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RESEARCH ARTICLE

DIAGNOSTICAL AND SOME IMMUNOLOGICAL PARAMETERS OF A TYPICAL PEDIATRIC PNEUMONIA

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Abstract

This study was planned to diagnosis of some bacterial causative agents of atypical pneumonia addition to clinical signs and symptoms and study some immunological biomarkers for pediatric pneumonia.This work isclinically 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.

By using the indirect immunofluorescent assay IIFA, the results showed that the specific (IgM) antibody were detected in serum of infection patients. Thebacterial agents 29 (29%) from all patients 100 which distributed to four genera *Mycoplasma pneumonia*(14%), *Legionella pneumophila*serogroup1(11%), *Coxiella burnetii*(2%) and *Chlamydophila pneumonia*(2%).

The WBCs count showed no significant increase at ($P<0.05$) of concentration in patient groups8.56±3.37cell/cu mm comparison with control8.17±1.83cell/cu mm.

A significant increase of neutrophil cells count patients 5.02±2.29cell/cu mm comparison with control 3.87±1.045 in bacterial causes 5.02±2.29 cell/cu mm.

Significant increase was shown in lymphocyte cells count of pneumonia patients 2.72±1.85cell/cu mm when camper with control 3.33±0.834cell/cu mm.A highly significant increase in patients C-Reactive Protein (CRP) 9.72±13.24mg/l comparison with control 3.40±1.08mg/l.

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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].

Mycoplasma are the smallest self-replicating organisms able to live outside the host cells. They do not have cell wall, but a cell membrane containing sterols and do not stain well with Gram stain[3].

Mycoplasma pneumoniae(MP) is an extracellular pathogen that attaches to ciliated epithelial cells of the respiratory tract mucosa and causes damage [4]. This agent is reported to cause 10%–30% of cases of community-acquired pneumonia (CAP) in pediatric patients[5].

Legionella bacteria are ubiquitous in nature and are often found in natural water sources as well as in man-made water systems. Humans may be infected through inhalation of contaminated aerosolized water droplets. Symptoms range from influenza-like disease (Pontiac fever) to severe pneumonia Legionnaires' disease (LD) with a high mortality rate [6]. More than 50 *Legionella* species are described, but *Legionellapneumophila* is the cause of more than 95% of LD cases [7]. High concentrations (104-1010 Legionella CFU/L) of *Legionella* in the water sources are considered a risk of infection [8].

*Chlamydophilapneumoniae*is an airborne obligate bacterial pathogen responsible for a significant number of respiratory infections around the world and implemented in the pathogenesis of atherosclerosis. [9]

Coxiella burnetii is an obligatory intracellular Gram negative bacterium which causes Q fever, an illness with multiple clinical manifestations in its acute presentation, including a flu-like respiratory process that could result in atypical pneumonia [10].

The airway epithelium of the human upper respiratory mucosa acts as the first physical barrier that protects against inhaled substances and pathogens [11]. Innate (natural) responses occur to the same extent however many times the infectious agent is encountered, whereas acquired (adaptive) 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[12].

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 [13].

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 age marched groups.

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

The diagnosis of atypical bacterial pneumonia by using indirect immunofluorescence test (IIFA)

The test used for simultaneous diagnosis in human serum of IgM antibodies of the main bacterial etiological agents ofinfectious diseases of atypical pneumonia which are *Mycoplasma pneumonia*, *Legionella pneumophila*serogroup1,*Coxiella burnetii*and *Chlamydophila pneumonia*.

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)

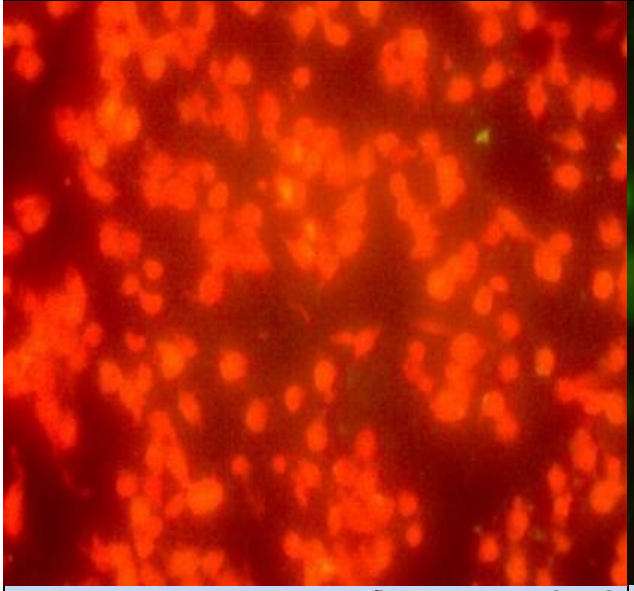
The CRP-latex particles are coated with antibodies to human CRP. The CRP-latex reagent has been standardized to detect serum CRP levels at or above 6mg/L which is considered the lowest concentration of clinical significance.

Results and Discussion

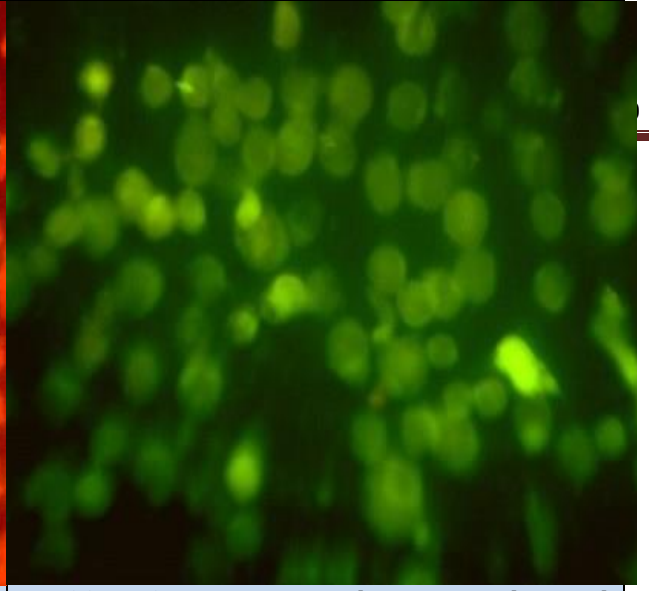
Diagnosis causes of pneumonia:

In current study was used indirect immunofluorescentassay (IIFA) as infigure (1) was showing the specific (IgM) antibody were detected in serum of infection patients they have clinical symptoms of pneumonia . The most bacterial causative agent of atypical pneumonia diagnosed is 29% as following:

Bacterial agents (29%) distributed to four genera *Legionella pneumophila*serogroup1(11%), *Mycoplasma pneumonia* (14%), *Coxiella burnetii* (2%) and *Chlamydophila pneumonia* (2%) table (1).



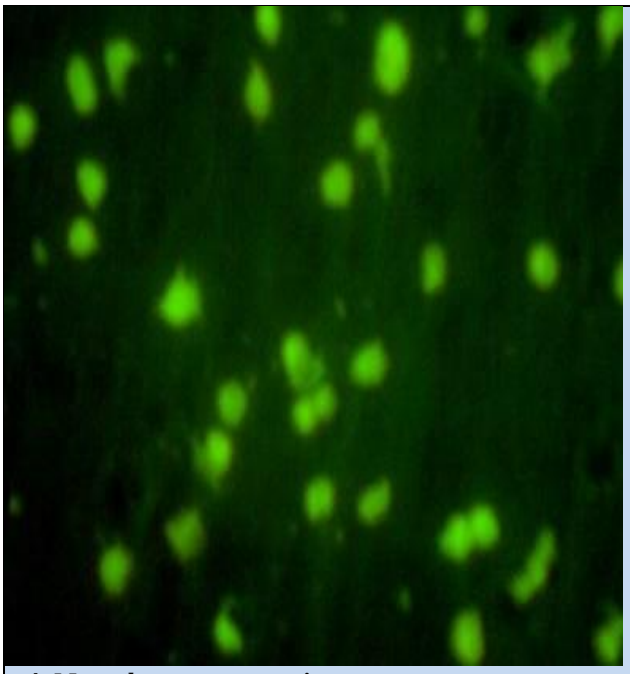
-Negative picture IgM: No fluorescence and red cellular pattern color after incubation with negative control that mean no reaction and negative result(power 400X).



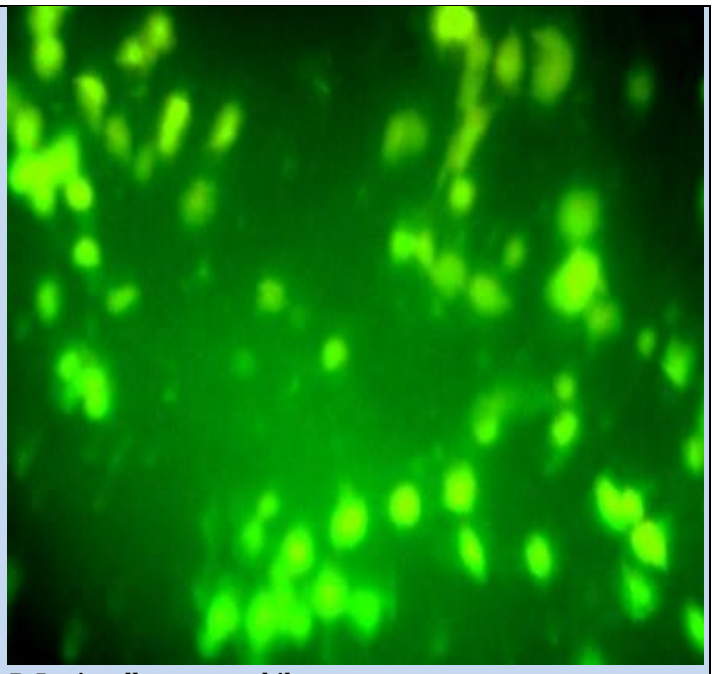
-Positive picture IgM: Apple green nuclear and cytoplasmatic fluorescence after incubation with positive control that mean reaction and positive result(power 400X).

Table (1) Main causes bacteria of atypical pneumonia by using(IIFA)

Figure (1A)Positive and Negative control fluorescent staining.



A-Mycoplasma pneumoniae



B-Legionella pneumophila

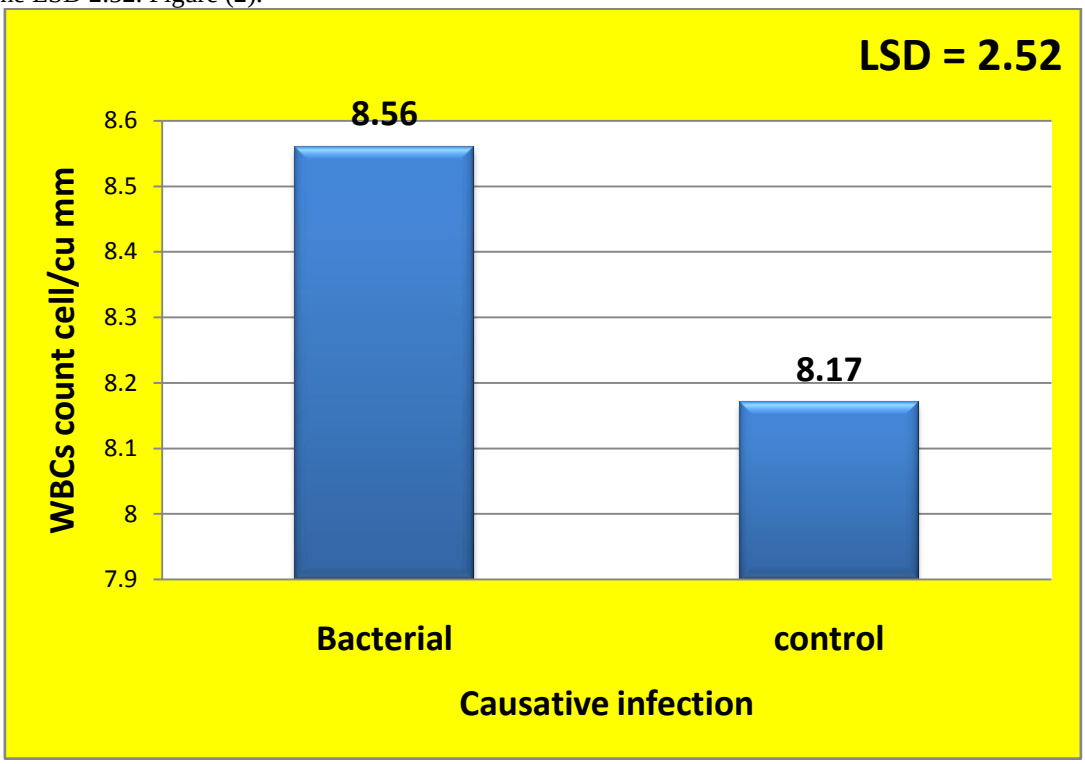
Total			100(100%)

Figure (1B)Positive fluorescent staining of some antibodies for bacterial infection causes.

Immunological parameters :

WBCs and differential count

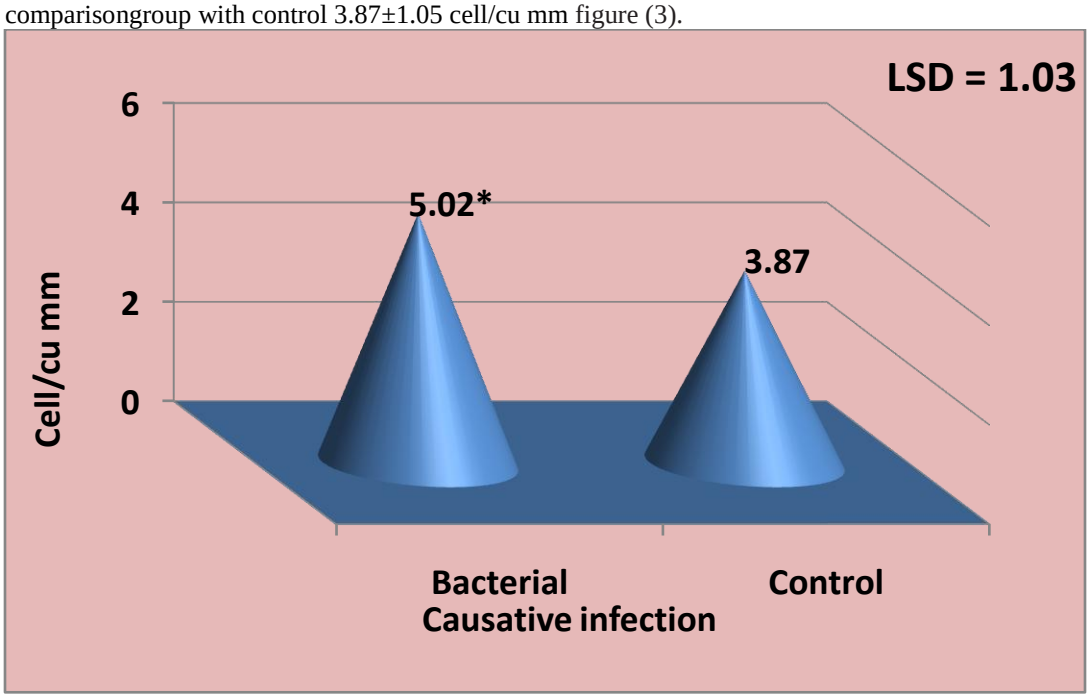
WBCs count in patients and control were carried out of counted asmean±SD. Result of patients were showno significant increase in concentration of patient group 8.56±3.37cell/cu mmcomparison with control 8.17 cell/cu mm at the LSD 2.52. Figure (2).



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

Figure (2) WBCs count in bacterialpneumonia patients according to the causative agent and control group.

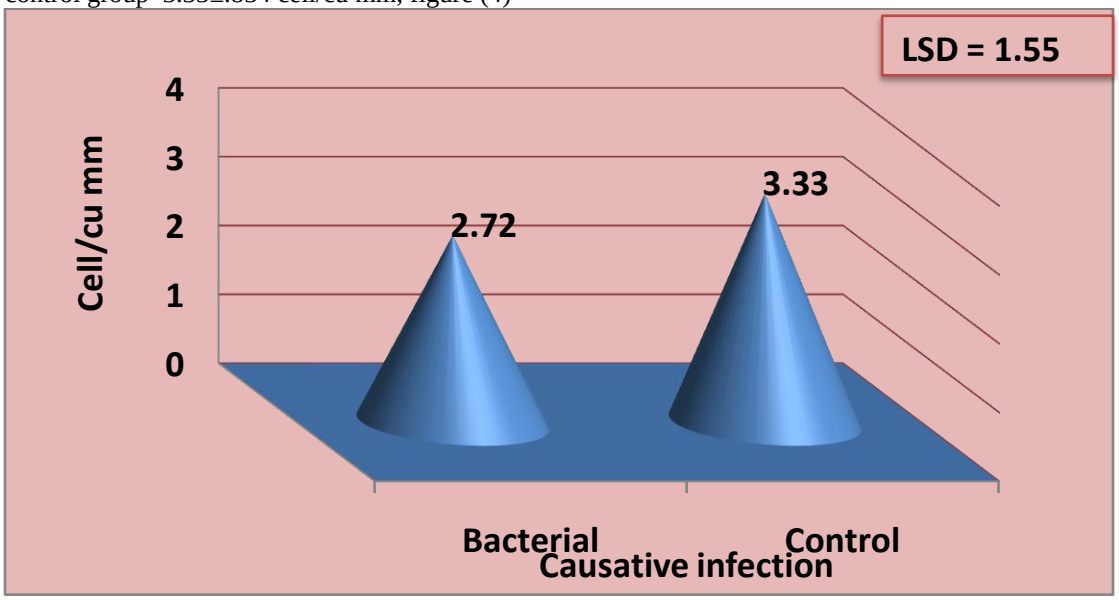
There are a significant increase of neutrophils count in the bacterialinfection 5.02±2.29cell/cu mmwhen comparisongroup with control 3.87±1.05 cell/cu mm figure (3).



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

Figure(3)Neutrophil cells in bacterialpneumonia patients according to the causative agent and control group.

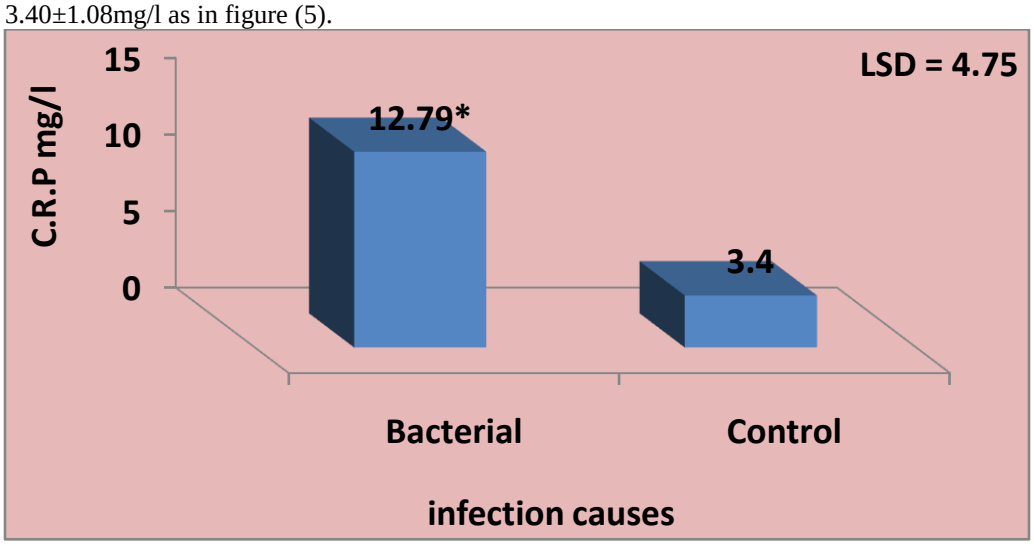
There are no significant increased of lymphocyte count in bacterial infection 2.72±1.85 when comparison with control group 3.33±.834 cell/cu mm, figure (4)



Figure(4)Lymphocyte cells in bacterialpneumonia patients and control group.

C- Reactive Protein Test(CRP)

The significant increased was showed inbacterial causes 12.79±16.89mg/l in comparison with control 3.40±1.08mg/l as in figure (5).



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

Figure (5) Concentrationof CRPin bacterialpneumonia patient according to the causative agent and control group.

Indirect immunofluorescentassay is used in diagnosis 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 in the 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 [14].

Pediatric patients <5 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 immunity as well as have large numbers of bacteria in the

air ways and tonsillitis.The result of [15] were agree with our results who mention to *Mycoplasma pneumonia* was more frequent than *Chlamydia pneumonia.*

Unknown agents: other causes not present in current study as in table(1)

In previous result show not found significant increase in WBCs count in bacterial causes because the bacteria in current study are intracellular which not appear to immune system , this result agree with [16] who mention that many as of patients might have an atypical illness, demonstrating the clinical variability of bacterial pneumonia.

The result of neutrophils count agree with [17]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.

The result is show no significant increase of lymphocyte in bacterial infection because bacterial infection induce neutrophils while viral infection induce lymphocytosis [18]who said most cases of lymphocytosis are reactive and commonly occur with viral infections.

The result might be show that the bacterial causes of pneumonia in children less than 5 year more induced of CRP production than other causes such as bacterial causes, This result agree with [19] which mention CRP concentration were higher in children with bacterial pneumonia than in children with pneumonia of viral etiology.

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