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DETECTION OF RHINOVIRUS AND SOME DNA/RNA VIRUSES BY REVERSE TRANSCRIPTASE REAL-TIME PCR AND THEIR IMMUNOLOGICAL PARAMETERS IN PATIENTS WITH ACUTE RESPIRATORY TRACT INFECTION IN IRAQ: MOLECULAR AND IMMUNOLOGICAL STUDY

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Keywords:

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ABSTRACT: Background and Objectives: Acute upper respiratory tract infections are the most frequent acute illnesses worldwide. This study has been undertaken for determining the diagnostic importance of Human Rhinovirus and other associated DNA/RNA viruses including human Respiratory Syncytial virus; Human Metapneumovirus; Human parainfluenza virus 1-4; human coronavirus; Human B, C, and E Adenovirus; and Human Bocavirus in common cold disease. Further, to detect the role of some immunological parameters including complement three and rhinovirus Ag (RhV-Ag) in rhinovirus infection. **Patients and methods:** The samples of 84 individuals infected with common cold were collected and studied. Viral nucleic acids either DNA or RNA were extracted and amplified using real-time multiplex PCR. **Results:** The most common type causative agent was hRv which accounted for 56.1% followed by hRv+hRSV-hMpv, 12.19% and hRv+hAdv-hBov, 9.75% respectively. Also, the most rarely infection was hRSV-hMpv which found only in one case. On the other hand, complement three was used as a potent immuno-parameter to detect the level in a sample of 80 tested individuals infected with human rhinovirus at which it was found that there is a simple linear correlation coefficient matrix of the variables C3 and RhV-Ag. **Conclusion:** The study suggested that multiplex RT-PCR is a rapid, cost-effective, specific and highly sensitive method for detection of respiratory viruses including rhinovirus. Further, this study has focused a highlight on the critical roles of C3 in the pathogenesis of rhinovirus infection. Furthermore, RhV-Ag was found to be significantly correlated with the complement C3 at $p<0.05$ and RhV-Ag cannot be predicted using complement C3.

INTRODUCTION: Human rhinoviruses (HRVs) are responsible for more than one-half of cold-like illnesses and cost billions of dollars annually in medical visits and missed days of work. HRVs are traditionally associated with upper respiratory tract infection, otitis media, and sinusitis.

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In recent years, the increasing implementation of PCR assays for respiratory virus detection in clinical laboratories has facilitated the recognition of HRV as a lower respiratory tract pathogen [1].

Viral pathogens play an important role in infants who present ARIs, respiratory syncytial virus (RSV) being the most important virus associated. Also, children with RSV infections are also exposed to a variety of other respiratory viruses with a similar seasonal pattern, mainly during winter months, such as influenza, rhinovirus (RV), human metapneumovirus (hMPV), and human bocavirus (HBoV) [2].

Human rhinovirus and influenza virus infections of the upper airway lead to colds and the flu and can trigger exacerbations of lower airway diseases including asthma and chronic obstructive pulmonary disease. Novel diagnostic and therapeutic targets are still needed to differentiate between the cold and the flu since the clinical course of influenza can be severe while that of rhinovirus is usually milder. The common cold is represented as a significant viral infection of the upper respiratory tract and the most commonly implicated viruses are rhinovirus (30-80%) following by human coronavirus (15%), influenza viruses (10-15%), adenoviruses (5%), human parainfluenza viruses, human respiratory syncytial virus, enteroviruses other than rhinoviruses and metapneumovirus. Also, Coronavirus-HKU1 accounted for 1.6% of adult respiratory infection [3]. Also, human coronavirus has pleomorphic spherical virion, coronavirus (60-220 nm); tubular nucleocapsid with helical symmetry; Linear plus sense ssRNA genome, three or four structural proteins: nucleoprotein, peplomer glycoprotein[4].

Adenoviruses the genus of mast adenovirus comprises all 47 human serotypes; has icosahedral virion (8-90nm), 252 capsomers, 12 fibers at vertices and 12 structural proteins; linear, dsDNA genome; complex program of transcription from seven early, intermediate and late promoters, splicing and transcription, DNA replication and virion assembly occur in nucleus[5]. Also, Human metapneumovirus is a genus of paramyxoviridae family isolated in 2001 in the Netherlands. Negative sense ssRNA and their genome analog to the RSV genome; the second most common cause of lower respiratory infection in children after RSV. It has been observed that co-infection with RSV and metapneumovirus can occur and cause 10% of respiratory tract infection and linked with severe idiopathic pneumonia in recipients of hematopoietic stem cells transplants [6].

Regarding human parainfluenza virus, it has pleomorphic spherical virion, 150-300 nm, sometimes filamentous; envelope containing two glycoprotein's: F (fusion protein) and attachment protein HN (hemagglutinin-neuraminidase) in addition to non-glycosylated membrane protein; helical nucleocapsid, 18nm (paramyxovirinae) or 13 nm diameter (Pneumovirinae), NP or N protein with associated transcriptase and phosphoprotein; linear minus sense ssRNA genome 15-16 kb with 6-7 genes encoding 10-12 proteins (paramyxovirinae) or 10 genes encoding 10 proteins (Pneumovirinae); cytoplasmic replication with budding from plasma membrane and syncytium formation and cytoplasmic inclusions [7]. Human bocavirus is a parvovirus that has been suggested to cause lower respiratory tract infection. HBoV is the fourth most common virus of respiratory disease; the virions are small (18-26 nanometers), icosahedral and non-enveloped; genomic analysis of human HBoV consist of linear ssDNA virus encodes the viral proteins, the coding sequences are flanked by imperfect terminal palindromes or inverted repeats to form hairpin-like structures responsible for genome replication [8].

It is well recognized that molecular methods for detection of an infectious agent are steadily becoming more widely applied concerning a vast array of microorganisms, including rhinoviruses. Different approaches based on PCR have been investigated to optimize the method for research as well as clinical purposes. A rapid and sensitive micro-well reverse transcription RT-PCR hybridization assay was developed to detect human rhinoviruses in clinical specimens and cell culture suspensions. RT-PCR test with oligonucleotide hybridization detection proved to yield the highest number of positive results more than half of negative by contemporary conventional culture [9].

Immunologically, both cellular and humoral immunity is activated in response to rhinovirus infection. Complement activation and cells under attack by rhinoviruses release cytokines to attract immune cells and warn neighboring cells that they have infected these cytokines including interferon gamma and interleukin-8. All of this accomplishment as the body attempts to clear and to turn the point of the viral infection [10]. The function of the complement system as an immune surveillance system that rapidly responds to infection and activation of the complement system by specific recognition pathways triggers a protease cascade, generating cleavage products that function to eliminate pathogens, regulate inflammatory responses and shape adaptive immune responses [11]. Therefore, this study aimed for the following purposes:-1) Detection of the reliability of the

multiplex-RT-PCR technique as a cut off molecular diagnostic tool in the detection of rhinoviruses in reverse transcriptase RT-PCR. 2) Also, to determine the diagnostic importance of other DNA/RNA viruses associated with this disease including human Respiratory Syncytial virus (hRSV); human Metapneumovirus (hMpv); human parainfluenza virus 1-4 (hPiv); OC43, E229, NL63 and HKUI human coronavirus (hCov); Human B, C and E Adenovirus (hAdv); Human Bocavirus (hBov). 3) Further, to detect the role of some immunological parameters in the serum of patients like Complement 3 (C3) level and rhinovirus antigen (Rhino-Ag) in rhinovirus infection.

PATIENTS AND METHODS:

Multiplex Real-Time PCR for DNA/RNA Detection: 84 nasal swabs specimen were collected from individuals infected with a common cold during the period from December 2012 to April 2013. They were subjected to real-time PCR (RT-PCR) after processing.

ARVI Screen Real-TM PCR kit, (Sacace biotechnologies, Italy) is an in vitro nucleic acid amplification test for multiplex detection and identification of specific nucleic acid fragments of pathogens that cause acute respiratory viral infections.

Procedure:

Isolation of RNA/DNA from Specimens:

1. (450 µl) Lysis solution and (10 µl) internal control RNC C+ (IC RNA) was added to each tube of the test.
2. Mixed by pipetting and incubated for 5 min at room temperature.
3. (10 µl) of samples were attached to the appropriate tube containing lysis solution and IC.
4. The tubes were vortexed.
5. Then, centrifuged for 5 sec at 5000 g. If the sample is not entirely dissolved it was recommended to re-centrifuge the tube for 1 min at maximum speed (12000 - 16000 g), then the supernatant was transferred into a new tube for RNA extraction.
6. The sorbent was vortexed vigorously and (25 µl) was added to each tube.
7. The mix was vortexed for 5-7 sec and incubated all tubes for 10 min at room temperature. They were vortexes periodically
8. All tubes were centrifuged for 1 min at 10000g, and a micropipette was used carefully to remove and discard supernatant from each tube without disturbing the pellet. The tips were changed between tubes.
9. To each tube, (400 µl) of washing solution was added. After that, the tubes vortexed vigorously, centrifuged for 1 min at 10000 g. A micropipette was used carefully to remove and discard supernatant from each tube without disturbing the pellet. The tips were changed between tubes.
10. (500 µl) was added of ethanol 70% to each tube. Vortexes vigorously and centrifuged for 1 min at 10000 g. A micropipette was used carefully to remove and discard supernatant from each tube without disturbing the pellet. The tips were changed between tubes.
11. Step 10 was repeated.
12. (400 µl) of acetone was added to each tube. Vortexes vigorously and centrifuged for 1 min at 10000 g. A micropipette was used carefully to remove and discard supernatant from each tube without disturbing the pellet. The tips were changed between tubes.
13. All tubes were incubated with an open cap for 10 min at 60 ºC.
14. The pellet was re-suspended in (30 µl) of RNA-eluent for 10 min at 60 ºC and vortexes periodically. Centrifugation of the tubes was achieved for two minutes at maximum speed (12000-16000 g)
15. The supernatant now contains RNA which was ready to use. The RT- PCR can be performed the same day [12, 13].

Reverse Transcription:

1. (20 µl) of the reaction, the mix was added into each sample tube.
2. (20 µl) of RNA samples was pipetted into the appropriate tube, all the tubes were centrifuged with extracted RNA for 2 min at maximum speed (12000 - 16000g), and the supernatant

was taken care for not disturb the pellet, (sorbent inhibit reaction). The mix was mixed carefully by pipetting.

3. Tubes were placed into thermal cycler and incubated at 37 ºC for 30 min.
4. Each obtained cDNA sample was diluted 1:2 with TE-buffer (40 µl TE-buffer was added to each tube). cDNA specimens could be stored at -20 ºC for a week or at -70 ºC during a year.

Amplification:

1. The required quantities of tubes or PCR plate were prepared. The tubes were thawed with the reagents.
2. For carrying out N reaction (including four controls: 3 PCR controls and one negative extraction control) in a new sterile tube, the following quantities were prepared: 10N µl of PCR-mix-1, 5.0N µl of PCR-mix-2-FRT and 0.5N of Taq polymerase. The tube was vortexed, then centrifuged shortly and marked with the name of the mix (for example hRSv-hMpv). This procedure with each PCR-mix-1 was repeated.
3. For each sample, six tubes were prepared, and 15 µl of the reaction mixture was added into each tube.
4. (10 µl) of cDNA sample was added to the appropriate tube with a reaction mix.
5. Prepare for each mix of three controls:

- A-(10 µl) of DNA-buffer was added to the tube labeled PCR negative control
- B-(10 µl) of cDNA, C+ was added to the tube labeled C_{pos}
- C-(10 µl) of IC DNA was added to the tube labeled IC DNA $_{post}$.

TABLE 1: TEMPERATURE PROFILE ADJUSTMENT IN A THERMAL CYCLER

	Plate or modular type instruments		
Step	**Temperature, ºC**	**Time**	**Cycles**
1	95	15 min	1
	95	10 sec	
2	54	25 sec	10
	72	25 sec	
	95	10 sec	
3	54	25 sec	35
		Fluorescence detection	
	72	25 sec	

Detection of the Level of Complement 3 (C3): Peripheral venous blood (5 ml) was aspirated by using (5 ml) disposable syringes from 80 clinical diagnosis rhinovirus patients (2- 70 years old) belonged to the urban & rural area of Ramadi city. A clear serum was separated from the blood sample. Complement 3 (C3) SRID plate from LTA, Italy was used to the quantitative determination of Complement 3 protein (C3) by Single Radial Immunodiffusion plate (Mancini test) [14].

Procedure: Endplates of Single Radial Immunodiffusion kits were used for the quantitative determination of human serum immunoglobulins as follows [14]:

1. The plate was removed from ziplock bag and leave for a few minutes so that any condensed water in the wells can evaporate.
2. Five µl of the sample and controls were applied into wells and wait until it has been utterly absorbing before handling the plate.
3. The lid closed firmly and incubated in the moist chamber in the cooled refrigerator at 23 ºC for 72 h.
4. Area of precipitation rings was measured with a suitable coulometer, and immunoglobulin concentrations were calculated according to the reference table.
5. The concentrations were representative for the precipitating ring diameter. The ring value was obtained from the reference table.

Detection of Human Rhinovirus Antigen (RhV-Ag) by ELISA: Rhinovirus antigen was detected using a double-antibody sandwich enzyme-linked immunoassay ELISA (My Biosource, California). This test was performed strictly according to the manufacturer's instructions:

1. Standards were diluted as follow:

12 pg/ml	Standard no. 5	120 µl Original Standard + 120 µl standard diluents
Six pg/ml	Standard no. 4	120 µl Standard no. 5 + 120 µl standard diluents
Three 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 µl Standard no. 2 + 120 µl standard diluents

2. Chromogen solution A, chromogen solution B and stop solution was added to the 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. The membrane was sealed, shacked gently and incubated for 60 min at 37 °C.
5. The plate was washed with 30X diluted wash solution.
6. Chromogen solution A 50 µl and Chromogen solution B 50 µl were added to each well. It was 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. According to standard' concentration and optical density (OD) values, the standard curve regression equation calculated out and the OD values of the samples on the regression equation to calculate the concentration of the corresponding samples.

RESULTS: The sample of real-time data contained 84 admitted cases divided into 41 males (48.8%) and 43 females (51.2%). Most of the accepted cases (approximately 69%) were below or equal 14 years old, whereas about 31% were above 14 years old. Age of the patients ranged between 1.17 years of old up to 35 years with a mean age of 14.1 years and standard deviation of 10.84 years. Females mean age was 15.7 years with a standard deviation of 11.4 years, and males mean age was 12.5 years with a standard deviation of 10.2 years. Although women mean age is higher than that of males patients, there is no statistical evidence point out significant difference since the p-value of the test was considerably larger than 0.05 both gender groups showed considerable variability in an age which is typical for this type of infection.

The study results revealed that 54 patients (64.3%) were from urban areas and 30 patients (35.7%) were from rural areas. Chi-square test (0.239) with df = 1 and p-value = 0.63, showed that residency has no significant effect on the infection with Rhinovirus.

TABLE 2: DISTRIBUTION OF PATIENTS CONCERNING THEIR RESIDENTIAL AREAS ON GENDER AND INFECTION GROUPS

Residency	RT-PCR								Total	
	Positive				Negative					
	Male		Female		Male		Female			
	<=14 yr	>14 yr	<=14 yr	>14 yr	<=14 yr	>14 yr	<=14 yr	>14 yr	<=14 yr	>14 yr
Rural	3	4	4	4	2	3	3	7	12	18
Urban	12	2	9	1	13	2	12	3	46	8
Total	15	6	13	5	15	5	15	10	58	26

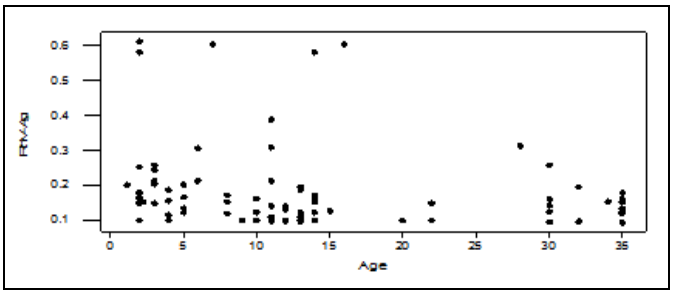

FIG. 1: SCATTER PLOT OF THE AGES VERSUS RT-PCR

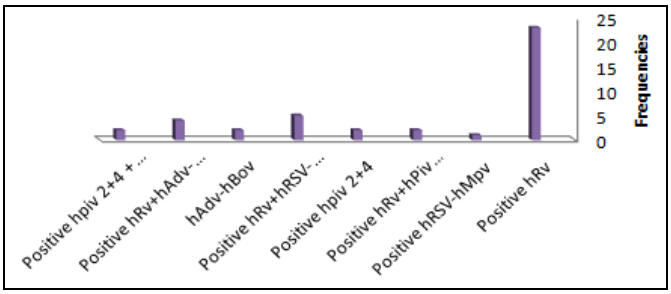

FIG. 2: BAR CHART REPRESENT THE FREQUENCIES OF VIRUSES CAUSING COMMON COLD FOR STUDY PATIENTS AS PRODUCED BY REAL-TIME PCR TECHNIQUE

To see whether readings of the RT-PCR is correlated with age or not, the simple linear correlation coefficient has been obtained for this purpose and found to be equals to (r = -0.14) with p-value >0.05.

Although the result of this measure is not significant, there is a slight indication of a negative relationship between age and readings as represented in **Fig. 1**.

The study results revealed that the most common type of infections is the hRv which accounted for 56.1% of total positive cases. This percentage was substantially larger than any other percentage of infection of this table. The next two percentages were for the (Positive hRv + hRSV - hMpv, 12.19%) and (Positive hRv + hAdv - hBov, 9.75%) respectively.

When considering the t-test of comparing two percentages, the result of this test revealed no significant difference between the two percentages of infections. It is, therefore, easy to comment that hRv is the most common infection among all other types of infection as represented in the following **Table 3**. The frequencies of infections are remarkably varied from 1 up to 23 which is constituted more than half the infections. The rarest infection was (Positive hRSV-hMpv) which found only in one case **Fig. 3**.

TABLE 3: THE FREQUENCIES AND PERCENTAGES FOR HUMAN RHINOVIRUS AND OTHER DNA/RNA VIRUSES CAUSING ACUTE RESPIRATORY TRACT INFECTION (COMMON COLD) AS PRODUCED BY REAL-TIME PCR TECHNIQUE

Result of RT-PCR	Frequency	Percentage
Positive hRv	23	56.10
Positive hRSV-hMpv	1	2.44
Positive hRv+hPiv 2+4	2	4.88
Positive hpiv 2+4	2	4.88
Positive hRv+hRSV-hMpv	5	12.19
hAdv-hBov	2	4.88
Positive hRv+hAdv-hBov	4	9.75
Positive hpiv 2+4 + hAdv - hBov	2	4.88
Total	41	100

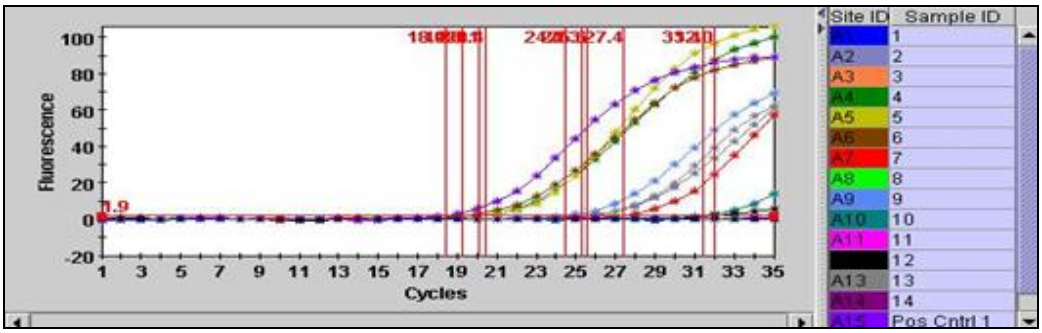

FIG. 3: OPTICS GRAPH SHOWS RT-PCR-RHINOVIRUSES RESULTS USING TxR FLUORESCENT STAIN. ACCORDING TO (PRIMARY CURVE=1.9) AND THRESHOLD=18.1:- POSITIVE SAMPLES NO. ARE 2, 4, 5, 6, 7, 9, 10 AND 13. POSITIVE CONTROL SAMPLE SITE ID.15

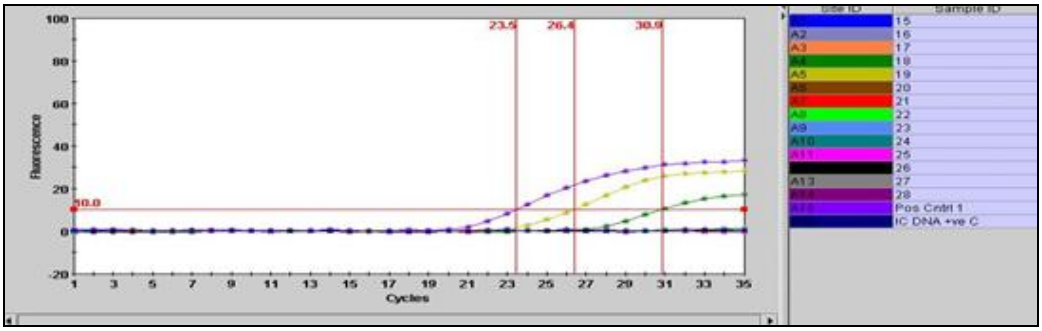

FIG. 4: OPTICS GRAPH SHOWS RT-PCR-HUMAN ADENOVIRUS-HUMAN BOCAVIRUS RESULTS USING TXR FLUORESCENT STAIN ACCORDING TO (PRIMARY CURVE=10.0) AND THRESHOLD=23.5: POSITIVE SAMPLES NO. IS 18 AND 19

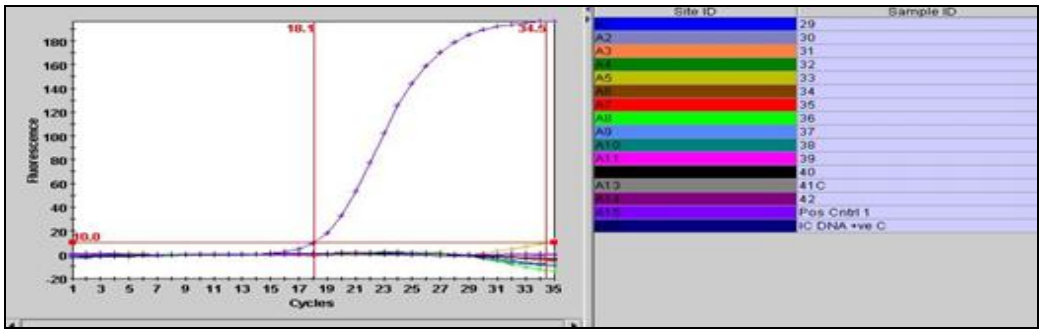

FIG. 5: OPTICS GRAPH SHOWS RT-PCR FOR HUMAN RESPIRATORY SYNCYTIAL VIRUS-HUMAN METAPNEUMOVIRUS RESULTS USING Cy3 FLUORESCENT STAIN. ACCORDING TO (PRIMARY CURVE=10.0) AND THRESHOLD=18.1: POSITIVE SAMPLES NO. 33. POSITIVE CONTROL: SAMPLE SITE ID. 15. INTERNAL CONTROL: SITE ID. 16

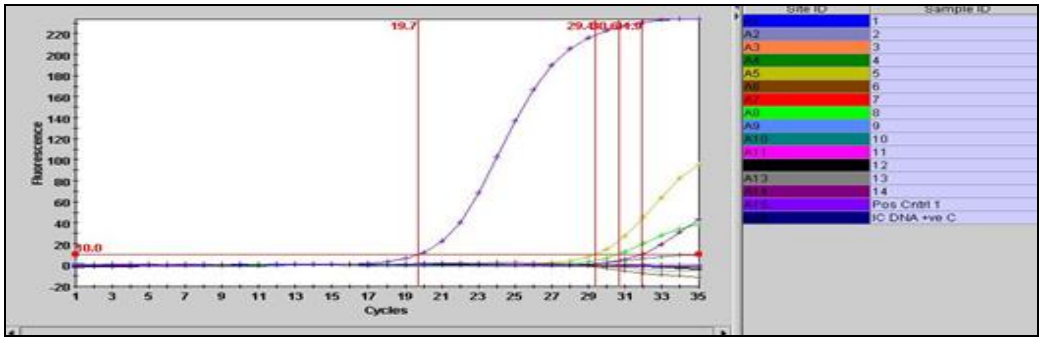

FIG. 6: OPTICS GRAPH SHOWS RT-PCR FOR HUMAN PARAINFLUENZA 2+4 RESULTS USING Cy3 FLUORESCENT STAIN. ACCORDING TO (PRIMARY CURVE=10.0) AND THRESHOLD=19.7: POSITIVE SAMPLES NO. IS 5, 8 AND 14. POSITIVE CONTROL: SITE ID. 15. INTERNAL CONTROL: SITE ID. 16

Regarding the immunological part of this study, the complement (C3) which was recorded for participated patients in this study, it was investigated once regarding their association with positive and negative infection, and another with similar readings of the RhV-Ag. It was found that there is a simple linear correlation coefficient matrix of the variables complement 3 (C3) and Rhinovirus antigen (RhV-Ag). RhV-Ag was found to be only significantly correlated with the complement C3 at $p<0.05$. On the other hand, the scatter diagram and proposed linear fitting for the scattering points in the following figure indicate that extreme readings for the RhV-Ag versus readings complement C3. Such extreme readings will not facilitate for a proper fitting. As a general trend, linear fitting is more appropriate for the complement observations. It can be concluded that RhV-Ag cannot be predicted using complement C3.

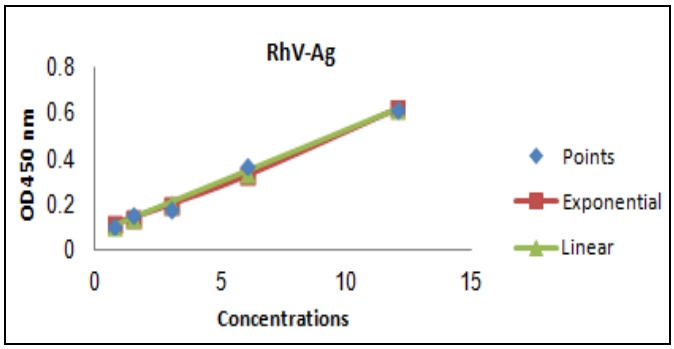

FIG. 7: LINEAR AND EXPONENTIAL FITTING OF THE RhV-Ag DATA

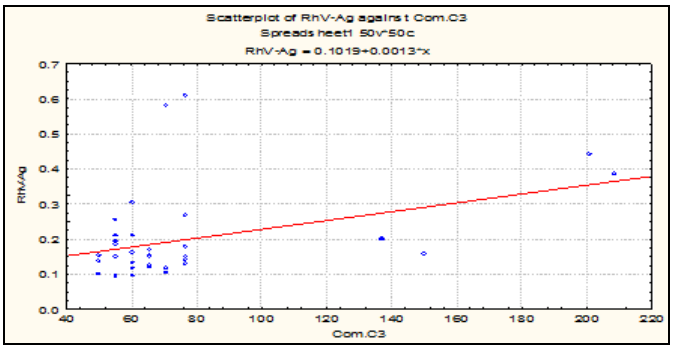

FIG. 8: SCATTER DIAGRAM OF COMPLEMENT C3 *vs.* RHINOVIRUS ANTIGEN

DISCUSSION: It is well known that acute respiratory viral infections are the most frequent illnesses experienced by most people globally. Most acute respiratory diseases are viral infections; it involves the upper respiratory tract infections (URI) including the nose, sinuses, pharynx, and larynx. This commonly includes tonsillitis, pharyngitis, laryngitis, sinusitis, otitis media and rhinovirus infection "the common cold [15]. Young children with ages between five and seven of these illnesses per year, with a general decrease in frequency with age increase and the adults, will have about two such diseases per year. Dual viral infections are common, and a significant proportion of children have evidence of viral-bacterial co-infection.

In adults, viruses are the putative causative agents in a third of cases of community-acquired pneumonia in particular rhinoviruses, coronaviruses, and influenza viruses are the causative agents. Since, rhinoviruses represent the most frequent cause of acute respiratory infections worldwide and in all age groups [16-18].

On the other hand, the availability of molecular assays has made laboratory diagnosis more efficient and has led to improved detection of a broad spectrum of respiratory viruses. This new multiplex technique dramatically shortens the hands-on time and much more straightforward than conventional routine methods which need many steps and a wide range of technical competence like

culture, PCR and immuno-fluorescence technique [19, 20]. The new version of the real-time RT- PCR assay used in this study has expanded the capacity for detecting seven viruses and thus increased the diagnostic potential of this test. A multiplex real-time PCR assays were used in this study for detection of respiratory RNA and DNA viral infections in 84 specimens; the respiratory specimens were collected from mixed groups of adults and children and the results obtained were analyzed. A very high percentage of the samples were positive giving viruses signature in nearly 39 (46.4%) This may be due to the immature immune response of children and more complex immune system of adults. The study results are in agreement with those obtained by Bhat and associates [21] who recorded that rate of detection in young children and infants is higher while in adults is lower. Detection of HRV by culture is slow and complex and the serological diagnosis not always possible due to the number of serotypes and not available rapid antigen test kits.

Thus, molecular methods such as the real-time RT-PCR appear to the most suitable method, combining short analysis time, high sensitivity, semi-quantification of viral load and the detection of the majority of respiratory viruses with multiplex methods [22]. Rhinoviruses were (56.10%) of total positive cases. It was the most prominent pathogen detected as the main cause of acute respiratory infection. The next two percentages were 12.9% for positive hRv+hRSV-hMpv and 9.8% for Positive hRv+hAdv-hBov respectively. Further, neither coronavirus HKU-1 and OC43 nor NL63 and 229E recorded positive results. It may be due to the site of the entry of this virus through the upper respiratory tract infection represented by the mouth and nose and the presence of the main receptor of rhinovirus in the epithelial cells of respiratory infection in this sites ICAM-1 for adhesion and initiate of infection that appear after 15 min after entry of virus [23]. The study was also assessed infection of respiratory viruses to determine whether age played a role in co-infection at which two multivariable logistic regression models of the age group was constructed under and above fourteen years old. Age was not significantly important (P value >0.05) in these infections and highlighting of infection was under fourteen years old.

Our study was also assessed residence variation in HRVs. It has included urban and rural groups. However, residency and seasonal variation were studied, residency show no significant effect on the infection with rhinovirus as described above most of the viral infection depends on community behavior. During this study more of HRVs infection were detected in December and April and the seasonal variations under comes the importance of studies to understand rhinovirus epidemiology fully. Leotte and co-workers [24] documented that most HRV-infected patients were younger than 2 years (57%).

Overall, patients infected with HRV had a lower frequency of severe acute respiratory infection than those infected with other CRVs (60% and 84%, respectively, P=0.006), but had more comorbidities (40% and 27%, respectively; P=0.043). However, in the adjusted analysis, this association was not significant. The mortality rate within the HRV group was 3%. Detection of HRV was more prevalent during autumn and winter, with a moderately negative correlation between viral infection frequency and temperature (r=-0.636, P value <0.001).

In this study, there are Six of cases infected with RSV 1.0 (2.44%) was hRSV+ hMPV and 5.0 (12.19%) was hRV+ hMPV+ hRSV of viral co-infection. Multiplex Real-Time PCR detected this co-infection with high sensitivity, and most of these cases were in children. Also, all of co-infection recorded cases were in patients under 14 years old, hRV+ hMPV+ hRSV of viral co-infection reported in this study and rhinovirus is a crucial virus that will be talking about it, metapneumovirus and respiratory syncytial virus consider the viral etiology of severe bronchiolitis. The study result revealed that specific virus combinations recorded in patients under 14 years old and this infection might affect clinical outcomes and warrant further investigation. Statistically, no significant difference observed between the two percentages of co-infections. Zhang and co-workers [25] reported that a high frequency of respiratory infections and co-infections was detected in children with acute respiratory symptoms. The multiplex RT- PCR assay was also able to directly detect Piv 1+3, Piv 2+4, hAdv, and hCov.

The study suggested that the multiplex RT- PCR assay can be used as a rapid and sensitive diagnostic method for major respiratory viruses. Also, Huey-Ling and co-workers[26] developed a highly specific and sensitive one-step triplex qRT-PCR assay to detect hMPV and RSV simultaneously. This assay (RT-PCR) offers a valuable tool for routine diagnosis of bacterial and viral infection [27]. Further, the use of real-time PCR as a diagnostic and genotyping tool for medically important viruses like HPV has the advantage for women who currently have high-grade cervical lesions and at high risk of developing cervical cancer in the future [28, 29].

According to our result about Complement 3 (C3) level which determined in the serum of rhinovirus-infected patients, this is considered the first study to establish that human rhinovirus 3C protease is the likely mediator of Complement 3 cleavage. HRV infection is catalyzed by binding to host cell plasma membrane receptors (usually, ICAM-1 or LDL receptors) followed by conformational changes in the human rhinovirus capsid leading to release of the viral genome inside the cytoplasm of the infected cell; the viral genome is the translated into a polyprotein [30]. The study result revealed that RhV-Ag was found to be only significantly correlated with the complement C3 at $p<0.05$. The presence of rhinovirus Ag meaning the presence of rhinovirus infection and this meaning the presence of 3C proteases activity that cleaves C3 leading to a deficiency in the level of C3 concentration in serum of rhinovirus infection patients.

So, many viruses disrupted cell nucleocytoplasmic trafficking and proteins appropriate it for their use probably as a strategy to limit anti-viral responses. There is a study result similar to our study observation, but work on 2A protease activity showed that 2A mediates the initial cleavage of nucleoprotein 98 early during infection (starting 3 h postinfection) with division of nucleoprotein 153 by 3C protease following at about 6 -9 hours post infection and 3C localized in the nucleus [31]. Tam and associates [32] demonstrated that during viral infection the pathogens traverse barriers during infection, including cell membranes and this transition pathogens carried covalently attached complement three into the cell triggering immediate signaling and effectors responses. This system could detect both viral and bacterial pathogens but be antagonized by enteroviruses such as rhinoviruses and polioviruses which cleave C3 using 3C protease activity. Therefore, complement C3 allows cells to determine and disable pathogens that have invaded the cytosol.

Regarding complement C3, it was found to be significantly correlated with RhV-Ag at $p<0.05$. C3 genes regulated by IFN- gamma, IFN- gamma stimulation increased C3, and C4 protein synthesis suggested that the increase in mRNA stability is a dominant effector mechanism by which IFN-gamma regulates C3 and C4 gene expression**.** Three distinct pathways of the complement system can initiate the complement cascade, namely classical, lectin, and alternative pathways; these three pathways of activation culminate with the generation of the C3 convertase enzyme complex, which cleaves complement C3 into C3a and C3b.

Besides their well-known function in the extracellular system of host defense, recent evidence indicates that complement C3 is also intracellularly activated in immune and no immune cell types, acting in metabolic organs such as adipose tissue, liver, and pancreas [33]. The airway epithelium, from the nasal cavity to the alveoli, persistently encounters toxins and pathogens. If the invader clears the mucus and breaches the epithelium, it must be contained by an innate immune system while awaiting the arrival of the adaptive immune system. The complement cascade is the prototype of such an innate process. It quickly amplifies based on the cleavage of its most abundant complement protein: C3 to C3a and C3b **Fig. 1A**. C3a is a vasodilator and chemoattractant for neutrophils and monocytes, whereas C3b is an opsonin for pathogens and debris [34].

CONCLUSION: The study suggested that multiplex RT- PCR is a rapid, cost-effective, specific and highly sensitive method for detection of respiratory viruses including rhinovirus. Further, this study has focused a highlight on the critical roles of the complement system (C3) in the pathogenesis of rhinovirus infection. Furthermore, RhV-Ag was found to be significantly correlated with the complement C3 at $p<0.05$ and RhV-Ag cannot be predicted using complement C3.

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REFERENCES:

1. Samantha EJ, Daryl ML, Kirsten SG and Thomas JW: Human rhinoviruses. Clinical Microbiology Review 2013; 26(1): 135-62.
2. Cristina C, Mar´ıa LG, Francisco P, Gallardo P, Mar M, Ana C, Mo´nica GE, Inmaculada C: Respiratory Syncytial Virus Co-infections With Rhinovirus and Human Bocavirus in Hospitalized Children. Medicine 2015; 94(2): 1-12.
3. Kanwar A, Selvaraju S and Espe F: Human Coronavirus-HKU1 infection among adults in Cleveland, Ohio. Open Forum Infectious Diseases 2017; 4(2): 052.
4. Taek-Kyun K, Anjali B, Yakang L, Koti S, Rachel A, Michael K, Charles C and Jay P: A systems approach to understanding human rhinovirus and influenza virus infection. Virology 2015; 486: 146-57.
5. Fehr AR and Perlman S: Coronaviruses: An Overview of Their Replication and Pathogenesis. Methods Mol Biol 2015; 1282: 1-3.
6. Zhang Q, Jing S, Cheng Z, Yu Z, Dehghan S, Shamsaddini A, Yan Y, Li M and Seto D: Comparative genomic analysis of two emergent human adenovirus type 14 respiratory pathogen isolates in China reveals similar yet divergent genomes. Emerging Microbes and Infections 2017; 6(92): 1-12.
7. Edwards KM, Zhu Y, Griffin MR, Geoffrey A, Weinberg GA, Hall CB, Szilagyi PG, Staat MA, Iwane M, Prill MM and Williams JV: Burden of human metapneumovirus infection in young children. The New England Journal of Medicine 2013; 368(7): 633-43.
8. Indumathi CP, Gunanasekaran P, Kaveri K, Arunagiri K, Mohana S, Khaleefathullah A, SureshBabu BV, Padmapriya P, Senthilraja R and Fathima G: Isolation and molecular characterization of human parainfluenza virus in Chennai, India. Indian J Med Res 2015; 142(5): 583-90.
9. Al-Ouqaili MTS, Jal'oot AS and Badawy AS: Identification of an OprD and blaIMP Gene-mediated Carbapenem Resistance in *Acinetobacter baumannii* and *Pseudomonas aeruginosa* among Patients with Wound Infections in Iraq. Asian Journal of Pharmaceutics. 2018; 12(3): 959-65.
10. Olli R, Elina L, Lance C, Jennings and David M: Viral pneumonia. Lancet 2011; 377: 1264-75.
11. Dani AC. Heesterbeek DAC, Angelier ML, Harrison RA and Rooijakkers SHM: Complement and Bacterial Infections: From Molecular Mechanisms to Therapeutic Applications. Innate Immun 2018; 27: 1-10.
12. Kristina AS and Thomas EM: Complement and viral pathogenesis. Virology 2011; 411(2): 362-73.
13. Green M and Sambrook J: Molecular cloning: A Laboratory Manual, 4th ed. Cold Springer Harbour Laboratory Press, Cold Springer Harbour, New York 2012.
14. Al-Ouqaili MTS, Al-Kubaisy SH and Aethwy AM: Detection of low-risk genotypes of human papillomavirus 6 and 11 in patients with cervicitis by Real-Time PCR assay. Al-Anbar Medical Journal 2016; 14(1): 47-57.
15. Delves PJ, Martin SJ, Burton DR and Roitt IM: Roitt's Essential Immunology. 13th ed. Chichester, Wiley-Blackwell 2017.
16. Ranieri V M, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, Fan E, Camporota L and Slutsky AS: Acute respiratory distress syndrome: the Berlin Definition. ARDS Definition Task Force". JAMA 2012; 307(23): 2526-33.
17. Creer DD, Dilworth JP, Gillespie SH, Johnston AR, Johnston SL, Ling C, Patel S, Sanderson G, Wallace PG and McHugh TD: Aetiological role of viral and bacterial infections in acute adult lower respiratory tract infection (LRTI) in primary care. Thorax 2006; 61(1): 75-9.
18. David L, Devora L, Avi S, Ayelet K, Rachel S and Yonat S: Identification of respiratory viruses in adults: nasopharyngeal *vs*. oropharyngeal sampling. J Clin Microbiol 2009; 47(11): 3439-43.
19. Torres A, Lee N, Cilloniz C, Vila J and Van der M: Laboratory diagnosis of pneumonia in the molecular age. Eur Respir J 2016; 48(6): 1764-78.
20. Otto CC, Kaplan SE, Stiles J, Mikhlina A, Lee C, Babady NE and Tang YW: Rapid Molecular Detection and Differentiation of Influenza Viruses A and B. J Vis Exp 2017; 30(119).
21. Eric AS, Champa P, Wing KS, Charlie WH, Kuan HL, Marilla L, Hanna N, Geraldine N, Pei LT, Chee WK, Yang SC, Jianmin M, Sebastian MS, Phyllis CL, Martin LH, Christopher WW and Arviac C: Pathogen chip for respiratory tract infections. J Clin Microbiol 2013; 51: 945-53.
22. Lokesh S, Ashley L, Thomas T, Charles S, Dela C and Chad R: Pneumonia updates on *Legionella*, *Chlamydophila*, and *Mycoplasma pneumonia*. Clin Chest Med 2017; 38(1): 45-58.
23. Callendo AM: Multiplex PCR and emerging technologies for the detection of respiratory pathogens. Clin Infect Dis 2011; 25(4): 5326-30.
24. Lessler J, Reich NG, Brookmeyer R, Perl TM, Nelson KE and Cummings DA: Incubation periods of acute respiratory viral infections: an asymptomatic review. Lancet 2009; 9(5): 291-00.
25. Leotte J, Trombetta H, Faggion H, Almeida B, Nogueira M, Vidal L and Raboni S: Impact and seasonality of human rhinovirus infection in hospitalized patients for two consecutive years. J Pediatr 2017; 93(3): 294-00
26. Zhang G, Hu Y and Wang H: High incidence of multiple viral infections identified in upper respiratory tract infected children under three years of age in Shanghai, China. Pols One. Pub Med 2012; 7(9): 445-68.
27. Huey-Ling Y, Shun-Jen C, Hong-Ren Y, Chia-Chin L, Chang-Han C and Wei-Ting L: BMC Pediatrics 2017; 17(89): 1-7.
28. Al-Ouqaili MTS, Al-Taei SA and Al-Najjar A: Molecular detection of medically important carbapenemases genes expressed by metallo-β-lactamase producer isolates of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Asian Journal of Pharmaceutics 2018; 12(3): 991-01.
29. Al-Ouqaili MTS and Al-Kubaisy SH: Molecular genetic study on high and intermediate risk genotypes of Human Papilloma virus among patients with benign and malignant cervical lesions. International Journal of Pharmaceutical Sciences and Research 2019; 10(4): 1000-09.
30. Ullmer W. and Semler BL: Diverse Strategies Used by Picornaviruses to Escape Host RNA Decay Pathways. Viruses 2016; 8(335): 1-19.

31. Erin J, Parisa Y, Alex J, Robert M, Belinda J, Philip G, David A and Reena G: Rhinovirus 3C protease facilitates specific nucleoprotein cleavage and mislocalization of nuclear proteins in infected host cells. Pols 2013; 8(8): 713-16.
32. Tam JC, Bidgood SB and McEwan WA: Intracellular sensing of complement C3 activates cell-autonomous immunity. Pub Med. US National Library of Medicine National Institutes of Health 2014; 5(345): 6201-35.
33. Reinaldo S, Laura M, Fabio A, Lorella M, Mara S, Stefan R, Piero M and Rasmus W: Protective Role of Complement C3 Against Cytokine-Mediated β-Cell Apoptosis. Endocrinology 2017; 158(8): 2503-21.
34. Hrishikesh S, Kulkarni HS, Liszewski MK, Brody SL and Atkinson JP: The complement system in the airway epithelium: An overlooked host defense mechanism and therapeutic target? J Allergy Clin Immunol 2018; 141: 1582-6.

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