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Detection of Respiratory Syncytial Virus infection in Clinical Samples Using Immunofluorescence Test

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Abstract
Current study aimed to investigate the Respiratory Syncytial Virus (RSV) in nasal secretion and throat swab samples of hospitalized patients with symptoms of respiratory tract infection using Immunofluorescence test. Previously these samples were tested for Respiratory Syncytial Virus infection by reverse transcriptase-PCR assay. The positive samples were tested by Immunofluorescence assay in monolayer confluent of Hep 2 cell line. The results showed that the positive samples using the RT-PCR test were positive in this test. These results reveal that Immunofluorescence test is sensitive method for detection the infection with RSV.

Keywords: Immunofluorescence, RSV, Tissue culture.

الكشف عن الأصابة بفيروس Respiratory Syncytial Virus في العينات السريرية بواسطة اختبار التألق المناعي

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الملخص
هدفت الدراسة الحالية للتحري عن الاصابة بفيروس(Respiratory Syncytial Virus (RSV في عينات من إفرازات الأنف و الحلق من المرضى الراقدين في المستشفى والذين يعانون من أعراض عدوى الجهاز التنفسي بواسطة اختبار التألق المناعي. في البداية تم اختبار هذه العينات لعدوى فيروس بواسطة reverse transcriptase - PCR.العينات الموجبة تم فحصها باختبار التألق المناعي في خطوط خلايا Hep 2. أظهرت النتائج ان العينات الموجبة بفحص RT-PCR كانت موجبة باختبار التألق المناعي. هذه النتائج تبين ان اختبار التألق المناعي ذو حساسية عالية لتشخيص الاصابة بفيروس RSV.

Introduction

Respiratory syncytial virus (RSV), causes infection of the lungs and breathing passages, is a major cause of respiratory illness in young children. The infants most at risk of severe disease are those under 6 weeks of age, those with bronchopulmonary dysplasia, congenital heart disease, or immunodeficiency, and those born prematurely [1]. In adults, it may only produce symptoms of a common cold, such as a runny nose, sore throat, mild headache, cough and fever. But in premature babies and kids with diseases that affect the lungs, heart, or immune system, RSV infections can lead to other more serious illnesses. RSV is highly contagious and can be spread through droplets containing the virus. It also can live on surfaces, on hands and clothing, so it can be easily spread [2].

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By 2 years of age, almost all children have been infected with respiratory syncytial virus (RSV) and over half have been infected twice. RSV induced bronchiolitis results in the hospitalization of >100,000 infants per year in the US. RSV is also a significant cause of respiratory illness in high-risk adults and the elderly [3]. Despite the importance of RSV as a respiratory pathogen, effective vaccines and efficacious post-infection therapies are not currently available. Risk factors for severe RSV disease include age less than 1 year, age less than 3 months at the start of the RSV season, bronchopulmonary dysplasia, congenital heart disease, and prematurity [4].

RSV is a negative-sense, single-stranded RNA virus. The virus belongs to the Pneumovirus genus within the family Paramyxoviridae [5-7]. Its name comes from the fact that F proteins on the surface of the virus cause the cell membranes on nearby cells to merge, forming syncytia [8, 9]. It is divided into two groups, A and B, based on variability in antigen reactions against the attachment (G) and fusion (F) glycoproteins [10].

The goal of this study is to detect infections with RSV in clinical samples of nasal secretion and throat swabs from patients hospitalized with signs and symptoms of an upper respiratory tract infection by Immunofluorescence assay using specific monoclonal antibodies.

Materials and Method

Samples Collection

This study was performed using (22) clinical samples of nasopharyngeal secretions and throat swabs. They were collected from different hospitals from patients of different ages hospitalized with respiratory distress symptoms.

The samples were collected in 5 ml of transport media (phosphate-buffered saline containing 10% glycerol, 1 mg/ml gentamicin together with 8 IU/ml of penicillin, 8 µg/ml of streptomycin, 0.02 IU/ml of amphotericin B (Invitrogen, Carlsbad, CA) and stored at 4°C.

Specimens were processed and tested for RSV infection by RT-PCR assay using specific primers of RSV N (N Forward primer CA TCC AGC A AA TA CA CCATCCA and N reverse primer GCATCTCTGAGTATTTTTATGG). Hep2 cell line was provided from durham university /Biochemistry department. Extraction of RNA from samples was done by using RNeasy Mini kit from QIAGEN and the procedure was done according to the kit instructions. Positive samples were used fo Immunofluorescence assay. The experiment was done using Hep2 cell line and specific monoclonal antibodies against RSV N protein (Ivitrogene) and the procedure was done according to Invetrogen easy manual as below:

Glass coverslips (13-mm) were sterilized by dipping in ethanol 70%, air-dried individually on tissue, before being used directly, one coverslip was added in each well of 24 wells plate. Hep2 cells in concentration of (5×10^4) were seeded in growth medium in each well; the plate was incubated overnight at 37ºC, 5% CO2 incubator to reach a confluent of (50 – 70) %. The growth medium was removed. 100 µl (1:3) diluted RSV virus sample in maintenance media was added to each well. Plate was shacked gently at regular interval (every 15 minutes) of 2 hours incubation at 33˚C, 5% CO2. Maintenance media (1ml) was added to each well, the plate was incubated for 20 hours in 33˚C, 5% CO2 incubator. The Media was removed and the coverslips were washed with PBS carefully. 1ml of fixation solution (PBS 19 ml, Formaldehyde 1 ml and Sucrose 0.4g) was added. The plate was left for 10 minuts at room temperature. The coverslips were washed briefly with PBS/1% calf serum. 1ml of Permealisation solution was added (PBS 19 ml, 10% NP40 1ml and Sucrose 2g) was added. The plate was left for 10 minutes at room temperature. The coverslips were washed briefly with PBS/1% fetal calf serum. The first Ab (Mouse IgG Ab - R2αm) was added (200µl) to each well; the plate was incubated for 1 hour at room temperature. The coverslips were washed briefly with PBS/1% fetal calf serum three times. 50µl of second Ab (Alexa Fluor 488 Goat Anti-Mouse IgG (H+L) Antibody) was added to each well; the plate was incubated for 1 hour at room temperature in the dark. The coverslips were washed briefly with PBS 1% calf serum three times. The DAPI stain 50 µl was added. A drop of the mounting medium was added on the sterile glass slide and the coverslip was added in a way that the monolayer encounter the slide. Slides were mounted in fluorescent mounting medium and analyzed by Zeiss confocal microscope.

Results and Discussion

Four positive samples in RT-PCR figure -1 were tested by IFA for direct detection of RSV antigen using monoclonal Ab against RSV N protein gave positive results for these samples. The figure -2 shows Hep 2 cells Stained by DAPI stain. The figure -3 shows RSV particles in infected Hep2 cells detected by IFA. These results revealed the high sensitivity of this method for detection of RSV infection as well

as it is a rapid technique because it takes only two days comparing with tissue culture which takes more than one week.

The results are comparable to the results of many studies like one done by [11] in which, the IFA test appears more sensitivity than cell culture particularly when no care is taken to maintain the specimens in the cold during transport. Another study found that the specificity of IFA in comparison to RT-PCR ranged between (99-100) %[12].

This study also found that this method is easy to perform and the results are available in a short time which agrees with many studies that also found that the antigen detection kits in pediatric specimens have sensitivity around 94% and specificities between (95- 100)% as compared to cell culture [13].

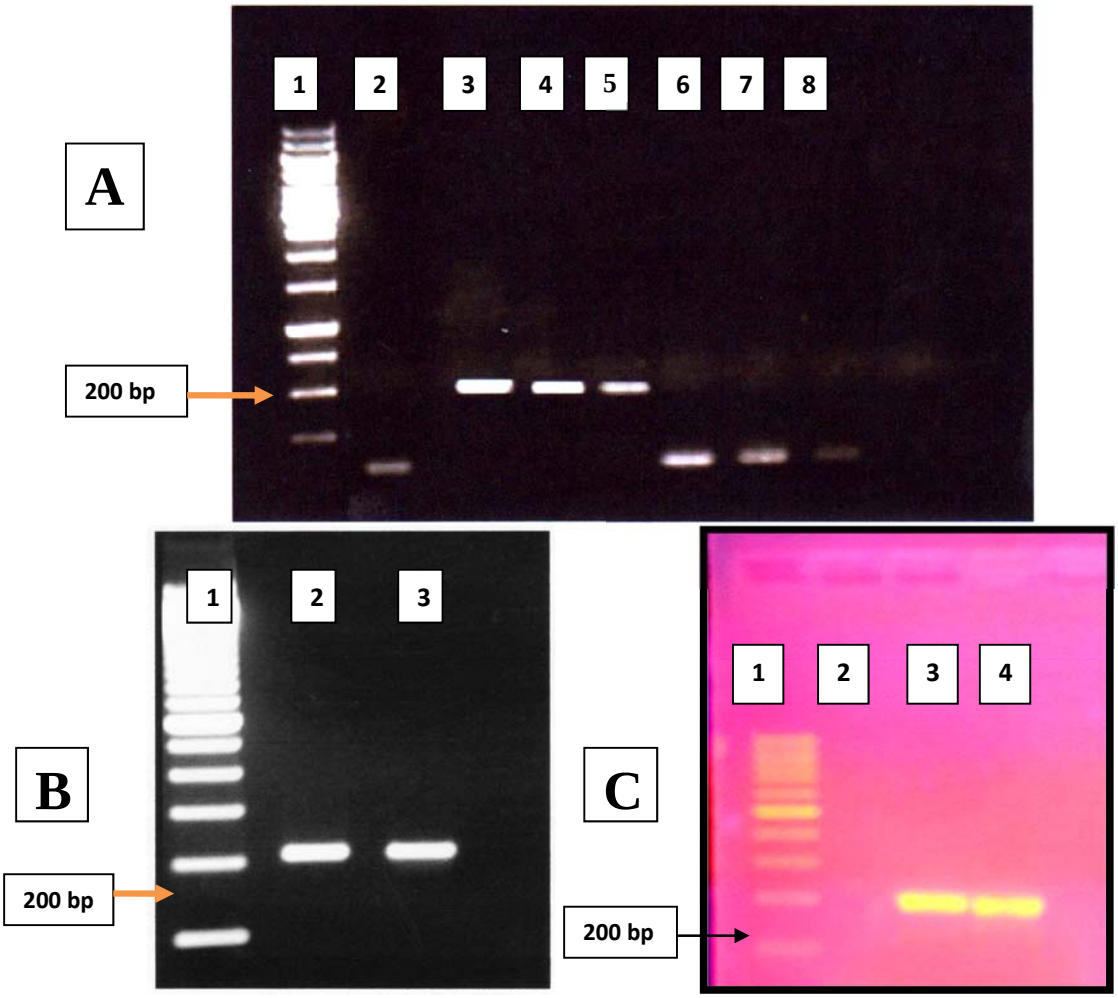

Figure 1- Gel-electrophoresis of the amplified samples of RSV N gene using 1.5% Agarose and 3 volt/ cm in TAE buffer. A: No. 1: 100 bp Marker, 2: negative control, 3: positive control, 4 and 5: positive sample for RSV 6, 7 and 8: are negative. B: 1: 100 bp Marker, 2 and 3: are positive. C: 1: 100 bp Marker, 2: negative control, 3 and 4 are positive.

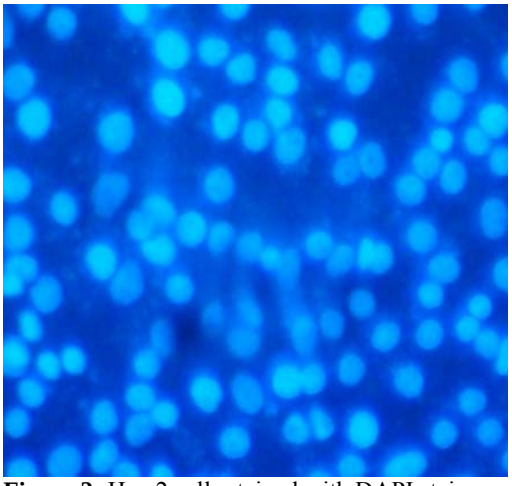

Figure 2- Hep 2 cells stained with DAPI stain

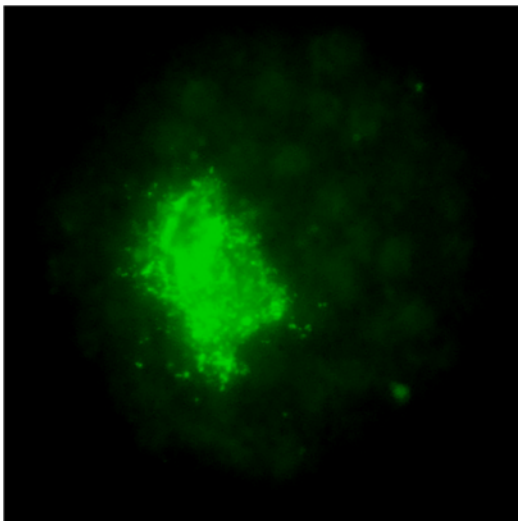

Figure 3- Cytoplasmic inclusions in respiratory syncytial virus-infected cells. RSV -infected HEp2 cells were fixed at 20 hours post infection and double stained with a mouse anti-N antibody and with goat anti-mouse IgG (whole molecule) conjugated to either FITC, followed by DAPI stain , Green fluorescence indicate inclusions of RSV N protein.

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