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SOME IMMUNOLOGICAL PARAMETERS OF VIRAL PEDIATRIC PNEUMONIA

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ABSTRACT

This study was planned to diagnosis of some viral causative agents addition to clinical signs and symptoms and study some immunological biomarkers for pediatric pneumonia. This work is clinically used diagnosed children patients (n =100) of pneumonia were chosen and in addition to (n = 40) apparently healthy children as a control group between October 2013 to July 2014. Specific diagnosis is clinically done by consultant physicians for all studied patients. The patients were distributed into three groups according to the age, <1 year (40) patient, 1-3 years (30) patient and 4-5 years (30) patient. The results of IIFA showed that the specific (IgM) antibody were detected in serum of infection patients. The viral causative agents of pneumonia diagnosed are 47%divided into single viral agents (37%) have five genera adenovirus(14%), parainfluenza (11%), respiratory syncytial virus(10%), influenza B(2%)and influenza A(0%) and mixedviral agents(10%) (viral with viral cause). Adenovirus with Respiratory Syncytial virus(6%), Adenovirus with Parainflunsa(4%), The WBCs count showed high significant increase at ($P<0.05$) of concentration in patient groups10.51±3.82cell/cu mm comparison with control8.17±1.83cell/cu mm, viral causes 10.93±3.75cell/cu mm and mixed causes 12.73±3.40cell/cu mm.

A significant increase of neutrophil cells count patients 4.54±2.41 cell/cu mm comparison with control 3.87±1.045 viral causes 3.78±2.49 cell/cu mm and mixed causes 5.81±2.03 cell/cu mm. A high significant increase was shown in lymphocyte cells count of pneumonia patients 4.65±2.56 cell/cu mm when camper with control 3.33±0.834cell/cu mm was appeared in viral causes 5.58±1.88cell/cu mm and mixed causes 5.39±3.14cell/cu mm. A significant increase in patients CRP 9.72±13.24mg/l comparison with control 3.40±1.08mg/l appear in mixed causative agents groups12.17±17.08mg/l but not observe in viral causes7.22±11.16.

KEYWORDS: Pneumonia, Viral Causes, Immunological Tests

INTRODUCTION

Pediatric pneumonia is a common and serious health care problem, responsible for one fifth of children's deaths around the world (2 million), 70% occurring in developing countries[1]. Its etiology can be viral, bacterial, or mixed infection, It is different according to age groups and during the various seasons of the year. And it also has an important impact on society and is a frequent cause of physician visits, work loss, and reduction of quality of life of the children and his/her family [2]. Wide range of viruses is known to be associated with respiratory disease in humans. Adenoviruses, coronaviruses, human enteroviruses (HEV), human rhinoviruses (HRV), influenza viruses, parainfluenza viruses (PIV), and respiratory syncytial viruses (RSV) are well-known causes of acute respiratory tract infections (ARTI) in both industrialized and developing countries. Over the last decade, modern molecular techniques have led to the discovery of several previously unknown respiratory tract viruses, including human metapneumovirus (hMPV) [3].

Respiratory syncytial virus (RSV) is an enveloped negative-sense single-stranded ribonucleic acid (RNA) virus of the Paramyxoviridae family, which is recognized as a leading cause of respiratory illness in young infants and children. No vaccine is currently licensed to prevent RSV infections. Each year in the United States, 75,000–125,000 hospitalizations related to RSV occur among children aged < 1 year, and RSV infection results in 1.5 million outpatient visits among children aged < 5 years [4].

The ADV is a non-enveloped virus composed of double stranded linear DNA. Virus particles range from 70– 90 nm in size and belong to the family Adenoviridae, genus mast Adenovirus. Based on characteristics such as haemagglutination, length of the fiber gene, and GC content of its genome, 68 types of ADV have been recently classified, which can be divided into seven different subgroups or species (A-G) [5].

Influenza virus belongs to Orthomyxoviridae family and is lipid enveloped with negative sense single stranded RNA segmented genome [6]. Influenza A viruses are responsible for both pandemic and seasonal epidemics, while influenza B and C viruses only cause epidemics [7]. The HPIV-1 infects the upper and lower respiratory tract and causes acute respiratory infections (ARIs), ranging from mild infections, such as the common cold and laryngitis, to severe infections, such as croup, pneumonia, and bronchiolitis. HPIV-1 is responsible for almost half of all hospitalizations due to ARIs both in patients younger than 5 years old and in the elderly; additionally, HPIV-1 is the most common cause of infectious laryngotracheitis (croup) in children [8].

The airway epithelium of the human upper respiratory mucosa acts as the first physical barrier that protects against inhaled substances and pathogens [9]. Innate (natural) responses occur to the same extent however many times the infectious agent is encountered, whereas acquired (adaptative) responses improve on repeated exposure to a given infection. The innate responses use phagocytic cells (neutrophils, monocytes, macrophages), cells that release inflammatory mediators (basophils, mast cells, and eosinophils) and natural killer cells. The molecular components of innate responses include complement, acute-phase proteins, and cytokines[10]. The CRP is whenever there is an infection or tissue inflammation, Within 4–6 hours of stimulation, CRP is secreted. Thereafter, its level doubles every 8 hours and reaches its maximum value at 36–50 hours. Once the stimulus is no longer present [11]. The complement system is composed of many proinflammatory proteins. Among those, C3 is critical for activation of the complement system as a whole. On the other hand, C4 is the major protein of the classical cascade. They play an important role in the immune/inflammatory response and are upregulated during pneumonia [12].

MATERIALS AND METHODS

Patients and Control

Clinically diagnosed children patients of pneumonia admitted in Babylon maternity and pediatric hospital for children and delivery. One hundred 100 blood sample were collected from children less than five years they were suffering from pneumonia in Babylon maternity and pediatric hospital after clinically diagnosed by consultant physician, and chest X-ray during the period between October 2013 to July 2014.

Forty as control samples which are apparently healthy children agemarched groups.

The time of study is limited in the period between October 2013and July 2014. Specific diagnosis is clinically done by consultant physician, and chest X-ray.

The Diagnosis of Viral Pneumonia by Usingindirect Immunofluorescence Test (IIFA)

The test used for simultaneous diagnosis in human serum of IgM antibodies of the main viral etiological agents of infectious diseases of pneumonia which are Adenovirus in HEp-2 cells, Respiratory syncytial virus in HEp-2 cells, Influenza A in LLC-MK2 cells, Influenza B in LLC-MK2 cells and Parainfluenza serotypes 1, 2 and 3 in LLC-MK2 cells.

IMMUNOLOGICAL PARAMETERS

WBCS and Differential Count

The cell-dyn Ruby instrument was used to measure, count and calculate the hematological parameters are discussed in sample analysis cycle overview and introduction to flow cytometry within this section according to manufacture instruction.

C. Reactive Protein Test (CRP)

The CRP-latex particles are coated with antibodies to human CRP. The CRP-latex reagent has been standardized to detect serum CRP levels at or above 6 mg/L which is considered the lowest concentration of clinical significance

RESULTS AND DISCUSSIONS

Diagnosis Causes of Pneumonia

In current study was used indirect immunofluorescent technique due to has several advantages, the first place the fluorescence is brighter than with the direct test since several fluorescent anti-immunoglobulins bind on to each of the antibody molecules present inthe first layer. Second, even when many sera have to be screened for specific antibodies it is only necessary to prepare (or, more usually the case, purchase) a single labeled reagent, viz. the anti-immunoglobulin [13].

Figure 1 was showing the specific (IgM) antibody were detected in serum of infection patients they have clinical symptoms of pneumonia. The most viral causative agent of pneumonia diagnosed is 47% as following:

- **Single Viral Agents (37%):** have five genera Adenovirus (14%), Parainfluenza (11%), Respiratory syncytialvirus (10%), Influenza B (2%) and Influenza A(0%).
- **Mixed or Co-Infection (Viral with Viral):** Adenovirus with Respiratory Syncytial virus (6%), Adenovirus with Parainflunza (4%)
- **Unknown Agents:** other causes not present in current study as in table 1

Table 1: Viral Causative Groups of Pneumonia

Causative Agents	Name of Genus	No. of Patients	Total
Viral agents	Adenovirus	14%	37(37%)
	Parainfluenza	11%	
	Respiratory syncytialvirus	10%	
	influenza B	2%	
	influenza A	0%	
Mixed viral agents	Adenovirus with Respiratory syncytialvirus	6%	10(10%)
	Adenovirus with parainfluenza	4%	
Unknown agents	other causative agents not included in present study.	53%	53(53%)
Total			**100(100%)**

Pediatric patients < 1 year have the major group than other. This result might be refer to that thus age group of children more susceptible to infection, because have low eduated immunity as well as have large numbers of viral in the air ways and tonsillitis.

Viral agents have a greaterrole in causing RTI as they are more commonly observed [14].

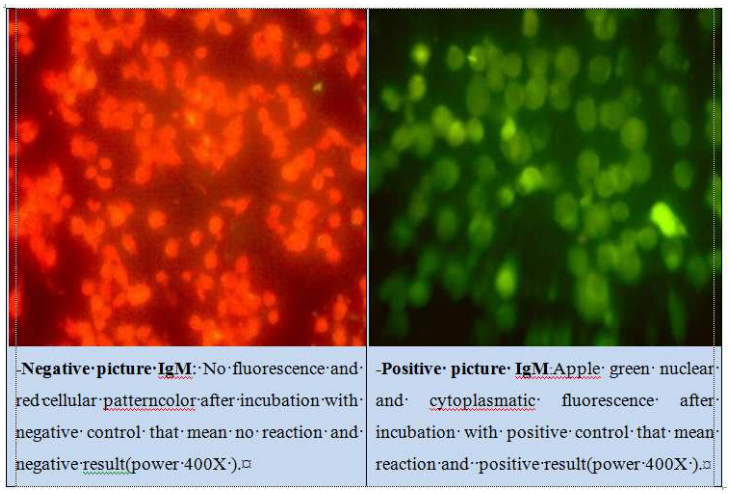


Figure 1A: Positive and Negative Control Fluorescent Staining

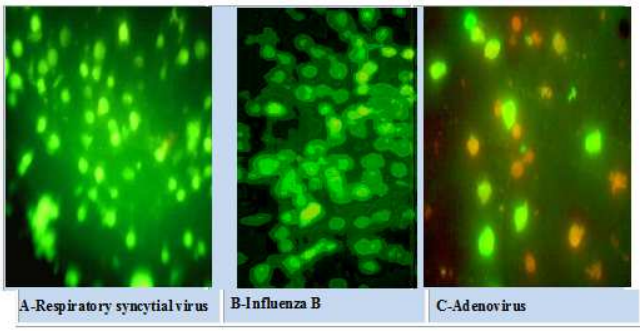
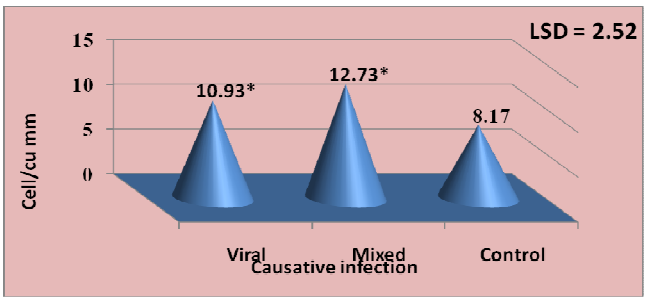


Figure 1B: Positive Fluorescent Staining of different Antibodies for Viral Infection Causes

IMMUNOLOGICAL PARAMETERS

WBCs and Differential Count

WBCs count in patients and control were carried out of counted asmean±SD. Result of patients were showa significant increase inviral infection 10.93±3.75cell/cu mm, mixed infection 12.73±3.40cell/cu mmcomparison with control 8.17 cell/cu mmat the LSD 2.52. Figure 2

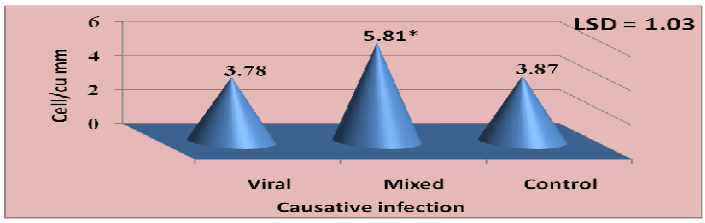


(*) mean significant difference in comparison with control at the 0.05 level

Figure 2: WBCs Count in Viral Pneumonia Patients According to the Causative Agent and Control Group

This result might be show that the leukocytosis is accounted with the acute pneumonia infection, the viral infection induce leukocytosis agree with [15] leukocytosis were detected in the patients withviral infection respiratory infection.

There are no significant increase in the viral infection 3.782.49± cell/cu mm but mixed infection 5.81±2.03 cell/cu mm have significant differences when comparison all groups with control 3.87±1.05 cell/cu mm figure 3.

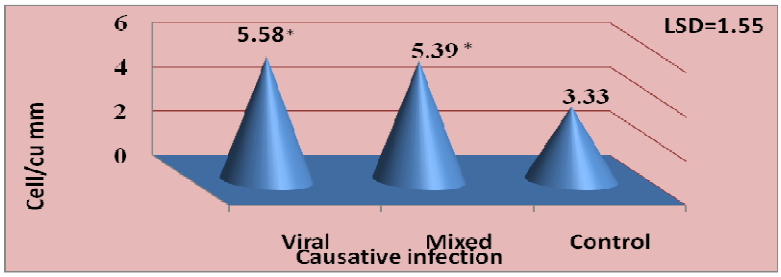


(*) mean significant difference in comparison with control at the 0.05 level

Figure 3: Neutrophil Cells in Viral Pneumonia Patients According to the Causative Agent and Control Group

The lowest neutrophils cells count in the patients with viral causes, because the viral infection induce lymphocytosis rather than neutrophils cells. The result agree with [16] which mention the neutrophil count is usually elevated in bacteremic infections. An alveolar infiltrate is used as evidence of bacterial, and an interstitial infiltrate as evidence of viral**.**

A significant increase was appeared in viral infection 5.58±1.88cell/cu mm and mixed infection 5.39±3.14cell/cu mm when comparison with control group 3.33±.834 cell/cu mm, figure 4



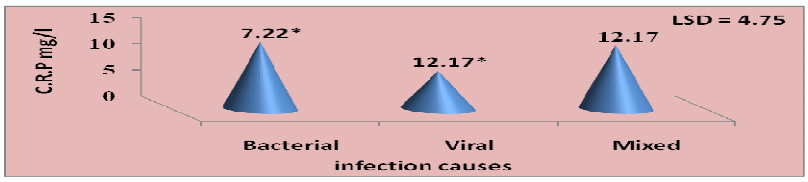
(*) mean significant difference in comparison with control at the 0.05 level

Figure 4: Lymphocyte Cells in Viral Pneumonia Patients According to the Causative Agent and Control Group

This result is show increase of lymphocyte in viral and mixed infection [17] who said most cases of lymphocytosis are reactive and commonly occur with viral infections.

C. Reactive Protein Test (CRP)

The significant increased was showed in mixed causes 12.17±17.08mg/l in comparison with control 3.40±1.08mg/l while no significant increase in viral infection7.22±11.16mg/l as in figure 5.



(*) mean significant difference in comparison with control at the 0.05 level

Figure 5: Concentration of CRP in Viral Pneumonia Patient According to the Causative Agent and Control Group

This result might be show that the viral causes of pneumonia in children less than 5 year less induced of CRP production than other causes such as bacterial causes, This result agree with [18] which mention CRP concentration were higher in children with bacterial pneumonia than in children with pneumonia of viral etiology.

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