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Real-Time PCR Detection of Respiratory Syncytial virus (RSV) among Adults with Pneumonia

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Abstract

This study aimed to confirm the presence of RSV using real-time PCR in nasal and throat swabs which had no visible cytopathic effect in tissue culture technique from adults of moderate-to-severe pneumonia with influenza-like illness. Results of real-time RT-PCR found that viral RNA in 11.63% (5/43) of adult with pneumonia and flu-like illness symptoms. A significant incidence of RSV infection in Dec. and Jan. 2014 was appeared among patients aged more than 45 years. The results showed that viral load significantly associated with disease severity. In conclusion, multiplex RT-PCR is recommended to diagnose RSV and influenza viruses in winter season in older patients with pneumonia and can decrease sever illness in the elderly.

Keywords: Real-Time RT-PCR, viral pneumonia, RSV and adult patients.

أستخدام تفاعل سلسلة البلمرة اللحظي (RT-PCR) في تشخيص ا لفيروس المخلوي التنفسي (RSV) بين البالغين المصابين بالالتهاب الرئوي

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الخلاصة:

هدفت الدراسة إلى تأكيد وجود فايروس المخلوي التنفسي (RSV) بأستخدام تقنية تفاعل سلسلة البلمرة اللحظي (Real-Time PCR) في مسحات الأنف والحلق للبالغين والتي أعطت نتائج سالبة بأستخدام الزراعة النسيجية من الالتهاب الرئوية المعتدلة إلى الشديدة وذو أعراض مشابهة للانفلونزا. أظهرت نتائج تفاعل سلسلة البلمرة اللحظي وجود الحامض النووي للفيروس في 11.63% (5/43) من البالغين المصابين بالتهاب رئوي. وقد ظهرت الإصابة معنوية من كانون الاول الى كانون الثاني 2014 بين المرضى الذين تتراوح أعمارهم أكثر من 45 عاما. وأشارت النتائج إلى أن المحتوى الفيروسي بالعينة يرتبط بشكل كبير ومعنوي مع شدة المرض. أستنتجت الدراسة بأستخدام تفاعل سلسلة البلمرة متعدد البريمرات في تشخيص الفيروس المخلوي التنفسي مع فايروس الانفلونزا في موسم الشتاء لمرضى كبار السن ومصابين بالالتهاب الرئوي لتقليل حدة المرض وخطورته.

Introduction

Respiratory syncytial virus (RSV) is a very contagious virus usually causes only a mild cold in healthy adults and elder children. After three to five days, the symptoms may worsen as the virus spreads to the lungs. These include breathlessness, rapid breathing, wheezing and a strong cough. RSV can also cause acute viral croup and viral pneumonia at any age, especially in infant, elderly, and those

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with chronic lung or heart diseases or compromised immune systems [1]. RSV is the main cause of bronchiolitis worldwide and can cause up to 70 or 80 percent of lower respiratory infections in high season [2]. There is evidence that severe RSV infection with lower respiratory tract involvement can occur, in particular in the elderly or those with underlying medical conditions [3]. In developing countries, it has been found that RSV is detected in 6-96% of cases of hospitalized lower respiratory tract infections. A difference in RSV rates in developing countries also depends on many social factors, and the peak for RSV correlates with the presence or absence of rainfall [4].

Viral culture is the gold standard by which other tests are compared; however, sensitivity in adults can be problematic. Adults generally shed lower titers and for a shorter period of time than children. Also, the virus is thermo labile and does not survive for long time. The benefit of tissue culture is its broad sensitivity for a range of viruses, the necessity for infectious virus particles and its relatively low cost. Human heteroploid cells, such as HEP-2 and HeLa cell generally provide the best tissue culture for RSV isolation. RSV produces a characteristic CPE consisting of syncytia formation which appears in 4 to 5 days [4]. Meanwhile, the detection of RSV antigens in respiratory secretions by IFA or EIA is widely used in children and removes the need to recover infectious virus but requires a significant viral load to generate a positive result, hence, these methods are not suitable for adults [5].

Furthermore, molecular diagnosis of isolated viruses in cell culture and using antigen detection assays have demonstrated superior sensitivity for nucleic acid tests [6]. Furthermore, molecular diagnostic assays are definite also more sensitive for older age groups as a result of the lower virus production in these patients [7]. An important benefit of real-time PCR is to allow kinetic quantification of viruses [8].

This study aimed to detect RSV presence in nasal and throat swabs by real-time PCR from adults of moderate-to-severe pneumonia with influenza-like illness.

Materials and Methods

Sample collection

A total of 43 swabs for each nasopharyngeal and throat, from adults aged from 35 to 60 years with pneumonia were placed in 2 ml of transport media (Sigma, USA) and stored at -20°C until use. All samples were collected from patients attending the national influenza laboratory/National Central Public Health Laboratory during the period from Dec. to Mar. 2014. The selected patient were clinically diagnosed with pneumonia and influenza were undergoing symptoms of rash, cough, fever to sever high fever, admission to intensive care unit for mechanical ventilation and bronchitis [9]. Previously, these samples checked negatively for influenza virus type A and B by applying RT-PCR with specific primers and probes. Also, for other viruses like RSV using the Hep-2 cell line.

RNA Extraction

RNA was extracted from both nasopharyngeal and throat swabs using QIA amp Viral RNA Mini Kit (QIAGEN), according to the manufacturer's instructions. The extracted RNA was stored at -70°C.

The RT-PCR assay

For RSV F gene detection, primers and probe were described by another study [10] for both RSV A and B detection. A forward primer sequence 5'- AACAGATGTAAGCAGCTCCGTTATC-3', reverse primer 5'-CGATTTTATTGGATGCTGTACATTT-3' and probe 5'-TGCCATAGCATG ACACAATGGCTCCT -3' that labelled with 5' reporter dye FAM and the 3' quencher dye TAMRA (Bio Crop, Canada). In one step RT-PCR, master mix reagents (QIAGEN) were added to 10 µl RNA templates (Conc.16-30.8 ng/ µl), 0.5 pmol of each primers and 0.3 pmol of probe in 20 µl reaction mixture. Amplification and detection were done with an Applied Biosystem 7500. Briefly, one cycle for 30 min at 50°C and 15 min at 94°C, followed by 45 cycles of 10 s at 95°C and 1 min at 60°C.

Statistical Analysis

Data were tabulated and analyzed using SPSS software. Chi-Square was used to investigate probable correlation between patient information and RSV infection. Values were considered statistically significant when $P \leq 0.05$.

Results and Discussion

RT-PCR have the ability to detect 1-1000 ng viral RNA and do not depend on intact viral particles, thus this assay is to confirm the identification of RSV infection in adults. In general, this study used the specific primers and probe of the F gene designing to detect both RSV types A and B.

Results of RT-PCR reported viral RNA in 11.63% (5/43) of adult with pneumonia which had no visible cytopathic effect in tissue culture technique to influenza virus type A and B. Of the remaining

percent, it may be an environment, bacterial and viral causes of pneumonia in adult other than RSV. Previous researches had similar results, in which pneumonia identification in adult that ranged 7.1-29% RSV of pneumonia [11-13].

This study emphasized that RT-PCR had more ability to identify RNA-RSV infections because these samples haven't been detected in tissue culture. Because of RSV reach the lung and their shedding in low titer, RT-PCR is more suitable for RSV detection in a low level of virus in clinical samples [14, 15]. Consequently, it improves patient management, infection containment and controls to reduce morbidity and mortality among patients with severe RSV infection [16].

The results of this study showed that all RSV positive cases appeared accompaniment in influenza season as observed with clinical impact of RSV, in some adult risk populations may be similar to that of seasonal influenza virus [17]. A significant incidence of RSV infection in Dec. and Jan. was appearing in this study ($P < 0.05$), this agreed with many studies found that RSV tends to occur in relation to the rainy season [18]. RSV infections occurred between November and February, with a peak in January [4]. RSV appears to be a predictable cause of wintertime respiratory illnesses among older adults living in congregate settings such as long-term care or attending daycare and is estimated to infect 5%–10% of nursing home residents per year, with rates of pneumonia and death of 10%–20% and 2%–5%, respectively [19]. RSV was strongly associated with low temperatures and relative humidity between 50-60% [20]. Therefore, RSV is an important pathogen contributing to the burden of influenza-like illness in the entire community in winter [16].

This study found that all positive samples of RSV-associated significant with respiratory problems observed among patients aged more than 45 years ($P < 0.05$). Within the host, cellular and humoral responses appear to have different roles in the protection against and results in RSV infection as well as disease pathogenesis. It is found that to be age-related differences in humoral immune response to RSV infection in adult [21].

The results of RT-PCR, this finding presented that endpoint of positive samples with a threshold cycle (Ct) value should be less than 39 as shown in Figure- 1.

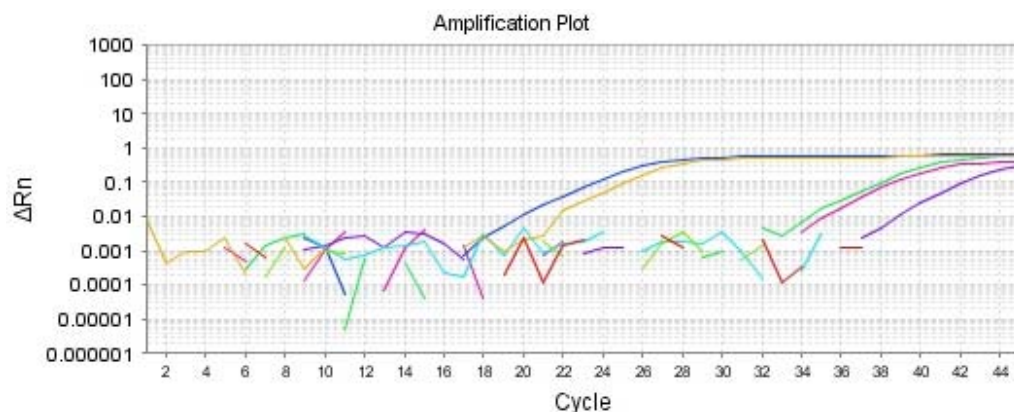


Figure 1- PCR amplification of one step real-time RT-PCR for RSV positive samples in adult with pneumonia

In a parallel study, the Ct values which indicated to the viral load in the detected sample [22]. The current study demonstrations of Ct values between 19.8 and 37.3 have been recorded. The results pointed out that the Ct values associated significantly with disease severity ($P < 0.05$). Meanwhile, the Ct values among studies ages were not statistical significant ($P > 0.05$).

Other investigators found a highly significant linear relationship between the log of the input target nucleic acid copy number and Ct values, hence the Ct values indicate for concentration (viral load) of unknown quantities of RSV-RNA in a clinical sample [15]. Early course of treatment reduce direct viral replication that may contribute to the low viral quantity in nasopharyngeal secretion [9]. The importance of preventing viral infection in these patients is based on avoiding the greater physical decline, greater hospitalization rates, and longer recovery time that viral exacerbation episodes bring compared to the exacerbations caused by bacterial infections [23].

In conclusion, multiplex RT-PCR recommend to diagnosis RSV and influenza viruses in winter season among patients with pneumonia can decrease sever illness in the elderly.

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