Evaluation of Immunoglobulin G4 (IgG4) Levels in Patients with Bronchial Asthma

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Abstract: Introduction: IgG4 has recently been a subject of great interest in human pathology. No data are available about the characteristics of asthma patients with different IgG4 levels. Subjects and methods: case control study was conducted from October 2015 to May 2016 on sixty asthma patients divided into HR and LR subgroups based on their response to bronchodilators (HR = FEV1 post bronchodilator≥ 20%) and (LR =FEV1 post bronchodilator below 20%), subgroups were subdivided into uncontrolled, partly controlled and controlled patients according to severity of symptoms, compared with twenty normal healthy nonsmoker subjects as control group. Blood eosinophilia percentage, total IgE and total IgG were compared between groups to better characterize asthma patients with different serum IgG4 levels. Results: the majority of patients in the HR and LR subgroups displayed poor disease control. HR was more frequently associated with uncontrolled patients and lower lung function than LR group (FEV1 49.57 ± 11.9% of predicted vs 64.07 ± 9.1% predicted; P <.001). Mean IgG4 concentrations were18.55± 9.4mg/dl, 20.73± 11.49mg/dl and 13.79 ± 4.69 mg/dl in high reversibility group, low reversibility group and control group respectively with significant difference between HR, LR groups and control group (p=0.023) and (p=0.005) respectively. Mean IgG4 concentrations were 24.30± 11.24mg/dl,10.89±4.mg/dl in partly controlled and uncontrolled patients in LR group respectively. Mean blood eosinophilia percentage was 8.17 ± 6.1%, 8.30 ± 4.9%, 5.50 ± 3.7% in HR group, LR group and control group respectively with significance difference between LR group and control group. The IgG4 level correlated to a certain extent with the level of serum IgE and blood eosinophilia percentage. **Conclusion**: Increased levels of IgG4 in asthmatic patients when compared with healthy nonsmoker individuals and increase levels of IgG4 in some asthmatic patients which characterized by partly controlled symptoms and there is negative correlation between IgG4 levels and blood eosinophilia percentage, total IgE.
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1. Introduction:

Asthma is a heterogeneous condition covering a multitude of diseases (Borish et al., 2008). Cluster analysis trials have revealed several phenotypes based on clinical and biological similarity (Haldar et al., 2008). These data highlight the importance of personalized treatment adapted to particular patients or small groups of patients with similar phenotypes (Fajt and Wenzel, 2015). To that end, phenotypes need to be better characterized, identifying new biomarkers.

The role of immunoglobulin G4 (IgG4), a subclass of immunoglobulin G (IgG), is not clearly understood in human pathology (Schroeder and Cavacini, 2010). Among all IgG subclasses, IgG1 has the highest concentration in the blood (>50%) and IgG4 the lowest concentration (classically < 5%) (Aalberse et al., 2009). IgG4 is described as non-inflammatory IgG with low affinity for Fc receptors (FcR) (Bruhns et al., 2009).

Little is known about different concentrations of IgG4 and their role in respiratory diseases. Elevated levels of serum and tissue IgG4 are found particularly in IgG4-related disease (IgG4-RD), which is characterized by a polyclonal lymphocyte and plasma cell infiltration and perivascular sclerosis (Kamisawa et al., 2015). Pulmonary involvement is described in 14-54% cases of IgG4-RD (Zen and Nakanuma, 2010).

Several clinical presentations have been identifiedsuch as inflammatory pseudotumours, idiopathic interstitial pneumonias, fibrosing mediastinitis and, inflammatory pleural lesions.

Elevated IgG4 is also observed in other diseases with lunginvolvement, such as vasculitis, connective tissue diseases and cancer, but its role has not yet been clearly identified (Aalberse et al., 2009).

To the bestof our knowledge, no data are available on the exact profile of asthma patients with different levels of IgG4.

The aim of this study is to measure the levels of IgG4 in asthmatic patients with different disease characteristics and to characterize some specific features for bronchial asthma patients associated with different levels of IgG4.

2. Subjects and methods:
2.1. Subjects:

This study was case control study which carried out on sixty bronchial asthma patients attended to chest department at Al-Azhar University Hospital (New Damietta hospital) at the period from October 2015 and May 2016.

Subjects were 60 patients with bronchial asthma (19 males and 41 females) which divided into two groups: 1-Group I (HR group) include 30 subjects with high airway reversibility which defined as a $\geq$ 20% increase in FEV1 following administration of a short acting bronchodilator during screening and baseline pulmonary function testing.

2- Group II (LR group) include 30 subjects with low airway reversibility those with reversibility below 20%.

Subgroups are further divided according to GINA 2014 guidelines into:

1-controlled. 2-partly controlled. 3-uncontrolled.

Another 20 subjects who are normal healthy nonsmoker individuals as control group of the same age and sex as matched to previous groups.
2.2. Methods:

2.2.1. Complete medical history: detailed medical history taking including age, sex, occupation and history of other medical diseases with special emphasis on bronchial asthma symptoms.

2.2.2. Full clinical examination: that divided into two stages, the first is general examination reviewing all systems of the body except the chest. The second stage included the chest and run in standard order starting by inspection, palpation, percussion and auscultation.

2.2.3. Lung function tests: lung function tests were performed using (ZAN 100 flow handy II) Forced Vital Capacity (FVC), Forced Expiratory Volume in 1 second (FEV1) and (FEV1/FVC) values were expressed as percentages of predicted values according to gender, weight and age. Measurements were performed following ATS/ERS recommendations (Laszlo, 2006). The predicted values were those of the ERS (Quanjer et al., 2006).

2.2.4. Laboratory investigation: serum level of IgG4 was estimated by an enzyme-linked immunosorbent assay (ELISA) kit (CUSABIO Biotech co. U.S.A).

Serum level of IgG was estimated by ELISA kit (RD-Westerbach stra Be- Frankfurt-Germany).

Serum level of IgE was estimated by ELISA kit (Bio check, Inc., Foster City, U.S.A).

The results were expressed ng/dl for IgG4 and mg/dl for both total IgE and total IgG.

Complete blood count using cell counter including percentage of eosinophil was estimated by (sysmexXSi 500).

Continuous variables are presented as means ±standard deviations (SD). Categorical variables are reported as number and proportions. The continuous data were checked for normality and equality of distribution, prior to any analysis being performed. Comparisons between the groups were made using independent t test for continuous normally distributed variables while chi-square test was used for comparison between categorical variables. All calculations were performed using SPSS 17.0 software for Windows. All analyses were 2-tailed.

Significance level (P) value was expressed as follows: P>0.05=Insignificant. P<0.05=Significant. P<0.01=High significant.

3. Results
3.1. Study and control subjects characteristics
3.1.1. Demographic characteristics:

A total of 60 asthmatic patients divided into two groups, 30 asthmatic patients in high reversibility group (HR group) and 30 asthmatic patients in low reversibility group (LR group), these groups subdivided into control, partly controlled and uncontrolled patients according to GINA 2014 guidelines, another 20 subjects who were normal healthy nonsmoker individuals as control group of the same age and sex as matched to previous groups. Mean age was 38.4 ± 12.1 years, 40.8 ± 11.2 years and 39.1 ± 11.5 years for HR group, LR group and control group respectively. There were more women than men in both study groups, with 66.7% and 70% in HR, LR groups respectively. There was no difference in sex or age between groups (Table 1). According to GINA 2014 guidelines there were 31 cases (51.66%) uncontrolled, 23 cases (76.7%) of HR group and 8 cases (26.7%) of LR group, there were 29 cases (48.33%) partly controlled, 7 cases (23.3%) of HR group and 22 cases (73.3%) of LR group, there was significant difference between HR and LR groups in severity of disease (Table 2).
3.1.2. Functional characteristics:

Bronchial asthma defined, defined as FEV1/FVC<70%, mean FVC was 82.37±5.5%, 82.00 ±6.1%, 90.25±4.7% in HR group, LR group and control group respectively, with significant difference between HR group, LR group and control group.

Mean FEV1 was 49.57 ± 11.9%, 64.07 ± 9.1% and 87.45 ± 4.1% in HR group, LR group and control

group respectively, with significant difference between HR group and LR group (p<0.001) (Table 3).

Table 1: Demographic data of studied groups:

Parameters / Groups	HR group(N=30)	LR group(N=30)	Control(N=20)	significance
Age(years)	38.4 ± 12.1	40.8 ± 11.2	39.1 ± 11.5	P1= 0.065 P2= 0.847 P3= 0.057
Sex Male: No (%) Female: No(%)	10 (33.3%) 20 (66.7%)	9 (30%) 21 (70%)	8 (40%) 12 (60%)	 0.67

P1(HR vs. LR) P2(HR vs. control) P3(LR vs. control)

Table (2): Comparison between two groups according to symptoms and grading of severity according to GINA:

	HR group		LR group		χ2	P
	No	%	No	%		
Daytime symptoms	30	100%	30	100%		
Limitation_Of_activity	3	10%	1	3.3%	1.071	0.301
Nocturonal_symptoms	3	10%	0	0%	3.158	0.076
Need_for_reliver	8	26.7%	22	73.3%	13.067	<0.001
Lung_funcations	30	100%	30	100%		
Excerbations	22	73.3%	7	23.3%	15.017	<0.001
Severity						
Uncontr.	23	76.7%	8	26.7%	15.017	<0.001
Partly contr.	7	23.3%	22	73.3%		

3.1.3. Immunoglobulin characteristics:

Mean IgG4 concentrations were 18.55±9.44mg/dl, 20.73±11.49mg/dl and 13.79±4.69mg/dl in HR group, LR group and control group respectively, with significant difference between HR, LR groups and control group. Mean total IgG concentrations were 1200.07±229.8mg/dl, 1173.90± 333.3mg/dl and 1134.35 ± 121.1mg/dl in HR group, LR group and control group respectively, without significant difference between three groups in concentration of total IgG. Mean total IgE concentrations were 181.50±245.2mg/dl,172.77± 248.8mg/dl and 82.40 ± 62.1 in HR group, LR group and control group respectively, with significant difference between HR group and control group (Table 4). Mean IgG4 concentrations were 19.34±10.44mg/dl, 15.94± 4.60mg/dl in uncontrolled and partly controlled patients respectively in HR group, Mean total IgG concentrations were 1210.39 ± 239.1mg/dl, 1166.14 ± 209.5mg/dl in uncontrolled and partly controlled patients respectively in HR group, Mean total IgE concentrations were 178.30 ± 268.1mg/dl, 192.00 ± 164.2mg/dl in uncontrolled and partly controlled patients respectively in HR group without significant difference between uncontrolled and partly controlled patients in HR group in concentrations of IgG4, total IgG, total IgE (Table 5). Mean IgG4 concentrations were 10.89 ± 4.15mg/dl,

24.30 ± 11.24mg/dl in uncontrolled and partly controlled patients respectively in LR group with significant difference between uncontrolled and partly controlled patients in LR group in concentration of IgG4, Mean total IgG concentrations were 1085.00 ± 380.2 mg/dl, 1206.23 ± 317.8 mg/dl in uncontrolled and partly controlled patients respectively in LR group without significant difference between uncontrolled and partly controlled patients in LR group in concentration of total IgG, Mean total IgE concentrations were 385.13±305.1mg/dl, 95.55± 176.2mg/dl in uncontrolled and partly controlled patients respectively in LR group with significant difference between uncontrolled and partly controlled patients in concentration of total IgE (Table 6).

3.1.4. Eosinophilia

Mean percentage of eosinophilia was 8.17 ± 6.1%, 8.30 ± 4.9%, and 5.50 ± 3.7% in HR group, LR group and control group with significance difference between LR group and control group (Table 7).

3.1.5. Correlation between the levels of IgG4 and IgE:

There was significant negative correlation between the levels of IgG4 and IgE in HR group but not significant, there was significant negative correlation between the levels of IgG4 and IgE in LR group, there was positive correlation between levels of

IgG4 and total IgG in both HR and LR group but not significant (Table 8), (Figure1).

3.1.6. Correlation between the levels of IgG4 and blood eosinophilia percentage:

There was negative correlation between the levels of IgG4 and blood eosinophilia percentage in HR group but non-significant, there was significant negative correlation between the levels of IgG4 and blood eosinophilia percentage in LR group (Table 9), (Figure 2).

Table (3): Comparison between three groups according to finding in respiratory function tests:

	HR group(N=30)	LR group(N=30)	Control(N=20)	Significance
FVC (%)	82.37 ± 5.5	82.00 ± 6.1	90.25 ± 4.7	P1=0.807 P2<0.001 P3<0.001
FEV1/FVC (%)	59.20 ± 7.6	65.27 ± 2.9	87.05 ± 3.9	P1<0.001 P2<0.001 P3<0.001
FEV1 (%)	49.57 ± 11.9	64.07 ± 9.1	87.45 ± 4.1	P1<0.001 P2<0.001 P3<0.001

P1(HR vs. LR)P2(HR vs. control)P3(LR vs. control)

Table (4): Comparison between three groups according to (Total IgE, Total IgG, IgG4):

	HR group(N=30)	LR group(N=30)	Control(N=20)	Test of significance
Total IgE(mg/dl)	181.50 ± 245.2	172.77 ± 248.8	82.40 ± 62.1	P1= 0.892 P2= 0.042 P3= 0.066
Total IgG(mg/dl)	1200.07 ± 229.8	1173.90 ± 333.3	1134.35 ± 121.1	P1= 0.725 P2= 0.195 P3= 0.556
IgG4(mg/dl)	18.55 ± 9.44	20.73 ± 11.49	13.79 ± 4.69	P1= 0.426 P2= 0.023 P3= 0.005

P1(HR vs. LR) P2(HR vs. control) P3(LR vs. control)

Table (5): Comparison between uncontrolled and partly controlled patients in HR group according to (Total IgE, Total IgG, IgG4):

	Uncontr.(N=23)	Partly contr.(N=7)	t	P
Total_IgE(mg/dl)	178.30 ± 268.1	192.00 ± 164.2	0.127	0.900
Total_IgG(mg/dl)	1210.39 ± 239.1	1166.14 ± 209.5	0.440	0.663
IgG4(mg/dl)	19.34 ± 10.44	15.94 ± 4.60	0.831	0.413

Table (6): Comparison between uncontrolled and partly controlled patients in LR group according to (Total IgE, Total IgG, IgG4):

	Uncontr.(N=8)	Partly contr.(N=22)	t	P
Total_IgE(mg/dl)	385.13 ± 305.1	95.55 ± 176.2	2.535	0.033
Total_IgG(mg/dl)	1085.00 ± 380.2	1206.23 ± 317.8	0.877	0.388
IgG4(mg/dl)	10.89 ± 4.15	24.30± 11.24	4.768	<0.001

Table (7): Comparison between three groups according to percentage of eosinophilic count:

	HR group (N=30)	LR group (N=30)	Control (N=20)	Test of significance
Presentage of eosinophils(%)	8.17 ± 6.1	8.30 ± 4.9	5.50 ± 3.7	P1=0.926 P2=0.061 P3=0.027

P1 (HR vs. LR)   P2 (HR vs. control)   P3 (LR vs. control)

Table (8): Correlation between total IgE, total IgG and IgG4 in LR group:

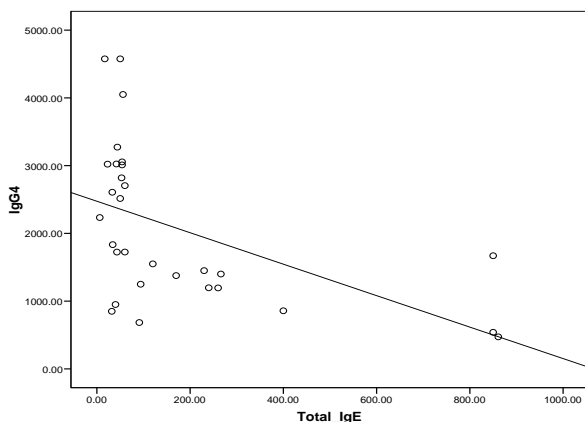|	LR group			
	Total_IgE		Total_IgG	
	r	P	r	P
Total_IgG	0.144	0.448		
IgG4	-0.502	0.005	0.130	0.495

Figure 1. Correlation between total IgE and IgG4 in LR group.
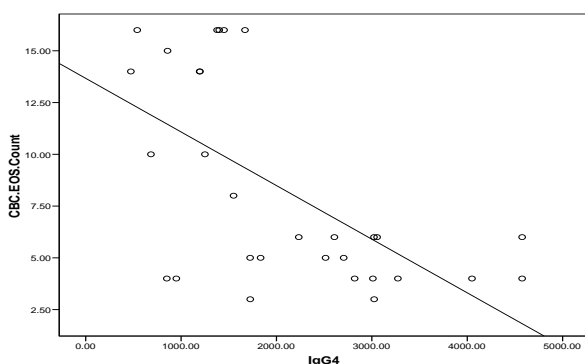

Figure 2. Correlation between IgG4 and percentage of Eosinophilic count in LR group.

Table (9): correlation between IgG4 and different parameters in LR group:

	LR group	
	IgG4	
	r	P
Eosinophilia percentage	-0.606	0.000

4. Discussion:

In the present study, there were 50% of cases with mean age 38.4 ± 12.1 years and there were 50% of cases with mean age 40.8 ± 11.2 years. One study reported that the mean age to be 42 years with age range from 10-68 years (Langley et al., 2003). This result was similar to those reported in the present work. In our study there were 60 cases of bronchial asthma, 19 of them were males (31.7%) and 41of them were females (68.3%), these results are similar to those reported by other study who reported that were 123 patients with acute asthma 47 males (38.2 %) and 76 females (61.8%), and 46 patients with severe chronic asthma 14 males (30.4 %) and 32 females (69.6%) (Cook et al., 1998). On the other hand, another study reported that they included 175 consecutive asthmatic patients, 54 women and 121 men, and these results are in contradiction to those reported in the present work, and this may be attributed to the different inclusion criteria between the two studies (Graif et al., 2002).

In the present work, patients from high reversibility group characterized by poor disease control and increase prevalence of uncontrolled patients in compare with cases from low reversibility group, patients from high reversibility group with lower lung function with FEV1 $49.57 \pm 11.9\%$ of predicted vs $64.07 \pm 9.1\%$ to cases from low reversibility group with significant p value <0.001. In accordance with the results of the present study, another study reported that the majority of patients in the HR and LR subgroups displayed poor disease control and lower lung function with FEV1 $63.5 \pm 7.7\%$ predicted for patients in high reversibility group vs $67.9 \pm 8.4\%$ predicted for patients in low reversibility group with significant p value <0.001 (Busseand Lemanske, 2015).

Total IgE level estimation provides evidence in support of atopy (Merret et al., 1980).

In the present work, patients from high reversibility group were associated with increased levels of IgE in compare with patients with low reversibility group.

In accordance with the results of the present study, another study reported that increase atopy between patients with high reversibility in compare with patients with low reversibility group(Busseand Lemanske, 2015).

In the present work, we found that increase levels of IgE in uncontrolled patients in compare with partly controlled patients with significant (p =0.033).

In accordance with these results, another study reported that mean value of serum total IgE levels in the three asthmatic groups was in the order severe (821IU/ml) > moderate (663 IU/ml) >mild (515 IU/ml), with the differences between the means of the

severe and moderate (p= 0.0004) and the moderate and mild (p= 0.0169) groups being significant (Shakib et al., 1994).

In the present work, we found that increase levels of IgG4 in partly controlled patients in compare with uncontrolled patients with the differences between the means of partly controlled and uncontrolled patients (p<0.001).

Other study reported that although asthma patients have significantly elevated levels of IgGI and IgG4 anti-lgE antibodies, the concentration of these autoantibodies is not related to the severity of asthma, this may be attributed to suppression of autoanti-IgE production as a result of treatment with systemic (in the severe group) and high doses of inhaled (in the moderate group) corticosteroids (Shakib et al., 1994).

In the present work, we found that there is negative correlation between eosinophilic percentage and IgG4 (p=0.000) and also negative correlation between total IgE and IgG4 (p=0.005).i.e that IgG4 tend to increase with decrease levels of total IgE and eosinophilic percentage and it means that IgG4 tend to increase in non-atopic asthma.

In accordance with these results another study reported that Patients from group non atopic asthma had significantly higher IgG4 subclass levels than patients from group atopic asthma (p= 0.02) (Kukhtnova et al., 2012).

These results are comparable to those reported by Tanizaki et al. (1987) who reported that the mean level of serum IgG4 showed an increasing tendency with the increase in serum IgE level, he reported that the mean level of serum IgG4 was 50.9 ± 33.2 mg/dl ($\pm$sd) in the patients under 100 IU / ml of serum IgE, The IgG4 level was highest (96.0 ± 79.4 mg/dl) in the patients over 1001 IU / ml of serum IgE and lowest in those under 100 IU / ml of serum IgE, it may be attributed to different demographic data, his study include 72 patients with bronchial asthma (37 females and 35 males), their mean age was 50.5 years (range,7 to 80 years).

In conclusion increased levels of IgG4 in asthmatic patients when compared with healthy nonsmoker individuals and increase levels of IgG4 in some asthmatic patients which characterized by partly controlled symptoms and there is negative correlation between IgG4 levels and blood eosinophilia percentage, total IgE.

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