Detection of Rhinovirus and Some DNA/RNA Viruses using Multiplex-Real-time PCR and both of Complement (C3) Level and Rhinovirus-Ag in Patients with Acute Respiratory Tract Infection: Molecular and Immunological Study

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Abstract: Acute upper respiratory infections, generally known as common colds are the most frequent acute illnesses world-wide. Depending on multiplex Real-Time PCR technique, our study has been undertaken for determining the diagnostic importance of Human Rhino virus (HRV) and other associated DNA/RNA viruses including, human Respiratory Syncytial virus (hRSV); Human Metapneumovirus (hMpv); Human parainfluenza virus 1-4 (hPiv); human coronavirus (hCov); Human B, C and E Adenovirus (hAdv) and Human Bocavirus (hBov) in common cold disease. Further, to detect the role of some immunological parameters in serum of patients like level of complement 3 and rhinovirus Ag in rhinovirus infection. The samples of 84 individuals infected with common cold were collected and studied during the period from December, 2012 to April 2013. The frequencies of infections are remarkably varied from 1 up to 23 which was constituted more than half the infections. 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 3 was used as a potent immuno-parameter to detect it's 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 complement 3 (C3) and Rhinovirus antigen (RhV-Ag). In conclusion, multiplex RT-PCR is a rapid, cost effective, specific and highly sensitive method for detection of respiratory viruses including rhinovirus. Further, this study was 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 by means of complement C3.

Keywords: Multiplex Real-Time PCR, Human rhino virus, Common Cold.

1. Introduction

The common cold refers to the upper respiratory tract infection. It is a viral infectious disease involve air passage above the lungs including the bronchi, trachea, throat, nose and sinuses. Symptoms include coughing, sore throat, runny nose, sneezing, headache and fever which usually resolve in seven to ten days with some symptoms lasting up to three weeks. Over 200 viral strains are implicated as causes of common cold disease because the human body does not develop effective immunity so, these organisms cause re-infection of human. The common cold is a viral infection of the upper respiratory tract and the most commonly implicated virus is rhinovirus (30-80%) following by human coronavirus (15%), influenza viruses (10-15%), adenoviruses (5%), human parainfluenza viruses, human respiratory syncytial virus, enterovirus other than rhinoviruses and metapneumoviruses. Rhinoviruses are small RNA viruses 30 nm, non-enveloped with single strand RNA genome in an icosahedral capsid belonging to the family Picornaviridae (pico=small+RNA). It has long been known as an etiological agent of common cold and can infect both of upper and lower respiratory tract and trigger asthma exacerbations in both adults and children. 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; Adenoviruses the genus of mast adenovirus comprises all 47 human serotypes; has icosahedral virion (8-90 nm), 252 capsomers, 12 fibers at vertices and 12 structural proteins; linear, ds DNA genome; complex program of transcription from seven early, intermediate and late promoters, splicing and transcription, DNA replication and virion assembly occur in nucleus. Also, Human metapneumovirus is a genus of paramyxoviridae family isolated in 2001 in Netherlands. Negative sense ssRNA and their genome analogue to 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 sever idiopathic pneumonia in recipients of hematopoietic stem cells transplants.

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 (12, 13). Human bocavirus is a parvovirus that has been suggested to cause lower respiratory tract infection; HBoV is the fourth most common virus of respiratory infection; 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 is flanked by terminal imperfect palindromes or inverted repeats to form hairpin-like structures responsible for genome replication (14, 15).

It is well recognized that molecular methods for detection of an infectious agent is steadily becoming more widely applied in regard to a vast array of microorganisms, including rhinoviruses. Different approaches based on PCR have been investigated in order 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 assay with oligonucleotide hybridization detection proved to yield the highest number of positive results more than half of negative by a concomitant conventional cell culture (16, 17). Immunologically, Both cellular and humoral immunity are 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 are infected these cytokines including interferon gamma and interleukin-8. All of this occurs as the body attempts to clear and turning point of the viral infection (18-20). The function of 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 (20). Therefore, this study has been undertaken for the following purposes:– 1) Detection the reliability of multiplex- RT-PCR technique as a cut off diagnostic molecular tool in the detection of rhinoviruses in reverse transcriptase RT-PCR. 2) Also, to determine the diagnostic importancy 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 serum of patients like Complement 3 (C3) level and rhinovirus antigen (Rhino-Ag) in rhinovirus infection.

2. Patients and methods

1) Multiplex Real- Time PCR for DNA/RNA detection

84 nasal swabs specimens were collected from individuals infected with common cold during the period from December 2012 to April 2013. They were subjected to the 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 (10μl) internal control RNC C+ (IC RNA) were added to each tube of test. Mixed by pipetting and incubated 5 min at room temperature

2) (10μl) of samples were added to the appropriate tube containing lysis solution and IC

3) The tubes were vortexed and centrifuged for 5 sec at 5000g. If the sample is not completely dissolved it was recommended to re- centrifuge the tube for 1 min at a maximum speed (12000 – 16000 g), then the supernatant was transferred into a new tube for RNA extraction

4) Sorbent was vortexed vigorously and (25μl) was added to each tube

5) The mix was vortexed for 5-7 sec and incubated all tubes for 10 min at room temperature. They were vortexes periodically

6) 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.

7) (400μl) of washing solution was added to each tube and then vortexed vigorously, centrifuged for 1 min at 10000g. A micropipette was used carefully to remove and discard supernatant from each tube without disturbing the pellet. The tips were changed between tubes.

8) (500μl) was added of ethanol 70% to each tube. Vortexes vigorously and centrifuged for 1 min at 10000g. A micropipette was used carefully to remove and discard supernatant from each tube without disturbing the pellet. The tips were changed between tubes.

9) Step 10 was repeated.

10) (400μl) of acetone was added to each tube. Vortexes vigorously and centrifuged for 1 min at 10000g. A micropipette was used carefully to remove and discard supernatant from each tube without disturbing the pellet. The tips were changed between tubes.

11) All tubes were incubated with open cap for 10 min at 60°C.

12) The pellet was resuspended in (30μl) of RNA-eluent for 10 min at 60°C and vortexes periodically. The tubes were centrifuged for 2 min at maximum speed (12000-16000 g)

13) The supernatant now contains RNA which was ready to use. The RT- PCR can be performed the same day (22).

3. Reverse Transcription

1. (20 μl) of reaction 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 minutes at maximum speed (12000 – 16000g) and supernatant was taken carefully 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°C for 30 minutes.

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°C for a week or at -70°C during a year.

1) Amplification

1. The required quantities of tubes or PCR plate were prepared. The tubes were thaw with the reagents.

2. For carrying out N reaction (including 4 controls: 3 PCR controls and 1 negative extraction control) in a new sterile tube the following quantities was prepared: 10N µl of PCR-mix-1, 5.0N µl of PCR-mix-2-FRT and 0.5N of Taq polymerase. The tube was vortexes, 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 15µl of reaction mix was added into each tube.

4. (10 µl) of cDNA sample was added to appropriate tube with reaction mix.

5. Prepare for each mix 3 controls:
   - (10 µl) of DNA-buffer was added to the tube labeled PCR negative control
   - (10 µl) of cDNA C+ was added to the tube labeled C_pos
   - (10 µl) of IC DNA was added to the tube labeled IC DNA_pos.

Table 1: “Temperature profile adjustment in a thermal cycler”

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature, °C</th>
<th>Time</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>95</td>
<td>15 min</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>95</td>
<td>10 s</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>95</td>
<td>10 s</td>
<td>35</td>
</tr>
<tr>
<td>4</td>
<td>54</td>
<td>25 s</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>72</td>
<td>25 s</td>
<td></td>
</tr>
</tbody>
</table>

2) Detection of the level of Complement3 (C3)

A 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 blood sample. Complement 3 (C3) SRID plate from LTA, Italy was used to quantitative determination of Complement 3 protein (C3) by Single Radial Immunodiffusion plate (Mancini test) (23).

Assay method:- Endoplates of Single Radial Immunodiffusion kits were used for the quantitative determination of human serum immunoglobulins according to Hudson and Hay (19) as follows:

1. The plate was removed from zip lock bag and leave for few minutes so that any condensed water in the wells can evaporate.

2. Five µl of sample and / or controls were applied into wells and wait until it has been completely absorbing before handling the plate.

3. The lid closed firmly and incubated in moist chamber in cooled refrigerator at 23°C for 72 hr.

4. Area of precipitation rings were measured with a suitable coulometer and immunoglobulin concentrations were calculated according to reference table.

5. The concentration value corresponding to the precipitating ring diameter. The ring value was obtained from the reference table.

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

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

1. Standards were diluted as follow:-

<table>
<thead>
<tr>
<th>Standard No.5</th>
<th>120 µl</th>
<th>Original Standard + 120 µl standard diluents</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 pg/ml</td>
<td>120 µl</td>
<td>Standard No. 5 + 120 µl standard diluents</td>
</tr>
<tr>
<td>3 pg/ml</td>
<td>120 µl</td>
<td>Standard No. 4 + 120 µl standard diluents</td>
</tr>
<tr>
<td>1.5 pg/ml</td>
<td>120 µl</td>
<td>Standard No. 3 + 120 µl standard diluents</td>
</tr>
<tr>
<td>0.75 pg/ml</td>
<td>120 µl</td>
<td>Standard No. 2 + 120 µl standard diluents</td>
</tr>
</tbody>
</table>

2. Chromogen solution A, chromogen solution B and stop solution were 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, shacked gently and incubated 60 minutes 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, 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 corresponding samples' concentration.

4. Results

Sample of real time data contained 84 admitted cases divided into 41 males (48.8%) and 43 females (51.2%). Most of the admitted 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 old


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with mean age of 14.1 years and standard deviation of 10.84 years. Females mean age was 15.7 years with standard deviation of 11.4 years, and males mean age was 12.5 years with standard deviation of 10.2 years. Although females mean age is higher than that of males patients but there is no statistical evidence point out significant difference since the p-value of test was considerably larger than 0.05 both gender groups showed a large variability in age which is common 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 have no significant effect on the infection with Rhinovirus.

Table 2: "Distribution of patients with respect to their residential areas on gender and infection groups"

<table>
<thead>
<tr>
<th>Residency</th>
<th>Male (&lt;14 yr)</th>
<th>Male (&gt;14 yr)</th>
<th>Female (&lt;14 yr)</th>
<th>Female (&gt;14 yr)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rural</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Urban</td>
<td>12</td>
<td>2</td>
<td>9</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>6</td>
<td>13</td>
<td>5</td>
<td>26</td>
</tr>
</tbody>
</table>

In order 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, but there is a slight indication for a negative relationship between age and readings as represented in figure 1.

Figure 1: " Scatter plot of the ages versus RT-PCR"

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, easily to comment that hRv is most common infection among all other types of infection as represented in the following table:-

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

<table>
<thead>
<tr>
<th>Result of RT-PCR</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive hRv</td>
<td>23</td>
<td>56.10</td>
</tr>
<tr>
<td>Positive hRSV-hMpv</td>
<td>1</td>
<td>2.44</td>
</tr>
<tr>
<td>Positive hRv+hPiv 2+4</td>
<td>2</td>
<td>4.88</td>
</tr>
<tr>
<td>Positive hPiv 2+4</td>
<td>2</td>
<td>4.88</td>
</tr>
<tr>
<td>Positive hRv+hRSV-hMpv</td>
<td>5</td>
<td>12.19</td>
</tr>
<tr>
<td>hAdv-hBov</td>
<td>2</td>
<td>4.88</td>
</tr>
<tr>
<td>Positive hRv+hAdv-hBov</td>
<td>4</td>
<td>9.75</td>
</tr>
<tr>
<td>Positive hPiv 2+4 + hAdv - hBov</td>
<td>2</td>
<td>4.88</td>
</tr>
<tr>
<td>Total</td>
<td>41</td>
<td>100</td>
</tr>
</tbody>
</table>

The frequencies of infections are remarkably varied from 1 up to 23 which is constituted more than half the infections. The most rarely infection was (Positive hRSV-hMpv) which found only in one case (figure 3).
Figure 2: 'Bar chart represent the frequencies of viruses causing common cold for study patients as produced by real time PCR technique.'

Figure 3: 'Optics graph show 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

Figure 4: 'Optics graph show RT-PCR-Human Adenovirus-Human Bocavirus results using TxR fluorescent stain'. According to (Primary curve=10.0) and threshold=23.5: Positive samples No. are: 18 and 19.

Figure 5: 'Optics graph show 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.'
Regarding to immunological part of this study, the complement (C3) which was recorded for participated patients in this study, it was investigated once in terms of their association with positive and negative infection, and another with corresponding readings of the Rhinovirus antigen (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 indicated that extreme readings for the RhV-Ag versus readings complement C3. Such extreme readings will not facilitate for a good fitting. As a general trend linear fitting is more appropriate for the complement observations. It can be concluded that RhV-Ag cannot be predicted by means of complement C3.
5. 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 as it involves the upper respiratory tract infections (URIs) including the nose, sinuses, pharynx and larynx. This commonly includes tonsillitis, pharyngitis, laryngitis, sinusitis, otitis media and rhinovirus infection "the common cold" (24). Young children have between five and seven of these illnesses per year, with a general decrease in frequency with increasing age, adults will have about two such illnesses per year. Dual viral infections are common and a large 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 (25, 26, 27). 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 hands on time and much simpler than conventional routine techniques which need many steps and a wide range of technical competence like culture, PCR and immuno-fluorescence technique (28, 29).

The new version of 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 assay was 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. These results are similar to those obtained by Bhat, et al (30) and Freymuth (31) 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, so 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 (32). Rhinoviruses was (56.10%) of total positive cases. Rhinovirus is a key virus that will be talking about it, rhinovirus capsid leading to release of the viral genome into adhesion and initiate of infection that appear after 15 minutes after entry of virus (33).

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 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's included urban and rural groups. However residency and seasonal variation was studied, residency show no significant effect on the infection with rhinovirus as described above most of viral infection depend 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 fully rhinovirus epidemiology. Sears and associates (34) concluded that HRV were detected in April and earlier reports have associated the September peak in asthma hospitalization with rhinovirus circulation.

In addition to that in cases of co-infection like HRV and RSV the severity of common cold was the same in the clinically diagnostic patients in this study. So, clinical features of both RSV and HRV indistinguishable however children infected with HRV suffer more than that recorded with RSV it's associated with atopy higher than that recorded in RSV infections (35, 36) illness with multiple virus detection were not correlated with severity of disease, Mrtin, and associates (37) not agreement with our result they recorded that the relationship between viral load and multiple virus infections was virus specific. In this study, there are Six of cases infected with RSV 1(2.44%) was hRSV+ hMPV and 5(12.19%) was hRV+ hMPV+ hRSV of viral co-infection. This co-infection was detected by multiplex Real-Time PCR 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 key virus that will be talking about it, rhinovirus is a key virus that will be talking about it, metapneumovirus and respiratory Syncytial virus consider the viral etiology of a severe bronchiolitis. The study result revealed that specific virus combinations recorded in patients under 14 years old and this infection may affect clinical outcomes and warrant further investigation. However, there is no significant difference between the two percentages of co-infections. Zhang and co-workers (38) 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. This suggests that the multiplex RT-PCR assay can be used as a rapid and sensitive diagnostic method for major respiratory viruses. In conclusion, RT-PCR is a rapid, cost effective, specific and highly sensitive method for detection of respiratory viruses.

According to our result about Complement 3 (C3) level which determined in the serum of rhinovirus infected patients this is consider the first study to clearly establish that human rhinovirus 3C protease is the likely mediator of Complement 3 cleavage. HRV infection is initiated 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 into...
the cytoplasm of infected cell, the viral genome is the translated into a polyprotein (39).

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 cleave C3 leading to deficiency in the level of C3 concentration in serum of rhinovirus infection patients. So, many viruses disrupted cell nucleocyttoplasmic trafficking and proteins appropriate it for their own use probably as a strategy to limit anti-viral responses (40, 41). 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 hours post infection) with cleavage of nucleoprotein 153 by 3C protease following at about 6-9 hours post infection and 3C localized in the nucleus (42). Tam and associates (43) demonstrated that during viral infection the pathogens traverse barriers during infection, including cell membranes and during this transition pathogens carried covalently attached complement 3 into the cell triggering immediate signaling and effector responses. This system could detect both viral and bacterial pathogens but was antagonized by enteroviruses such as rhinoviruses and polioviruses which cleave C3 using 3C protease activity. Thus, complement C3 allows cells to detect and disable pathogens that have invaded the cytosol.

Regarding to 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 major effector mechanism by which IFN- gamma regulates C3 and C4 gene expression (44). Other interrupted our result that the production of C3 may be extrahepatic locally in the monocytes/ macrophages, fibroblast, renal tubular epithelial cells and endothelial cells and this production play an important role in host defense and inflammatory responses at site sequestered from the blood stream. C3 produced in the lung by alveolar epithelial cells and because alveolar epithelial cells have only been identified as a pulmonary source of C3, factors regulating C3 production by alveolar epithelial cells investigated to be IFN-\( \gamma \) as cytokine produced by T-cell and IFN-\( \gamma \) would regulate C3 gene expression by alveolar epithelial cells (45, 46).

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 was 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 by means of complement C3.

References


