



Original Research Article

Role of *C. Pneumoniae* in nasal Polyp formation: PCR in tissue and serology: a cross sectional study: Tehran, Iran

Mohammad Reza Shokrollahi¹, Mohammad Farhadi², Samileh Noorbakhsh^{3*},
Shima Javad Nia⁴, Sahar Ghavidel⁵, and Ali Reza Shamshiri⁶

¹MD, Department of medicine, Qom University of medical sciences and Health services, Qom, Iran & Research Center of Pediatric Infectious Diseases Iran University of Medical Sciences

²Professor of ENT, ENT & Head and Neck Surgery Research Center, Iran University of Medical Science

³Professor of Pediatric Infectious Disease, Research center of pediatric infectious diseases, Iran University of Medical Sciences, Iran

⁴Research Center of Pediatric Infectious Diseases, Iran University of Medical Sciences, Iran

⁵ENT -Head & Neck Surgery Research Center, Iran University of Medical Sciences

⁶MD; Epidemiologist; Department of Epidemiology and Biostatistics, School of Public Health, Iran University of Medical sciences

*Corresponding author

ABSTRACT

Nasal polyps are considered to result from chronic inflammation, but the initial or persisting stimulus for the inflammation is not known. Goal of this study is to determine the role of *C. Pneumonia* in nasal Polyp formation. A cross sectional study done in Ear, nose, throat ward in Rasul Hospital in Tehran (2010-2012) upon 51 cases (age=12 -72 year, mean=35 year) with nasal polyp and 19 healthy control (age=18 -41 year, mean=23 year). Specific serum *C. Pneumonia* antibodies (IgG & IgM- ELISA) and Tissue PCR compared between 2 groups. Positive PCR detected in 19.6% (10/51) of cases and none of controls with significant difference (OR=9.9%; Pvalue=0.05). Positive IgM detected in 9.8% (5/51) of cases and none of the controls (non significant differences ;P value = 0.31; OR =0.99).; positive IgG determined in 47.1% (24/51) of cases and (9/19) 47.4 % of controls (non significant differences;(P value = 0.1; OR =2.499).The chance for positive IgM- ELISA (recent infection) is 1.3 times more than chance for positive PCR ; good agreement between IgM and PCR tests (Kappa =0.17) ,non significant differences: P value =1 .Positive -IgG was 30 times more than positive PCR ;week agreement ;Kappa index =0.07 and significant differences (P value=0.001). It showed that *C. pneumonia* infection has a moderate role (near %20) in nasal polyp formation. Although PCR tests are reliable and more specific and sensitive but invasive method for diagnosis of active *C. pneumonia*. Determination the *C. pneumonia* infection in polyp formation is possible before surgery by specific IgM -ELISA test. In the future placebo-controlled studies are necessary to validate the effect of macrolides (8 weeks) before surgery on reducing the nasal polyp diseases.

Keywords

Nasal polyp;
PCR
(Polymerase
Chain
Reaction);
C.pneumonia;
IgG;
IgM.

Introduction

Nasal polyps are benign pedunculated masses of nasal or sinus mucosa which

affect between 1 and 4% of the population (Norlander *et al.*, 1993). The aetiological

factors associated with the occurrence of nasal polyps include infection, inflammation or an imbalance of a metabolic pathway, such as the arachidonic acid pathway (Kozak *et al.*, 1991). A variety of bacteria and fungi have been cultured from nasal polyps, but approximately 35% have sterile cultures. (Bucholtz *et al.*, 2002).

C. pneumoniae is a common respiratory pathogen (Brook and Shah, 2001; Kumar and Hammerschlag, 2001; Cervin, 2001; Cultrara *et al.*, 2003; Apan *et al.*, 2007; Storgaard *et al.*, 2004; Engstrand *et al.*, 2001). Routine laboratory-based detection of *C. pneumoniae* infection is slow and insensitive; it is largely based on serological detection and bacterial cell culture (Brook and Shah, 2001; Kumar and Hammerschlag, 2001). More recently, a number of authors have reported the amplification and detection of in clinical samples (Kumar and Hammerschlag, 2001). Although many patients with community-acquired pneumonia caused by *C.pneumoniae* have symptoms suggestive of sinusitis, isolation of the organism from the maxillary sinus of a patient with sinusitis reported only in one (Cultrara *et al.*, 2003). Rapid detection and diagnosis may lead to the efficient treatment of infection focused on the eradication of a micro-organism which is insensitive to [beta]-lactam antibiotics, but which responds to tetracycline and erythromycin. Treatment with erythromycin for at least 8 weeks showed a reduction in chronic rhinosinusitis with nasal polyps by 52% (Cervin, 2001). *C. pneumoniae* is a common respiratory pathogen in paediatric populations in our country tract (Noorbakhsh *et al.*, 2009; Noorbakhsh *et al.*, 2008; Noorbakhsh *et al.*, 2004; Ah Torshizi1 *et al.*, 2008). Previous studies in our center detected the probable role for

C. pneumoniae in upper and lower respiratory tract (Noorbakhsh *et al.*, 2009; Noorbakhsh *et al.*, 2008; Noorbakhsh *et al.*, 2004).

Goal of this study is to determine the role of *C. pneumoniae* in nasal Polyp formation.

Materials and Methods

This cross sectional study done in ENT ward of Rasoul Akram Hospital in Tehran (2010-2012).The study was approved by the Ethical Committee in the ENT & Head and Neck Syrgery Research Center, Tehran University of Medical Science

Cases consisted 51 cases with nasal polyp surgery (age: 12 -72 year, mean=35 year). And 19 healthy control with nasal fracture (age: 18 -41 year, mean=23 year) who were hospitalized for elective repair surgery.

All of the controls were visited by an ENT specialist before surgery to ascertain the absence of nasal polyp. Also an internist visited all cases and controls before surgery for other disorders (immune deficiencies, Diabete mellitus, renal failure; etc)

Consent Letters were obtained from patients and controls. Initially a questionnaire was completed by an authorized physician for each case and control followed by complete clinical exams. Blood samples (2 ml) obtained from all persons, centrifuged and transferred to our research laboratory. In the controls, the extra blood which was taken for routine blood tests before their respective surgery was used. The serum was restored in -20°C temperature freezer until the serologic examination was

performed. The centrifuged blood specimens were screened using an assay for *C.pneumonia* antibodies (IgM and IgG). Serological test: The evaluation of specific *C.pneumoniae* IgG and IgM antibody were carried out with commercial kits (Chemicon-Germany). Both kits were used and the results were interpreted as suggested by the Manufacturer. Results were calculated quantitatively.

During surgery 2 cubic centimeter of nasal polyp tissues (cases) and inferior nasal turbinate mucosa (controls) removed and put down in sterile tube by surgeon. Those tubes preserved in -80 centigrade refrigerator, and tested for *C. Pneumonia* DNA. PCR template Purification Kit (Roche; Germany) was used for all prepared tissue samples. The binding column tube transferred to a new 1.5 ml tube and add 200 μ l of elution buffer; centrifuge at 8000 rpm for 1 min. The integrity of the DNA was assessed by gel electrophoresis (1% agarose). Primers for *C.pneumoniae* (Roche, Germany): The major outer membrane protein genes (ompA) of *C.pneumoniae* was chosen as the target for amplification in a PCR. 333 base 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 GGC 3' 373-393

Statistical analysis

All analyses were conducted using SPSS11.5 software. The Student's t test was used to determine significant differences in means for all continuous variables. Chi square values ($p < 0.05$) were calculated for all categorical variables. Kappa (5%) were calculated for comparison between PCR and serological results.

Results and Discussion

Positive PCR determined in 19.6% (10/51) of cases and none of the controls with significant differences. (P value = 0.05; OR = 9.9). Table-1

Positive IgM (recent infection) observed in 9.8% (5/51) of cases and none of the controls without significant differences (P value = 0.31; OR = 0.99). Table-1

Positive - IgG (Previous infection) detected in 47.1% (24/51) of cases and 47.4 % (9/19) of controls without significant differences. (P value = 0.1; OR = 2.499).

The chance for positive IgM- ELISA (recent infection) is 1.3 times more than chance for positive PCR; good agreement between IgM and PCR results (Kappa = 0.17) without significant differences between 2 tests (P value = 1). Positive -IgG (ELISA) was 30 times more than positive PCR; with weak agreement (Kappa index = 0.07) and significant differences (P value = 0.001)

In present study positive PCR and IgG, IgM in studied polyp cases was 9.8%; 47.1% ; 19.6% respectively. It showed that *C. pneumoniae* infection had a moderate role (near 20%) in nasal polyp formation if it searched by a reliable but invasive method (PCR).

Although serologic exams (positive-IgM; IgG) for *C.pneumoniae* infection had not significant differences between cases and controls (P value = 0.31; 0.1) but the positive PCR had significant differences (P value = 0.05).

Table.1 Comparison of results between cases and control"

Variable	Cases (n=51)	Control n=(19)	Odds Ratio	p-Value
Positive <i>C.pneumoniae</i> -IgG	47.1%	47.4%	0.99	0.1
Positive <i>C.pneumoniae</i> -IgM	9.8%	0	0.90	0.31
Positive <i>C.pneumoniae</i> -PCR	19.6%	0	0.92	005

Table.2 Correlation between PCR and ELISA tests

Variable corelation	Positive ELISA and Negative PCR (n=70)	Negative ELISA and positive PCR (n=70)	OR (p-Value) (McNemar test)	Kappa (p-Value)
IgM & PCR results	4	3	1 (1.33)	0.17 (0.15)
IgG & PCR results	30	1	30 (0.001)	0.07 (0.25)

Poor agreement observed between IgG and PCR in cases, the chance for positive IgG (previous infection) in cases was 30 times more than positive PCR (P value < 0.001), but a good agreement reported between positive IgM and PCR. The chance for positive IgM was 1.5 times more than positive PCR. So, the searching the *C.pneumoniae* -IgM in sera is a valuable and non-invasive method (instead of PCR) for diagnosis of active infection in polyp cases.

The serologic results in present study is very close to Kozak *et al.*, (1991) study; positive-IgM; IgG had not significant differences between cases and controls. They concluded that chronic bacterial infection is not a major component of nasal polyp etiology (Kozak *et al.*, 1991) Apan *et al.*, (2007) explained probable role for infection in cases with nasal polyps. Like here, they determined in the polyp tissue (indirect immunofluorescence) in 53.3% of polyp cases and 26.6% of

controls (P = 0.034). In contrast to us, IgG antibodies were positive in 53.3% of cases and 22% of the controls; P = 0.065. (Apan *et al.*, 2007).

The presence of *C. pneumoniae* in near 20% of nasal polyp tissues shows a probable role for this organism in nasal polyp formation. Previous studies indicate Real-time PCR is the most sensitive method for finding the *C.pneumoniae* in tissue samples in compare with serology (Engstrand *et al.*, 2001; Noorbakhsh *et al.*, 2009; Noorbakhsh *et al.*, 2008; Noorbakhsh *et al.*, 2004; Ah Torshizi1 *et al.*, 2008). The incidence rate of positive PCR in polyp tissue is near to *C.pneumoniae* rate in children with adenoid surgery (Nourbakhsh *et al.*, 2008). *C.pneumoniae* - PCR was positive in 15.9% of 51 adenoid samples. Recent infection (positive IgM); and previous infection (positive IgG) in cases was 2%; 11.8% respectively without significant difference

between cases and controls (Nourbakhsh *et al.*, 2008). *C.pneumoniae* cultured from nasopharyngeal epithelial cells in 47.6% exacerbation and 35% of patients with chronic stable asthma (vs 14.3% and 5% of control) in Iran (Ah Torshizi *et al.*, 2008) Clinical improvement reported in cases with successful eradication of *C. pneumoniae* (Ah Torshizi *et al.*, 2008) Cervin *et al.*, (2001) reported that some patients with recurrent nasal polypoidosis, who do not respond to surgery or steroids might benefit the long-term, low-dose macrolide treatment (Cervin, 2001). Future larger studies are needed before macrolides are used to treat recurrent nasal polypoidosis.

C. pneumoniae infection has a moderate role (positive PCR = 19.6%) in nasal polyp formation if it searched by PCR, a reliable method. PCR tests are more specific and sensitive but invasive method for diagnosis of active *C.pneumoniae*. Determination the *C.pneumoniae* infection in polyp formation are possible before surgery by specific IgM -ELISA test. In contrast to IgM serology, IgG test is not suitable test instead of PCR before surgery. In the future placebo-controlled studies are necessary to validate the effect of macrolides (8 weeks) before surgery on reducing the nasal polyp diseases.

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Ethical Considerations

Ethical Committee in the ENT and head & Neck Surgery Research Center in Iran University of Medical Sciences has

reviewed and approved the Waiver of Authorization for use of protected health information (PHI) for research purposes for the following study. Principal Investigator: Dr Mohamad Farhadi , MD.

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