - 95% agreement between the specific IgE antibody assays and SPT results [25]. Demir *et al*. (2011) stated that all allergens had significant correlations between their specific IgE levels and skin test results with the exception of molds allergens [26]. Cho *et al.* found that there was 81-97% agreement between SPT and individual specific IgE test in allergen detection [27]. The validity of IgE varies with the quality of the allergen and the system being used. Over all IgE specificity ranges from 30% to 95% and sensitivity ranges from 60% to 95% [28, 29].

In our study there was high sensitivity to mite (100, 86.66,100 and100%) and pollen (91.42, 96.77, 92, and 85.71%) in all the groups respectively. Sensitivity to mould was (74.07, 76.92, and 50%) in the first three groups respectively (AKC group had no allergy to mould).

In our study there was high specificity to mite (100, 100, 100, and 100%) and pollen

(100, 100, 100% and 100) in the all groups respectively, and to mould (100, 100, and 100%) in the first three groups respectively (AKC group had no allergy to mould). This was in agreement to the results of Attia *et al*. who found specific IgE sensitivity, specificity, positive predictive value and negative predictive value were 93.1, 88.9, 96.2, and 93.7 respectively [30]. Values of 100% specificity and more than 90% sensitivity were recorded by Williams *et al.* for specific IgE of pollens of grasses, dust mites, and cat hair [31]. In contrast to our results Popielet *et al.* founded that the specificity of the group of home dust mites was only 40% and sensitivity was 85%. He also found that the sensitivity of storage mites was 90.24% however the specificity was only 25%. [32].

In evaluation of allergic patients, skin tests are the most clinically appropriate techniques because of their ease, natural application in the patient's own skin, little time of performance, low cost, and high sensitivity [33]. As skin tests are physiologic procedures, they necessitate a degree of expertise by the observer to interpret the results and link them with the history and physical findings. Improperly performed or interpreted skin test can lead to false negative or false positive results. Also skin responsiveness varies between patients, so positive result will be clearer in some patients than others. Therefore other factors may play a role in correct interpretation of the test such as patient's history and the patient's response to antigens and controls [21].

Other limitations to skin tests include that the test should be performed with a physician available to treat side effects, including anaphylaxis and also skin testing should generally be performed on normal skin [34]. Some antihistamines, tricyclic antidepressants and tranquilizers suppress the wheal and flare response and should be stopped before kin test for up to 10-19 days or even more [35]. In patients taking beta blocking agents, skin testing was considered potentially problematic because of blunted response to treatment such as epinephrine or a risk of an exaggerated adverse reaction [36]. Long-term corticosteroid treatment may alter skin test sensitivity although short time corticosteroid does not have the same effect. Skin prick test is safe in pregnancy but if anaphylaxis occurs, the fetus might be adversely affected [37]. It is difficult to interpret skin test results in patients with pigmented skin. We should not perform SPT in areas where any skin lesions are present as they may interfere with the skin test reactivity [21]. In addition patients with chronic disease such as diabetes, renal failure or patients with spinal cord injuries may have a decrease in skin test sensitivity [38].

In conclusion, the correlation between tear specific IgE and the skin prick test in different types of allergic conjunctivitis suggests that tear specific IgE has a high sensitivity and specificity in relation to the skin prick test. Tear IgE concentrations can be measured in small tear samples easily and less frightening especially to a young patient. It also does not carry the risk of anaphylaxis or other adverse effects of the skin prick test. Unlike skin test, tear specific IgE doesn't depend on the observer's experiences, nor affected by individual variation in response. In addition, no need to stop medications before IgE testing and during pregnancy there is no possible undesirable effect on the fetus if adverse reaction occurs during skin testing. On the other hand skin test reactivity or interpretation can be affected by patient's age, skin lesions, skin color and chronic

diseases. As regard allergic conjunctivitis, tear film specific IgE has high sensitivity and specificity and less complications and can be a good substitute to skin brick test in diagnosis of the causative allergens.

References

1. Dumavibhat P, Sri Supan V, YenjitW, Hanson KT. Research strategy: ophthalmic diseases. In: Bharnarapravati N, Ton tisirin K, Porapakkham Y. eds, Strategic plan for health research. Bangkok, P A Living Co, 1998; pp. 194-211.
2. Bielory L, Katelaris CH, Lightman S, Naclerio RM. Treating the ocular component of allergic rhinoconjunctivitis and related eye disorders. Med Gen Med 2007; 9:35.
3. Pepose JS, Holland GN, Welhelmus KR eds, Ocular infection and immunity. St. Louis, Mosby, 1996; pp. 345-90
4. Stazi MA, Sampogna F, Montagano G, Grandolfo ME, Couilliot MF, Annesi-Maesano I Early life factors related to clinical manifestations of atopic disease but not to skin-prick test positivity in young children. Pediatr Allergy Immunol 2002; 13:105-12.
5. Holt PG. Development of sensitization versus tolerance to inhalant allergens during early life. Pediatr Pulmonol Suppl 1997; 16:6-7.
6. Oryszczyn MP, Annesi-Maesano I, Campagna D, Sahuquillo V, Huel, Kauffmann. Head circumference at birth and maternal factors related to cord blood total IgE. Clin Exp Allergy 1999;29:334-337
7. O'Brien RM. Skin prick testing and in vitro assays for allergic sensitivity. Australian Prescriber 2002; 25:91-3.
8. Celedon JC, Soto-Quiros ME, Hanson LA, Weiss ST. The relationship among markers of allergy, asthma, allergic rhinitis, and eczema in Costa Rica. Pediatr Allergy Immunol 2002; 13:91-7.
9. Friedlander MH. Ocular Allergy .CurrOpin Allergy ClinImmunol. 2011; 11(5):477–482.
10. Bielory L. and Frohman L. Allergic and immunologic disorders of the eye. J Allergy ClinImmunol. 1992 ; 86:1–20.
11. Bielory B., Bielory L. Atopic dermatitis and keratoconjunctivitis. Immunol Allergy Clin North Am. 2010; 30:323–336.
12. Aas, K., Belin, L. Standardization of diagnostic work in allergy. Int Arch Allergy ApplImmunol. 1973; 45:57-60
13. Nomura K., Takamura E. Tear IgE concentrations in allergic conjunctivitis. Eye. 1998; 12,296-298.
14. Verlato G, Corsico A, Villani S, Cerveri I, Migliore E, Accordini S. Is the prevalence of adult asthma and allergic rhinitis still increasing. Results of an Italian study. J Allergy Clin Immunol, 2003; 111:1232–1238.
15. Mario La Rosa, Elena Lionetti , Michele Reibaldi, Andrea Russo, Antonio Longo, Salvatore Leonardi. Allergic conjunctivitis: a comprehensive review of the literature. Italian J Pediat. 2013; 39:18
16. Tolga Kocatürk, ÖzlemKocatürk, Volkan Dayanir. Skin prick test and immunotherapy in children with allergic eye disease. Turk J Med Sci. 2012; 42 (3): 485-490.
17. Hesselmar B, Aberg B, Eriksson B, Aberg N. Allergic rhinoconjunctivitis, eczema, and sensitization in two areas with differing climates. Pediatr Allergy Immunol 2001; 12: 208–15.
18. Kosrirukvongs P, Visitsunthorn N, Vichyanond P, Vichyanond P and Bunnag C. Allergic conjunctivitis. Asian Pac J Allergy Immunol 2001; 19: 237–44.'
19. Gradman J, Wolthers OD. Allergic conjunctivitis in children with asthma, rhinitis and eczema in a secondary outpatient clinic. Pediatr Allergy Immunol 2006; 17: 524–526.
20. Fokkens W, Lund V, Bachert C, Clement P, Helllings P, Holmstrom M. EAACI position paper on rhinosinusitis and nasal polyps executive summary. Allergy 2005; 60:583-601
21. AAAAI Work Group Report: Allergy Diagnosis in Clinical Practice. Joint Task Force on Practice Parameters representing the American Academy of Allergy, Asthma & Immunology, the American College of Allergy, Asthma and Immunology, and the Joint Council on Allergy, Asthma and Immunology. Ann Allergy Asthma Immunol. 2006; 97-111.

22. Kari O, Salo O, Bjorksten F, Backman E. Allergic conjunctivitis, total and specific IgE in the tear fluid. Acta Ophthalmol (Copenh) 1985; 63:97-9.

23. Kelly D. Stone, CalmanPrussin, Dean D. Metcalfe. IgE, Mast Cells, Basophils, and Eosinophils.J Allergy ClinImmunol. 2010; 125(2 Suppl 2): S73–S80.

24. Freidhoff LR, Meyers DA, Marsh DG. A geneticepidemiologic study of human immune responsiveness to allergens in an industrial population. II. The associations among skin sensitivity, total serum IgE, age, sex, and the reporting of allergies in a stratified random sample. J Allergy Clin Immunol 1984; 73:490-9.

25. Wohrl S, Vigl K, Zehetmayer S, Hiller R, Jarisch R, Prinz M, Stingl G, Kopp T. The performance of a component-based allergen-microarray in clinical practice. Allergy 2006; 61(5):633–639.

26. Esen Demir, Levent Midyat, Figen Gulen. correlation of serum specific IgE and skin prick tests in children with respiratory allergy. Yeditepe Med J 2011;5(19): 421-428

27. Cho J H, Suh J D, Kim J K, Hong SC, Park IH, Lee HM. Correlation between skin-prick testing, individual specific IgE tests, and a multi allergen IgE assay for allergy detection in patients with chronic rhinitis. American Journal of Rhinology & Allergy 2014;28 (5): 388-391(4)

28. Nolte H, DuBuske LM. Performance characteristics of a new automated enzyme immunoassay for the measurement of allergenspecific IgE. Summary of the probability outcomes comparing results of allergen skin testing to results obtained with the HYTEC system and CAP system. Ann Allergy Asthma Immunol 1997; 79:27-34. 26)

29. Williams PB, Dolen, WK, Koepke, JW, Hiller TY. Comparison of skin prick testing and three in vitro assays for specific IgE in the clinical evaluation of immediate hypersensitivity. Ann Allergy 1992;68:35

30. Attia MY, Mohamed HS. Assessment of Commercial Specific IgE Assay for Detection of Allergens in Allergic Patients. Journal of Analytical Sciences, Methods and Instrumentation, 2013; 3: 110-114.

31. Williams PB, Barnes JH, Szeinbach SL, Sulivan TJ. Analytic precision and accuracy of commercial immunoassays for specific IgE: Establishing a standard. J Allergy Clin Immunol 2000; 105:1221-30.

32. Popiel J. and Cekiera A. Comparison of IgE test results with intradermal skin tests for dust mites and storage mites in atopic dogs. Polish J Vet Sci 2015; 18(2): 351–356

33. Lucie Heinzerling, Adriano Mari, Karl-Christian Bergmann. The skin prick test – European standards. ClinTransl Allergy 2013; 3: 3.

34. Rao KS, Menon PK, Hilman BC, Sebastian CS, Bairnsfather L. Duration and suppressive effect of tricyclic antidepressants on histamine-induced wheal-and-flare reactions in human skin. J Allergy Clin Immunol. 1988; 82:752-7.

35. Almind M, Dirksen A, Nielsen NH, Svendsen UG. Duration of the inhibitory activity on histamine-induced skin weals of sedative and non-sedative antihistamines. Allergy 1988;43(8):593-6.

36. Hepner MJ, Ownby DR, Anderson JA, Rowe MS, sears-Ewald RD, Brown EB. Risk of systemic reactions in patients taking beta-blocking drugs receiving allergen immunotherapy injections. J Allergy Clin Immunol. 1990; 86:407-11.

37. Schatz M, Hoffman CP, Zeiger RS, Barnes MH. The course of management of asthma and allergic diseases during pregnancy. In: Middleton E Jr, Reed CF, Ellis EF, eds. Allergy: Principles and Practice. St Louis, MO: CV Mosby Co., 1988, 1092-1155.

38. Lockey RF, Benedect LM, Turkeltaub PC, Bukantz SC. Fatalities from immunotherapy (IT) and skin tests (ST). J Allergy Clin Immunol. 1987;79:660-77.