Frequency of Chlamydia pneumonia infection in asthmatic patients in northeast of Iran

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Abstract

Background: The role of Chlamydia pneumonia in asthma has drawn much attention in recent years. Considering conflicting data about frequency of C. pneumonia in asthmatic subjects and, regarding that no such study has been done in Middle East, we assess the prevalence of C. pneumonia infections in patients with chronic stable and acute exacerbation of asthma and compared it with normal subjects. **Methods:** 20 adult patients with chronic stable asthma and 21 patients with acute exacerbations of asthma and 41 matched control subjects were studied for presence of C. pneumonia using PCR and IgA and IgG assay. **Results:** This study suggests that positive results of C. pneumonia IgA antibody are associated with both chronic stable and acute exacerbation of asthma, while IgG antibody and PCR are not. **Conclusions:** Regarding PCR results which were statistically insignificant, it could be concluded that in our region C. pneumonia is not a major risk factor for either development or exacerbation of asthma.

Keywords: Asthma, Chlamydia pneumonia, PCR, IgG, IgA

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INTRODUCTION

The worldwide high prevalence of asthma and the impact of the disease on quality of life, have led to numerous investigations on the cause of the disease. However, the cause and pathophysiology of this syndrome are presently not completely defined. Viral infections have been linked to the acute exacerbation of asthma in approximately 50% of patients [1]. Recently Chlamydial infection has been suggested to participate in the pathophysiology of asthma [2-4].

C. pneumonia is a relatively new respiratory pathogen, which first described in 1986[5]. The possible link between asthma and *C. pneumonia* was first published by Hahn et al, [4] then, with use of different methods, multiple studies have been led in this subject mostly with conflicting results [6-14]. However, multiple serologic evaluation studies suggest that *C pneumonia* may be associated with chronic stable asthma [2-4, 6, 8, 9].

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The present study copulate serologic evaluation with PCR among well defined asthmatic subjects to study the role of *Chlamydia pneumonia* in asthma.

MATERIALS AND METHODS

Study population: From Feb. 2005 to May 2005, we identified 48 patients; from whom 28 were admitted as asthma exacerbation and 20 patients with chronic stable asthma from out-patient clinics in Ghaem Medical Center, Mashhad University of Medical Sciences, Iran. The asthmatic patients fulfilled criteria for asthma, exhibiting a recurrent attacks of dyspnea, wheezing, cough and >15% reversibility of lung function with bronchodilator. An asthma exacerbation was defined as an abrupt and/or progressive worsening of shortness of breath, wheezing and chest tightness, while asthmatic patient in chronic group did not have such an attack during last 4 week. Seven patients out of 28 case of asthma exacerbation which were participated in this study were excluded from study due to heavy smoking habit or persistent productive cough, and the remaining 21 patients were selected as case group 1. Case group 2 consisted of 20 asthmatic patients diagnosed as chronic stable asthma. Pair matched healthy control subjects were also chosen for each of these groups. As sex is not important in asthma pathogenesis, the female to male ratio of participants in our study, was merely based on actual hospital visit and not the true ratio in asthma, besides by matching exact sex (and similar age) subjects for each case, we neutralize any potential effect of it.

Study design: After local ethical committee approval was granted and informed consent was obtained for this study, subjects were examined by the physician and routine questionnaire were completed. Subjects underwent spirometric evaluation. Chest radiography was carried out to rule out infiltrates. Measurements of total serum immunoglobulin levels (IgG, IgA, and IgM) was also performed to ensure subjects were not hypogammaglobulinemic and were thus capable of mounting an appropriate serologic response to infection.

Table 1. Demographic and clinical characteristics of the study population.

Variable	Case group 1	Control group 1	Case group 2	Control group 2
Subject Number	21	21	20	20
Mean age (years)	44.42±14	44.42±14	42.00±17	42.00±17
Sex (M/F)	8/13	8/13	7/13	7/13
FEV_1 pre BD L	2.31 (1.72-2.82)	3.41 (3.20-3.71)	3.31 (3.1-3.62)	3.42 (3.18-3.81)
FEV_1 post BD L	3.01 (2.55-3.41)	3.43 (3.21-3.74)	3.41 (3.20-3.68)	3.44 (3.20-3.82)
FEV_1 percentage of predicted pre BD	58.2% ± 10.3%	85.6% ± 14.5%	78.5% ± 12.5%	81.3% ± 15.2%
FEV_1 percentage of predicted post BD	70.8 ± 11.2%	86% ± 15.2%	80.7% ± 10.8%	82% ± 13.4%

21 asthma exacerbation patients were selected as case group 1. Case group 2 consisted of 20 patients diagnosed as chronic stable asthma. Pair matched healthy control subjects were also chosen for each of these groups (Control group 1 & Control group 2). The FEV_1 pre BD L and FEV_1 post BD L were shown for each group in table.

Nasopharyngeal epithelial cells, which were obtained with a swab, were used for PCR as Boman et al found nasopharyngeal swab is reliable indicator of lower respiratory tract infection with *C. pneumonia* [15].

For PCR assay, specimens were placed in 1×PCR transport buffer and the DNA isolation and amplification were performed using *Chlamydia pneumonia PCR Detection Kits (Pajohesh Azma, Iran)* according to manufacturer's instruction. PCR products were analyzed in agarose gel and were visualized by etidiumbromide (ETBr) under UV light. All positive results were reprocessed and retested for confirmation of their results.

C. pneumonia-specific IgA and IgG were determined in the serum samples using Chlamydia pneumonia-IgG and IgA EIA assay (*SEI-ISA medac, Germany*) according to the manufacture's instruction.

For increasing reliability, a single lab was used for all samples, and all process was performed blinded.

Statistical analysis

In this study the differences between groups were determined using the Chi square, Cochran and Fisher tests (SPSS 11.5). For all analyses, all tests were 2-tailed, with the level of significance defined as a P value of .05 or less.

RESULTS

The mean age in exacerbation and chronic stable asthmatic groups were 44.42 and 42.0 years old respectively. The mean FEV_1 was 2.31^L and 3.31^L in these two groups respectively while it was 3.41 and 3.42 in the paired control groups. Demographic and clinical characteristics of patients are summarized in table 1.

While P-value for IgA was statistically significant in asthmatic exacerbation patients, IgG and PCR results were only slightly different in this group from its matched control group in favor of more C. pneumonia infection in the case group 1 than its control group (table 2).

The same has been true about the chronic stable asthma group in comparison with its control group (Table3).

DISCUSSION

Concerning the growing interest in *C. pneumonia* role in exacerbations of COPD, ischemic heart disease and more recently asthma, we assessed the frequency of *C. pneumonia* in asthmatic patients and matched control subjects. In

Table 2. The result of tests among asthma exacerbation and its control group

Tests	Asthma exacerbation positive result numbers (percent)	Control group of positive result numbers (percent)	P-value
PCR	10 (47.6%)	7 (33.3%)	0.34
IgG	17 (81%)	14 (66.7%)	0.29
IgA	12 (57.1%)	4 (19%)	0.011

Positive results of C. pneumonia by IgA, IgG and PCR in study group were found in 12(54.1%), 17 (81%) and 10 (47.6%) patients respectively, while in matched control group the positive results were in 4 (19%), 14 (66.7%) and 7 (33.3%) individuals respectively. [P value for IgA test was < 0.05 while for the IgG and PCR tests were both > 0.10] In comparison between study group and its control group, the differences were statistically significant only for IgA. IgG and PCR results were slightly different in this group from its matched control group in favor of more C. pneumonia infection in the study group than its control group.

Table 3. The result of tests among Chronic stable Asthma and its control group

Tests	Chronic stable Asthma positive result numbers (percent)	Control group of positive result numbers (percent)	P-value
PCR	9 (45%)	5 (25%)	0.18
IgG	16 (80%)	14 (70%)	0.46
IgA	9 (45%)	4 (20%)	0.09

In study group, positive results were obtained in 9 (45%), 16 (80%) and 14(70%) patients by IgA, IgG and PCR respectively, while in the matched group, positive results were obtained in 4 (20%), 14 (70%) and 5 (25%) individuals respectively. [P value for IgA test was < 0.10 but for both IgG test and PCR were > 0.10] In comparison between study group and its control group, IgA, IgG and PCR results were slightly different in favor of more C. pneumonia infection in the study group than its control group but the differences were statistically insignificant.

this study, in addition to PCR, we also assessed specific *C. pneumonia* IgA and IgG for detection of *C. pneumonia* in both exacerbation and chronic stable asthma.

While many studies like Foschino Barbara M.P [7], Tuuminen T [11], Mills G.D [16], and Pasternack [17], raised question about possible association of *C. pneumonia* seroprelavence and asthma pathogenesis and even some like Brouard et al [18], discredit any association between *C. pneumonia* and asthma exacerbation, many others found a significant relationship between asthma with this microorganism [3, 8, 11, 19-23]. More over Araafa R.M [24] and Tyl G. [25] found *C. pneumonia* to be related with more severe forms of asthma.

The rate of *C. pneumonia* infection in both case and control groups were significantly higher in our study than similar studies in developed countries, while asthma rate were not much higher in our region. Comparison between case and control group showed that, although 47.6% and 45% of asthmatic groups had positive PCR results while about 33.3% and 25% of control subjects were positive on PCR, but the difference was not statistically significant. The same was true about IgA and considering past infection (IgG assay) the frequency was even higher but no significant difference was observed.

Our study has yielded conflicting data; while our result for IgA was slightly significant, the results of PCR and IgG examination were not significantly different among asthmatic patients and control subjects. Considering the positive IgA results could occur in B cells response to other stimulants; and high IgA level can in turn, activate eosinophils and degranulate them so that, causing bronchospasm and other asthma manifestation, therefore an IgA positive result per se is not indicative of a definite cause and effect relationship between *C. pneumonia* and asthma pathogenesis [26].

In conclusion, considering insignificant difference of *C. pneumonia* infection rates, among asthmatic patients compare with control subjects and also, regarding very high C. pneumonia infection rate in both case and control groups, which not accompanied with significantly higher rate of asthma prevalence (nationwide), we deduced C. pneumonia isn't a major and significant contributing factor in asthma pathogenesis in our region, so its eradication is not recommended in all asthmatic patients in northeast of Iran.

It should be mentioned that, we did not consider severity of asthma in our study, while some authors like Von-HL [27], Claus Kroegel [28], Araafa RM [24] and Tyl J [25] studied *C. pneumonia* role in sever asthma, so we are not to question or discredit the relationship between *C. pneumonia* and severe asthma pathogenesis.

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