Detection of Human Rhinovirus Antigen (RhV-Ag) and Total IgE in Serum Samples from Common Cold Patients

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Abstract

Background: Human rhinovirus infections account for approximately 50% of common colds and up to 80% or more of all infections during respiratory epidemic outbreaks. Rhinovirus infections are considered as a major trigger of asthma exacerbations. Almost all children have experienced at least one rhinovirus infection by the age of two years. An enzyme linked immunosorbent assay (ELISA) can detect rhinovirus specific antibodies in both sera and nasal secretions of patients with a rhinovirus infection. An enzyme linked immunosorbent assay was shown to be more sensitive and reliable than the traditional neutralization test.
Patients and Methods: A cross sectional study was carried out in Al- Ramadi city from the first of December, 2012 to the end of April 2013. The number of patients recruited in this study who having a common cold was 150. All of them were tested for the RhV-Ag and IgE tests. An interview was carried out with these patients using questionnaire prepared by the investigators and it's include the following: name, age, residency and occupation …etc.
Results: Out of 150 patients, RhV-Ag was found in 104 (69.33%) as detected by the technique of Enzyme Linked Immunosorbent assay. The simple linear correlation coefficient between RhV-Ag and IgE readings was calculated and found to be significant ($r=0.242$, $p<0.05$). The two-sample t-test was used in order to compare means RhV-Ag and IgE between adults and children of the considered sample, the t-test revealed that means RhV-Ag and IgE of the children were significantly lower than that of the adults.
Conclusion: Means of RhV-Ag and IgE were compared with respect to age groups (adults and children), in both comparisons adults found to have significantly higher means than children. IgE and RhV-Ag were found to be significantly linearly correlated.

Key words: RhV-Ag, IgE , Common cold infection, Allergy.

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Introduction:

Rhinovirus is a member of the picornaviridae family "pico" meaning small and "RNA" signifying that those are RNA viruses around 27 nm in diameter in size, icosahedral viruses, non-enveloped and contain one positive strand RNA[(1)] according to the International Committee on Taxonomy of virus in July 2009, three species of human rhinoviruses A, B and C were identified. Rhinovirus C unlike A and B species, may be able to cause severe infection [(2)] Human rhinoviruses (HRVs) are the most common causative agent of upper respiratory tract infection (acute viral rhinitis, acute coryza or common cold). They are also associated with more severe disease such as pneumonia, bronchiolitis and acute asthma in children. The nose is the main portal for rhinovirus entry eyes and possibly the mouth may also serve as entry routes. The nasal epithelium is the primary site of infection and virus replication[(3,4)] Rhinovirus capsid

is highly organized, noncovalent assembles of hundreds of proteins subunits which play a substantial role in many stages of the virus life cycle like attachment, entry and release of the genome. The rhinovirus capsid is composed of 60 identical subunits arranged in 12 pentameters in an icosahedrons each subunit consist of all four structural proteins of the virus named VP1, VP2, VP3 and VP4. VP1, VP2 and VP3 are surface protein interacting with antibody, while VP4 is closely associated with viral RNA[5,6] Rhinoviruses are worldwide account for more than 80% of common cold infection during high prevalence seasons in autumn and spring and can affect all age groups. Rhinoviruses are host specific (host range human only) the major route of transmission of the virus which can enter the body through the respiratory tract by the nose and mouth. Transmission through direct and indirect contact also occurs and is most common via the hand – nose – hand route [7,8,9]

The attachment of rhinovirus to the surface of epithelial cells is mediated by cell surface receptors, which are either intercellular adhesion molecule 1 (ICAM 1. or a member of the low-density lipoprotein (LDL) receptor family. These receptors are important in inflammation, immune responses and intracellular signaling events[10] A specific humoral immune response can be detected following rhinovirus infection in both serum and nasal secretions represented by IgA and IgG antibodies. In contrast to the high specificity of humoral immunity, rhinovirus specific T – cells can recognize serotypes with an increased in production of IL–2 and IFNγ after mitogen stimulation[11,12].

An enzyme linked Immunosorbent assay (ELISA) can detect rhinovirus specific antibodies in both sera and nasal secretions of patient with rhinovirus. ELISA was shown to be more sensitive and reliable than the traditional neutralization test[13] According to our information, no data is available about rhinoviruses status in Al – Anbar Governorate. This study is a first study conducted to evaluate the seroprevalence of human rhinovirus in acute respiratory viral infection.

Patients and Methods:

Collection of samples:

From December 2012 to April 2013, a peripheral venous blood (3 – 5 ml) was separated by using 5 ml disposable syringes from 150 patients of an average 2 – 65 years old together with another 20 samples used as control group. Samples collected from both the urban and rural parts of Al–Anbar Governorate who presented to Al – Ramadi Teaching Hospital (ENT unit and Allergy and Asthmatic unit), Maternity and Children Teaching Hospital and primary and secondary schools for boys and girls in the city. A clear serum was separated from blood samples.

Detection of Human Rhinovirus Antigen (RhV-Ag) by ELISA test

Rhinovirus antigen was detected using a double – antibody sandwich enzyme linked immunoassay ELISA (MyBiosource, California). The test was performed strictly according to the manufacturer's instructions:

1. standard was diluted according to the instruction as follows:

12 pg/ml	**Standard No.5**	**120 µl Original Standard + 120 µl standard diluents**
6 pg/ml	**Standard No.4**	**120 µl Standard No. 5 + 120 µl standard diluents**
3 pg/ml	**Standard No.3**	**120 µl Standard No. 4 + 120 µl standard diluents**
1.5 pg/ml	**Standard No.2**	**120 µl Standard No. 3 + 120 µl standard diluents**
0.75 pg/ml	**Standard No.1**	**120 Standard No. 2 + 120 µl standard diluents**

2. Chromogen solution A, chromogen solution B and stop solution was added to blank well
3. Standard (50 µl) , Streptavidin-HRP (50 µl) and (10 µl) of RhV-Ag-antibody labeled with biotin were added to standard wells.
4. Sample (40 µl), Streptavidin-HRP (50 µl) and (10 µl) of RhV-Ag- antibody labeled with biotin were added to samples wells. Then membrane was sealed, gently shacked and incubated 60 minutes at 37°C.
5. The plate was washed with 30X diluted wash solution.
6. Chromogen solution A 50 µl then Chromogen solution B 50 µl were added to each well, mixed gently and incubated for 10 min at 37°C away from light.
7. Stop solution 50 µl was added into each well to stop the reaction (the blue changes into yellow immediately).
8. The optical density (OD) was measured under 450 nm wavelength which should be carried out within 15 min after adding the stop solution.
9. According to standard' concentration and OD values, the standard curve regression equation calculated the OD values of the samples on the regression equation to calculate the corresponding samples' concentration.

Measurement the level of total IgE by ELISA test

The ELISA test was used also for the quantitative determination of immunoglobulin E in human serum using kit from (DRG International Inc., USA). This assay was used for the assessment of and allergic reactions in patients. The test was performed according to the manufacturer's procedure.

The mean absorbance value (OD 450) was calculated for each set of reference standards, controls and samples.

Table 1. Concentrations and corresponding optical densities of RhV-Ag and IgE

RhV-Ag		IgE	
Conc.	**O.D.**	**Conc.**	**O.D.**
0.75	**0.104**	**0**	**0.110**
1.5	**0.149**	**10**	**0.205**
3	**0.172**	**50**	**0.510**

A standard curve was constructed by plotting the mean absorbance obtained for each reference standard against its concentration in IU/mL on graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.

Results:

The result showed that exponential and linear models were the best among other proposed models that used to predict OD readings of RhV-Ag when standard readings are known. Linear model found to perform better than exponential model. Table 2 showed the scatter diagram of the original data and the two fittings are stated in table 2 for the RhV-Ag variable (Figure 2).

The quadratic and linear models were the best among other proposed models used to predict OD readings of IgE when standard readings are known. In this context, quadratic model was found to perform better than linear model for IgE data. Figure 3 showed the scatter diagram of the original data and the two fittings as stated in Table 2 for the IgE variable.

Table 1 showed the standard readings of RhV-Ag and IgE and their corresponding optical density (OD) readings. The relationship between standard and OD for both RhV-Ag and IgE were examined under different hypothesized mathematical models.

Table 2. Linear and nonlinear fitting of the data in Table 1

	RhV-Ag		IgE	
	Exponential	**Linear**	**Quadratic**	**Linear**
Equation	$OD = -0.9143 + e^{0.03583*conc.}$	$OD = 0.0675 + 0.04578\ conc.$	$OD = 0.175 + 0.00598 * conc. - 0.000003333 * conc.^2$	$OD = 0.3132 + 0.003385\ conc.$
R-square	**0.9856**	**0.9899**	**0.997**	**0.952**
RMSE	**0.0292**	**0.0244**	**0.0773**	**0.2399**

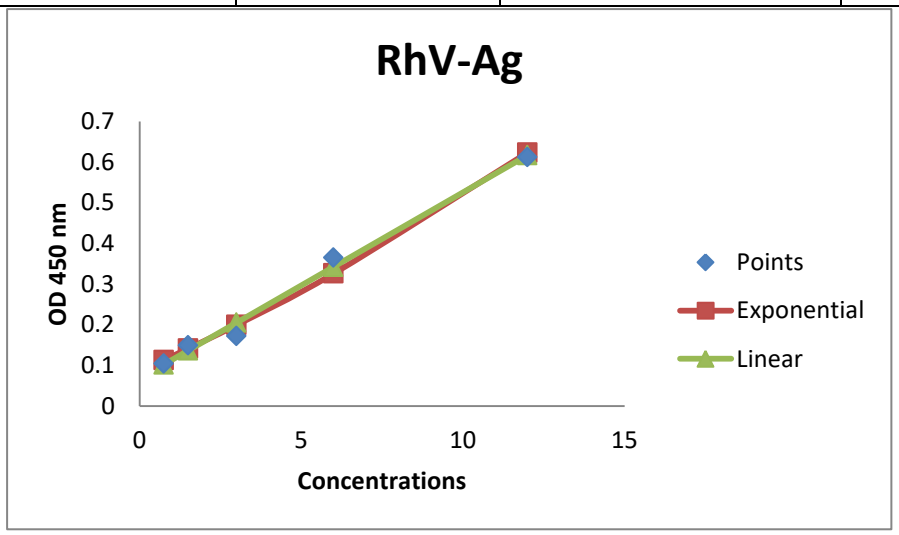

Fig.2. Linear and exponential fitting of the RhV-Ag data in Table 1

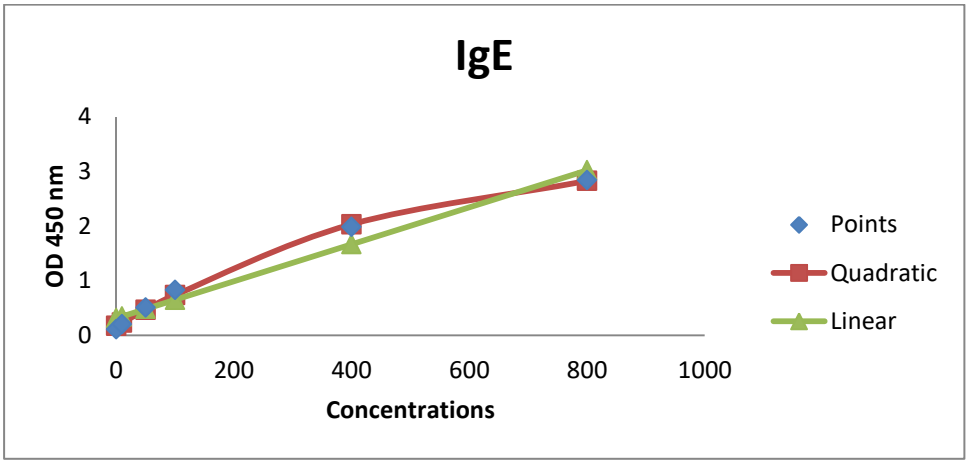

Fig.3. Linear and quadratic fitting of the IgE data in table 1

The simple linear correlation coefficient between RhV-Ag and IgE readings was calculated and found to be significant ($r=0.242$, $p<0.05$). Linear and nonlinear fittings (Figure 4) were also performed between RhV-Ag and IgE.

The two-sample t-test was using in order to compare means of RhV-Ag and IgE between adults and children of the tested samples. In this context the results revealed that means of RhV-Ag and IgE in children were significantly lower than that of the adults, Table 3.

Table 3. Two-sample t-test for the comparisons of means RhV-Ag and IgE with respect to age groups.

Factor	Adults			Children			t-test	p-value
	N	Mean	Sd	N	Mean	Sd		
RhV-Ag	28	0.435	0.176	122	0.316	0.177	3.227	<0.01
IgE	28	1.177	0.933	122	0.308	0.291	8.677	<0.01

The criteria that used to judge the best fitting was root mean square error. Both fits linear and nonlinear showed no and fail to model the relationship between the two variables. Such a result is definitely due to huge fluctuation between readings of both variables.

The distribution of positive cases from both RhV-Ag and IgE with respect to age groups showed in Table 4. With respect to adults, both IgE and RhV-Ag showed higher percentages than that found in children.

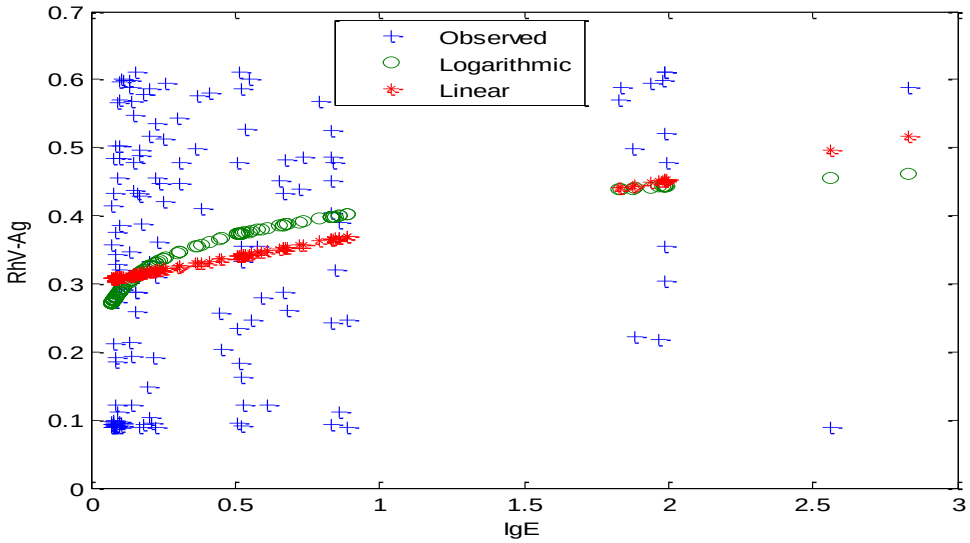

Fig.4. Linear and nonlinear fitting of the RhV-Ag versus IgE.

Table 4. Distribution of positive cases from RhV-Ag and IgE with respect to age groups.

Test	Age				Total positives	
	Adults (No.=28)		Children (No.=122)			
	No. (+ve)	% (+ve)	No. (+ve)	% (+ve)	No.	%
RhV-Ag	24	85.71	80	65.57	104	69.33
IgE	16	57.14	42	34.43	58	38.67

In both groups of persons, RhV-Ag found in a higher percentages of positive infection than that of the IgE. It is logical to conclude that both tests do not perfectly agreed to isolate either positive or negative cases. In this context the two tests are not identical or even fairly similar.

The cross classification between cases of the RhV-Ag test against that of the IgE test was tested Table 5. The percentage of agreement in both tests can be obtained by dividing the sum of the diagonal cells on the total sum of the sample individuals. In this context this percentage is 57.33.

Table 5. Cross classification of cases from RhV-Ag against cases from IgE.

RhV-Ag	IgE		Total
	Negative	Positive	
Negative	37	9	46
Positive	55	49	104
Total	92	58	150

Discussion:

Viral aetiology of the common cold was demonstrated for 69.33% (104 of 150 patients included in this study as show in table 4. Rhinovirus were detected in 105 patients, rhinoviruses were the causative agent of the common cold in more than half of the cases in this investigation. This observation agrees with a recent study in which rhinoviruses was detected in 276 of 346 (80%) common cold patients during epidemic months in Iraq. In this study rhinovirus were detected only during months of Spring and Winter seasons. This reflects that the presence of virus related with the runny and cold months of our country Iraq [14, 15]

According to the presented results individuals with asthma are more susceptible to infection with rhinoviruses. In this study (Rhv-Ag) ELISA screening test was used as a first step for the detection of rhinovirus infection among clinically diagnosis common cold patients. Out of 150 patients, 104 (69.33%) were positive who were divided into 24(85.7) % of adults and 80(65.6%) of children appeared to have rhinovirus infection. Rhinoviruses were the causative agents of the common cold in more than half of the cases in this investigation. This observation agrees with a recent study in which rhinoviruses was detected in 276 out of 346 (80%) common cold patients during epidemic months [16, 17] .

With respect to adults, RhV-Ag showed higher percentages of positive infection than children as shown in table 4 and this can be explained that adults are more susceptible to this viral infection than children it may be due to some deficiency in the immune system of them or may be those patients have some chronic pulmonary and cardiac diseases especially in the elderly patients or patients with a past or current history of smoking.

This fact indicats that HRV infections can no longer be considered a minor nuisance and its diagnostic search is justified. Alberto F *et al.*[18] showed the same result that HRV is more frequently encountered in symptomatic adult patients than in others age group demonstrating its pathogenic relevance.

However, the HRVs detected in this study were not sequenced to identify specific serotypes, previously HRV serotypes were classified into group A, B and the novel C group by many studies using RT-PCR technique. Human rhinovirus C were significantly more likely to have underlying high risk condition of asthma. The common cold was detected in 38.67% patients have asthmatic whereas 84.4% of patients have this respiratory condition in this study.

Measurements of IgE level supported by clinical diagnosis of patients aid in the determination of asthmatic patients group. There is simple linear correlation coefficient between RhV-Ag and IgE readings. It was found that there is significant correlation between IgE and RhV-Ag with higher percentages of positive infection in adult than children in this study. This high percentage of infection may be due to that the virus behavior through induced wheezing episodes instead revealing a preexisting tendency for asthmatic infection to impaired lung physiology or antiviral response, therefore the combination of allergic sensitization and human rhinovirus infection induced wheezing was associated with the highest risk of developing asthma. Other reasons that airway hyper responsiveness is the main feature of asthma due to inhaled substances such as histamine. Also, viral respiratory infections can transiently increase airway responsiveness in human and this gives chance to asthmatic development.

Salvolainen and Kathryn [19, 20] concluded that HRVs play an important role in asthmatic infection especially the HRV.

Depending on above facts, the study concluded individuals with asthma are more susceptible to infection with rhinovirus, viral respiratory infections can have a profound effect on many aspects of asthma including it's exacerbations and severity of many viral respiratory infections that influence asthma. Rhinovirus has emerged as the most frequent illness associated with exacerbation and other aspects of asthma. Many cell types are involved in the immune response to rhinovirus but the most important are respiratory epithelial cells and macrophages and this can be explained by the rate of rhinovirus receptors ICAM-1 and LDL found in the epithelial cells, so infection of epithelial cells generates a variety of pro-inflammatory mediators to attract inflammatory cells to the airway and the epithelial airway antiviral response to rhinovirus is not effective in asthma. Rhinovirus infection induces the release of chemokines from airway epithelial cells, thereby attracting inflammatory cells to the airways. In patients with preexisting airway inflammation the influx of additional inflammatory cells caused by rhinovirus infection would lead to additive or synergistic effects and exacerbation of airway disease. There is evidence that rhinovirus infection induces airway epithelial cells to express pro-inflammatory chemokines. Rhinovirus increases expression of neutrophil, eosinophil, T- cell attracting chemokines including CXCL8, CCL5, CCL11 and CXCL10. Therefore, the understanding of the immune response to rhinovirus is a key step in defining mechanisms of asthma and most importantly improved treatment [23, 24].

Infection with rhinovirus play an important role in the increase the level of IgE but overall positive cases 9 patients showed positive IgE and negative RhV-Ag. This may be due to other causes like asthmatic patients with bronchitis or patients exposure to dust and stress. Another study suggested that experimental rhinoviruses infection of subjects with allergic rhinitis induced a rapid increase in serum IgE without evidence of elevation in antigen- specific IgE, whether total IgE or rhinovirus- specific IgE plays a role in lower airway response to rhinovirus infection remains to be demonstrated [24]. HRV are associated with a significant burden of asthma in young children and adult. Our study is the first comprehensive population based assessment of group HRV patient with clinical information collected in the context of history with asthma.

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