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ORIGINAL ARTICLE

Role of Chlamydia pneumoniae in the pathogenesis of hypertrophy and adenoid tissue inflammation in children

Authors' Contribution:
A – Study Design
B – Data Collection
C – Statistical Analysis
D – Data Interpretation
E – Manuscript Preparation
F – Literature Search
G – Funds Collection

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ABSTRACT:

Objective. A tropism to epithelial cells and lymphocytes, an inhibition of apoptosis in host cells, an ability to occurrence in persistent form resistant to antibiotic treatment are the features of Chlamydia pneumoniae, which can have connection with chronic inflammation of an adenoid tissue and adenoid hypertrophy. This study aimed to (1) detect the C. pneumoniae in an adenoid in children undergoing adenoidectomy, (2) estimate a connection between C. pneumoniae occurrence and the size of adenoid, (3) demonstration in which of adenoid cells C. pneumoniae occurs most often.

Material and methods. The examined group consisted of 200 children aged from 2 to 16 years (mean age 6,4) undergoing adenoidectomy. In all children during qualification for adenoidectomy a fiberoscopic examination of the nasopharynx was performed. A part of removed adenoid tissue was analysed by real-time PCR for C. pneumoniae. Adenoids from children with positive PCR examination and from 10 children with negative PCR examination were examined using immunohistochemistry (IHC).

Results. C. pneumoniae in the adenoid was present in 5,5% children. Positive results were obtained most frequently (24,14%, 7/29) in the eldest group (10-16 years). A statistical analysis demonstrated the correlation between C. pneumoniae occurrence in an adenoid tissue and the size of adenoid. In immunohistochemistry C. pneumoniae was found the most frequently in lymphocytes and in epithelial cells.

Conclusions. A presence of C. pneumoniae in lymphocytes and epithelial cells of the adenoid first of all in older children with adenoid hypertrophy confirms the participation of this bacteria in adenoid pathology.

KEYWORDS: chlamydia pneumoniae, adenoid, PCR, child

INTRODUCTION

In recent years, there have been single reports in the literature focusing on a possible role of *Chlamydia pneumoniae* infection in adenoid pathology [7,8,16,28,26]. A limited number of such publications may be due to difficulties sustaining cultures of *C.pneumoniae* and low accessibility to other methods confirming infection (PCR, immunohistochemistry). *C.pneumoniae* is classified as a so-called atypical pathogen and is a common cause of airborne respiratory infections. In the course of a respiratory infection, *C.pneumoniae* can reach circulatory system and be carried away to other organs by blood lymphocytes [25] and monocytes [10]. Development of new diagnostic methods contributed to discovering connection between *C.pneumoniae* and asthma [6,9], atherosclerosis [20,22], seronegative arthritis [13], Alzheimer's disease [2], multiple sclerosis [23] and other chronic conditions.

Respiratory infection due to *C.pneumoniae* often resolves spontaneously, however, the resolution of clinic symptoms is not necessarily associated with complete eradication of the pathogen. *C.pneumoniae* can persist in monocytes [10], epithelial cells [19], and nervous cells [4]. Ineffectiveness of therapy may

be caused by pathogen's transformation into a dormant form, instead of antibiotic resistance [11].

The affinity to epithelial cells and lymphocytes, impaired motility of cilia [21], ability to cause chronic infection [12] and to stimulate increased production of proinflammatory cytokines, as well as increased expression of molecules facilitating leukocyte adhesion [15], inhibition of host cell apoptosis [1,19] and insensitivity to antibiotics acting intracellularly in lymphocytes and monocytes [10,24] – these are the features of *C.pneumoniae*, which can be related to chronic inflammation of tissues infected by the bacterium. The adenoid, situated in the nasopharynx, is an immunological organ with a characteristic fold pattern which increases its epithelium-lined surface, and can be a site of an on-going infection by this pathogen.

The aim of this study was to determine:

- 1. Is *C.pneumoniae* present in the adenoid of children qualified for adenoidectomy?
- 2. Is there a relationship between *C.pneumoniae* presence in the adenoid and its size?
- 3. In which cells of the adenoid does *C.pneumoniae* reside most often?

MATERIALS AND METHODS

The study included 200 children (114 boys and 86 girls) undergoing adenoidectomy. On each child, fiberoscopy of the nasopharynx was performed during qualification for the procedure. Children with adenoid hypertrophy (the adenoid occupying more than 50% of the lumen of the choanae) and associated:

- 1) symptoms of upper respiratory obstruction (open mouth, snoring, apnea) and/or
- 2) recurrent acute (≥6 times a year) or chronic upper respiratory infections were qualified for adenoidectomy.

The following groups of children were excluded from the study: children who had received antibiotics within 2 months prior to surgery, children with a history of immunodeficiency and children with viscerocranial abnormalities.

The adenoids removed during surgery were cut in half. One piece was placed in 10% formaldehyde solution buffered with PBS (phosphate buffered saline) and was sent for IHC testing. The other half was frozen and kept in -70°C before performing PCR.

DNA isolation of *C.pneumoniae* was conducted according to the protocol of nucleic acid isolation for animal tissues, utilizing High Pure PCR Template Preparation Kit (Roche Diagnostics, Mannheim, Germany). Detection of *C.pneumoniae* DNA was performed using real-time PCR method with real-life analysis of

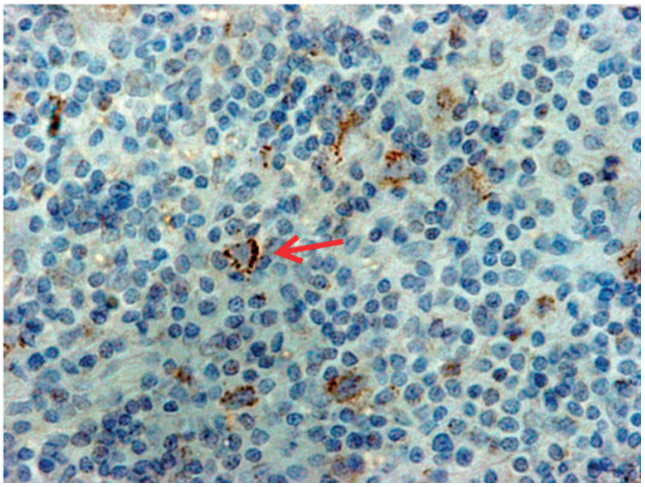

Fig. 1. Positive reaction for C.pneumoniae in lymphocytes of the adenoid. EnVision staining, 200x.

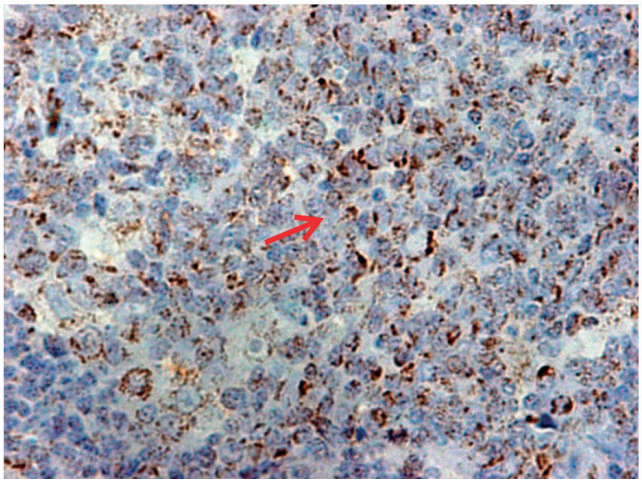

Fig. 2. Positive reaction for C.pneumoniae in multiple cells of a germination center in lymphatic follicle of adenoid.

product quantity. For the reaction, the primers were specific for J38 region fragment of C.pneumoniae genome, their length being 140 base pairs.

The DNA particles obtained during the reaction were detected using fluorescent probes complementary to the amplified DNA sequence, which were included in the LightCycler® FastStart DNA Master HybProbe Set (Roche Diagnostics, Mannheim, Germany).

Immunohistochemical staining of the tonsil tissue was performed using monoclonal antibodies *Anti-Chlamydia pneumoniae* by Thermo Scientific and revealing solution EnVisionTM+/HRP Mouse or EnVisionTM+/HRP Rabbit Detection System, Dako Denmark A/S, Glostrup, Denmark (DAKO).

Tab. I. Number of PCR(+) children in respective age groups.

AGE (YEARS)	NUMBER OF CHILDREN (%)	NUMBER OF PCR(+) CHILDREN (%)
2-5	95 (47.5)	1 (1.1)
6-9	76 (38.0)	3 (4.0)
10-16	29 (14.5)	7 (24.1)

Tab. II. Comparison of adenoid size and clinical symptoms of upper respiratory obstruction in PCR(+) and PCR(-) children.

	PCR(+) CHILDREN	PCR(-) CHILDREN
Degree of obturation of the choanae by the adenoid on fiberoscopic examination		
50-70%	0 (0.0%)	46 (24.3%)
71-90%	8 (72.7%)	117 (61.9%)
Over 90%	3 (27.3%)	26 (13.8%)
Symptoms of upper respiratory obstruction		
Apnae	2 (18.2%)	51 (27.0%)
Snoring	11 (100.0%)	176 (93.1%)
Open mouth	11 (100.0%)	182 (96.3%)

For the statistical analysis, SPSS PASW Statistics 18 software (18.0.3 edition) was utilized. Pearson's chi-squared test was used, and the mean age of children was compared using t-test. P<0.05 was considered statistically significant.

The permission of the Bioethcs Committee of the Regional Chamber of Physicians and Dentists in Warsaw to conduct the study (2010-2011) was obtained (KB/701/10).

RESULTS

In the studied group (n=200), the *C.pneumoniae* DNA was detected using PCR method in adenoids of 11 children, which constituted 5.5% of all participants. *C.pneumoniae* infection was found more frequently in older children. The mean age (±SD) of PCR(+)children (n=11) was 10.5 (±3.6), and the mean age of PCR(-) children (n=189) was 6.2 (±2.8). The difference was statistically significant (P<0.01). Among the youngest children (2-5 years old, n=95), the percentage of positive results was 1.1%, however, among the oldest children (10-16 years old, n=29), positive results were found in 24.1% (Table 1).

The relationship has been established between the presence of *C.pneumoniae* in the adenoid and its size assessed on fiberoscopic examination. In all children, with confirmed *C.pneumoniae* infection, the adenoid occupied more than 70% of the lumen of the choanae, while among PCR(-) children –in 75.7%. The differences were statistically significant (P<0.05) (Table 2).

In all adenoids testing positive with PCR (n=11), the presence of *C.pneumoniae* was confirmed by immunohistochemical staining. In 10 randomly chosen adenoids with negative PCR, the presence of *C.pneumoniae* was not detected using IHC staining. In the IHC study, it seemed worth noticing that cells displaying positive reaction for *C.pneumoniae* were not evenly distributed in terms of both type of cell and their locations throughout the adenoid. *C.pneumoniae* was most commonly found in lymphocytes of the tonsil (n=5) (Fig. 1). In 2 patients, *C.pneumoniae* was found in most cells forming lymphatic follicles of the adenoid (Fig. 2). In other tonsils, *C.pneumoniae* was detected only in epithelial cells (n=2) or single epithelial cells and small subepithelial lymphocytes (n=2).

DISCUSSION

Reports on the presence of *C.pneumoniae* in adenoid tissue vary, and the percentage of its detection in various studies spans from a few to 100% [3,7,8,16,18,26]. It depends on detection method and correlation between time of study and rate of epidemic infections caused by *C.pnemoniae*. In our study, we found *C.pnuemoniae* DNA in 5.5% of children. Similar results (4.8%) were obtained by Drago. [7] Piacentini detected *C.pnuemoniae* DNA in removed adenoids in 15.15% of patients (n=38) [18]. Discrepancies in DNA detection rates may be due to different types of PCR method (nested PCR, real-time PCR). *C.pneumoniae* was far more often detected while using immunohistochemistry (IHC) method. The results of studies on *C.pneumoniae* presence in the adenoids obtained by other authors, as well as in our own study, are summarized in Table III (Table III).

On immunohistochemical study, it was noticeable that a positive reaction for *C.pneumoniae* was not evenly distributed within each cell and between different parts of the adenoid. *C.pneumoniae* was most commonly detected in lymphocytes of the adenoid (5/11). In 2 children, massive infection of the adenoid tissue was observed, along with positive reaction in the majority of lymphatic follicle forming cells.

In our study, *C.pnumoniae* infection was found far more commonly (24.1%, 7/29) among the oldest children (10-16 years old), compared to younger children (2-5 and 6-9 years old, 1.1% and 4.0% respectively), and the difference was statistically significant (P<0.05). The youngest patient with positive PCR was 3 years old and it was the only child with a positive result in the age group of 2 to 5, despite the fact that children under 5 constituted the largest group of surgically treated children (n=95). The low frequency of *C.pneumoniae* detection among the youngest children (1.1%) may be caused by the fact that the young children had not yet been exposed to the pathogen.

Tab. III. Summary of study results regarding detection of *C.pneumoniae* in the adenoid.

AUTHOR'S NAME	YEAR	NUMBER OF PARTICIPANTS	AGE (YEARS)	TYPE OF TEST	POSITIVE RESULTS
Normann E.	2001	69	1-15 (mean 5)	IHC (adenoid tissue) PCR (throat swab)	98.5% 7%
Engstrand I.	2001	20	3-10 (mean 5)	IHC (adenoid tissue) Nested PCR (adenoid tissue)	100% 30%
Zalesska-Kręcicka M.	2006	110	3-15 (mean.6,1)	Direct immunofluorescence (nasopharyngeal swab)	26.4%
Drago L.	2008	44	mean 5,78	Nested -PCR (adenoid tissue)	4.8%
Piacentini GL.	2010	33	2-16 (mean 5)	Nested-PCR (adenoid tissue)	15.15%
Our study	2011	200	2-16 (mean 6,4)	Real-time PCR (adenoid tissue)	5.5%

Although the percentage of positive PCR results confirming presence of *C.pneumoniae* in the adenoid tissue is relatively low (5.5%) for the entire studied group, the presence of the bacterium was confirmed in 1 in 4 of patients (24.1%) presenting adenoid pathology. Detection of this pathogen mainly in older children showing clinical symptoms of adenoid hypertrophy may suggest the association of *C.pneumoniae* with adenoid pathology. This phenomenon is probably based on the ability of *C.pneumoniae* to cause chronic infection [5,12]. Recent studies proved that, inside chlamydial inclusion bodies, the pathogen may transform from a replicating to dormant form, which does not reproduce and is insensitive to antibiotics [10,14]. *C.pneumoniae* can remain in a dormant form in monocytes [10], epithelial cells [19] and nervous cells [4]. Resistance to antibiotics presented by *C.pneumoniae* residing inside B cells [24] can contribute to its prolonged presence in adenoid tissue. Inside the inclusion body, *C.pneumoniae* is separated from the host cell cytoplasm and is therefore resistant to host's cell-mediated immunological response. At the same time, however, the bacterium itself changes regulatory signals within the host's cells, thus influencing e.g. inhibition of apoptosis [1,14,19]. *C.pneumoniae* modifies natural signals leading to apoptosis of host's cells by means of cytokine excretion through type III secretion system of the pathogen.

Our study confirmed the presence of *C.pneumoniae* in the adenoid tissue. The survival of some organisms in specific tissues or cells may present as a chronic infection without rapid destruction of host's cells. Abnormal or partial immunological response to *C.pneumoniae* infection can promote chronic antigen stimulation, thus sustaining inflammation, which in turn contributes to hypertrophy or chronic inflammation of the adenoid [7].

CONCLUSIONS

1. The relationship has been established between the presence of *C.pneumoniae* in the adenoid and the child's age.
2. The relationship has been established between the presence of *C.pneumoniae* in the adenoid and its size.
3. It has been established that *C.pneumoniae* is found most commonly in lymphocytes and epithelial cells of the adenoid.

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