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

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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 in Ear, nose and throat ward and clinics in Rasal 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 was 12-72 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=9.9%; 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. Pneumoniae*-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 of execution and exquisite sensitivity of such techniques 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

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Steps for DNA - Extraction included transferring 25-50 mg of the specimen after homogenization by Polytron homogenisator 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) were calculated 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.

<table>
<thead>
<tr>
<th>p-Value</th>
<th>Odds Ratio</th>
<th>Control(n=19)</th>
<th>Case(n=51)</th>
<th>C Variable</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.10</td>
<td>2.4</td>
<td>47.4%</td>
<td>68.6%</td>
<td>Positive M. pneumonia -IgG</td>
</tr>
<tr>
<td>1</td>
<td>0.99</td>
<td>15.8%</td>
<td>15.7%</td>
<td>Positive M. pneumonia -IgM</td>
</tr>
<tr>
<td>0.05</td>
<td>9.9</td>
<td>0</td>
<td>19.6%</td>
<td>Positive M. pneumonia -PCR</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Kappa (p-Value)</th>
<th>OR (p-Value) (McNemar test)</th>
<th>Negative ELISA and positive PCR (n=70)</th>
<th>Positive ELISA and negative PCR (n=70)</th>
<th>Variable</th>
</tr>
</thead>
<tbody>
<tr>
<td>(0.001) 0.50</td>
<td>(1) 1.25</td>
<td>4</td>
<td>5</td>
<td>Comparison of Positive M. pneumonia-IgM &amp; PCR</td>
</tr>
<tr>
<td>(0.23) 0.08</td>
<td>(&lt;0.001) 18</td>
<td>2</td>
<td>36</td>
<td>Comparison of Positive M. pneumonia-IgM &amp; PCR</td>
</tr>
</tbody>
</table>

**Table 2:** Correlation between PCR and ELISA tests.
In our center (15.7% vs 15%) but, the rate of previous *Mycoplasma* infection (IgG) in polyp cases is higher than its rate in rhinosinusitis cases and normal adults (68.6% vs 11% 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.

**Acknowledgment**

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