

Chlamydomphila pneumoniae Infection Assessment in Children With Adenoid Hypertrophy Concomitant With Rhino Sinusitis

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Background: Since adenoids may act as a reservoir for bacteria, they can cause ear infection, recurrent otitis and recurrent adenotonsillitis. Therefore, adenotonsillectomy is an efficient method for reducing the number and severity of subsequent infections.

Objectives: This study aimed to determine the *Chlamydomphila pneumoniae* infection by serological tests and performing PCR in the adenoid tissue, in children undergoing adenoid surgery and compare the results with normal children.

Patients and Methods: This case control study was conducted in the ENT and pediatric wards of Rasoul Akram Hospital in Tehran (2008-2011). We studied 53 patients who had undergone the adenoid surgery (adenoid hypertrophy concomitant with rhino sinusitis) and 31 other patients undergoing elective surgeries in the general surgery ward (like orthopedic surgeries, hernia, etc.) without any infection (like rhinosinusitis), as the control groups. We searched for *C. pneumoniae* by PCR kits (Chemicon, Germany) in resected adenoid tissues and nasopharyngeal swabs of controls (ethics restrictions in controls). We also looked for specific IgM & IgG antibodies (ELISA, Biochem Immuno Systems, Italy) and compared them between the two groups. A P value < 0.05 was considered statistically significant.

Results: Positive PCR results were observed in 13.5% of cases and none of the controls. The condition prevalence was higher in warm seasons (P = 0.05). No correlation was found between positive PCR results and patients' gender. IgM presence was observed in 11% (6.51) of cases and 6.5% (2.31) of controls (P = 0.7). IgG results were not positive in any of the cases (0.51), whereas positive results were seen in 13.3% of the controls (P = 0.007). IgM results showed no significant difference with PCR results (P = 0.7) but IgG results did (P = 0.05).

Conclusions: The results of the study showed a positive correlation between PCR and serology (IgM) results in the case group. Recent *C. pneumoniae* infection was proved to exist by PCR and serology (IgM) in patients who had undergone adenoidectomy. In contrast to the controls, none of the patients in the study group showed previous immunity. Findings indicate the probable role of *C. pneumoniae* infection, in inducing adenoid hypertrophy in nearly 13% of cases.

Keywords: Rhinosinusitis; Adenoid Tissue; Adenoid Hypertrophy; *Chlamydomphila pneumoniae*

1. Background

Adenotonsillectomy is usually performed to resolve obstructive symptoms and recurrent infections. Chronic infection is the third most common indication for surgery (1, 2). Adenoid may act as a reservoir for bacteria, causing ear infection and cholesteatoma and result in development of recurrent and secretory otitis and recurrent adenotonsillitis (3). Usual and unusual bacterial load in the adenoids contributes to the etiology of adenoid hypertrophy. Some experts consider this as the common etiology for rhino-sinusitis and adenoid hypertrophy. Adenotonsillectomy is efficient in reducing the number and severity of subsequent episodes of infection for at least 2 years (1, 2). *Chlamydomphila pneumoniae* is an obligate intracellular organism and its culture requires long incubation

time and is difficult and insensitive to perform. Serologic results are difficult to interpret because *C. pneumoniae* specific antibodies are prevalent in the general population and IgM antibodies are often absent in reinfection (3, 4). Optimal serologic testing depends on the age of the patient, time of testing, obtaining paired (acute and convalescent) sera, appropriate technique and experience of the laboratory technician.

Molecular methods like the PCR are always reliable for *C. pneumoniae* rapid detection in clinical samples. Besides, PCR is a rapid and highly sensitive method in patients previously treated with antibiotics (4, 5). *C. pneumoniae* infection may present as asymptomatic upper or lower respiratory tract infections (LRTIs) in children (6,

Implication for health policy/practice/research/medical education:

Findings indicate the probable role of *Chlamydomphila pneumoniae* infection in adenoid hypertrophy in nearly 13% of cases. In our opinion adenoid tissue might act as a reservoir for *C. pneumoniae* and cause rhino sinusitis concomitant with adenoid hypertrophy in infected children. To establish the effect of suitable antibiotics on *C. pneumoniae* eradication, before submitting to adenoidectomy, further RCT studies are essential in the future.

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7). *C. pneumoniae* had been found in adenotonsillar tissue of children with community acquired infections (7, 8). A number of reports defined the role of other atypical infections like *C. pneumoniae* in children with rhinosinusitis and adenoid hypertrophy (8, 9). Huminer et al. found *C. pneumoniae* in adenoids and tonsils of children undergoing adenoidectomy or tonsillectomy (7). Diagnosis of *C. pneumoniae* infection is usually made by serology or PCR, but to confirm the active *C. pneumoniae* infection only cultures are helpful (9, 10). Engstrand et al. (10) Isolated *C. pneumoniae* from adenoid tissue in children with and without secretory otitis media, using immunohistochemistry and PCR. A prevalence of 2-5% was determined for *C. pneumoniae* in LRTI, using culture and validated real-time PCR. Early and effective antibiotic treatment is necessary to reduce the infectious period, mucosal injuries and complications.

2. Objectives

Rhinosinusitis is common in the Iranian population (10, 11). For *C. pneumoniae* detection, PCR is a more sensitive method than serology (12, 13). Little is known about the role of *C. pneumoniae* in children's adenoid hypertrophy, accompanied with rhinosinusitis. The goal of this study is to determine *C. pneumoniae* infection in adenoid tissue by PCR and serology in children undergoing adenoid surgery and compare the results with that of the normal children.

3. Patients and Methods

This case control study was conducted in the ENT and pediatric wards of Rasoul Akram Hospital, Tehran (2008-2011). It was approved by the Ethical Committee of the ENT Department of Hazrat Rasul Hospital, Tehran University of Medical Sciences. We studied 53 patients undergoing adenoid surgery (adenoid hypertrophy concomitant with rhinosinusitis) and 31 controls. From a total 53 patients 48% (25) were male and 52% (28) were female. The age range was 3-14 years, with a mean of 8 ± 2 years. Most subjects were between 6-9 years old (71.5%). The diagnosis of rhinosinusitis was based on clinical and imaging diagnostic criteria (2). Written consents were obtained from patients and controls. Initially a questionnaire was completed by an authorized physician and then complete clinical exams were performed.

A total of 31 children undergoing elective surgeries in the general surgery ward (like appendicitis, hernia, etc.) were selected and studied as the control group. They were age matched with the cases and then visited by a pediatrician before surgery to be assessed for rhinosinusitis and LRTI symptoms. Only after appropriate physical exams with proof of no present disease manifestations, they were selected as the controls. The normal adenoid tissue is small and not easily reachable for examination. The extra blood from pre-elective surgery routine blood tests was used for running the serological tests. All cases with

known proven pathological causes of adenoid hypertrophy other than infection (like malignancy or immunodeficiency) and those treated by any type of antibiotics, at least two weeks prior to the surgery, were excluded. Blood samples (2 mL) were obtained from cases and controls and centrifuged. The sera were then transferred to our lab and kept frozen at a -20°C refrigerator.

Specific *C. pneumoniae* (IgM and IgG) antibodies were looked for by the ELISA assay (Biochem Immuno Systems, Italy). ELISA reader at 450 nm was used in this study. The results were interpreted by cut-off control as suggested by the manufacturer. To detect *C. pneumoniae*-DNA, nasopharyngeal swabs were used in the control group (ethics restrictions in controls). In the study group, 1 cm³ resected adenoid tissues, cut by the surgeon, during the surgery, were used. All samples were then transferred to the research laboratory right away. They were kept frozen at -80°C until the DNA was extracted by the PCR template Purification Kit (Roche; Germany). The binding column tubes were transferred to a new 1.5 mL tube and 200 μL of elution buffer was added to them and then centrifuged at 8000 rpm for one minute. The integrity of the DNA was assessed by gel electrophoresis (1% agarose).

Primers for *C. pneumoniae* (PCR kits, Chemicon; Germany) were used for detecting the *C. pneumoniae*-DNA, based on the constructions provided by the manufacturer. The results of positive PCRs were confirmed by positive and negative DNA controls of *C. pneumoniae*. The main outer membrane protein genes (Omp A) of *C. pneumoniae* were chosen as the amplification target for the PCR, 333 bases pair product:

CPI (sense) 5' TTA CAA GCC TTG CCT GTA GG 3' 61-80

CP2 (anti-sense) 5'GCG ATC CCA AAT GTT TAA GGC3' 373-393

The reactions were performed in a final volume of 50 mL, containing 0.1 μM *C. pneumoniae* probe and 10 mL DNA. To determine any significant difference regarding continuous variables, the Student's t test was used. Chi-square values (CI 95%; $P < 0.05$) were calculated for all categorical variables and a P value < 0.05 was considered of a significant value. All analysis was conducted using SPSS version 13.5, USA.

4. Results

In 77.6%, 9% and 3% of cases, the maxillary, frontal and ethmoid sinuses were involved, respectively and in 10.4% pan sinusitis were detected. Regarding time of the surgeries, 23% of cases went under adenoid surgery in spring, 17% in summer, 28% in autumn and 32% in winter. Positive *C. pneumoniae* PCR results were detected in adenoid samples of 13.2% (7/53) of cases, but none of the controls. Although most cases with positive PCR were older than the negative cases (> 5 years old), there was no significant difference between the two (2/53 vs 2/3; $P = 0.1$). The mean age of the subjects did not show a significant difference between cases with positive and negative PCR results (6.8 ± 1.9 years vs 8 ± 2 years, $P = 0.1$). Positive PCR results in

Table 1. Demographic Distribution in Cases With Positive and Negative PCR Results^a

Results/Variables	Positive PCR	P Value ^b
Gender		0.4
Male	4	
Female	3	
Seasons		0.05
Cold	5	
Warm	2	
Age, y		0.1
> 5	5	
< 5	2	

^a Abbreviation: PCR: polymerase chain reaction.

^b P value < 0.05 was considered statistically significant.

Table 2. Comparison of the PCR and Serological Results Between Cases and Controls

Variable results Test	Case, (%)	Control, (%)	P Value ^a
Positive PCR	13.5	0	0.05
Positive IgM	11	6.5	0.7
Positive IgG	0	13.3	0.007

^a P value < 0.05 was considered statistically significant.

adenoid tissues and the season in which adenoid surgery was performed were related ($P = 0.05$). However, no correlation was found between positive PCR results and patients' gender (Table 1). Anti *C. pneumoniae* IgM was detected in 11% (6.51) of patients in the study group and 6.5% (2.31) of controls, without any significant difference ($P = 0.7$). Even though IgG was not detected in any patients in the study group, (0.51) 13.3% of controls revealed IgG in their blood ($P = 0.007$) (Table 2). Positive IgM did not have a significant difference with PCR results ($P = 0.7$). Positive IgG results had a significant difference with PCR results ($P = 0.05$).

5. Discussion

We had positive PCR results in 13.2% of adenoid samples in cases (mean age = 6.8) but in none of the controls ($P = 0.05$). The condition was more prevalent in warmer seasons (spring & summer = 5/7; $P = 0.05$). Although, based on our findings, recent infection (positive IgM) was seen twice as frequently in cases, but it still was of no significance (11% vs 6.5%, $P = 0.7$).

Positive IgG (previous immunity) was detected in none of the cases (0.51) and 13.3% of the controls, without any significant difference ($P = 0.007$), suggesting that patients in the study group did not have a *C. pneumoniae* infection history and thus, the immunity against it. Finding IgM in the sera correlated well with positive PCR results in the cases. The results of the study showed a positive correlation between PCR and serology (IgM) results in the case group. The positive correlation between PCR and IgM test results indicates the probable role of recent *C.*

pneumoniae infection in at least 13% of cases with adenoid hypertrophy. None of the studied cases showed previous immunity (positive IgG) against *C. pneumoniae*, however, 13.3% of controls were immune to the disease ($P = 0.007$). It is not unreasonable to assume that Iranian children get infected with *C. pneumoniae* between the ages of 6-8 and thus, get immune against *C. pneumoniae*. In fact, the absence of a protective immunity in some infected cases leads to organism reservation in the adenoid tissue and rhinosinusitis concomitant with adenoid hypertrophy. For the diagnosis of *C. pneumoniae* infection, culture is the gold standard diagnostic method. However, *C. pneumoniae* culture is not considered as the optimal method due to limitations like slow growth, technical difficulty and limited viability of the bacteria.

Serological tests are the most common methods for of *C. pneumoniae* infection Diagnosis. Detection of *C. pneumoniae*-specific antibodies is also possible by micro immunofluorescence, ELISA and EIA. As recommended by disease control and prevention centers, for acute *C. pneumoniae* infection diagnosis, a single IgM titer of greater than or equal to 1:64 or a fourfold increase in the IgG titer in acute and convalescent serum, measured 4 weeks apart from each other, is enough. The use of single IgG or IgA titers is discouraged due to their relatively high overall seroprevalence in healthy populations (4, 5, 9).

Positive PCR results in the present study were two times higher than those reported by Normann et al. (7%) (10) and 5% Cultrara et al. (5%) (6), but very lower than in children with pneumonia (14). Cultrara et al. did not isolate *C. pneumoniae* from sinus specimens in children, with

the use of sensitive culture methods (6). According to Volanen et al. (15) *C. pneumoniae* infection probably occurs at an early age, asymptotically, resulting in the consecutive high IgG and IgA antibody concentrations at the ages of seven and eight years. Multiple Iranian studies (using serology and PCR methods) defined a higher incidence of *C. pneumoniae* infections in respiratory tracts.

The incidence of *C. pneumoniae* in our population is higher than that of the developed countries (11, 16). *C. pneumoniae* played a prominent role in pediatric *C. pneumoniae* (mean age of 3.8 years) (13). Most of the children were seropositive (IgG) at the age of five. The previous immunity (IgG) was present in 57% of children with pneumonia but had similar results in cases and controls. Recent *C. pneumoniae* infection (IgM) was significantly higher in pneumonia cases ($P > 0.001$) (13). *C. pneumoniae* is a common respiratory pathogen in our pediatric populations (< 5 years). *C. pneumoniae* can occur commonly at an early age, often asymptotically but may colonize in the adenoid tissue in children. (12, 13). *C. pneumoniae* was also separated from nasal polyps in adult cases (14).

In recent years, a new role was reported for *C. pneumoniae* in asthma exacerbation, in North east of Iran (16). *C. pneumoniae* was separated from 7.6% of nasopharyngeal epithelial cell cultures of patients with asthma exacerbation and 35% of patients with chronic stable asthma and from 14.3% and 5% of their control groups, respectively. Successful eradication of *C. pneumoniae* was accompanied with clinical improvement (16). Most studies performed in Iran, except for one study, detected *C. pneumoniae* infection by serological tests and PCR or cultures were not used to confirm the active *C. pneumoniae* infection (16). Using the most sensitive culture methods, Piacentini et al. (8) were not able to isolate *C. pneumoniae* from sinus specimens of children. Due to the higher sensitivity of PCR for *C. pneumoniae* detection, in comparison with serological tests, detection of DNA in adenoid tissues would exaggerate and magnify even the smallest differences between the two groups.

Theoretically, the use of suitable antibiotics to eradicate the *C. pneumoniae*, before performing adenoid surgery in cases with rhinosinusitis might be helpful. However, it needs further randomized controlled trials before being approved as a treatment measure. The positive PCR and serological (IgM) tests proved the existence of recent *C. pneumoniae* infection in patients undergoing adenoidectomy. None of these patients had any sign of previous immunity, unlike the controls. All these findings indicate the probable role of *C. pneumoniae* infection in adenoid hypertrophy in nearly 13% of cases. In our opinion adenoid tissue might act as a reservoir for *C. pneumoniae* and cause rhinosinusitis concomitant with adenoid hypertrophy in infected children. To establish the effect of suitable antibiotics in *C. pneumoniae* eradication, before submitting for adenoidectomy, needs more randomized clinical trials in the future.

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Authors' Contribution

Shima Javadi Nia: coordinator; Vida Zarrabi: radiologist, reporting the CT scan; Samileh Noorbakhsh, pediatric clinician; Mohammad Farhadi ENT clinician; Sahar Ghavidel Darestani: English edition confirmed that they have not any relevant financial interests or financial conflicts within the past 5 years and for the foreseeable future. They have no financial interests related to the material in the manuscript.

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