Chlamydia and Mycoplasma Pneumoniae Infections in Children with Bronchial Asthma

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Abstract: The worldwide increase in asthma incidence and the impact of the disease on public health care have led to new investigations of the cause of the disease. A relationship between respiratory Chlamydia pneumoniae (C. pneumonia), Mycoplasma pneumoniae (M. pneumonia) and bronchial asthma is under discussion. In the present study we aimed to evaluate the seroprevalence of M. pneumoniae and C. pneumoniae infections in children with stable asthma. One hundred and twenty asthmatic and 50 healthy Children (aged 1.5-12 years) were included in this study. All cases were subjected to: Full history and thorough clinical examination, skin prick tests for common allergens were performed. Blood samples were taken for complete blood count, serum total IgE; detection of C. pneumoniae IgG, IgM, IgA antibodies and M. pneumoniae IgG, IgM antibodies using an enzyme linked immunosorbent assay (ELISA). In this study, M. pneumoniae and C. pneumoniae antibodies were detected significantly in children with bronchial asthma than controls. C. pneumoniae antibodies were detected in 55(45.83%) of asthmatics versus 3 (6%) of the controls. C. pneumoniae IgG serology suggestive of persistent infection was significantly higher in chronic persistent asthma group than in the control group. Thirty six patients (30.0%) were seropositive for specific IgG of, while only one healthy control (2%) was IgG seropositive. C.pneumoniae IgM was found in 13 (10.83%), IgA in 6(5%) asthmatics versus 1(2%), 0(0%) in controls respectively. M. pneumoniae antibodies were detected in 13(17.5%) of asthmatics versus none of the controls. Six (5%) patients were seropositive for specific M. pneumoniae IgG antibody and 15(12.5%) had positive M.pneumoniae IgM antibody. The incidence of infection due to both pathogens were higher in males than females and increased with age. As regards asthma severity, seropositivity was more in moderate persistent group than in the mild one. We concluded that there was a significant relationship between bronchial asthma and either M.pneumoniae or C.pneumoniae.

Key words: Asthma, mycoplasma, chlamidia, pneumonia, children

INTRODUCTION

Asthma is an inflammatory disease of airways, with a worldwide-unexplained increased incidence. Marked inflammation of the bronchial mucosa is a common feature of the asthma and leads to structural changes of the lung tissue[1]. The fact that the airways are exposed to a large number of respiratory infectious agents could explain the frequency of respiratory infections and their causal effect in bronchial inflammation[2]. It was found that atypical organisms as C. pneumoniae and M. pneumoniae are very common pathogens of pediatric upper and lower respiratory tract infection among children in many countries[3]. C. pneumoniae infection is less common in young children, but rises sharply during school-age years. M. pneumoniae is the first cause of community-acquired pneumonia in children older than five years of age[4]. These atypical organisms are particularly important due to their capacity to provoke immune dysfunction and chronic inflammation[5].

These atypical organisms have been recently linked to asthma in various ways, as infection with any of them may precede asthma onset, exacerbate asthma[6] or make asthma control more difficult, suggesting that these organisms can play an important role in the natural history of asthma. Furthermore, animal models of acute and chronic infection with these organisms indicate that they have the ability to modulate allergic sensitization and pulmonary physiologic and immune response to allergen challenge[7].

Further evidence for the role of these pathogens in asthma comes from observation of improvement in asthma symptoms after antimicrobial therapy active against M. pneumoniae and C. pneumoniae[8]. In some studies C. pneumoniae seems to be more important in
asthma pathogenesis and exacerbations than *M. pneumoniae*; in other reports the role of *M. pneumoniae* appears to be more significant\(^9\).

The aim of this study was to investigate the relationship between *M. pneumoniae* and *C. pneumoniae* and bronchial asthma in Egyptian children.

**MATERIALS AND METHODS**

One hundred and twenty children with established asthma were included in this study (aged 1.5-12 years). Fifty healthy children with the same socioeconomic standard and age group were evaluated as control group. A written consent was obtained from the parent of each child at the beginning of the study. The diagnosis, classification of asthma, asthma severity and asthma exacerbation were based on the criteria of the new Global Initiative for Asthma (GINA) guidelines\(^10\). All cases and controls were subjected to: Full history including the onset of diagnosis of asthma and methods used, use of asthma medicines and thorough clinical examination.

**Laboratory Investigations:** Routine Laboratory investigations including: Urine and stool analyses for detection of any parasitic infection. Complete blood count and absolute eosinophilic count (AEC). Ten mL of peripheral blood was collected and serum was separated by centrifugation and stored at -20°C until used for serology.

**Serum Total IgE:** Quantitative determination of serum total IgE using solid phase chemiluminescence immunometric assay (IMMULITE, DPC).

**C. pneumoniae and M. pneumoniae Antibodies:** Quantitative determination of *C. pneumoniae* IgG, IgA and IgM antibodies and *M. pneumoniae* IgG and IgM antibodies were performed by ELISA using Vircell Kit (Vircell, Santa Fe-Granada, Spain)\(^11\). According to the manufacturer’s instructions, samples with antibody index (ABI) below 9 were considered as not having anti *C. pneumoniae* or anti *M. pneumoniae* antibodies (negative). Samples with (ABI) above 11 were considered as having specific anti *C. pneumoniae* or anti *M. pneumoniae* antibodies (positive). Samples with ABI of 9-11 were considered equivocal.

**Statistical Analysis:** The asthmatics and control group were compared for the presence or absence of Mycoplasma or Chlamydia infection and the presence of positive serology using Chi square test with Yates correction and Fisher exact test. Continuous variables were compared using t test. The p-value is considered significant if less than 0.05.

**RESULTS AND DISCUSSION**

**Results:** Table (1) shows the demographic, clinical and laboratory characteristics of the studied population. No significant difference was observed in sex and age. Among children with bronchial asthma, 75 (62.5%) children had a history of pediatrician – diagnosed recurrent episodes of wheezing (i.e., at least four acute episodes of wheezing in the last 12 months preceding enrollment), where as it was the first episode of wheezing for 45 (37.5%) children. None of the controls showed a history of recurrent episodes of wheezing. There was significant difference in prevalence of atopy between asthmatic children and controls.

There was statistical significant difference in the mean AEC and total serum IgE between asthmatics compared to controls (p<0.05).

Table (2) shows the incidence of *C. pneumoniae* antibodies and *M. pneumoniae* antibodies in the studied population.

* *C. pneumoniae* antibodies were significantly higher in asthmatic children 55(45.83%) than controls 5(10%). Acute *C. pneumoniae* infection was demonstrated in 13(10.83%) of 120 children with bronchial asthma having positive IgM antibodies. Among controls, only 1 (2%) child showed evidence of acute *C. pneumoniae* without any respiratory symptoms. Past *C. pneumoniae* infection was demonstrated in 36 (30.0%) children with bronchial asthma having positive IgG antibodies. Among the control group, 3 children were seropositive for IgG antibodies. Chronic infection with *C. pneumoniae* was detected in 6 (5%) of 120 children with bronchial asthma having positive IgA antibodies versus one child of the controls (2%). *M. pneumoniae* antibodies were significantly higher in 21 (17.5%) of 120 children with bronchial asthma versus none of the controls. Acute *M. pneumoniae* infection was demonstrated in 15 (12.5%) of 120 children with bronchial asthma versus none of the controls. Past *M. pneumoniae* infection was demonstrated in 6 (5%) children having positive IgG antibodies versus none of the controls.

Combined infection with *C. pneumoniae* and *M. pneumoniae* was detected in 6 of asthmatic children. Cp IgG + Mp IgM in 1, Cp IgM + Mp IgM in 5 and none of the controls (0%).

Table (3) summarizes the incidence of *C. pneumoniae* and *M. pneumoniae* infection in asthmatic children in different age groups. The significant differences in the incidence of infection due to either
In asthmatic children, 5 of them > 4 years and with 4 years than children < 4 years. Combined infection with *C. pneumoniae* and *M. pneumoniae* was detected in 6 of asthmatic children, 5 of them > 4 years and one < 4 years. Table (4) summarizes the incidence of *C. pneumoniae* and *M. pneumoniae* infection according to sex. The incidence of infection due to both pathogens was higher in males than females. Combined
infection with both *M. pneumoniae* and *C. pneumoniae* was detected only in 6 boys but not in girls.

Table (5) shows the prevalence of atopy in *C. pneumoniae* and *M. pneumoniae* infections. No significant difference was found between children with and without atopic manifestations regarding infection. Table (6) shows the relation between specific *C. pneumoniae* and *M. pneumoniae* antibodies and grades of asthma. The percentage of positive patients with moderate asthma was higher than those with mild asthma with no statistical significance (p > 0.05). Combined infection with both pathogens was detected in 2 children with severe asthma. Table (7) shows the correlation between ABI and different clinical and laboratory parameters in asthmatic patients. There was significant correlation between asthma severity and *C. pneumoniae* IgM and between total IgE and *C. pneumoniae* IgA.

**Discussion:** Asthma is one of the most common chronic diseases which cause school absenteeism of childhood[22]. The potential role of atypical bacterial infection in the pathogenesis of asthma is a subject of continuing debate. There is a growing body of basic and clinical science concerning the association between atypical bacteria *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* and asthma pathogenesis, although their exact contribution to asthma development and/or persistence remains to be determined[7].

In our study, we investigated the relationship between *C. pneumoniae* and *M. pneumoniae* and bronchial asthma in Egyptian children. The incidence of *C. pneumoniae* (45.83%) and *M. pneumoniae* (17.5%) infection in asthmatic children was significantly higher than that in healthy controls (10%, 0%). Silva et al found a lower prevalence of these agents, *M. pneumoniae* being found in 16.4% and *C. pneumoniae* in only 2.7% of the cases[13]. Our results are also in agreement with Martin et al and Silva et al who showed that *C. pneumoniae* and *M. pneumoniae* positive rates in the bronchial asthma group were significantly higher than control group[12] and[13]; however, Park et al showed no statistical significance and they attributed that to the low number of enrolled patients and/or geographic factors[15].

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In some studies *C. pneumoniae* seems to be more important in asthma pathogenesis and exacerbations than *M. pneumoniae*; in other reports the role of *M. pneumoniae* appears to be more significant[16]. In our study *C. pneumoniae*(45.37%) seems to be more frequent in asthmatic children than *M. pneumoniae* (17.5%). Combined infection with *C. pneumoniae* and *M. pneumoniae* was detected in 6 of asthmatic children. Cp IgG + Mp IgM in 1 Cp IgM and Mp IgM in 5 and none of the controls (0%). Silva et al found a case of *C. pneumoniae* detection corresponded to a co-infection with *M. pneumoniae* in a child presenting severe-persistent asthma[13]. We studied the association between IgG antibodies against *C. pneumoniae* and asthma. A significant association was found between anti-*C. pneumoniae* IgG antibodies in asthma, as compared to the control group (30.0% vs. 6.0%, p <0.05). The prevalence of *C. pneumoniae*-specific IgM, and IgA was significantly higher in asthmatic children than in control subjects (10.83%, 5% vs. 2% and 2% respectively, p <0.05). Jiang et al demonstrated Anti-*C. pneumoniae* IgM in (18.3%) and anti-*C. pneumoniae* IgG (26.7%) in asthmatic patients[17]. These data are similar to our findings and may suggest that *C. pneumoniae* infection may trigger the development of asthma.

In agreement with our study, Wazir et al found that *C. pneumoniae* IgG serology suggestive of persistent infection was significantly higher in chronic persistent asthma group than in the control group[18]. Meanwhile there was no evidence of acute *C. pneumoniae* infection (IgM serology) in acute exacerbations. The increased level of IgG class anti-*Chlamydia pneumoniae* antibodies in the group of patients with asthma seems to support the hypothesis about the role of Chlamydial infections in an etiopathogenesis of bronchial asthma. However Sato et al and Anagür et al did not find significant difference in *C. pneumoniae* IgG between asthma and control groups[19] and[20]. There was a statistically significant difference for Chlamydia IgM among groups, suggesting that *C. pneumoniae* infection may trigger acute exacerbations of childhood asthma.

In an Egyptian study in adults, there was a significant difference between cases and controls regarding specific *C. pneumoniae* IgG and IgM. A significant correlation between asthma and *C. pneumoniae* was found especially in moderate and severe, long standing, steroid dependent asthma[21].

In contrast to our results, Larsin et al could not support the speculative theory that *C. pneumoniae* is a cause of bronchial asthma[22]. Also, a study of newly diagnosed asthma in children showed no difference in *C. pneumoniae* serology between asthmatic and control groups, regardless of the serologic method used[23]. The conflicting data, in part may be due to the difficulty in accurately diagnosing infection with these atypical pathogens[24].

Despite numerous studies demonstrating an association between asthma and signs of *C. pneumoniae* infection, the role of *C. pneumoniae* in the
pathogenesis of asthma remain still unclear. Investigators suggested that the immune response to organism might play a pathological role in asthma. *C. pneumoniae* infects the human bronchial tree causing ciliary dysfunction and epithelial damage. It also generates inflammatory cytokines\[^{25}\]. Additionally *C. pneumoniae* might infect bronchial smooth muscle and promote bronchial hyperactivity \[^{26}\]. Nisar *et al*
suggested that *C. pneumoniae* infection might act as a
cofactor, possibly rendering asthmatic children more
susceptible to other stimuli such as allergens or viruses
or both[27].

Many studies have been carried out trying to
clarify the link between *M. pneumoniae* and asthma
with varying method and varying results as well. In the
present study *M. pneumoniae* (17.5%) infection in
asthmatic children was significantly higher than that in
healthy controls. None of the controls had positive anti
*M. pneumoniae* antibodies. If the positivity in
asthmatics were only due to persistence after an
infection with *M. pneumoniae* that was present in the
community, we would have expected a positive
antibody response in control subjects.

In the present study, 6(5%) patients had a positive
ELISA test for serum specific IgG *M. pneumoniae*
antibody and 15(12.5%) had positive anti *M.
pneumoniae* IgM antibody. Our results were in
accordance to Gil et al who found evidence of *M.
pneumoniae* in 24.7% of asthmatics versus 5.7% of
non-asthmatic controls[28]. However, Kondo et al
demonstrated a higher percentage of seroconversion to
Mycoplasma infection in association with acute asthma
exacerbations[29]. Cunningham et al on the contrary, had
a smaller percentage of *Mycoplasma* infection as it was
only found in 2 (0.7%) of the asthmatic samples[30].

This study suggested that *M. pneumoniae* appeared
to be involved in some asthma exacerbations as we
found IgM antibodies in 12.5% in asthmatic children.
Annagür et al., demonstrated positive *Mycoplasma* IgM
in 8.1% of asthmatic children, they found a statistically
significant difference for *Mycoplasma* IgM between
asthmatic and control groups, but had not found
significant difference for *M. pneumoniae* IgG among
both groups[20].

An important issue to remember when evaluating
these studies is that organisms are difficult to culture,
which may underestimate the incidence. In addition, the
titer range that defines an acute or chronic infection
varies from one study to another, making them difficult
to compare. The lack of antibody response to *M.
pneumoniae* has been previously noted in both pediatric
and adult populations with community-acquired
*pneumoniae*. Lack of a response perhaps owing to
genetic differences may in fact contribute to the
organism’s persistence[31]. Immunoglobulin E levels
predict onset and clinical expression of atopy and asthma.
In this study, there was no significant
correlation between level of IgE and levels of *M.
pneumoniae* antibodies (IgG, IgM). However, we found
significant correlation with C.pneumonia IgA
antibodies. *C. pneumoniae* antigenic stimulation leads
to specific IgE production[32]. In the study done by
Nagayama and Skurie transient elevation of total serum
IgE levels has been demonstrated during the acute
phase of viral as well as M.pneumoniae infections,
even in the absence of wheezing, demonstrated the
presence of anti-C.pneumoniae IgE in 85.7% of culture
positive children with wheezing, in contrast to only
9.1% of culture-positive patients with community
acquired *pneumoniae* who were not wheezing[33]. Thus
the IgE response seems to be integral part of the host
response to a variety of infections. The role of

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*P<0.05 significant
immunoglobulin E-related hypersensitivity and induction of T helper type 2 immune response leading to inflammatory response in M. pneumoniae-infected patients with asthma have also been proposed[27].

Our results have demonstrated significant increase in the incidence of infection due to either pathogen in children with bronchial asthma more than 4 years compared to less than 4 years. Combined infections with C. pneumoniae and M. pneumoniae were detected in 6 of asthmatic children, 5 of them > 4 years and one < 4 years. These findings were in accordance with other studies that have shown the highest incidence of atypical bacterial infections in children of more than 5 years[9,24].

We found no significant statistical difference between males and females as regards positivity for C.pneumoniae and M. pneumoniae (but more in males). This difference may be secondary to other factors such as the increased ability to produce IgE, airway caliber that has a clear gender difference or the possibility of inherited vulnerability to bronchial asthma. In quite similarity to this result, Kaledy showed that prevalence of antibodies to C.pneumoniae was more common in males than in females, a difference that increased with age[34]. Combined infections with both M.pneumoniae and C. pneumoniae were detected only in 6 boys with no girls. Similarly, Megias et al. found that most combined infection occurred in boys [35]. However, Normann et al suggested that infection with C.pneumoniae may be an important risk factor for wheezing and possibly for non-atopic asthma, predominantly in girls[36].

The present study highlights the link between asthma and atopy defined by positive skin-prick tests and atypical bacterial infections. No significant difference in the prevalence of atopy was found between asthmatic children with and without infections due to both pathogens. Similarly, Ronchetti et al found no association between atopy or history of atopic illness and the presence of antibody production[37]. Normann et al suggested that infection with C.pneumoniae may be an important risk factor for wheezing and possibly for non-atopic asthma[38]. One controlled study in Finland found that elevated IgG levels were significantly associated with asthma, particularly long-standing asthma[38]. Analysis by atopic status revealed that C. pneumoniae infection was most strongly related to the risk of long-standing, non-atopic asthma. The relationship between atopic asthma, irrespective of asthma past history, and IgG titres was not significant. The authors concluded that asthma is significantly associated with elevated IgG antibody levels to C. pneumoniae in patients with non atopic longstanding asthma. However, a population-based study in Italy found a significant association between C. pneumoniae seropositivity and atopy in young adults[39]. However, further studies of the relationship between M. pneumoniae and C. pneumoniae infection and atopy are needed to provide a more comprehensive understanding of how these triggers for wheezing interact[37].

As regards asthma severity, the percentage of positive patients for C. pneumoniae and M. pneumoniae with moderate asthma was higher than that of mild but it didn't reach statistical significance. In this study IgA antibodies to C. pneumoniae were significantly associated with asthma severity markers. This raises the possibility that chronic infection with C. pneumoniae leads to an increase in the severity of asthma.

In accordance to our findings Biscione et al and Wasir et al reported that persistent C. pneumoniae infection had occurred more frequently in patients with moderate and severe asthma than in ones with mild asthma[18,40]. Von et al reported significantly associated elevated IgA antibody levels to C. pneumoniae with asthma severity[38].

Several studies have assessed the correlation of C. pneumoniae serology and airway obstruction[14,41]. One study provided statistical evidence of a linkage of the severity of asthma with high IgA and Ig G in adults. Strachan et al showed associations between C. pneumoniae IgA antibody than with C. pneumoniae IgG, and decline of pulmonary function in adult patients with chronic obstructive pulmonary disease[41]. Tumerelle et al reported that persistent clinical features were more frequently associated with atypical bacterial infection[14]. Jiang et al also suggested that C. pneumoniae IgG seropositivity was associated with impaired lung function in children[17]. In this study Combined infections with both M. pneumoniae and C. pneumoniae were detected in 2 patients with severe asthma.

Eosinophils are believed to be key effector cells in producing the bronchial mucosal inflammation characteristic of allergic asthma. In our study there was no significant correlations between levels of absolute eosinophilic count (AEC) and levels of C. pneumoniae (IgA, Ig G,IgM) antibodies and M. pneumoniae antibodies (Ig G.IgM). This can be attributed to the fact that eosinophilic count in sputum and markers of eosinophil degranulation (such as blood eosinophilic cationic protein) can reflect the disease severity rather than blood eosinophilic count[42].

In conclusion, the present preliminary results show a possible association of infection with Mycoplasma pneumoniae and Chlamydia pneumoniae and bronchial
asthma. We recommended that in children whose asthma related symptoms remain poorly controlled, a careful search for evidence of *M. pneumoniae* and *C. pneumoniae* infection might be indicated. Ongoing research into the importance of atypical pathogens in asthma will further elucidate whether these infections are important in disease development or whether their prevalence is increased in asthmatic subjects due to chronic airway inflammation or other, yet unidentified, predisposing factors.

REFERENCES


