SEROPREVALENCE OF CHLAMYDIA PNEUMONIAE ANTIBODIES AMONG CHILDREN ADMITTED FOR RESPIRATORY INFECTIONS IN SULAIMANI PEDIATRIC TEACHING HOSPITAL

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ABSTRACT

Background

The incidence of severe acute respiratory tract infections in children caused by *Chlamydia pneumoniae* varies greatly from year to year and place to place around the world. *Chlamydia pneumoniae*, a bacterium that causes respiratory infections, is probably under-diagnosed, it's of worldwide distribution. The seroprevalence of *C. pneumoniae* antibody is age dependent

Objectives

This study examined the prevalence of *C. pneumoniae* infections, their clinical manifestations and demographic features in hospitalized children with respiratory tract infections. The study also tested two different immunological techniques for detection *C. pneumoniae* infections.

Methods

This cross sectional study was carried out in Sulaimani Pediatric teaching hospital. One hundred and twenty seven children aged less than 5 years, hospitalized for respiratory tract infections were enrolled in this study. Serum anti-*C. pneumoniae* specific IgM and IgG antibodies were measured using an enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescent assay technique (IFA). The clinical and demographic data were compared between positive *C. pneumoniae* antibodies and negative *C. pneumoniae* antibodies.

Results

Acute *C. pneumoniae* infections were detected using IgM anti-*C. pneumoniae* antibodies which were positive in 5 (3.9%) children by ELISA technique and the same result was obtained by IFA technique. IgG anti-*C. pneumoniae* antibodies were positive in 32 (25.2%) and 33 (25.9%) by ELISA and IFA respectively, the results of the two tests were similar and the differences were statistically not significant. Clinical and demographic data of patients with *C. pneumoniae* infections were comparable to those of negative IgM anti-*C. pneumoniae* antibodies.

Conclusion

C. pneumoniae has a role in children hospitalization with respiratory tract infections in Sulaimani Governorate. ELISA technique is a good alternative for IFA in serological diagnosis of *C. pneumoniae* infections.

Keywords: *Hospitalized children, Anti-Chlamydia pneumoniae antibodies, ELISA technique, IFA technique, respiratory tract infections.*

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INTRODUCTION

Chlamydia pneumoniae (*C. pneumoniae*) is an obligate intracellular bacterium that has been associated with a variety of acute and chronic diseases[1]. In 1999, a new taxonomic classification was proposed, renaming the bacterium as *Chlamydophila pneumoniae*[2]. Acute respiratory infections due to *C. pneumoniae* occur worldwide in different age groups, while persistent *C. pneumoniae* infections have been associated with chronic diseases like asthma, chronic pulmonary obstructive diseases, and coronary heart diseases [3, 4]. The acute respiratory infections occur in children and adults and involve upper and lower respiratory infections. Mostly, the clinical respiratory course among children progress slowly and has no specific features [5].

The seroepidemiological studies have shown that epidemics and endemics of *C. pneumoniae* infections occur in different parts of the world and the antibody titers are rising with age in adult population [6].

The diagnosis of acute infections with *C. pneumoniae* is mostly done by serological assays to detect antibodies specific to this bacterium. Among immunoglobulin classes, IgM antibodies are very helpful for diagnosis of acute infections because of their early serological response to *C. pneumoniae* infections compared to the times of response of IgG and IgA antibodies [7]. IgM antibody titer is elevated after acute respiratory tract infections and usually falls within 2 months of infection and normalized within 4 to 6 months. IgG antibodies are elevated for several years after infections and can be detected in different age groups.

The specific laboratory diagnosis of *C. pneumoniae* infections is useful for patient management as curative antimicrobial therapy is available for *C. pneumoniae* infections [8].

C. pneumoniae is not uncommon respiratory pathogen among children. *C. pneumoniae* causes acute upper respiratory tract infections as well as lower respiratory tract infections in children such as pharyngitis, sinusitis, acute otitis media, and pneumonia [9].

To our knowledge, there is no previously reported data about *C. pneumoniae* infections among children in Sulaimani governorate. The present study investigated infections that are caused by this pathogen among hospitalized children in Sulaimani governorate.

MATERIALS AND METHODS

Children aged less than 5 years with acute respiratory tract infections of unknown etiology admitted to Pediatric Teaching Hospital in Sulaimani city were enrolled in this study. Exclusion criteria included children more than 5 years old whatever the cause of their admission to hospital and children less than five years old admitted to hospital for causes rather than acute respiratory tract infections. The study was performed for one hundred and twenty seven patients from November 2011 to March 2012; children were chosen by random sampling.

The parents of studied children signed informed consent for giving permission to collect blood samples from their children in this study. A questionnaire was designed to collect demographic and clinical data from all hospitalized children enrolled in the study.

About 5 milliliters of venous blood were collected from each child enrolled in the study. Blood of each child was centrifuged and serum was separated in two labeled tubes and stored at – 20°C till starting the procedures. Sera were tested for IgM and IgG anti – *C. pneumoniae* antibodies using indirect immunofluorescent assay (IFA) and ELISA techniques.

IFA for detection of IgM anti-*C. pneumoniae* antibodies was performed by applying ready-to-use slides (Euroimmune Company/Germany) coated with epithelial cells infected with *C. pneumoniae*. In addition IgM dilution buffer, fluorescein – labeled anti- IgM antibodies conjugate, PBS – Tween washing buffer, and mounting medium were also used in the procedure. Briefly, the patients' sera were diluted with IgM sample buffer diluents (1:101 dilutions), then 25 µl of diluted serum was added to the reaction field of *C. pneumoniae* slide. The slide was incubated at 37°C for 1 hour then it was washed with washing buffer. IgM Conjugate was added and then the slide was incubated, thereafter washing step was done and followed by the addition of the mounting medium and the slide was covered with cover slip. The fluorescence was detected with the fluorescence microscope by using fluorescein isothiocyanate (FITC) filter. The presence of green cytoplasmic fluorescence in the cells indicated positive staining and a positive result.

The procedure for detection of IgG anti- *C. pneumoniae* antibodies was done in the same manner as for detection IgM anti- *C. pneumoniae* antibodies with one exception is that the fluorescein – labeled anti –

IgG antibodies was the conjugate . The positive IgG anti- *C. pneumoniae* antibodies were showing green cytoplasmic fluorescence similar to the corresponding IgM anti- *C. pneumoniae* antibodies slides.

In addition to IFA technique, two ELISA kits, one for detection serum IgM anti- *C. pneumoniae* antibodies and another for detection serum IgG anti- *C. pneumoniae* were used in this study; the ELISA procedure was done according to the manufacturer instructions (Euroimmune company/Germany).

The Statistical Package for Social Science (SPSS, Chicago, II, USA), version 16 was used for data entry and analysis. Chi-square test (X^2) and Fisher's exact test were used to test the association between categorical variables. P value of ≤ 0.05 was considered as statistically significant.

RESULTS

Children who met the inclusion criteria of this study were one hundred and twenty seven. The main characteristics of the studied children are shown in table 1; the mean age of the children was 1.8 years (SD 1.4) and most of them (54%) were below 2 years.

Fifty two percent of children were males and 48% were females, 87% were from urban areas and 13% were from rural areas. The hospital stay ranged from 1-7 days with mean stay of 4 days (SD 1.3 days).

Table 1. Main characteristics of the patients.

Characteristics	No.	Percent
Total	127	100
Age in years		
0 – 1	68	53.5
1 – 3	35	27.6
3 – 5	24	18.9
Sex		
Male	66	52.0
Female	61	48.0
Residence		
Urban	111	87.4
Rural	16	12.6
Onset of disease		
Insidious	71	55.9
Sudden	56	44.1
History of chronic illness		
Yes	3	2.4
No	124	97.6
Diagnosis		
Pneumonia	77	60.6
Acute bronchiolitis	18	14.2
Acute bronchitis	12	9.5
Acute Tonsillitis	8	6.3
Croup	7	5.5
Acute pharyngitis	5	3.9
Mortality rate	1	0.8
Mean age in years (SD)	1.8 (1.6)	
Mean hospital stay in days (SD)	4 (1.3)	

Patients who were positive for IgM anti-*C. pneumoniae* antibodies were considered acutely infected with this bacterium, while IgG positive children were regarded as an old infection. The ELISA technique showed that the overall IgM anti-*C. pneumoniae* antibodies were positive in 3.9% of all hospitalized children enrolled in the study, while IgG anti-*C. pneumoniae* antibodies were positive in 25.2%; the results of IFA showed that IgM seropositivity was as that of ELISA technique, while IgG was positive in 25.9% (table 2).

The differences in results of both techniques were statistically not significant ($p>0.05$). A positive IgM and IgG anti-*C. pneumoniae* slides of IFA have similar microscopic appearance with cytoplasmic fluorescence and faded nuclear region (figure 1).

Table 2. Frequency and percentage of *C. pneumoniae* seropositivity in different respiratory infections using ELISA and IFA methods

	Upper respiratory infection (n=20) No. %	Pneumonia (n=77) No. %	Bronchitis/ Bronchiolitis (n=30) No. %	All patients (n=127) No. %	P value
IgM anti-*C. pneumoniae* seropositive (ELISA)	0 0.0	5 6.5	0 0.0	5 3.9	χ^2=3.4 P=0.19
IgM anti-*C. pneumoniae* seropositive (IFA)	0 0.0	5 6.5	0 0.0	5 3.9	χ^2=3.4 P=0.19
IgG anti-*C. pneumoniae* seropositive (ELISA)	4 20.0	22 28.6	6 20.0	32 25.2	χ^2=1.2 P=0.55
IgG anti-*C. pneumoniae* seropositive (IFA)	4 20.0	22 28.6	7 23.3	33 25.98	χ^2=0.75 P=0.69

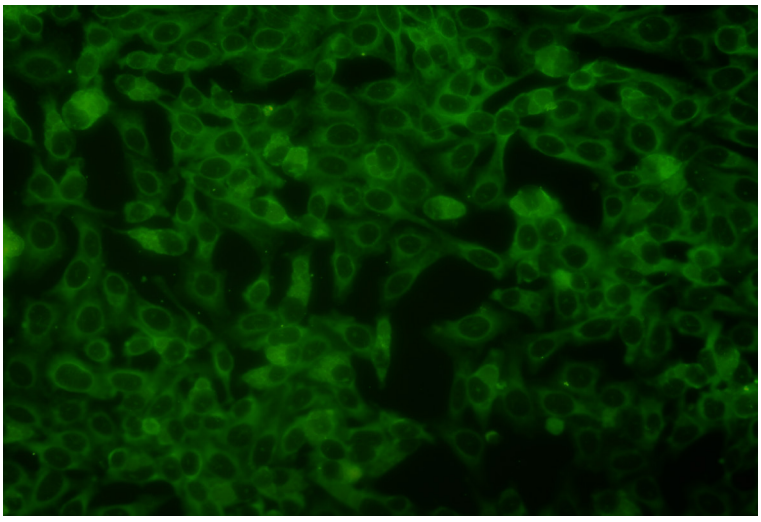

Figure 1. Microphotograph of immunofluorescent technique for serological diagnosis of *C. pneumoniae* antibodies. Human cells infected with *C. pneumoniae* showed positive IFA slide for detection IgM anti-*C. pneumoniae* antibodies. The cells have green cytoplasmic fluorescence (magnification x400).

The ELISA technique for detection of IgM anti-*C. pneumoniae* antibody seropositivity showed that children were suffering from bronchiolitis, pneumonia, or other acute respiratory tract infections in a frequency of 0 , 5, and 0 respectively, while the remaining one hundred and twenty two seronegative children for IgM anti-*C. pneumoniae* antibodies were diagnosed as suffering from the same diseases in frequencies of 17, 72, and 33 respectively; these results of the two groups were statistically not significant (P value > 0.05).

The results of ELISA technique showed that seropositive children for IgG anti-*C. pneumoniae* antibodies were suffering from bronchiolitis, pneumonia, or other acute respiratory tract infections in a frequency of 6, 22, and 4 respectively, while the remaining 95 seronegative children for IgG anti-*C. pneumoniae* antibody were diagnosed as suffering from the same diseases in frequencies of 14, 55, and 26 respectively, these results were also statistically not significant (P value > 0.05), table 1. The results of IFA technique were nearly the same of ELISA technique except that one more child suffered from bronchiolitis was having positive IgG anti-*C. pneumoniae* antibody result. The results were statistically not significant (P value > 0.05), tables 2.

The results of ELISA technique showed that children in age group 0 – 1 year were having the highest percent of IgM anti- *C. pneumoniae* antibody seropositivity whereas the age group 3-5 years was negative for IgM anti- *C. pneumoniae* antibodies. The results of the three age groups of seropositive and seronegative children were statistically not significant (P >0.05), table 3.

The results of IFA technique in different age groups were the same as that of ELISA technique, table 4.

It's clear that in both techniques, ELISA and IFA, the age group of children 3 – 5 years has the highest percentage of IgG anti- *C. pneumoniae* antibody seropositivity whereas, the age group 0 – 1 year has the lowest IgG anti- *C. pneumoniae* antibody seropositivity. The results of different age groups of seropositive and seronegative children were statistically not significant (P >0.05), tables 5 and 6.

The results of clinical and demographic data were not statistically significant comparing between IgM anti-*C. pneumoniae* positive and negative groups, as shown in table 7.

Table 3. Seropositive and seronegative IgM anti- *C. pneumoniae* antibody in patients enrolled in the study according to the age groups using ELISA technique.

Age group in years	Negative	Positive	Total
0-1	64 (94.12%)	4 (5.88%)	68 (100%)
1-3	34 (97.14%)	1 (2.86%)	35 (100%)
3-5	24 (100%)	0 (0%)	24 (100%)
Total	122 (96.06%)	5 (3.94%)	127 (100%)

Table 4. Seropositive and seronegative IgM anti- *C. pneumoniae* antibody in patients enrolled in the study according to the age groups using IFA technique.

Age group in years	Negative	Positive	Total
0-1	64 (94.12%)	4 (5.88%)	68 (100%)
1-3	34 (97.14%)	1 (2.86%)	35 (100%)
3-5	24 (100%)	0 (0%)	24 (100%)
Total	122 (96.06%)	5 (3.94%)	127 (100%)

Table 5. Seropositive and seronegative IgG anti- *C. pneumoniae* antibody in patients enrolled in the study according to the age groups using ELISA technique.

Age group in years	Negative	Positive	Total
0-1	55 (80.88%)	13 (19.12%)	68 (100%)
1-3	25 (71.43%)	10 (28.57%)	35 (100%)
3-5	15 (62.5%)	9 (37.5%)	24 (100%)
Total	95 (74.8%)	32 (25.2%)	127 (100%)

Table 6. Seropositive and seronegative IgG anti- *C. pneumoniae* antibody in patients enrolled in the study according to the age groups using IFA technique.

Age group in years	Negative	Positive	Total
0-1	53 (77.94%)	15 (22.06%)	68 (100%)
1-3	26 (74.29%)	9 (25.71%)	35 (100%)
3-5	15 (62.5%)	9 (37.5%)	24 (100%)
Total	94 (74.02%)	33 (25.98%)	127 (100%)

Table 7. The frequencies and percentages of demographic and clinical data between IgM anti-*C. pneumoniae* positive group and negative group.

Variable	Patients with positive IgM anti- *C. pneumoniae* (n=5)	Patients with negative IgM anti- *C. pneumoniae* (n=122)	P value
Fever	5 (100%)	92 (75%)	0.5911
Dyspnoea	2 (40%)	58 (48%)	1.0000
Cough	4 (80%)	71 (62%)	0.6478
Sputum	1 (20%)	39 (32%)	1.0000
Wheezing	1 (20%)	35 (29%)	1.0000
Tachypnoea	3(60%)	41 (34%)	0.3401
Runny nose	0 (0%)	22 (18%)	0.5863
Cyanosis	0 (0%)	14 (11%)	1.0000
Male : Female ratio	1.5:1	1.9:1	1.0000
History of chronic disease	0 (0%)	4 (3%)	1.0000
History of previous similar condition	0 (0%)	15 (12%)	1.0000
Mean hospital stay (days)	5	4	1.0000
Condition at discharge:			
Complete recovery	3	70	
Discharged with medication	2	51	0.9753
Death	0	1	

DISCUSSION

C. pneumoniae has high affinity for respiratory epithelium, it causes both upper and lower respiratory tract infections [10]. This study investigates the seroprevalence of *C. pneumoniae* infections among hospitalized children using IFA and ELISA for measuring titers of antibodies specific to this bacterium. The percentage of *C. pneumoniae* infections was 3.9% depending on measuring IgM anti-*C. pneumoniae* antibodies, this percentage is lower than that reported in comparable studies some countries like Poland, China, Austria, and Germany [11, 12, 13, and 14], the differences in results might be due to geographic and environmental variations, the type of diagnostic test, and/or the sample size. The current study showed that hospitalization due to *C. pneumoniae* infections is more common in infants than other age groups; this result is contrast to studies conducted in various countries [15, 16, 17, 18 and 19]. The high family density in Suleimani city which increases the exposure and the chance of infection may be an important influence in acquiring this infection.

Bacterial isolation by cell culture, antigen detection methods, molecular techniques for diagnosis of *C. pneumoniae* infections, and serological methods are used for clinical testing of *C. pneumoniae* infections and the last three techniques are currently the most often applied method. In the current study, two techniques, ELISA and IFA, were used to detect serum anti-*C. pneumoniae* antibodies. Immunofluorescence test is the current "gold standard" for serological testing for *C. pneumoniae* infections worldwide [20, 21]. The results of the two techniques were comparable and this indicates that ELISA technique in a good alternative to IFA for the diagnosis of *C. Pneumoniae* infections, a result is comparable to that reported by Vainas, et al.[19].

The clinical features and demographic data of *C. pneumoniae* respiratory infections showed no significant differences when compared to other respiratory infections, similar results were also obtained in an Egyptian study [22], this demonstrate that there is no specific clinical feature that can be useful to predict *C. pneumoniae* infections, and there is a need of laboratory technique that can simultaneously detect different respiratory pathogen from single specimen for diagnosis of different respiratory microorganisms.

In the present study, seroprevalence of IgG anti-*C. pneumoniae* antibodies was 25.9% which is higher than that reported by Kumar et al [23], and another study by Tamara et al.[24], this seroprevalence was spreading in all age groups. The high seroprevalence of IgG anti-*C. pneumoniae* antibodies reflect the wide spectrum of infections in children from asymptomatic infections to severe lower respiratory tract infections.

Limitations of this study include the use of only serological tests in detection of *C. Pneumoniae* infections, the samples were only taken from hospitalized children and not from outpatients, and the period of sample collection was relatively short to assess the epidemiology of *C. pneumoniae* infections.

In Conclusion, *C. pneumoniae* has a role in children hospitalization with respiratory tract infections in Sulaimani Governorate. *C. pneumoniae* infections are mostly affecting children equal to or less than 1 year of which hospitalization is due to pneumonia, and the clinical features of these infections are similar to respiratory infections due to other microorganisms. ELISA technique is as good as IFA in serological diagnosis of *C. pneumoniae* infections.

We recommend the use of ELISA technique as a diagnostic technique for detection of Chlamydia pneumoniae, and testing the respiratory specimens for other respiratory pathogens to get better idea about the prevalence of each microorganism as a causative agent in respiratory tract infections.

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