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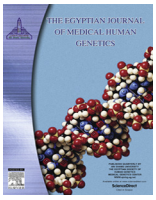

Original article

Detecting *Mycoplasma pneumoniae* infections in nasopharyngeal specimens from Paediatric patients with asthma exacerbations in Baghdad: A Polymerase Chain Reaction – Gene based study

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ABSTRACT

Background: Numerous viral infections have triggered acute asthma exacerbations. Despite the fact that diagnosis of *M. pneumoniae* infection is based on sero-prevalence studies but molecular diagnostic techniques, such as PCR, have offered improvements in sensitivity, specificity and rapidity over the latest methods.

Objectives: The aim of this molecular study is to determine the infection rates of *M. pneumoniae* in acute asthma exacerbation in a group of Iraqi children from Baghdad and also to examine the correlation of the disease with different variable characteristics and symptoms.

Methods: This study included 94 children between 2 and 13 years old; Fifty in-patient asthmatic children and 44 non-asthmatic children as control group who were out-patients of the same hospital. Throat and nasal swab samples were taken for DNA extraction and PCR procedures.

Results: PCR results show that 33.3% asthmatic patients were positive for *M. pneumoniae* while 66.7% were negative ($p < 0.001$). 53.8% of *M. pneumonia*-positive asthmatic children were 2–5 years while 46.2% were 6–14 years old. Among asthmatic patients with positive PCR, 30.8% had positive history of seasonal pattern ($p = 0.026$) and 69.2% have positive family history of atopy ($p = 0.05$).

Conclusions: Family history of atopy has strong association with asthma ($p = 0.005$), while factors such as sex, residence, seasonal allergen, animal allergen, passive smoking, mode of delivery or consanguinity has not been associated with asthma. *M. pneumonia* in a respective bulk among pediatric patients with asthma constituted an important risk factor for asthma exacerbation presented as cough and wheezy chest without fever or chest X-ray findings.

1. Introduction

Respiratory infections were reported to either cause wheezing episodes in children or to influence the onset as well as severity of asthma through both complex and intersecting mechanisms. Infections can also trigger atopic asthma while atopy in turn cause wheezing during airway infections and can modify the course of the disease [1].

Mycoplasma pneumoniae (*M. pneumoniae*) is an exceptionally small prokaryote, with no cell wall, insensitive to β-lactam antibiotics and Gram negative. It is a fastidiously growing bacterium, requiring the presence of a variety of substances such as nucleotides and sterols, for replication both in host and culture [2].

In humans, *M. pneumoniae* is among the most common organisms to cause acute respiratory infections in either children or adults [3]. It is also a major cause of community acquired pneumonia affecting 10–40% of children and young adults with clinical manifestations ranging from asymptomatic infection to fatal pneumonia or extra pulmonary diseases. However, the prevalence and association of *M. pneumoniae* in asthma is still not clear, especially in developing countries [4,5].

It is logical to believe, as others did, that infections with *M. pneumoniae* could play a greater role than it is readily considered

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for, regarding both the pathophysiology of asthma and acute exacerbations of bronchial asthma. In addition, we think that large numbers of infections with *M. pneumoniae* have been overlooked and not treated, and often it has been misdiagnosed as viral infections. Therefore, it seems important that this prospective molecular study made an attempt to assess the incidence and role of *M. pneumoniae* infection in children with acute exacerbation of bronchial and to make a correlation between bench and bedside such as making connection between the result of PCR for detecting *M. pneumoniae* DNA and the clinical and radiological findings.

2. Patients and methods

A prospective case-control study was performed on 94 children with age range of 2 to 13 years old. Fifty asthmatic children were admitted to the Children Welfare Teaching Hospital and 44 non-asthmatic children as outpatient visitors to the same hospital were included as age-matched control group. The study period was from July 1, 2013 to December 31, 2013.

This work has been carried out in accordance with The Code of Ethics of The World Medical Association (Declaration of Helsinki) for experiments in humans.

For each child, a questionnaire form was filled asking the information about age, sex, growth parameter, number of wheezing attacks, response to bronchodilator, exposure to allergen, family history of asthma and atopy. Exclusion criteria of this study were those patients with failure to thrive as well as patients having associated diseases other than asthma. Each patient aged more than 6 years of age was sent for clinical tests such as chest X-ray, echocardiography, and pulmonary function tests. Throat and nasal swab samples were collected by taking stringent precaution and using sterile methods for collecting the samples. Samples were kept in special ready-to-use viral transport media (kept in collecting container filled with ice bags) and transferred immediately to the molecular laboratory of Clinical Communicable Diseases Research Unit/College of Medicine/University of Baghdad, for DNA extraction and PCR testing.

GENEKAM DNA ISOLATION KIT (Germany) was used to isolate DNA from the swabs containing the nasopharyngeal specimens, following manufacturer's instructions. Then *M. pneumoniae* amplification kit (manufactured by GENEKAM Biotechnology/Germany) was utilized to amplify the isolated DNA, following manufacturer's instructions. The cycling steps were as follows: initial denaturation at 95 °C for 9 min, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 62 °C for 30 s, and extension at 72 °C for 30 s, and a final extension of 7 min at 72 °C. PCR fragments were separated on 2% agarose gel, stained with etidium bromide and visualized under UV light with trans-illuminator apparatus. Picture was taken with a gel documentation system.

Statistical analysis: Data were analysed using SPSS (Statistical Package for Social Science) program version 20. Chi-square was used as a test of significance for the qualitative data; P value ≤ 0.05 was considered to be statistically significant and a P value < 0.01 was considered highly significant.

3. Results

Thirty-nine asthmatic patients with a mean age of (6.33 ± 3.608) years old were included in this study along with forty-four non asthmatic patients as control group with mean age of 6.05 ± 3.444 years old. No statistical difference was found between means of age of cases and control (P value = 0.711). More than half (51.28%) of asthmatic children are 2–5 years old while the remaining 48.72% of asthmatic children were 6–14 years old.

The study shows that asthma is more common in males (27/39) than females (12/39) with male to female ratio were 2.2:1. No statistical significant difference as compared to control group as shown in Table 1.

The association of family history of allergen (including history of asthma, atopic dermatitis, and food allergy) in those asthmatic patients had showed statistically significance ($p \leq 0.05$) as compared to the control group while there was no statistical significance regarding the family history of allergic rhinitis in both groups as shown in Table 2.

PCR results show that *M. pneumoniae* was positive (Fig. 1) in 13/39 (33.3%) of asthmatic patients while negative in 26/39 (66.7%) asthmatic patients and all control group (44 out of 44; 100%) showed negative PCR results as shown in Table 3.

More than half (53.8%) of asthmatic children with *M. pneumonia*-had positive PCR results and were in the range of 2–5 years old age group, while 46.2% of them are in the 6–14 years old age group (Table 4). Among 13 asthmatic patients with positive PCR for *M. pneumoniae*, the male (9/13) to female (4/13) ratio was 2.2:1 (p = 0.648), where 7/13 were living in urban areas and the others have lived in rural areas (p = 0.199), 4/13 showed positive history of seasonal pattern (p = 0.026) and 9/13 showed positive family history of atopy (p = 0.05) (Table 5).

Table 6 shows the no of PCR-positive patients (i.e. having *M. pneumonia* infections) and number of PCR-negative data in asthmatic patients according to their signs and symptoms of pneumonia where from 13 asthmatic patients with positive PCR for *M. pneumoniae*, 6/13(46.15%) were presented with fever (p = 0.135), 10/13 (76.93%) have presented with cough (p = 0.023) and 11/13 (84.62%) have presented with wheezy chest (p = 0.006).

4. Discussion

4.1. Risk factors of asthma

Asthma as a chronic inflammatory disease of airways is characterized by hyper-responsiveness of airways to a multiple stimuli as well as a reversible airway limitation producing recurrent respiratory symptoms which are in the form of shortness of breath, cough and wheeze. The asthma pathogenesis seems to be the result of the influence of a complex mixture of several known factors, such as genetic, environmental, dietary change and occupation, recognized as predisposition factors to asthma [6,7].

Our study has found that family history of asthma is an important risk factor for this disease as showed up in 35.9% of asthmatic children in comparison to control group which showed only in 18.2% (p = 0.05). This result were consistent with other studies such as Burke in 2003 [8] Aws in 2005 [9], Nebal in 2007 [10] and Mahdi in 2010 [11].

Family history of atopic dermatitis also correlated as statistically significant (p = 0.005) and as a risk factor for childhood asthma where this result was detected in 41.1% as compared to 13.6% in the control group. This result is consistent with the results obtained by Aws in 2005 [9] and Ronchetti in 2001 [12] who found that a family history of atopy not only persisted but also strengthened over time. Our study did not found a significant correlation between childhood asthma and family history of allergic rhinitis (p = 0.26) which was 12.8% as compared to control group 20.5%, this result is consistent with the result reported by Nebal in 2007 [10].

It is generally agreed that atopy is an important risk factor for allergic diseases, such as asthma, rhinitis, and eczema, however the extent to which this atopy accounts for each of these diseases is controversial [13].

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