

Immunoglobulin Isotypes, C3 – C4 Complement Components, Absolute Eosinophils and Allergen Specific IgE as Biomarkers Correlated to Preschool Wheeze Children

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Abstract

Wheeze chest is a common problem among preschool children, and represents a common disorder characterized by airways obstruction. Almost half of children manifest wheeze chest symptoms before the age of six. Their parents report at least one attack within this period. This study aimed to assess the relationship between the immunoglobulin (IgA, IgM, IgG, and IgE), complement, and the level of eosinophils with children's wheeze chest. Moreover, the level of IgE with the numbers of the attacks per year, age, family history, and eosinophilic count.

A total number of a randomized (n=73) preschool children were involved in this study, divided into 2 groups, (n=52) patients with recurrent wheezy chest attacks and (n=21) controls. Samples were analyzed for immunoglobulin isotypes; complement C3, C4, Eosinophils, and specific IgE allergen test.

Number of attacks were strongly correlated with IgE with a P-value of (P=0.001), as well as the manifestation with a P-value of (P=0.002), while age was weakly correlated with IgE with a P-value of (P=0.005). The other variables in this study were found to be insignificant, correlating with IgE.

Regarding specific allergens, 20 different types were evaluated in patients who had elevated levels of IgE, and the findings were that all patients were not only allergic to 1 type but unlikely to many. Most definitely the majority of the cases shared Dermatophagoides Peter (8 patients) and Dermatophagoides farinae (7 patients) allergens and so the list goes descending in the number with 2 allergens were not found, Cladosporium herbarum and Penicillium notatum.

Keywords: Immunoglobulin Isotypes; C3 – C4 Complement Components; Absolute Eosinophils; Allergen; Specific IgE; Biomarkers; Preschool Wheeze Children

Introduction

Wheeze chest is a common problem among preschool children, and represents a common disorder characterized by airways obstruction. Almost 30% of children manifest wheeze chest symptoms before the age of three and 50% before the age of six [1].

In correlation of IgA with wheeze chest children, it is established that infants who had suffered from upper or lower respiratory infections before the acute bronchiolitis, IgA was significantly higher than in infants without previous respiratory infections, which gives the hypothesis of IgA involvement with children who suffer from wheeze chest. Immunoglobulin A, secretory IgA and immunoglobulin G are the major components of respiratory secretions. Meanwhile, IgG

mainly provides a primary protection against systemic and local infections but in the lower respiratory tract [2].

Deficiencies of IgG limited to one or more subclasses have been recognized in children with recurrent infections and one small study found evidence of IgG subclass deficiency in non-allergic children with chronic chest symptoms. As other forms of immunodeficiency have been associated with atopic diseases, a study under the name of IgG subclass deficiency in asthma studied IgG subclass concentration and have found low concentrations of IgG among 28 asthmatics children, disagreeing with the hypothesis of the correlation between IgG and asthma [3].

Since IgM has a major role in mucosal defense, it is believed that the presence of high levels of IgM might have a role developing symptoms that associate with bronchial asthma and may also be associated with the elevated immunoglobulins [4].

A significantly strong association has been proven between asthma and total IgE or specific IgE. Several studies found a close correlation between serum IgE levels and self-reported asthma. Alternatively, it was found that allergic rhinitis to be independent of serum IgE concentration but associated with cutaneous reactivity to common seasonal aeroallergens in most individuals tested, and by that, it is clearly understood that asthma is almost always associated with some type of IgE-related reaction and therefore has an allergic basis [5].

Population studies have shown an association between prevalence of asthma or bronchial hyper-responsiveness and total serum immunoglobulin E (IgE) levels, meaning that IgE levels increase among asthma or bronchial hyper-responsiveness [6].

Some studies have reported increased plasma complement levels (C3 and C4) since the complement system plays an important role in immune responses of the host. The role of the complement system in asthma has been suggested, possibly through initiation and/or amplification of the inflammatory response in the airways through the complement activation cascade. Accordingly, C3 was more noticeable in many studies, whereas others have demonstrated no significant changes [7].

An increased number of eosinophils in the tissues and/or blood representing the so-called eosinophilia. Atopic dermatitis may produce a more significant eosinophilia if affecting a large part of the body and if associated with significant atopy. While allergic rhinitis and asthma often produce a mild eosinophilia [8].

Aims of the Study

Given the findings for the role of immunoglobulins, complement (C3 and C4), and eosinophils in wheeze chest, the major aims were to assess the correlation between the immunoglobulins (IgA, IgM, IgG, and IgE), complements, and the level of eosinophils and development of wheeze chest. Moreover, correlation of the level of IgE with, IgA, IgM, IgG, C3, C4, the numbers of the attacks per year, age, family history, and eosinophilic count.

Materials and Methods

This is an observational case-control study, University's ethical committee approved it and the nature of it was explained in details to the parents of involved participants before being consented. Samples were obtained for this study on Feb 2019. The research subjects were invited to the buildings of Libyan International Medical University where all blood samples were collected by an expert healthcare professionals and examined.

A total number of a randomized 73 cases were involved in this study, divided into 52 patients with recurrent wheezy chest attacks and 21 controls. Five milliliters of blood sample were equally collected and transported into EDTA and plain test tubes immediately.

Total IgE concentration was determined by ELISA using (EUROIMMUN total IgE kit) according to manufacturer's instructions. While specific IgE was Determined by immunoblotting technique using EUROLINE inhalation middle east (IgE) kit.

Determination of IgA, IgG, IgM, C3 and C4 has been performed by immunodiffusion kits from LTA Company (Italy). Complete Blood Count (CBC) tests were done by the 3 parts CELLTAC and in order to count the total leukocytes count then Giemsa blood films had to be done for giving the absolute eosinophil count manually. Clinical and Laboratory data were statistically analyzed using a computer program (Minitab 13 for Windows).

Results

Gender and age distribution

Meanwhile analyzing the data, children gender was equally selected among cases and controls. The number male children were 48 (which represents 66% of the total number) and compared to 25 (which represents 34% of the total number) (Figure 1). Among them children with recurrent wheezy attacks (52 in total), 34 were males (which represents 65% of the patients' number) and 18 were females which represent 35%.

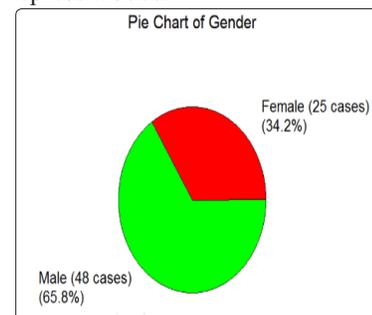


Figure 1: Gender distribution among samples

According to the age group that was required for this study, 6 years old children were the oldest to accept and younger than 1-year old children were rejected. Using histogram, ages were ranging from months to 6 years showing a majority for the age 3 mainly and the age 5 closely (Figure 2).

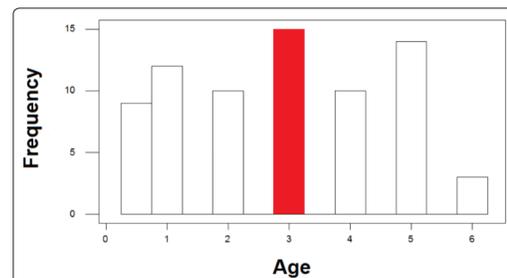


Figure 2: Age distribution among the children

Estimation of IgE and Eosinophils

There were only 29 cases who had elevated levels of IgE while the others are having a normal range. For absolute Eosinophils count, it was revealed that only 4 cases had elevated levels of eosinophils while the others are having a normal range.

Type of Allergen

As a part of this study, testing the type of the allergen to those who had elevated IgE was mandatory and a bar chart was done to show a representative figure3 for the number of cases.

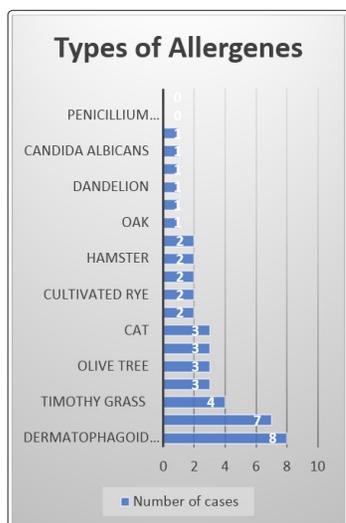


Figure 3: Type of Allergen

All IgE positive were screen for inhalation middle east (IgE) showing

individuals not only allergic for one allergen, but many. Most of the cases shared Dermatophagoides Pter allergen and so the list goes descending in the number with 2 allergens were not found Cladosporium herbarum and penicillium notatum

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Statistical Correlation

Correlation and regression analysis were applied to data to define and quantify the relationship between our study variables in which, Three out of twelve variables have been found to be **significant** according to the correlation with total IgE levels. Number of attacks strongly correlated with a P-value of (P=0.001), as well as the manifestation with a P-value of (P=0.002), while age was weakly correlated with IgE with a P-value of (P=0.005). Furthermore, the other 9 variables in this study were found to be insignificant, correlating with IgE. As shown (Table1).

Table 1: The correlation of IgE with most effective variables

IgE	P-value	IgA	IgG	IgM	C3	C4	Gender
			0.471	0.505	0.917	0.590	0.463
IgE	P-value	Age	Residence	Number of attacks	Manifestation	Family history	Eosinophilic count
		0.005	0.874	0.001	0.002	0.463	0.094

*P-value < 0.05

On applying one-way classification analysis of variance (ANOVA), with null hypothesis (Ho) “there were no differences of IgE according to the variability of attacks”, the results gave P-value (P=0.011) which means that the variability of IgE based on the number of attacks was **significant** and this indicates that at least one level of attacks differs compared to the other levels.

For more details, it was founded that the number of attacks levels differences in IgE are not significant, except for control (no attacks) and (levels 3 and 4), also (levels 2 and 4). (0 for controls, 1 one attack per year, 2 two attacks per year, 3 three attacks per year, 4 more than three attacks per year).

Table 2: This test designed to show the significant differences of IgE according to the number of attacks

Table 3		Number of Attacks				
		0	1	2	3	4
Number of Attacks	0		0.188	0.185	0.032 Significant	0.001 Significant
	1			0.620	0.855	0.233
	2				0.323	0.017 Significant
	3					0.170
	4					

On applying ANOVA, the Ho says that there are no differences of IgE according to the variability of manifestation. The result of this test gave P-value (P=0.006) which means that the Ho should be rejected and it concludes the variability of IgE is based on the manifestation is **highly significant** and this indicates that at least one level of manifestation differs compared to the other levels.

Table 3: This test designed to show the significant differences of IgE according to the manifestation

IgE vs manifestation	P - value	Comment
	0.006	Significant

According to the family history, on applying ANOVA, the result of this test gave P-value (P=0.019) which means that variability of IgE is based on the family history is **significant** and this indicates that at least one level of family history differs compared to the other levels.

Table 4: This test designed to show the significant differences of IgE according to the family history

IgE vs family history	P - value	Comment
	0.019	Significant

T-test used to determine if there is a significant difference between the means of two groups, in this study the two groups are the controls and effected individuals. All thirteen variables were included in the test showing that all are insignificant, except for IgE (p>0.001) highly significant, number of attacks (p>0.001) highly significant, manifestation (p>0.001) highly significant, family history (p>0.001) highly significant and IgM (p=0.059) significant as shown in (table 5).

Table 5: T-test for variables associated with wheeze chest

Factor	P-value
IgE	0.000
IgA	0.190
IgG	0.990
IgM	0.059
C3	0.443
C4	0.218
Gender	0.919
Age	0.406
Residence	0.862
No. of attacks	0.000
Manifestation	0.000
Family history	0.000
Eosinophilic count	0.095

P<0.05

Discussion

Almost 30% of children known for their wheezing symptoms, which was a very important area to focus the lights on, specially that these symptoms happen to occur before their third birthdays. Accordingly, these facts were documented by many population-based birth cohort studies while all participants were in a respiratory infection status. Immunologic and molecular influences are not as good in comparison to older children or even adults, by which, infants are prone to wheeze due to anatomic factors related to the lung and chest wall. Immunocompetent and immunocompromised infants may or may not develop immunologic derangements that cause wheezing, which it was originally led by viral infections. Anatomic causes of wheeze may be extrinsic or intrinsic to the

airway. Development of asthma in persistent wheeze children is possible; however, not every wheeze is indicative of asthma. Testing for allergy in these infants is worthwhile and can be of significant value in avoidable allergens [9].

As previously mentioned, almost 30% of children manifest wheeze chest symptoms before the age of three and 50% before the age of six. Their parents report at least one attack within this period [1].

Recurrent attacks of wheeze chest have a significant morbidity and have been estimated that about one third of school-age children manifest the symptom during the first 5 years of life. In young children, wheezing is associated with a poor quality of life [10]. Some types of IgE-related reaction are known to be usually associated with asthma and therefore has an allergic basis. In contrast, various epidemiologic studies have shown a highly significant relationship between asthma and interaction to various allergens as were demonstrated by skin tests or the specificity of IgE in the serum presence [11].

In the present study, total number of participants was (n = 73) divided into patients and controls, represented by 34.2% females (n = 25) and 65.8% males (n = 48). According to the patients group, 34.6% were females (n= 18) and 65.4% were males (n = 34). Ages varied from months to 6 years, showing a majority “highest number” for the age 3 mainly and the age 5 closely, along the other age groups with a minority “lowest number” for the age of 6. Moreover, residence was one of the variables of this study, and it has shown that almost all participants were urban residents with a percentage of 94.5% (n = 69) and only 5.5% (n = 4) were rural residents.

Regarding the immunoglobulins levels, IgE concentrations were high in 29 patients while relatively normal ranges to the rest of the patients and a totally normal concentration to all controls. IgA levels were high only in 17 cases of the total number of this study, while the rest were had normal levels. IgG levels were elevated in 30 cases in this study while the rest were had normal levels. IgM was the second least to be noticed elevated in all cases with a number of only 12 cases, while the rest were had normal levels. Meanwhile, C3 was the highest levels to be noticed with a number of 50 cases been positive for C3. Complement 4 levels were high in 39 cases in this study while the rest were had normal levels. The first least to be noticed elevated in all cases with a number of only 4 patients was eosinophilic count.

There are few studies investigating the relationship between recurrent wheezy chest and the prevalence of immunoglobulins in general and IgE in specific. In this study, the aim was to estimate all immunoglobulins, complements, eosinophils and specific allergens in infants and children younger than 6 years of age with recurrent wheeze.

Regarding specific allergens, 20 different types were evaluated in patients who had elevated levels of IgE, and the findings were that all patients were not only allergic to 1 type but unlikely to many. Most definitely the majority of the cases shared *Dermatophagoides Pter* (8 patients) and *Dermatophagoides fariaeni* (7 patients) allergens and so the list goes descending in the number with 2 allergens were not found, *cladosporiumherbarum* and *penicillium notatum* (figure 3). Eight patients had *Dermatophagoides Pter* allergen in their serum making it the highest one to be present in all cases, this finding

supports the reports of “Ella et al” in which their study included 132 cases, *Dermatophagoides Pter* and *Dermatophagoides fariaeni* allergens were found in 48 cases making them the commonest allergens. Moreover, IgE levels were correlated to all other variables, including: IgA, IgG, IgM, C3, C4, family history, manifestation and age. Many other researches hypothesized that other immunoglobulins may correlate significantly with IgE; however, this study disagrees, since the correlation was insignificant for all variables. The only significant ones are age, number of attacks and manifestation. Respectively, IgA, IgG and IgM correlated insignificantly with IgE (P=0.471) (P=0.505) (P=0.917) to support these findings, reports by “Gu et al” shown that the serum IgG, IgA and IgM levels showed no correlation to serum IgE level, meaning that immunologist are considerably independent from each other [12, 13].

On the other hand, findings by “Liphaus BL et al” concluded that in the 69 allergic patients, 39 had high IgE levels, those were significantly correlated with IgA levels with a p-value of (P=0.003). Meanwhile, there was no correlation observed between IgE and IgM or IgG [14].

Regarding the complement system, C3 and C4 levels were correlated to IgE and it has been found that the correlation was insignificant for both of them C3 (P=0.590) C4 (P=0.463). Despite the results of C4, the previously mentioned findings by “Liphaus BL et al” found that IgE correlated insignificantly with C3 as well, therefore, it correlated significantly with C4 with a P-value of (P >0.03), meaning that somehow when IgE levels elevate, C4 levels elevate as well [14].

Blood eosinophilia and total serum IgE level have been proposed as screening tests for allergies. Regarding the heterogeneous disorder allergic rhinitis, it is characterized by one or more symptoms including sneezing, itching, nasal congestion, and rhinorrhea. The key to diagnosis of allergic rhinitis is awareness of signs and symptoms. IgE antibody tests to detect specific allergens are the standard method used today; in addition, diagnosis must be confirmed with a positive history and demonstration that the symptoms are the result of IgE-mediated inflammation [15].

According to “El-Asheer et al” findings, eosinophilic count correlated significantly with IgE, by which, these findings contradict with this present study findings, since eosinophilic count correlated insignificantly with IgE (P<0.095) [1].

According to the number of attacks variable, first it was correlated with IgE and shown a significant correlation (P=0.001). And for a further statistical testing, the significant differences of IgE according to the number of attacks test was done via one-way ANOVA, to represent an even confirmative result that the variability of IgE based on the number of attacks is significant (P=0.011). Moreover, comparing the patients group to the controls using T-test, a highly significant result was found with a p-value of (p>0.001), which means that values of IgE of patients are significantly high comparing to the healthy controls. In contrast, “Peat et al” study included 3 groups of early atopics, late atopics and nonatopics. The non-atopic group had a mean serum IgE level of 45.9 IU/ml, which was significantly lower than in the other two atopic groups. This difference between groups was significant at the (P <0.001) [16].

According to the manifestation variable, it was correlated with IgE and shown a significant correlation (P=0.002). And for a further

statistical testing, one-way ANOVA was done, to represent an even confirmative result that the variability of IgE based on manifestation is highly significant (P=0.006). Comparing the patients group to the controls using T-test, a highly significant result was found with a p-value of (p>0.001), which means that values of IgE of patients are significantly high comparing to the healthy controls. Relatively, findings by “J. Sunyer et al” found out that the difference between patients with current wheezing symptoms and IgE levels (in the terms of allergic rhinitis only) was significant with a p-value of (p>0.01) in which it supports this study’s findings [17].

According to the family history variable, it was correlated with IgE and shown an insignificant correlation (P=0.463). For a further statistical testing, one-way ANOVA was done, to represent an even confirmative result that the variability of IgE based on family history is significant (P=0.006) and it was unlikely different from correlation. Comparing the patients group to the controls using T-test, a highly significant result was found with a p-value of (p>0.001), which means that values of IgE of patients are significantly high comparing to the healthy controls. Moreover, a study by “Dávila I, et al” showed that 2 groups were involved, patients with and without a family history and patients with a family history. When patients with and without a family history of asthma were analyzed separately, a significant correlation was only detected in the former (P <0.001) in patients with a family history vs P=0.412 in patients with no family history. By this, we can establish that this study and the mentioned one both agree on family history being a significant variable when compared patients to the healthy controls [10].

In this study, it has been found that IgE levels were significantly higher in infants and children with recurrent wheeze in patients group than those of healthy controls (P>0.0001). These findings are in agreement with “El-Asheer et al” as they also found significantly high IgE levels in patient groups with closely the same P-value [1]. In addition, IgA, IgG and IgM were found to be insignificant in comparison between patients and healthy individual, IgA (P=0.190), IgG (P=0.990), IgM (P=0.059) and by these findings suggesting that other immunoglobulins do not differ in the 2 groups (i. e. both patients and controls might have high/low levels and not demanding on whether the case is affected or healthy). The present study’s findings come in contradiction with “F.I.E. Najam et al”, as the study shown a significant elevation of IgG in patients as compared to controls (P>0.005), therefore, they have also shown a significant elevation of IgE in patients as compared to controls (P< 0.001), IgA and IgM were within the normal ranges, and there were no significant differences were between controls and patients, in which both of these results come in agreement with the present study findings [18].

According to the complements (C3 and C4), both of them were found insignificant in this study C3 (P=0.443) C4 (P=0.218), meaning both patients and controls might have high/low levels and not demanding on whether the case is affected or healthy. These findings are supported by “F.I.E. Najam et al” study on Complement Components in Childhood Asthma, in which it has been found that C4 was no different when compared patients to control (P>0.05). Therefore, C3 was found to be significantly high in patients group (P<0.001) by which, this finding disagrees with this study’s findings [19].

Conclusion

This study aimed to assess the correlation between the immunoglobulins (IgA, IgM, IgG, and IgE), complements, the

level of eosinophils and development of wheeze chest. Insignificant correlation was found for all variables, except for manifestation, the numbers of the attacks and family history, which were found significant to IgE. Measuring IgE, IgM, and IgA in both control and effected children, approved only for IgE only, while disapproved for IgM, IgA, IgG, C3, C4 and eosinophil since there was no significant differences between patients and controls groups. Furthermore, all IgE positive patients were tested for almost 20 specific allergens, given the result of *Dermatophagoides Pter* and *Dermatophagoides farinae* to be the most abundant allergens among these patients.

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