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Evaluation the Efficacy of Immunotherapy in Treatment of Bronchial Asthma

*Seham El-Mansi,**Abd Allah El-Bialy,*** Mohsen El-Shafey,*Shaimaa Mohamed Ebrahim El-Sayed
Zoology Department, Faculty Of Science Damietta*,Faculty Of Medicine Zagazig University**, Faculty Of Medicine Mansoura University***

Abstract

The study was carried out on 50 allergic asthmatic children in rural district in sherbin,dakahlia and received their medication and immunotherapy from clinical immunology unit,zagazig university hospital.fifty patients were divided into 40 patients as active immunotherapy group and 10 patients as control group.
Immunotherapy was administered according to preseasonal schedule six month before pollen season.all patients have positive skin prick test and were evaluated clinically and immunologically pre and post immunotherapy (for six month),

Results
significant decrease in total serum IgE level P=0.018 ($p<0.05$)and significant increase in total serum IgG ($P<0.001$) after immunotherapy in all patients So immunotherapy is considered line of treatment for allergic patients.

Key words
Asthma,Chlidren,Total IgE,Total IgG.

Introduction

Asthma and allergies are among the most chronic disease,asthma is reported to be the most frequent cause of childhood disability(Martin Munoz, 2004)
Pollen allergy

Life-threatening reactions may be observed with high quality extracts and exceptional deaths have been reported (Bousquet *et al.*, 1994a). The rate of systemic reactions is greater with standardized pollen extracts than with either non-standardized extracts or high molecular weight preparations (Bousquet *et al.*, 1989,Grammer *et al.*, 1986and Grammer *et al., 1987).* However, using SIT with care it has been shown that standardized extract can be used safely (Hejjaoui *et al.*, 1992).
Allergen immunotherapy in children is effective and well tolerated.It has been shown to prevent the new onest of allergen sensitivities in monosensitized patients as well as progression from allergic rhinitis to asthma(lind *et al*.,2010)

Immunotherapy induces a switch of the preferential differentiation of native t helper cell from TH2 type effector cells to TH1 type cells(Till *et al*.,2004)

• Allergen immunotherapy works like a vaccine. Patients receiving allergen *immunotherapy are injected with increasing amounts of an allergen over several months. The body responds to the injected amounts of the allergen by developing an immunity or tolerance to it. As a result, allergy symptoms can* be decreased or minimized when the patient is exposed to that allergen in the future.

- Immunotherapy acts by modifying T cell responses either by immune derivation (increase in Th_0/Th_1) or T cell energy (decrease Th_2/Th_1) or more likely both, depending on a number of factors including the nature of the allergen, the allergen dose, adjuvants used.
- Shortly after initiation of

immunotherapy there is an increase in CD4+CD25+ regulatory T-Iymphocytes secreting IL-10 and TGF-B

- Associated with immunologic tolerance, defined as a long-lived decrease in allergen-specific T-cell responsiveness. With continued immunotherapy there is some warning of this response and immune deviation from Th_2 to Th_1 cytokine response to the administered allergen predominates.
- Immune deviation from a Th_2 in favor of aTh_1 cytokine profile. Data indicate that increases in production of IL-12, a strong inducer of TH1responses, may contribute to this shift(lind *et al*.,2010)
- During conventional immunotherapy serum IgE concentrations initially rise and gradually fall to normal over months(Lichtenstein,1973) .
- An increase in serum allergen-specific IgG levels, particularly of the IgG4 isotope, has also been associated with immunotherapy..
- Blocking antibody theory (Golden *et al*.,1982)IgG compete with IgE for allergen binding thereby blocking IgE-dependent activation of mast cell .

Patients and Methods:

Fifty extrinsic asthmatic children sensitive to grass pollen were enrolled in this study (30male and 20 female).Their age ranged from 5-12years(mean8.5years).the fifty patients were divided into 40 patients as active immunotherapy group and 10 patients as control group.All patients lived in rural district in sherbin,dakahlia and received their medication and immunotherapy from allergy and clinical immunology unit,zagazig university hospital.All patients have positive skin prick test to grass pollen.Clinical and immunological evaluation was performed to every patient.Clinical evaluation include symptoms ,medication score and peak expiratory flow rate.

Immunological evaluation include skin test,determination of total serum IgE by ELISA,and total serum IgG by ELISA (radial immunodiffusion method)

1.Determination of total serum IgE (Silva *et al*.,2001):

heparinized plasma from patients were collected and stored at 20c° until the time of assay .The quantizyme immunoassay kit was used(Silva *et al*.,2001)

Sample preparation(dilute patient sample 1/5 with normal saline)

1-Add 50 of IgE zero calibration standard to zero tubes.
2- Add 50 of undiluted IgE calibration standards and control to the appropriately labeled tubes.
3- Add 50 of each diluted sample to the appropriate labeled tubes.
4- Add 200 of IgE zero calibration standard to all tubes .
5- Mix reagents in tubes thoroughly by vortexing.
6- Incubate all tubes one hour at 37c°±2 c°.
7- Wash all tubes 3 times with 2000 of normal saline. Aspirate
thoroughly after each wash.
8- Add 250 of total IgE conjugate to all tubes.
9- Incubate all tubes one hour at 37c° ±2 c°.
10- Wash 3 times as described in step 7.
11- Add 250 of prepared substrate working solution to each tube in carefully timed intervals.
12- Incubate *a*ll tubes one hour at room temperature .
13- Add 1000 of stopping solution to each tubes at the same time interval as the addition of the substrate working solution.
14- Mix reagents in tubes thoroughly by vortexing
15-Read absorbance on ELISA wavelength reader at 450nm.

2- Determination of serum IgG level by ELISA(radial immunodiffusion *method)(Mancini et al., 1965):*

By using single radial immunodiffusion method (Kallestad Endoplate IgG) .
This technique based on the fact that antigen - antibody interaction is manifested

as precipitation ring and can be visualized in gels such as agar. If an antigen is placed in a well within antibody containing agar gel, it diffuses into the agar. Then diameter of precipitation ring becomes manifested around the antigenic well. The diameter of precipitation ring is directly related to the concentration of the antigen.

Procedures:

- The immunodiffusion plate was opened and allowed to remain uncovered at room temp. for 5 minutes (to allow evaporation of any water condensation).
- 5 microL of patient's serum was dispensed onto wells and the plate was covered tightly and incubated for 48 hours at room temp.
- After the diffusion time had elapsed, the diameter of each precipitation ring was measured using the magnifying lens against black ground.
- The corresponding concentration of the antigen (IgG) was measured from a standard table for conversion of ring diameter.

Statistical analysis:

The statistical analysis was done using IBM compatible computer using the SPSS / PC + statistical package (SPSS Inc. Chicago, IL) The statistical methods were done for the data of this work according to *(Barnett*, 1979).

Results

We recruited 50 patients, 40 patients in the active treatment group (group A) and 10 patients as control group (group B).

The results of the study are shown in the following tables.

Table (1): Demographic data of the studied groups.

	Cases (N= 40)	Control (N= 10)	Test of sig.	P
Age (years) X±SD	7.37 ± 2.8	7.1±2.8	T=0.77	0.28 N.S.
(Range)	(4-12)	(4-12)		
Gender	No%	No. %		
Male	24 60.0	6 60.0	X2=0.0	1.0 N.S.
Female	16 40.0	4 40.0		
Duration X±SD	5.4 ± 3.3	5.2 ± 3.3	T= 0.18	0.8 N.S.
(Range)	1-12	2-12		
Family history % of atopy	40 100.0	10 100.0	N.S.	

There is no significant difference as regard age, sex, duration of illness.
SD = standard deviation.
N = number. N.S. = non significant.

Table (2): Severity of asthma of cases and control before immunotherapy.

	Cases		**Control**		X2	p
	No.	O/O	No.	O/O		
Moderate	23	57.5	6	60.0	0.02	0.8
Severe	17	42.5	4	40.0	N.S.	

There is no significant difference between clinical types among cases and control.

Table (3): Symptoms and medication scores of cases and control before immunotherapy.

	Cases	**Control**	T	p
Nasal symptoms	71 ± 10.5	67 ± 6.7	1.48	> 0.05 N.S.
Bronchial Symptoms	23 ± 5.1	25 ± 6.1	0.95	> 0.05 N.S.
Medication score	2.1 ± 0.5	2.05 ± 0.7	0.2	> 0.05 N.S.

There is non significant difference in symptom and medication scores between case group and control group before immunotherapy.

Table (4): Immunoglobulin levels before immunotherapy.

	Cases N=40	Control N= **10**	T	p
IgE X±SD	176.8 ± 39.0	177.5±21.9	0.05	0.95
(Range)	130 - 280	155-220		N.S.
IgG X±SD	1695.7 ± 642.4	1495 ± 592.3	0.89	0.62
(Range)	900 - 3000	900 - 2750		N.S.

There is no significant difference between the two groups before immunotherapy.

Table (5): The relation between the IgE level and the severity of asthma among cases before immunotherapy.

	Moderate N=23	Severe N= 17	T p
IgE X±SD	149.3 ± 12.4	213.9 ± 30.8	9.1 < 0.001 Sig.
Range	130 - 170	180 - 280	
Median	150	195	

There is significant increase in the IgE level according to severity among cases before immunotherapy.

Table (6): The relation between the IgG level and the severity of asthma among cases before immunotherapy.

	Moderate	Severe	T P
IgG X±SD	1209.6 ± 283.5	2271.4 ± 423.8	10.6 < 0.001 Sig.
Range	900 - 2200	1620 - 3000	
Median	1110	2200	

There is significant increase in the IgG level according to severity among cases before immunotherapy.

Table (7): The correlation between IgE and clinical parameters before immunotherapy.

	r	p
Age	-0.63	<0.001
Symptom score	0.6	<0.001

There is significant positive correlation between IgE level and symptom score while there is significant negative correlation between IgE level and age of the patients.

Table (8): Correlation between IgG and clinical parameters before immunotherapy.

	r	p
Age	-0.63	<0.001
Symptom score	0.7	<0.001

There is significant positive correlation between IgG level and symptom Score while there is significant negative correlation between IgE level and age of the patients.

Table (9): The relation between IgE level and duration of illness before immunotherapy.

	> 5 years N= **18**	< 5 years N=22	T P
IgE X±SD	164 ± 25.1	187.3±45	1.94 0.056 N.S.
Range	130 - 232	135 - 280	
Median	160	190	

There is no significant difference in IgE level among cases according to duration of illness.

Table (10): IgG level according to duration of illness before immunotherapy.

	>5 years	< 5 years	T	p
	N= **18**	N=22		
IgG X±SD			206	0.01
	1921.8±672	1419.4±491.3	Sig.	
Range	1100 - 3000	900 - 2400		
Median	1905	1240		

There is significant relation between IgG level and duration of illness (natural immunotherapy)

Table (11): Symptom and medication scores of cases and control after immunotherapy.

	Cases X±SD	Control X±SD	T P
Nasal symptoms	44.9 ± 6.5	67 ± 6.7	9.3 < 0.001 Sig.
Bronchial symptoms	13 ± 5.1	25 ± 6.1	5.5 < 0.001. Sig
Medication score	1.07 ± 0.3	2.05 ± 0.7	4.2 < 0.001 Sig.

There is significant difference in symptom and medication scores between case group and control group after immunotherapy.

Table (12): The changes in immunoglobulin levels after immunotherapy.

	Before N=40	After N=40	T	P
IgE				
X±SD	176.8 ± 39.0	170.6 ± 40.4	2.426	0.018
(Range)	130 - 280	100 - 250		Sig.
IgG				<0.001
X±SD	1695.7 ± 642.4	1832.5 ± 715.9	5.45	H.S
(Range)	900 - 3000	1000 - 3500		

There is significant decrease of IgE and sign Increase of IgG after immunotherapy.

Table (13): The IgE level according to severity among cases after immunotherapy.

	Moderate	Severe	T	P
	N=23	N=17	35.92	0.001
IgE X±SD				
	152.2 ± 13.1	211 ± 29	Sig.	
Range	130-180	180 - 280		
Median	150	195		

There is significant difference of IgE level according to severity among cases after immunotherapy.

Table (14): The IgG level according to severity among cases after immunotherapy.

	Moderate	Severe	T	p
	N=23	N= 17	9.3	<0.001
IgG X±SD				
	1240.4±301	2311.7 ± 424	Sig.	
Range	900 - 2200	1620 - 3000		
Median	1180	2300		

There is significant difference of IgG level according to severity among cases after immunotherapy.

Table (15): Adverse reactions to immunotherapy:

Adverse reactions	**No. of cases**
Local reactions.	**15**
Urticaria.	**6**
Allergic symptoms of eye.	**3**
Allergic symptoms of nose.	**5**
Anaphylaxis	**0**

Discussion:

In our study there is significant positive correlation between IgE level and asthma severity. Before immunotherapy, IgE is higher in sever cases than In moderate cases. Furthermore, there is also significant difference in IgG level according to severity of asthma before immunotherapy (table 5 & 6). This agreed with *Soda et al.* (1992) who reported that serum levels of IgG was higher in sever asthmatics than those with mild asthma. This is in agreement with *Corrigan and Kay (1990)* who found that T-cell activation correlates with the severity of asthma, also *Motojima et al.* (1995) found a significant difference of the serum IL-2R concentration between patients with severe and moderate asthma.

This can be explained on the basis that allergenic stimulation to the T lymphocytes results in their activation which can be expressed by more IL-2 R and hence the release of cytokines IL-4 & IL-5. IL-4 cause the release of more IgE and IL-5 recruits eosinophils to the site of inflammation with release of major basic proteins, PAF and leukotrines. The last 3 substances which are released by eosinophils result in bronchospasm and damage of respiratory epithelium giving more symptoms *(Kuo and Leiden, 1999).*
Moreover we detected significant decrease *in* IgE level and significant increase in IgG after immunotherapy as shown in (table 12). Our results showed increased IgG level following six months of SIT this in agreement with *(Djurup,* 1988) who found

a significant increase *in* serum specific IgG. Also, it agrees with *Frostad et al. (1983)* who found that after long term immunotherapy specific IgE shows slow decline.

Michel et al. (1990) concluded that the IgG increased in response to allergen injection immunotherapy is

Called blocking antibody because it successfully competes for antigen with cell bound IgE, thereby reducing

the effective concentration of antigen that can react with mast cell bound IgE, thus interrupting the initial triggering event in the allergic response.

Serum IgE is also altered by immunotherapy in patients undergoing specific immunotherapy, the IgE antibody titer directed against the injected allergen frequently rises initially but then gradually declines *(Mc Hugh et al., 1990).*

Our study revealed significant difference in IgE and IgG level according to severity among active immunotherapy group after immunotherapy when compared with the same groups before immunotherapy (table 13& 14).

Several mechanisms have been postulated to account for the clinical effectiveness of immunotherapy.

They include:

- Increased allergen-specific IgG antibody that competes with cell **bound IgE for circulating allergen *(Djurup, 1988).***
- Decreased total serum IgE level and blunted seasonal rises of allergen **specific IgE antibody *(Eggleston et al., 2004).***
- **A reduction of *in-vitro* lymphocyte responsiveness to *allergens* as**

- Generation of allergen - specific Suppressor cells *(Gurka and **Rocklin, 1988).***
- Reduced expression high affinity IL-2 receptors and decreased sensitivity of helper T cells *(Hsieh et al., 2003).*
- Switch of the preferential of Native T helper cell from TH2- type effector cells to the TH 1- type *(Nouri-Aria et al., 2004),* and this switch results in

- Inhibition of IL-4 which is a mandatory cytokine for release of IgE from B cells *(Gillis et al.,* 1989), and thus the activity of mast cells is reduced and results in a decreased release of allergic mediators responsible for allergic symptoms,
- Reduced production of IL-5, the activity of eosinophils is decreased, resulting in less inflammation and destruction *(Feria et al., 2004),*
- Increased gamma interferon (INF-gamma) by THI which has an inhibitory effect on B growth, inhibiting the action of IL-4 and inducing a switch to gamma 4 in immunoglobulin gene, which favors an IgG4 response *(Tapia, 2004).*

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تقييم كفاءة العلاج المناعى فى علاج مرضى الربوالشعبى

سهام السيد منسى* ، عبدالله عبدالقادرالبيلى** ، محسن محمد الشافعى** ، شيماء محمد إبراهيم السيد****
*أستاذ المناعة ووكيل الكلية السابق-علوم دمياط-جامعة المنصورة
**أستاذ مساعد المناعة والميكربيولوجى الطبية-طب الزقازيق
***أستاذ مساعد طب الأمراض الصدرية-طب المنصورة
****باحث ماجستيرفى علم الحيوان

كان دور العلاج المناعى فى علاج مرضى الربو الشعبى موضع دراسة ومناقشة على مدار السنوات الماضية ولكن أصبح هناك تقدم واضح فى قياس واستخلاص مسببات الحساسية واستخدامها فى العلاج.

وتهدف هذه الدراسة الى تقييم دور العلاج المناعى بالحقن فى الأطفال المصابين بحساسية الربو الشعبى لحبوب اللقاح ولقد أجريت هذه الدراسة على 40طفل مصابين بهذا المرض يقيمون فى المنطقة الريفية بشربين (محافظة الدقهلية) ويتلقون علاجهم المناعى من وحدة أمراض الحساسية والمناعة بقسم الميكربيولوجى بكلية الطب-جامعة الزقازيق و10 اطفال أخرين كمجموعة ضابطة. وقد تم عمل متابعة دورية مستمرة لهم على مدار6شهوروتم تدوين كل متابعة بملفاتهم بالوحدة قبل وأثناء العلاج المناعى وبعد 6 أشهر من بدايته.

وقد تم عمل الأتى لكل طفل من هؤلاء الأطفال:

1-الفحص الاكلينكى الشامل متضمنا الأعراض والأدوية التى تعاطها ومازال يتناولها كل طفل والتاريخ المرضى .
2- الفحص المناعى:

أ- دراسة حساسية جلدية باستخدام التوفرمن المواد المسببة للحساسية من حبوب اللقاح المتاحة بالمنطقة.
ب- تم تدوين الملاحظا ت التى ظهرت على المريض كأعراض جانبية للحقن.
ج- تم تعيين الأجسام المناعية(Total IgE) بواسطة جهازELISA.
د- تم تعيين الأجسام المناعية(Total IgG) بواسطة جهاز
ELISA(radial immunodiffusion method).

وقد خلص هذا البحث الى أن :
مجموعة المرضى التى خضعت للعلاج قد أبدت تحسنا ملحوظا بالقياس إلى المجموعة الضابطة.