

# Unusual infections in resected adenoid of children: PCR for *C. pneumonia*, *M. pneumonia*, *H. pylori*.

Mohammad Farhadi<sup>a</sup>, Samileh Noorbakhsh<sup>b\*</sup>, Farideh Ebrahimi Taj<sup>c</sup>, Naser Javahertrash<sup>d</sup>, Azardokht Tabatabaei<sup>e</sup>, Masomeh Bakhshyeh<sup>f</sup>

<sup>a</sup>Department of Pediatric Infectious Disease, Research Center of Pediatric Infectious Diseases, Iran University of Medical Sciences

<sup>b</sup>Department of ENT and Head & Neck Research Center, Iran University of Medical Sciences.

<sup>c</sup>Department of Pediatrics, Research Center of Pediatric Infectious Diseases, Iran University of Medical Sciences.

<sup>d</sup>Department of Pediatric Allergy and Immunology, Research Center of Pediatric Infectious Diseases Iran University of Medical Sciences

<sup>e</sup>Department of Microbiology, Research Center of Pediatric Infectious Diseases, Iran University of Medical Sciences

<sup>f</sup>Department of Microbiology, Iran University of Medical Sciences

**Abstract.** Recurrent or chronic adenotonsillar infections mainly affect children. The possible role for infectious agents in adenoid hypertrophy have reported. Searching the DNAs (PCR) of *M. pneumonia*, *C. pneumonia* and *H. pylori* in resected adenoid of children with adenoid surgery. A cross-sectional study done in ENT and Pediatric Department of Rasul Akram Hospital during 2006-2008. 53 children with recurrent or chronic adenotonsillar infections candidate for adenoid surgery were selected. The resected adenoid tissues (1cm) during surgery removed by surgeon. The tissue samples were centrifuged and homogenized, DNAs were extracted and searched for DNAs of *M. pneumonia*, *C. pneumonia* and *H. pylori* by qualitative PCR. Mean age of cases was  $8 \pm 1.9$  years. 48% male; 51.9% female 23%. Most cases aged between 6-9 years (71.5%). Most adenoid surgery was done in winter (32%). *M. pneumonia*- DNA detected in 28%; *C. pneumonia* in 13.2%; *H. pylori* in 15% of tissue samples with no relation to sex and age of cases. Most positive PCR results for *C. pneumonia* and *H. pylori* ( $p=0.05$ ;  $0.02$ ) were seen in spring and summer but not for *M. pneumonia* ( $p=0.5$ ) We could detect at least 1 of these 3 unusual infectious agents (*M. pneumonia*, *C. pneumonia* and *H. Pylori*) in adenoid tissues of 60% cases. These unusual infections may have a relative role in etiology of adenoid hypertrophy. Chronic sinusitis and ear infection might be added to infected adenoid tissue as a reservoir for these unusual bacteria. The search by more specific method such as Real time-PCR; or specific culture may elucidate better the role of these unusual infections in adenoid hypertrophy in future. The decision for use of antibiotics to eradicate these unusual infections in recurrent or chronic adenotonsillar infections before adenoid surgery (with or without rhinosinusitis or chronic ear infection) needs Randomized Controlled Trial studies.

Key words: Adenoid tissue; Adenoid hypertrophy; *C. pneumonia*; *M. pneumonia*; *H. pylori*

## 1. Introduction

Tonsillar tissue is a component of mucosa-associated lymphoid tissue (MALT), which has evolved to protect vulnerable mucosal surfaces. Adenotonsillectomy is usually performed for obstructive symptoms, recurrent infection. Chronic infection is the third indication for surgery (1,2).

In opinion to some authors chronic rhino sinusitis (with otitis media) and adenoid hypertrophy in children are due to one etiologic factor (1-3). Brook et al. (4) highlighted the importance of the usual bacterial load in the adenoids in contributing to the etiology of recurrent otitis media, recurrent adenotonsillitis, and obstructive adenoid hypertrophy.

Adenotonsillectomy is efficacious in reducing the number and severity of subsequent episodes of throat infection for at least 2 years (1-4). The adenoid, which has a central role in the development of secretory otitis media, may act as a reservoir for bacteria causing ear infection and chronic rhinosinusitis (3,4).

*H. pylori* is a gram negative bacteria and is the etiologic agent of some gastrointestinal and extra

\*Correspondence: Dr. Samileh Noorbakhsh  
Research center of pediatric infectious diseases, 4<sup>th</sup> floor  
Hazrat Rasul Hospital, Niayesh Street, Satarkhan Avenue,  
Tehran, 14455 Islamic Republic of Iran  
Email: samileh\_noorbakhsh@yahoo.com  
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gastrointestinal diseases. Colonization of *H. pylori* has been found in dental plaques, saliva, tonsils, sinus mucosa and adenoids (5). *Mycoplasma* species are found in naturally occurring adenotonsillitis (6-8). Engstrand et al (9) detected *C. pneumonia* as a common finding in the adenoids of children undergoing adenoidectomy. Some studies demonstrated the prevalence of *C. pneumonia* in sinusitis, adenotonsillar infection in children (9,10).

Many investigators detected *H. pylori* from palatine tonsils, adenoid tissues and middle ear effusion (11-14).

Seroprevalence to *H. pylori* infection is high in Iranian population (15-19). At least in one study in Iran, *H. pylori* was detected in 48.2% of adenoid specimens by Rapid Urease (CLO) Test (16).

Some studies in Iran reported the *C. pneumonia* and *M. pneumonia* in respiratory tract of patients (20-23). Little is known about the true colonization and the localization of these bacteria in the adenoid tissue of children in Iran.

Goal of the study: Searching the DNAs (PCR) of *M. pneumonia*, *C. pneumonia* and *H. pylori* in resected adenoid of children with adenoid surgery.

## 2. Method and material

This cross-sectional study was done in ENT and Pediatrics Department of Rasul Akram Hospital in Tehran (2006-2008). This study was approved by the Ethical Committee in the ENT and Head & Neck Research Center in Iran University of Medical Sciences. (Ethical Considerations detail in the end of article)

Our study group consists of 53 children (< 14 years) with recurrent or chronic adenotonsillar infections candidate for adenoid surgery selected continuously. Exclusion Criteria: We excluded all cases with immunodeficiency states; patients received macrolide antibiotic at least 2 weeks before surgery. All cases with known malignancy or other diseases proved in pathology were excluded.

Table 1. Age distribution in cases

| Age group of cases<br>(years) | Number | Percent |
|-------------------------------|--------|---------|
| 3-5 years                     | 5      | 9.5%    |
| 6-9 years                     | 38     | 71.5%   |
| >10 years                     | 10     | 19%     |
| Total                         | 53     | 100%    |

Initially a questionnaire was completed by an authorized physician for each case, followed by complete clinical exams. During surgery, 1cm of adenoid tissue was resected and put down in a sterile tube by surgeon. The samples were centrifuged and homogenized. Those tubes were preserved in -80 centigrade refrigerator. DNAs of *M. pneumonia*, *C. pneumonia* and *H. pylori* were searched by qualitative PCR. PCR template Purification Kit (Roche; Germany) was used for all prepared tissue samples. Steps for DNA – Extraction were done. The binding column tube transferred to a new 1.5 mL tube. The integrity of DNA was assessed by gel electrophoresis (1% agarose). *M. pneumonia* PCR- ELISA kits were used as manufacturer order (Roche, Germany) in Roche Diagnostics. 40 µL denaturation reagent was added into reaction tube (DNA extraction). Absorbance was measured with ELISA reader at 450 nm.

### 2.1. *M. pneumonia* primer:

*M. pneumonia* AT P as operon gene,

MP5-1 (GAAGCTTATGGTACAGGTTGG)

MP5-2 (ATTACCATCCTTGTTGTAAGG)

Amplification product of 144 base-pairs. Specific PCR primers qualitative diagnostic kit (QIA quick P<sup>®</sup> QIAGEN; Germany) for Detection of *C. pneumonia* and *H. pylori* DNAs was used.

Diagnostic kits included a ready to use PCR mix Kits, positive and negative controls and other qualified reagents along with an easy to follow protocol for detecting as low as 10 copies/mL of *H. pylori* & *C. pneumonia*–genome.

### 2. 2. *H. Pylori*:

Primers 93089 and 93261 were selected from consensus regions of the two available CA g A gene sequences (Gen Bank accession no. L11714 and EMBL accession no. 70039) 400bp product  
Statistical analysis: All analyses were conducted using SPSS 11.5 software. The Student's T test was used to determine significant differences in means for all continuous variables.

Table 2. Season (North hemisphere) of adenoid surgery in cases

| Season of surgery (North hemisphere) | Number of cases | Percentage |
|--------------------------------------|-----------------|------------|
| Spring ( April to June)              | 12              | 23%        |
| Summer                               | 9               | 17%        |
| Autumn                               | 15              | 28%        |
| Winter                               | 17              | 32%        |
| Total                                | 53              | 100%       |

CA g A gene:

Forward: AAT ACA CCA ACG CCT CCA AG

Reverse: TTG T TG CCG CTT TTG CTC TC

### 2.3. Primers for *C. pneumonia*:

Chi square values ( $p < 0.05$ ) were calculated for all categorical variables.  $p$  value  $< 0.05$  was considered statistically significant.

### 3. Results

48% (25/53) of cases were male; 52% (28/53) female. The age range of the cases ( $n=53$ ) was 3 - 14 years, Mean= $8 \pm 2$  year. Distribution of age and season for adenoid surgery in cases are shown in Table-1&2. Most cases aged between 6-9 years (71.5%): Table-1; Most adenoid surgery PCR results: Positive PCR results found in adenoid tissue included: *M. pneumonia*-DNA: 28% (12/44); *H. pylori*-DNA: 15% (8/53); *C. pneumonia*-DNA: 13.2% (7/53). Mean age of cases between positive and negative PCR (for all 3 organisms) had not meaningful differences: Table-3.

The major outer membrane protein genes (OMP A) of *C. pneumonia* was chosen as the target for amplification in a PCR. 333 base pair product CP 1 (sense) 5' TTA CAA GCC TTG CCT GTA GG 3' 61-80 CP2 (anti-sense) 5' GCG ATC CCA AAT GTT TAA GGC 3' 373-393

Cases with positive *M. pneumonia* (mean=8.2 years) was slightly older than cases with positive *C. pneumonia* (6.8y) and positive *H. pylori* PCR (7.5 y) but without meaningful differences ( $p=0.06$ ). No correlation was found between positive PCR results and sex predilection in cases (Table-3).

Positive PCR for *C. pneumonia* and *H. pylori*-DNAs in adenoid tissues were related to season of adenoid surgery ( $p=0.05$ ; 0.02) but not for *M. pneumonia*-DNA ( $p=0.5$ ): Table 4.

### 4. Discussion

Most adenoid surgeries (71.5%) had been done upon children between 6-9 years old in cold weather (winter&Autumn=60%) which is acceptable epidemiologically for respiratory infections in our country (20,21). 60% of cases in early age had at least 1 of these 3 unusual infections which is more frequent than children in developed countries (24). We found *M. pneumonia*-DNA (28%) as the most common unusual infections which was studied. *H. pylori* (15%); *C. pneumonia* (13.2%) was found in adenoid tissues of studied children. Positive PCR for 3 agents had not had differences between two sex. Cases with positive *M. pneumonia* (mean age: 8.2 years) was slightly older than cases with positive *C. Pneumonia* (mean age: 6.8 y) and

Table 3. Age &amp; sex distribution in cases with positive and negative PCR results.

| PCR Results         | Age: (years) Mean $\pm$ SD               | Sex: Male/Female (ratio) |
|---------------------|--|--------------------------|
|                     | Positive / Negative                      | Positive / Negative      |
| <i>H. pylori</i>    | 7.5 $\pm$ 1.8 y / 7.9 $\pm$ 2 y (p=0.6)  | 5/3 vs 20/25 (p=0.2)     |
| <i>M. pneumonia</i> | 8.2 $\pm$ 2.5y / 7.4 $\pm$ 1.6 y (p=0.2) | 6/7 vs 16/15 (p=0.5)     |
| <i>C. pneumonia</i> | 6.8 $\pm$ 1.9 y/ 8 $\pm$ 2 y (p=0.1)     | 4/3 vs 21/25 (p=0.4)     |

p value <0.05 was considered statistically significant

Table 4. Positive PCR and seasons of adenoidectomy in cases.

| PCR Results         | Positive/negative PCR        |        |        |        | p value |
|---------------------|------------------------------|--------|--------|--------|---------|
|                     | Spring<br>(April to<br>June) | Summer | Autumn | Winter |         |
| <i>H. pylori</i>    | 4/12                         | 4/9    | 0/15   | 0/17   | 0.02*   |
| <i>M. pneumonia</i> | 4/12                         | 4/9    | 1/8    | 4/15   | 0.5     |
| <i>C. pneumonia</i> | 4/12                         | 1/9    | 0/15   | 2/17   | 0.05*   |

\*p value<0.05 was considered statistically significant

*H. pylori* infection (mean age 7.5 y) but without meaningful differences (p=0.06). Similar results were demonstrated in a previous case control study in rhinosinusitis children (serology) in our center “(20) Positive *M. pneumonia*-IgM (ELISA) seen in 15%; *H. pylori*-IgA in 13%; *C. pneumonia*-IgM in 13.2% of children with confirmed rhino sinusitis (20).

*M. pneumonia*-DNA was detected in adenoid tissues of 28% children between 6-8 years old; without correlation to season of adenoid surgery (p=0.5); and no meaningful difference was observed between children more and less than 5 years old. It is very close to previous case/control study in rhinosinusitis children; acute *M. pneumonia* infection (positive serum IgM) was observed in 15% of cases (20). Rhinosinusitis

children with positive *M. pneumonia*-IgG (immune) were older than non immune cases (6 vs 4 year, p<0.05) (20). *M. pneumonia* may present as asymptomatic upper or lower respiratory infection in children (7,8) Storgaard et al (7) defined *C. pneumonia*, *M. pneumonia* in otitis media with effusion. In contrast to Storgaard et al and the present study, Sprinkle et al (8) reported Mycoplasma species cause acute adenotonsillitis but *C. pneumonia* and *M. pneumonia* could not induce recurrent or chronic adenotonsillitis (8).

*C. pneumonia*-DNA was found in 13.2% of adenoid samples of children between 5-9 years old. Surprisingly most cases with positive *C. pneumonia* PCR in adenoid tissues were seen in warmer seasons (spring & summer =5/7) (p=0.05).

Table 5. Positive PCR results and age group in cases.

| PCR Results         | < 5 years old      | >5 years old       | p value |
|---------------------|--------------------|--------------------|---------|
|                     | Positive /negative | Positive/ Negative |         |
| <i>H. pylori</i>    | 1 / 4              | 7/41               | 0.5     |
| <i>M. pneumonia</i> | 1/3                | 12/28              | 1       |
| <i>C. pneumonia</i> | 2 / 3              | 5/43               | 0.1     |

p value < 0.05 was considered statistically significant.

Positive *C. pneumonia* PCR was 2 times more than 7% reported by Normann et al (10) and 5% Cultrara et al (6). It is very close to serologic results in rhinosinusitis study in our center (13.2% vs 12%) (20), but very lower than children with pneumonia (21).

Variation in methods are the primary reason for different results but older age of cases with adenoid surgery (8.2 years) vs (4.2 years) in rhino sinusitis; and (3.8 years) in pneumonia study might be the other reason. Recent studies using culture and validated real-time PCR in children reported 2-5% prevalences for *C. pneumonia* in LRTI (24)

Multiple Iranian studies by using the serology & PCR defined higher *C. pneumonia* & *M. pneumonia* infection in respiratory tract systems (pneumonia; rhinosinusitis, adenoid, polyp) (20-23). In developed countries, *C. pneumonia* and *M. pneumonia* are generally considered to peak in children 10-12 years vs 6-8 years in Iranian children (7,8,24). This pattern may have wide variation in different populations. Probably, *M. pneumonia* and *C. pneumonia* can occur commonly at an early age in our cases (20). These infections sometimes are asymptomatic but may be colonized in adenoid tissue of children. (11-12); or both are detectable in nasal polyp of adult cases (22).

*C. pneumonia* is a common respiratory pathogen in our pediatric populations (< 5 years). The incidence of *C. pneumonia* in our population is higher than developed countries (20-23). In recent years, a role for *C. pneumonia* in asthma was reported in North east of Iran. *C. pneumonia* was cultured in nasopharyngeal epithelial cells in 47.6% exacerbation and 35% of patients with chronic stable asthma while about 14.3% and 5%

of control subjects. Successful eradication of *C. pneumonia* was accompanied with clinical improvement (23). Most above studies in Iran except 1 reported the *C. Pneumonia* infection serologically but PCR or culture for confirmation of active *C. pneumonia* infection was not used (23). Cultrara et al (6) did not isolate *C. pneumonia* from sinus specimens of children by using the most sensitive culture methods. In our opinion, rhinosinusitis and adenoid hypertrophy in our children might have a common etiology but the role of *M. pneumonia* in adenoid is twice more than *C. pneumonia* (28% vs 13.2%).

*H. pylori* is the second common unusual infection which obtained in adenoid tissues of 15% cases between 5.5-9 years old in warmer seasons (spring & summer = 8/8) (p=0.02). PCR results for *H. Pylori* is very close to positive serology results. Positive *H. pylori*-IgA was found in 15% rhino sinusitis vs 13% healthy children. 11% of cases with rhino sinusitis were immune (positive *H. pylori*-IgG). Variation in methods and higher age in adenoid study (7.5 vs 4.2 years) could explain these differences. Present results are close to other Iranian studies (15-19). Khademi et al (16) showed *H. pylori* infection (positive urease test) in tonsils and adenoid tissues of 48.2% cases (3-43 years) in Shiraz. Although *H. pylori* infection varies between countries and often within a country, higher age for cases with adenoid surgery in Khademi study could explain this difference. Probably, *H. pylori* infection happened in 15% of studied cases before 9 years and increases to 48% in higher ages (16). Saffari et al (15) studied *H. pylori* antibodies in population in Shiraz (south of Iran). 28.3% of persons between 20-40 years; 32% of population between 41-80 years had

positive *H. pylori*-IgG; positive *H. pylori*-IgA was observed in 16.7 % and 53.5% respectively (15) All above data showing initial infection occurs at an early age but prevalence of *H. pylori* infection increases with age. 15% of studied persons were infected near 7th years. The infection will increase to 30% in 2nd and 53.5% after 4th decade of life (15).

Therefore adenoid tissue may act as a reservoir for these unusual bacteria just like as usual bacteria; and may be causing sinusitis and chronic ear infection in some cases. The use of suitable antibiotics at least 2 weeks before adenoid surgery would be helpful in some cases (1-4).

#### 4. 1. Limitations of the study:

We are not able to differentiate the colonization from recent active infection in studied cases. Whether or not these unusual infections play a pathogenic role, could not be determined from the data obtained in this investigation. The search by more specific methods such as Real time-PCR; or specific culture may elucidate better the role of these unusual infections in adenoid hypertrophy in future.

## 5. Conclusion

We could detect at least 1 of these 3 unusual infectious agents (*M. pneumoniae*, *C. pneumoniae* and *H. pylori*) in adenoid tissues of 60% cases. These unusual infections may have a relative role in etiology of adenoid hypertrophy. Chronic sinusitis and ear infection might be added to infected adenoid tissue as a reservoir for these unusual bacteria. The search by more specific methods such as Real time-PCR; or specific culture may elucidate better the role of these unusual infections in adenoid hypertrophy in future. Erythromycin, tetracycline or other new macrolides (azithromycin, clarithromycin) are antibiotics recommended for *M. pneumoniae*, *C. pneumoniae* for *H. pylori* dependent to antibiotic sensitivity test in each country. The decision for the use of antibiotics to eradicate these unusual infections in recurrent or chronic adenotonsillar infections before adenoid surgery (with or without rhinosinusitis or chronic ear infection) needs RCT studies.

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