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# Searching the *M. Pneumonia* in Cases with Nasal Polyp Accompanied by Chronic Rhinosinusitis using Polymerase Chain Reaction in Tissue and Serology: a Case Control Study

**Shima Javad Nia[1], Mohammad Farhadi[2], Sahar Ghavidel Darestani[3], Azardokht Tabatabaei[4], Ali Reza Shamshiri[5] and Samileh Noorbakhsh[6]***

[1]*Research center of pediatric infectious diseases,Tehran University of Medical sciences, Iran*
[2]*ENT, ENT and Head and Neck Surgery ResearchCenter, Tehran University of Medical Science, Iran*
[3]*ENT and Head and Neck Surgery Research Center Tehran University of Medical sciences, Iran*
[4]*Research center of pediatric infectious diseases Tehran University of Medical Sciences, Iran*
[5]*Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical sciences, Iran*
[6]*Infectious Disease, Research center of pediatric infectious diseases, Tehran University of Medical Sciences, Iran*

## Abstract

**Background:** Infectious organisms might have some role in nasal polyp formation.

**Objective:** Goal of this study was to look for *M. Pneumonia* in cases with nasal polyp accompanied by chronic rhinosinusitis using Polymerase Chain Reaction in tissue and serology.

**Material and methods:** This case control study was done inEar, nose and throat ward and clinics in Rasul Hospital in Tehran (2007-2008). We studied 51 cases with nasal polyp accompanied by chronic rhinosinusitis and 19 healthy controls who were only suffering from nasal fractures. Specific *M. Pneumonia* antibodies (IgG and IgM-ELISA) were measured in blood samples.

Nasal polyp tissues in cases and inferior nasal turbinate mucosa in controls were evaluated for Mycoplasma-DNA (PCR).

**Results:** The range of age in cases was12-72 years with a mean of 35 years; in controls it was 18 -41 years with a mean of 23. *M. Pneumonia*-DNA was detected in 19.6% (10/51) of cases and none of the controls showing a significant difference (OR=9.9%; P<0.05).

Acute and previous immunities (*M. Pneumonia*-IgM and IgG) were observed in 15.7% (8/51) and 68.8% (35/51) of cases; and 15.8% (3/19) and 47.4% (9/19) of controls, respectively, indicating no significant differences (OR=99%; P=0.1).

**Conclusion:** We determined the possible role of *M. Pneumoniae* in nasal polyp formation; and for diagnosis of active infection, we recommend use of *M. Pneumonia*–IgM instead of DNA–*M. Pneumonia* (PCR). Specific treatment with new macrolides at least for 8 weeks will reduce the size of nasal polyps. Future placebo-controlled studies to validate the effects of macrolides on polyposis are paramount.

**Keywords:** Nasal polyp; PCR (polymerase chain reaction); *Mycoplasma Pneumonia*; IgG; IgM; ELISA

## Introduction

Nasal polyps inflict between 1 and 4% of the population. They are benign pedunculated masses of nasal or sinus mucosa [1], believed to arising from chronic inflammation, with reasons unknown. Infection, inflammation or an imbalance of a metabolic pathway, such as the arachidonic acid pathway are considered as the aetiological factors [2]. Although allergy does not necessarily predispose patients to nasal polyps, mast cell reaction and eosinophil activation, with subsequent inflammation, are of importance in their development and may explain why corticosteroids are useful in their treatment. Bucholtz et al. [3] reported association between the nasal polyps and chronic sinusitis. A range of various disorders similar in nasal and paranasal sinuses' mucosa inflammation for as long as at least 12 consecutive weeks are called chronic rhinosinusitis. *M. Pneumonia* specific DNA was previously detected in human nasal polyps using polymerase chain reaction (PCR) techniques, which implicates *M. Pneumoniae* as a causative agent in the etiology of nasal polyps [2]. Other studies did not show similar results [3].

Although a variety of bacteria and fungi have been cultured from nasal polyps in previous studies, approximately 35% had sterile cultures [4,5]. Nasal polyps are well described as for cell and cytokine contents; nonetheless its origin is still not understood. Extra pulmonary infections due to *M. Pneumonia* were reported by some authors as the source [6,7].

Kai et al. [8] detected an *M. Pneumoniae* specific fragment in 0.05 pg of *Mycoplasma* DNA and Tjhie et al. [9] detected one colony forming unit. The specificity, relative speed ofexecutionand exquisite sensitivity of suchtechniques argues well for the diagnosis of *M. Pneumoniae* infection. More recently, a number of authors have reported amplification and detection of *M. Pneumoniae* in clinical samples (artificially seeded and clinical throat swabs, bronchoalveolar

***Corresponding author:** Samile Noorbakhsh, Research center of pediatric infectious diseases, 4th floor Hazrat Rasool Hospital, Niayesh Street, Satarkhan Avenue, Tehran, 14455 Islamic Republic of Iran, Tel: 66525328; Fax: 66516049; E-mail: Samileh_noorbakhsh@yahoo.com

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lavages and nasopharyngeal aspirations) using different PCR primers [9]. Stelmach reported existence of humoral and cellular Immunity in children with *M. Pneumoniae* Infection [10]. Since routine laboratory-based detection of *M. Pneumoniae* infection is largely based on serological detection and bacterial cell culture, not only is it slow but also insensitive. The widely-used complement fixation test is comparatively insensitive, especially in infection detection in young children. ELISA (enzyme-linked immunosorbant assay), the more sensitive method, requires paired sera for antibody detection [11]. To eradicate a micro-organism insensitive to beta-lactam antibiotics albeit sensetive to tetracycline and erythromycin, rapid detection and diagnosis might be of help [11]. Treatment with erythromycin for at least 8 weeks showed a reduction in prevalence of chronic rhinosinusitis accompanied by nasal polyps by 52% [12]. Tissue cell culture is insensitive and time-consuming; it takes 3-4 weeks to obtain a result. Therefore, the need for rapid and sensitive assays to detect *M. Pneumoniae* in a timely manner in small amounts of tissue is great.

Previous studies in our center detected the significant role for *M. Pneumonia* in upper and lower respiratory tract [13-15].

The aetiology and microbial flora of nasal polyps in our cases are not well understood.

Goal of this study was to determine the role of M *Pneumonia* in Polyp formation.

## Materials and Methods

This case control study was performed in ENT ward and clinics of Rasul Hospital in Tehran (2007-2008), and was approved by the Ethical Committee in the ENT department, Rasul Hospital.

Our study group consists of 51 cases with nasal polyp accompained chronic rhinosinusitis. Patients older than 12 years were selected by ENT surgeons. The control group consisted of 19 admitted cases for elective repair surgery for nasal fracture.Initially a questionnaire was completed by an authorized physician for each participant then they undergone a complete clinical exam.

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 before surgery for other disorders (immune deficiency states; Diabetes mellitus, renal failure; etc).

Nasal polyp tissues in cases and inferior nasal turbinate mucosa in the control group were tested for DNA-M. pneumonia (PCR). In the control group, extra blood taken for routine blood tests before surgery was used for serologic tests.

Blood samples (2 ml) were centrifuged and transferred to our research laboratory. The serum was restored in -20°C freezer until the serologic examination was performed. The centrifuged blood specimens were screenedusing an assay for *M. pneumonia* antibodies (IgM and IgG).

The evaluation of specific *M. pneumonia*-IgG and IgM antibodies 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 cm of resected tissue was removed and put in a sterile tube by the surgeon. Those tubes were preserved in -80°C. PCR template Purification Kit and PCR- ELISA kits (Roche; Germany) were used for all prepared tissue samples as suggested by the manufacturer in Roche Diagnostics.

Steps for DNA -Extraction included transferring 25-50 mg of the specimen after homogenization by Polytrom homgenisator to a 1.5 ml sterile tube with 200 µl digesting buffer and lysis buffer. 20 µl proteinase k was then added and the solution was incubated at 60°C for 1hr. afterwards, 200 µl of binding buffer was added and mixed with vortex and incubated at 60°C for 10 min. Then, 100 µl Isopropanol was added and carefully transferred to upper reservoir of the binding column tube and centrifuged at 8000 rpm for 1 min. It was then mixed with 500 µl of washing buffer and centrifuged at 8000 rpm for 1 min. Washing buffer was used for a second time and the solution was centrifuged at 12000 rpm for 1 min. The binding column tube was transferred to a new 1.5 ml tube and 200 µl of elution buffer was added and then centrifuged at 8000 rpm for 1 min. The integrity of DNA was assessed by gel electrophoresis (1% agarose).

PCR ELISA for *M. Pneumonia* was done as suggested by the manufacturer in Roche Diagnostics. 40 µl of denaturation reagents were added into reaction tube (DNA extraction). 10 µl of the amplification product per tube was incubate at 15-25˚C for 10 min. 200 µl of the mixture was transferred to every well of the microplate. The wells were then covered with the self- adhesive cover foil and incubated at 37˚C on a shaker (300 rpm) for 3 h. Hybridization mixture was removed and washed 3 times with 250 µl of washing buffer per well. 200 µl of anti-pod working dilution was added per well. Covered wells were incubated at 15-25°C on a shaker (300 rpm) for another 30 min. After removing the anti-pod, it was washed 5 times by the washing buffer. 100 µl of TMB substrate solution was added. Covered wells were incubated again at 15-25°C on a shaker (300 rpm) for 20 min.100 µl of stop reagents were added per well and absorbance was measured with ELISA reader at 450 nm.

**Statistical analysis:** All analyses were conducted using SPSS13 software.

Chi square values (CI 95%, $p<0.05$) werecalculated for all categorical variables. Kappas (5%) were calculated for comparison between PCR and serological results.

## Results

Demographic pattern: Patients with polyposis were between 12 and 72 years old with a mean age of 35. These numbers for the control group were 18-41 years and mean of 23 years.

| p-Value | Odds Ratio | ControIn=(19) | Casae (n=51) | C Varable |
|---|---|---|---|---|
| 0.10 | 2.4 | 47.4% | 68.6% | Positive *M. pneumonia* -IgG |
| 1 | 0.99 | 15.8% | 15.7% | Positive *M. pneumonia* -IgM |
| 0.05 | 9.9 | 0 | 19.6% | Positive *M. pneumonia* -PCR |

**Table 1:** Comparison of the results between cases and controls.

| Kappa (p-Value) | OR (p-Value) (McNemar test) | Negative ELISA and positive PCR (n=70) | Positive ELISA and Negative PCR (n=70) | Variable |
|---|---|---|---|---|
| ( <0.001) 0.50 | (1) 1.25 | 4 | 5 | Comparision of Positive *M. pneumonia*-IgM & PCR |
| (0.23) 0.08 | (<0.001) 18 | 2 | 36 | Comparision of Positive *M. pneumonia*-IgG & PCR |

**Table 2:** Correlation between PCR and ELISA tests.

### PCR results

*M. Pneumonia*- DNAs were detected in 19.6% (10/51) of polyp cases and none of the controls which show a significant difference (P<0.05).

### Serologic results

Acute infection (IgM) was detected in 15.7% (8/51) of cases and 15.8% (3/19) of controls. These numbers for previous immunity (IgG) were 68.8% (35/51) and 47.4% (9/19), respectively, without any significant difference in either one (P=1,0.1) (Table 1).

Detected acute *Mycoplasma* infection (IgM-ELISA) was reported 1.25 times more than DNA–PCR in tested samples, with accepatable agreement (Kappa index =0.50; P<0.001) without any significant differences between the 2 tests (p=1); (Table 2).

Previous *M. Pneumonia* infection (IgG) was reported 8 times more frequently with ELISA than DNA–PCR methods in tested samples (P<0.001) with a weak agreement index, although the actual agreement was 90% (Kappa index=0.08; P=0.2).

Ergo, we can use serum Mycoplasma–IgM instead of DNA -PCR for diagnosis of active *Mycoplasma* infection in polyp cases (Table 2).

## Discussion

Mycoplasma-DNA was detected in 20% (10/51) of polyp cases and none of the controls (P<0.05). *Mycoplasma* antibodies in serum (IgM and IgG) had not shown significant differences (P<1, 0.1) between cases and controls. Current study shows that *M. Pneumoniae* has a possible ethiologic role in development of nasal polyps. In cases with nasal polyp previous immunity (Mycoplasma-IgG) was detected more frequently (18 times) than *Mycoplasma* -DNA (PCR) in polyp tissues (P<0.001). We did not observe any agreement between previous immunity (Mycoplasma-IgG) and *Mycoplasma* - DNA (PCR) detection tests in polyp cases.

Acute *Mycoplasma* infection (IgM) was detected 1.25 times more by ELISA than DNA –*Mycoplasma* (PCR) in tested samples. There was acceptable agreement between the 2 tests (Kappa index=0.50; P<0.001). No significant differences were reported between the two tests (p=1).

In our opinion Mycoplasma-DNA (PCR) detection test is a reliable and specific test for diagnosis of active *Mycoplasma* infection in patients with polyp but serum Mycoplasma-IgM (ELISA) test in comparison has a lower specify for diagnosis. Due to acceptable agreement between these tests (Kappa index=0.50; P=0.001) we can use serum Mycoplasma–IgM test instead of DNA –PCR for diagnosis of active *Mycoplasma* infection in polyp tissues.

The incidence rate of DNA-*Mycoplasma* in polyp tissue in our study is lower than that of Gurrstudy [2]. Gurr et al. [2] detected *M. Pneumoniae* in 13/14 (93%) of nasal polyps, 4/5 (80%) of rhino sinusitis mucosal samples but it was seen only in 1/7 (14%) of the controls (obstructive turbinates).

Bucholtz study was not successful in defining *M. Pneumoniae*-specific DNA in polyp tissues [3]. They concluded that chronic bacterial infection does not play a determinant role in development of nasal polyps.

The incidence rate of DNA-*Mycoplasma* detection in polyp tissue was close to its incidence rate in adenoidectomies children (28%; 12/43 cases) in our center [15]. Indeed, the results of acute *Mycoplasma* infection (IgM) in polyp cases study is very close to adenoid study in our center (15.7% vs15%) but, the rate of previous *Mycoplasma* infection (IgG) in polyp cases is higher than its rate in rhinosinusitis cases andnormal adults ( 68.6% vs11% and 47.4%, respectively) [15].

We concluded that the presence of *M. Pneumoniae* might be of importance in the etiology of nasal polyps. Macrolides` potential to reduce the virulence of some bacteria may be an important feature in reducing tissue damage in cases with chronic infection in polyps.

Treatment with low-doses of macrolide over a long period can probably award most polyposis patients and severe chronic rhinosinusitis cases who do not respond to surgery or steroids, with symptomatic relief [12].

Placebo-controlled study shortage should be considered before this treatment method is used on a larger scale to treat therapy-resistant chronic sinusitis concomitant with polyp.

## Conclusion

In our opinion, this study defines the plausible role for *M. Pneumoniae* in nasal polyps. However, more studies are needed to evaluate this correlation. The results show that PCR amplification is useful for *M. Pneumoniae* detection in polyp tissues. We recommend using *Mycoplasma* –IgM (not IgG antibody) instead of DNA–PCR for diagnosis of active infection in polyps. Specific treatment with new macrolides for at least 8 weeks will reduce nasal polyps.

More placebo-controlled studies are needed to evaluate this hypothesis.

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