REVIEW ARTICLE

Microbiology

KEY WORDS: conventional PCR, diagnostic accuracy, real-time PCR, respiratory syncytial virus

DETECTION OF RSV INFECTION IN ADULTS BY REAL-TIME PCR

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ABSTRACT

Respiratory syncytial virus (RSV) is emerging as a significant problem in some elderly immunocompromised . These include the elderly, persons with cardiopulmonary diseases, and immunocompromised hosts. Those appear to be at increased risk for serious diseases include adults with underlying cardiopulmonary disease, frail elderly persons living in long-term care facilities or at home, and the severely immunocompromised. Epidemiological evidence indicates that the impact of RSV in older adults may be similar to that of non pandemic influenza. It is observed that diagnosis of RSV infection is difficult because viral culture and antigen detection are relatively insensitive, due to low viral titers in nasal secretions. Real-time PCR had a significantly improved turnaround time compared with conventional PCR and required minimal sample handling, which reduces the possibility of cross-contamination due to PCR products. In spite of being more expensive, the added costs of real-time PCR can be offset by more accurate diagnosis that in turn can help in better patient management, decreased hospital costs and length of stay.

INTRODUCTION

RSV identified as child pathogen now recognize as a significant problem in certain adult population. It causes about 2 to 5% of adult community-acquired pneumonias. Clinical features include nasal congestion, cough, wheezing, and fever (low-grade) (1). Although RSV infection was reported in adults with pneumonia in the 1960s, it has only been during the last decade that the potential for widespread occurrence with serious clinical impact in this population has been recognized (2). Despite growing appreciation of this problem, there are significant gaps in our understanding of RSV infection in adults, especially with regard to immunology, diagnosis, treatment, and prevention. Management of RSV in the elderly is supportive, whereas early therapy with ribavirin and intravenous gamma globulin is associated with improved survival in immunocompromised patients (1). An effective RSV vaccine has not yet been developed, and thus prevention of RSV infection is limited to standard infection control practices such as hand washing and the use of gowns and gloves. Development of an RSV vaccine for both pediatric and adult immunization offers the best hope to reduce disease burden, although three decades of effort have yet to yield an effective and safe vaccine. This review will discuss the diagnosis and importance of real time PCR assay.

DIAGNOSTIC METHODS:

RSV does not have any distinctive clinical symptoms in adults, thus laboratory confirmation required for specific RSV diagnosis. Methods of diagnosing RSV infection in adults are Culture, antigen detection by immunofluorescence assay (IFA) or enzyme immunoassay (EIA), RNA detection by reverse transcription-PCR (RT-PCR), and serologically by demonstrating RSV-specific IgM in sera (3).

Culture, the definitive gold standard upon which all other methods are judged, is highly sensitive and specific however it is not a rapid diagnostic test as PCR and required technical expertise & perfect handling (4). Detection of RSV antigen by Immunofluorescence assay (IFA) or Enzyme Immunoassay (EIA) is more sensitive in infants and children, as compared to elderly adults (5). Various serological methods, including complement fixation and EIA, have been employed for diagnosis of RSV infection in adults with variable results (6).

Molecular assays have revolutionized the diagnostic procedure in respiratory virus detection. When compared with cell culture or antigen assays, it gives high specificity and sensitivity (Nearly 100%) (3,7,8,9).

PCR technology has developed novel chemistry and detection methods (3,10). Most of these are detected by gel analyses, enzyme hybridization and fluorescent probes to enable real-time (RT) monitoring, such as Taqman, molecular beacon, eclipse, and scorpion probes (real-time RT-PCR). These assays are rapid (3–4 hours) and sensitive in their ability and able to detect minute quantities of viral nucleic acid (11).

Real-Time PCR is more sensitive than culture and antigen testing. World Health Organization (WHO) and Centers for Disease Control and Prevention (CDC) also recommends real –time PCR for detection of RSV [12,13]. The use of real-time PCR has allowed large epidemiologic studies of pathogens, such as RSV, Influenza viruses (14).

A study carried out on applicability of a TaqMan-based real-time quantitative PCR assay for diagnosis of respiratory syncytial virus infection in immunocompromised adults. They compared real-time PCR with cell culture, shell vial culture and nested PCR, of these RSV- positive samples, 31% were detected by cell culture and none were detected by shell vial culture. All samples positive by real-time PCR were confirmed by the in-house nested PCR (100%) and they concluded that the TaqMan real-time PCR and nested PCR was rapid and sensitive diagnostic tool as compare to cell culture, shell vial culture (15).

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A study carried out at the Department of internal medicine,(university hospital Bergmannsheil, Bochum, Germany). The aim of study was to evaluate a sensitive and rapid RSV-A specific qPCR assay for the determination of viral load in different reparatory diseases. Samples of hospitalized children with acute respiratory tract infection (ARI) and of adults with chronic obstructive pulmonary disease (COPD) were analyzed. They found samples from children with ARI were 62 and adults with COPD were 125. A total of 47% RSV-positive were found in the ARI study group and 28% in the COPD study group. Compared with conventional nested PCR, qPCR have 30-times more sensitive. They concluded RSV-A specific TaqMan real-time PCR assay is sensitive and rapid method for the determination of viral load in clinical samples. (16).

A prospective and observational study conducted in fifty-seven sites in nine countries in the northern hemisphere. The study aim was to determine the incidence of RSV -related

medically attended acute respiratory illness (MARI) in adults with severe chronic obstructive pulmonary disease (COPD) and congestive heart failure (CHF) by polymerase chain reaction (PCR) and seroresponse. Result showed 445 subjects were enrolled between October 2011 and May 2012. Overall 99 RSV infection were documented by PCR or serology, of these majority having severe COPD (77.5%), 16.2% had advanced CHF, and 6.3% had both severe COPD and advanced CHF . The study concluded that RSV infection was common in adults with severe chronic obstructive pulmonary disease and advanced congestive heart failure (17).

A study on military recruits receiving basic training at HMS Raleigh, a large Royal Navy new entry training establishment on the south-west coast of Britain. Aimed to estimate the prevalence of clinically significant RSV infection, to characterize the illness associated with RSV and other common viruses which were determined by culture, serology and real-time PCR. They found among 54 Royal recruits with respiratory symptoms adenovirus was identified in 35% influenza viruses in 19% and RSV in 14%. They concluded that the culture is not optimal diagnosis for RSV detection. Real-Time PCR is more sensitive and specific than serology (18).

A study carried out in Leiden University Medical Centre, Leiden The Netherland. The study aim was to design two multiplex RNA PCRs for detection of respiratory viruses and compared with viral culture retrospectively. They detected respiratory viruses in these samples was 67 of 358 (19%) and 87 of 358 (24%) by culture and real-time PCR respectively. They concluded The application of real-time PCR to clinical samples increases the sensitivity for respiratory viral diagnosis. (19).

A study was conducted in a university hospital and teaching hospital in Utrecht, The Netherlands. The finding of study was to evaluate the feasibility and clinical impact of the use of TaqMan PCR for detection of respiratory viruses and atypical pathogen in patients with lower respiratory tract infection. Results showed a total of 107, the pathogen detected most frequently were influenza virus (14), corona virus (six), rhinovirus (five). Real-time PCR increased the diagnostic yield from 21 % to 43% compared with conventional diagnostic tests. This technique also implemented for the etiological diagnosis of lower respiratory tract infection (20). One other study from The Netherlands analyzed 267 hospitalized patients with respiratory symptoms by real-time PCR, viral culture and direct immunoflourescence for respiratory viruses. They compared with conventional diagnostic tests; real –time PCR increased the diagnostic yields for these viruses from 3.5 to 36% in adults (21).

A study carried out in Southeast Michigan hospitals and aim of study was to describe the epidemiology and clinical severity of RSV compared to influenza in hospitalized adults ≥18 years, they tested samples by Real-Time PCR. Result showed that RSV was detected in 84 (7%) of 1259 patients. The highest frequency was found in RSV-associated hospitalizations among adult 50-64 years old; 98% of RSV cases in this age group had chronic co morbidities (22). In a recent retrospective analysis of respiratory samples from 2225 subjects with medically attended acute respiratory illness (MAARI) in Marshfield, Wisconsin. RSV was identified in 8.2% of those 50–64 years old, 10.2% of those 65–79 and 10.5% of those ≥ 80 years old (23).

A study reported an outbreak of respiratory synctyial virus (RSV) in a long-term care facility (LTCF) during ongoing routine respiratory illness surveillance. They compared rapid antigen test, viral culture, direct fluorescent antibody (DFA) test, and reverse transcriptase polymerase chain reaction (RT-PCR) .RSV was detected using RT-PCR in seven (32%) of the 22 cases. None of the seven cases had positive RSV rapid antigen testing, and only two had positive culture or DFA results. They concluded RSV may cause outbreaks in LTCFs that traditional diagnostic methods do not detect. RT-PCR can provide a more timely and accurate diagnosis of outbreaks, which allows for early symptomatic treatment, rational use of antibiotics, and improved infection control. (24).

One study carried out in Vitória, Southeast Brazil. The study aim was to determine the frequencies for a large range of respiratory viruses using a sensitive molecular detection technique (Real-Time PCR) and indirectimmunofluorescence assay (IIF) in specimens from outpatients of all ages with ARIs and found a total of 162 samples 33 (20.4%) samples were identified with IIF while through real-time PCR assay 88 (54.3%) positive samples were identified. concluded that the application of PCR assays in a real-time multiplex format for respiratory pathogens considerably increases the pathogen detection rate when compared to conventional methods (IIF) (25).

A Study carried out in a tertiary care centre in Southern India showed that the respiratory syncytial virus is a significant cause of contagious acute respiratory infections in children and older adults. Since there are contradictory reports regarding the efficacy of different methods to detect RSV, the authors evaluated the performance of the conventional PCR versus Real-Time PCR in patients with acute respiratory infections. Conventional PCR had a very poor sensitivity of 40% and failed to detect RSV in respiratory samples with low viral load. Thus, it may be prudent to replace it with real-time PCR to achieve precise diagnosis (26).

A retrospective cohort study was conducted at four tertiary care adult teaching hospitals in the university of Toronto,Canada with RSV identified by qualitative real-time reverse-transcriptase PCR assay.eighty-six subjects were identified as required hospitalization for RSV infection. Median age was 74 year; 34% were< 65 year,97% had underlying chronic medical conditions. they showed the advantage used RT-PCR for detection when compared with previous testing for RSV has been done using antigen immunoassay or culture and serology which lake sensitivity in immunocompromised patients or the frail elderly(6, 24, 27, 28).

A Prospective,observational study was conducted at two general hospitals in HongKong during a 12-week seasonal RSV outbreak in 2013(29).Adults aged >18 year with symptoms of acute respiratory infection and were hospitalized because of potentially serious medical condition, exacerbation of underlying chronic illness or severe symptoms impossible to manage at home were included in this study(30) samples tested by quantitative real-time PCR assay, in addition to an antigen assay performed during routine care irrespective of its result. 123 patients were confirmed to have RSV infection by real-time PCR. Lower respiratory tract complications causing respiratory insufficiency (52.8%). They showed the efficacy of real-time PCR for detection of RSV infection compared with antigen detection (31,32,33).

A study was conducted 3 continents in the northern hemisphere: America, Europe and East Asia.

They used multiplex RT-PCR to detect viral etiologies of moderate-to- severe Influenza- like-Illness (ILI) in elderly influenza-vaccinated community-dwelling elderly adults during one winter season from November 2008 to April 2009 in a large international study. They detected Viruses in 57.6% (320/556) of moderate-to-severe ILI episodes, of which RSV detected in 7.4% of samples. This study reported virus

detection rate 57.4% which was higher than that of reported in previous studies (34) and efficacy of RT-PCR that have different analytical sensitivities for the different viruses, which can affect the diagnostic yield (35).

CONCLUSION :

It is evident that real-time PCR technique is superior to traditional virus detection techniques due to increased sensitivity and specificity(24) which helps to detect RSV infection in all ages of adults as well as adults have chronic underlying illness(28,30). It was seen that real-time PCR had a significantly improved turnaround time compared with conventional PCR and required minimal sample handling, which reduces the possibility of cross-contamination due to PCR products. In spite of being more expensive, the added costs of real-time PCR can be offset by more accurate diagnosis that in turn can help in better patient management, decreased hospital costs and length of stay.Thus, to conclude, precise data regarding morbidity and mortality of respiratory syncytial viruses should be collected on a routine basis through a rapid and sensitive method. Conventional PCR showing significant false negatives and poor sensitivity cannot be considered as an accurate method to detect RSV, and it may be prudent to replace it with real-time PCR to achieve a specific diagnosis and to document true incidence of RSV in acute respiratory infections.

Aknowkedgement-

Dr. Anil Prakash, Professor and Hod Microbiolgy, Barkatullah University Bhopal (M.P.)

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