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Case Study

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Viral Pneumonia Causative Agents Diagnosis by using Indirect Immune Fluorescent Assay

Samer Raheem Obaid

Corresponding author: Samer Raheem Obaid

Introduction:

Viral pneumonia is a disease characterized by pulmonary dysfunction, and it is caused by the downward spread of the virus and the inflammation of the lung parenchyma. In recent years, the incidence of viral pneumonia has gradually increased, especially in infants under 2 years old (YJM A, et al,2015)

Viral pneumonia in children is one of the common diseases in pediatrics, and it is commonly caused by respiratory syncytial virus, influenza virus, adenovirus and so on(Xi S, et al,2020) It is easy for these viruses to damage the respiratory system, and to invade the circulatory system and central nervous system. If it is not controlled in time, it can cause serious damage to the body(Kurvers RAJ, et al,2013) The disease has two characteristics, namely rapid onset and rapid progression. Most of the clinical manifestations include fever, cough, expectoration, wheezing and other symptoms(Zimmermann P, et al,2020)

Although chest radiography (CXR) is frequently used to diagnose pneumonia, it has important limitations including poor interobserver agreement, decreased accessibility in resource-poor settings, and most importantly for this analysis, an inability to distinguish bacterial pneumonia from viral pneumonia (Elemraid et al,2014)

This study aimed to develop new method for diagnosis of viral pneumonia causative agent

Subjects and study design

This is a case-control study. A total of 80 individual were involved in this study, with age ranging from (1 to 36) months.

Subjects: A 40 patients divided according to disease. And 40 health individuals as control, who were observation in pediatrics hospitals in Babel governorate during the period from September 2019 to February 2020 by helping of clinicians in diagnosis of pneumonia patients.

The sample are whole blood were collected from each participant, noting that the sera were used determination of causative agent of viral Pneumonia for all specimens.

Ethical considerations

The approvals were obtained from all the participants (patients and healthy) and also agreed to study scientifically and morally by the medical committee in the department of pediatric for pediatric hospitals in Babel governorate.

Materials and Methods

Materials of Indirect Immune Fluorescent Assay Kits

IgM kits

1.Vircell Pneumoslide Slide: each kit have10 slides and each slide have 10 wells with the following antigens:

1-Adenovirus in HEp-2 cells.

2. Respiratory syncytial virus in HEp-2 cells.

3. Influenza A in LLC-MK2 cells.

4. Influenza B in LLC-MK2 cells.

5. Parainfluenza serotypes 1, 2 and 3 in LLC-MK2 cells.

6. Cell control.

2. Vircell PBS: 1 vial of PBS pH 7.2 powder to reconstitute with 1 l of distilled water.

3. Vircell Pneumoslide IgM Positive control: 500 µl of positive control serum, containing sodium azide.

4. Vircell Pneumoslide IgM Negative control: 500 µl of negative control serum, containing sodium azide.

5. **Vircell Anti human IgM FITC conjugate**: 2 vials with 1.1 ml of fluorescein-labeled anti-human IgM fluorescein conjugate in a phosphate buffer containing Evan´s blue, sodium azide and a protein stabilizer.

6. Vircell MOUNTING MEDIUM: 3 ml of mounting medium: buffered glycerol, containing sodium azide.

7. Vircell Anti human IgG globulin (sorbent): 1 vial of 1.65 ml of sorbent (goat anti-human IgG, containing sodium azide).

Method of Diagnosis of causative agents of pneumonia

3.2.4.1.IIF assay procedure:

1. All reagents was putted to room temperature before use. Allow the slides to reach room temperature before opening.

2. Prepared a 1/128 and 1/256 dilution of samples by added 10 µl of sample to 1270 and 2550 µl of PBS 2 (1/128 and 1/256 dilution).The control sera 3 and 4 should not be diluted.

3. Applied 20 µl of 1/128 dilution into the wells 1, 2, 3 and 4 and of 1/256

dilution into the wells 5, 6, 7, 8, 9 and 10. Added 20 µl of non-diluted positive control 3 to each well of a slide and 20 µl of non-diluted negative control 4 to each well of another slide.

4. slide was incubated in a humid chamber for 30 minutes at 37ºC.

5. Rinsed slide 1 briefly with a gentle stream of PBS 2 (avoid directing PBS at wells) and immerse in PBS while shaking gently on a shaker, for ten minutes. Dip wash slide briefly in distilled water.

6. Slide was allwed to air dry.

7. A20 µl was added of anti-human IgG FITC conjugate solution 5G to each well. (No dilution required).

8. A steps 4, 5 and 6WAS repeated

9. Added a small drop of mounting medium 6 to each well and carefully

cover with a coverslip.

10. The slide was read as soon as possible in a fluorescence microscope at 400x magnification. If this is not possible, store in the dark at 2-8ºC up no more than 24 hours, until observation.

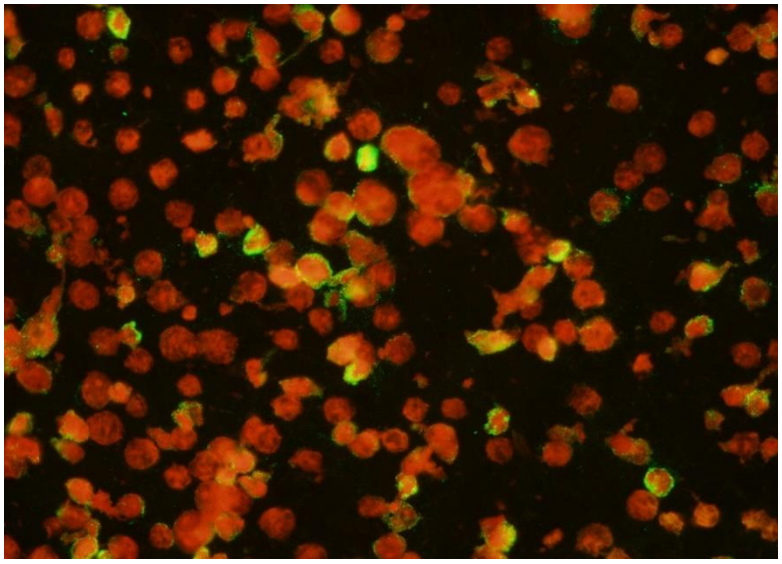

Figure1: IIFA for Adenovirus.

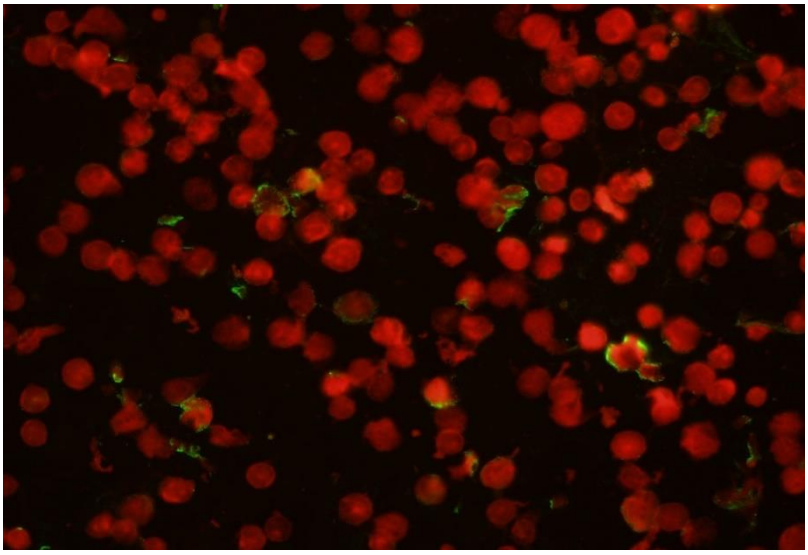

Figure 2: IIFA for Parainfluenza 1,2 and 3

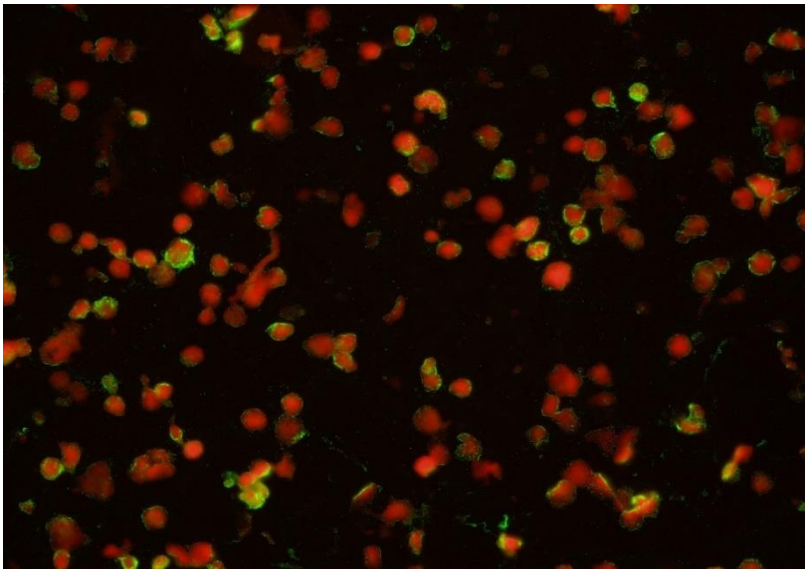

Figure3: IIFA for Influenza

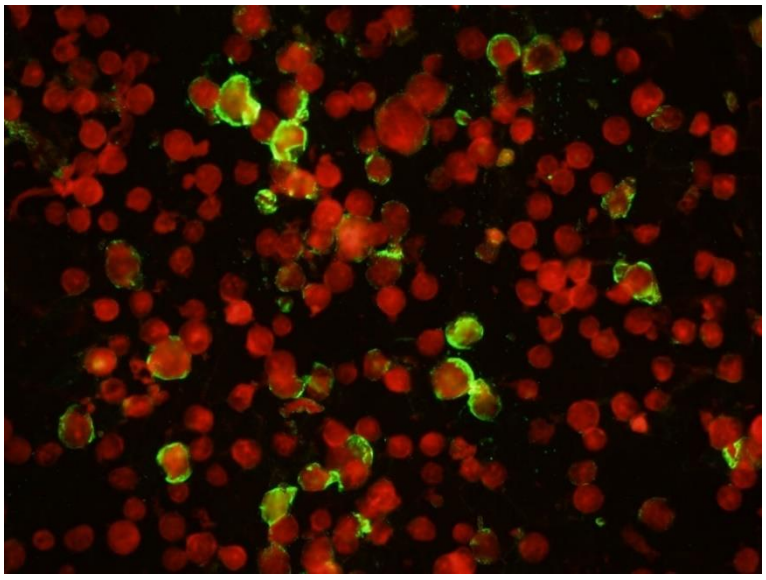

Figure 4: IIFA Influenza_A

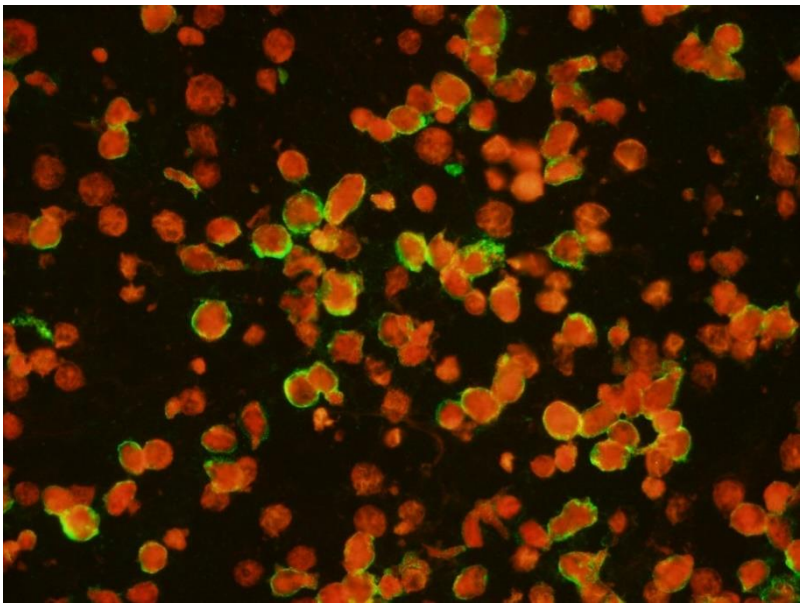

Figure 5: IIFA for RSV

Results and Discussion

In the current study, the following viral agents were isolated from patients with their corresponding frequencies: Respiratory syncytial, 8 (20%), Influenza A, 8 (20 %), Parainfluenza, 4 (10%), Adenovirus, 3 (7.6 %), combined Adenovirus and Parainfluenza, 2 (5 %) and combined Influenza B and Parainfluenza, 1 (2.6 %).

thViruses are responsible for a large proportion of acute respiratory tract infections (ARTIs). Human influenza, parainfluenza, respiratory-syncytial-virus, and adenoviruses are among the leading cause of ARTIs(Umuhoza, T., et al,2021) so agree with our study.

According to (Leung et al., 2018) the most common viruses Respiratory syncytial and this is in line with our finding, also some of the most frequent viral agents implicated in CAP in the United States include influenza virus followed by respiratory syncytial virus, parainfluenza virus, and adenoviruses (Jain et al,2015).

The burden of IAV infection is highest in children, the elderly, and persons with chronic

medical conditions. Each year, IAV infection affects up to 40%of children younger than 5 years in the United States and 90 million worldwide(American Academy of Pediatrics,2003).

It has been stated that mixed viral-bacterial infection is seen in 3-30% of the pneumonia cases (Korppi et al., 2002). It appears that mixed infections are highly variable and depends on the technique used in the diagnosis (Mathew et al., 2018)which in line in our study.

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