

Original Research Article

Detection of Human Rhinovirus (HRV) in Upper Respiratory Tract Infections by RT- PCR Technique

Kassim Raisan Dekhil^{1*} Anwar Salih Saihood¹ Hassan Hachim Naser²

¹ College of Medicine, University of Al-Qadissiya , Al-Diwaniya , IRAQ

² College of Veterinary Medicine, University of Al-Qadissiya , Al-Diwaniya , IRAQ

* E-mail : kassim_dekhill@yahoo.com

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Abstract

Human rhinovirus (HRV) is the most common respiratory microorganism, which causes acute respiratory infections especially in healthy young children, elderly people, and the immunocompromised person. The diagnosis of rhinovirus infection by clinical and convenient means is more complicated because of the lack of sensitive, accurate tools for differentiation of the rhinovirus from other respiratory tract viral infections. Here, we had developed a rapid, specific and sensitive one step Reverse Transcription Real-Time PCR molecular method for the detection of human rhinovirus (HRV) from nasopharyngeal secretion specimens. The Primers designed from the highly conserved polyprotein gene of human rhinovirus. Our results that had shown a positive RT-PCR assay were specific in detection of HRV and observed in 41 out of 50 specimens (82%). We conclude that one-step RT-PCR assay was a highly specific and rapid technique for the detection of HRV RNA in nasopharyngeal secretions of patients with acute respiratory tract infections.

Key words: Human rhinovirus (HRV), nasopharyngeal secretions, RT- PCR Technique

الخلاصة

الفايروس الانفي البشري (HRV) هو من اكثر الجراثيم التنفسية شيوعا والذي يسبب الالتهابات التنفسية الحادة وخاصة في الاطفال الكبار الاصحاء, الكهول والاشخاص المصابين بضعف المناعة. ان تشخيص التهابات الفايروس الانفي بالطرق السريرية والوسائل المناسبة المعروفة تعتبر معقدة بسبب عدم توفر الادوات والاجهزة الحساسة والمضبوطة لتمييز الفايروس الانفي من الفايروسات الاخرى المسببة للالتهابات التنفسية. ونحن هنا قدمنا طريقة سريعة ومعينة وحساسة تسمى

one step Reverse Transcription Real-Time PCR molecular method للكشف والتحري عن الفايروس الانفي البشري (Human rhinovirus) من عينات مفرزات البلعوم الانفي. البرايمرات (primers) مأخوذة من جينات البروتينات المتعددة المحفوظة للفايروس الانفي البشري. النتائج التي كانت ايجابية باستخدام (RT-PCR assay) كانت محددة في التحري عن الفايروس الانفي البشري وكانت ايجابية في 41 حالة من اصل 50 عينة اعدت للدراسة اي بنسبة 82%. نستنتج بان هذه الطريقة هي طريقة خاصة ومحددة وسريعة للكشف عن الفايروس الانفي البشري من مفرزات البلعوم الانفي للمرضى المصابين بالتهابات المجاري التنفسية العليا الحادة.

مفاتيح الكلمات : الفايروس الانفي البشري, مفرزات البلعوم الانفي, تقنية RT- PCR

Introduction

Rhinovirus is a single-stranded RNA virus in the picornavirus family. There are more than one hundred serotypes of rhinovirus exist. The transmission of the virus starts through a large particle aerosols that

firstly affect the ciliated nasal epithelium. The invasion of the virus to the nasal epithelium is not direct, but rather causes an acute inflammatory reaction. Inflammatory mediators subsequently cause edema & hyperemia of the nasal mucosa that extends into the

pharynx [1]. Replication is limited to cells of the upper respiratory tract with optimal growth at lower temperatures of about 33 °C. In the 1950s, the isolation of HRV were found in patients with upper respiratory tract infections [2, 3], and from that time the HRVs have become known as the common cold virus because these viruses are responsible for about 50% of upper respiratory tract infections [4].

Typically, Human rhinovirus (HRV) associated with mild upper respiratory tract infections & exacerbation of asthma in children [5,6,7,8].

In the immunocompetent patients, most of the viral-induced infections are simple and self-limiting while in immunocompromised patients there are a possibility of serious complications [9].

Previously, the diagnosis of HRV had many difficulties due to the viral culture was limited [10], But due to the major advances in nucleic acid amplification and other facilities like molecular biology, we used Reverse Transcription PCR technique to detect HRV & decide when HRV is the causative and responsible agent [11,12,13,14].

Materials and Methods

Specimen collection

Fifty nasopharyngeal secretion specimens were collected from adult patients with acute upper respiratory tract infection from Al-Diwanyia Teaching Hospital. The samples were placed in sterile 25ml container and directly transported into laboratory and stored in freezer until used in viral RNA extraction.

Viral RNA extraction

The extraction of viral RNA from nasopharyngeal secretion specimens by using (AccuZol™ RNA extraction kit Bioneer. Korea). A 250µl nasopharyngeal sample was placed in 1.5

ml microcentrifuge tube, and then 1ml trizol reagent was added and mixed well by vortex for one minute. After that, 200µl chloroform was added and mixed strongly for 15 seconds, and then the contents had been incubated for 5 minutes on ice. The tubes placed in cold centrifuge 4°C at 12000 rpm for 15 minutes. We transferred the supernatant to a new microcentrifuge tube, then a 500µl of isopropanol added and the contents mixed by inverting the tube 4 to 5 times and had been incubated at 4°C for 10 minutes. The tubes returned back to centrifuge at 12000 rpm for 10 minutes, and then the supernatant was discarded. The RNA pellet was washed by adding 1 ml 80% Ethanol with DEPC and mixed again, and then, placed in centrifuge at 12000 rpm for 5 minutes. After that, the supernatant discarded and the RNA pellet exposed to air for drying. Finally 50µl DEPC water added to elution of RNA pellet, then the extracted RNA sample was checked by Nanodrop spectrophotometer and stored in -20 °C freezer until used in RT-PCR assay.

Reverse-Transcription PCR

RT-PCR was performed by using one step RT-PCR kit (AccuPower® RT-PCR PreMix from Bioneer. Korea). The RT-PCR primers which were designed in this study were from Human Rhinovirus type 14 (HRV14) strain (NCBI-Genbank: K02121.1) that is specific for amplification of HRV protease gene, forward primer (GGTGGCATGCCCTCAGGGTG) and reverse primer (AGGTCCAACCAGCGCCTCCT). The primers were provided from Bioneer Company. Korea).

RT-PCR master mix prepared by using the company instructions as the table below:

RT-qPCR master mix	Volume
RNA template	5 μ L
HRV Forward primer (10pmol)	2 μ L
HRV Reverse primer (10pmol)	2 μ L
DEPC water	11 μ L
Total	20 μ L

These RT-PCR master mixture reaction components that mentioned in previous table added into standard RT-PCR premix tube which contains (RocketScript reverse transcriptase enzyme, DNA polymerase, dNTPs, and 10X buffer as well as loading dye). Then all RT-PCR master mix tubes placed in Exispin vortex centrifuge for 3000 rpm

for 3 minutes, after that transferred into Mygene PCR thermocycler.

RT- PCR Thermocycler conditions

Reverse-Transcription step and PCR amplification was done in one tube reaction by applying the following RT-PCR thermocycler conditions as in the following table:

Step	Condition	Cycle
Reverse transcriptase	50 °C for 15 min	1
Initial denaturation	95 °C for 5 min	1
Denaturation	95 °C for 20 sec	45
Annealing	55 °C for 30 sec	
Extension	72 °C for 1 min	
Final Extension	72 °C for 10 min	
Hold	4 °C	forever

RT- PCR Product Analysis

RT- PCR product had been analyzed by using agarose gel electrophoresis. Where, 2% agarose was prepared using 1X TBE buffer and stained with ethidium bromide at 100volts and 80 mA for 1 hour. After that the RT- PCR bands were seen under U.V transilluminator.

Results and Discussion

The Reverse Transcription PCR had appeared as highly sensitive and specific assay that can be used in

detection of human rhinovirus from nasopharyngeal secretions of upper respiratory tract infection patient samples. RT-PCR assay results showed 41 positive samples out of 50 samples (82%).

RT-PCR assay used in amplification of HRV protease gene from RNA extracted samples in one step reverse transcription of viral RNA into cDNA and then PCR amplification at one tube reaction appeared as specific detection of HRV (Figure 1).



Figure 1: Agarose gel electrophoresis of RT-PCR assay shows the positive results of HRV protease gene. Where, Lane (M) DNA marker 100-10000bp), Lane (1-12) positive for HRV at 530bp RT-PCR product and lane (N) negative control no RNA template.

The detection ability for HRV in nasopharyngeal secretions samples had increased many times when a validated RT-PCR assay had been used. In this study, there were frequent detections of human rhinovirus by RT-PCR, these results were consistent with Xiang ZD [15] who explained that one-step RT-PCR system was highly specific, rapid and convenient for the detection of HRV RNA in nasopharyngeal secretions of patients with acute respiratory tract infections and that the genome of HRV viruses was highly variable.

However, human rhinovirus diagnostic procedures using tissue culture are limited by the expertise of the diagnostic laboratory and the time required obtaining a result as such, viral culture has a limited place in routine diagnostic microbiology [16]. Human rhinoviruses (HRV) are the major cause of common cold symptoms in adults and children, the adult patients are also the most frequent to be exposed to upper respiratory tract infections caused by Human rhinoviruses [17]. Over one hundred serotypes of human rhinoviruses (HRV), which are members of the family Picornaviridae, identified through community surveillance of respiratory common cold illness [18].

Human rhinovirus (HRV) is the most common respiratory pathogen, which causes not only acute respiratory

infection and community acquired pneumonitis in children, but also asthma episode and deterioration [15]. The absence of detection method limits the clinical understanding of HRV pathogenicity, and causes unreasonable use of antibiotics by serological tests. Our study aimed to build a one-step reverse transcription (RT) PCR system for specific detection of human rhinoviruses RNA. Therefore, we conclude that one-step RT-PCR system can be considered a highly specific, rapid and convenient for the detection of HRV in nasopharyngeal secretions of patients with upper respiratory tract infections and the molecular diagnostic method may be suitable for a large-scale clinical and epidemiologic studies.

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