Chlamydophila pneumoniae infection and its Heat shock protein 60 (Hsp60) in childhood asthma

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Abstract: Background: Asthma is the most common chronic inflammatory disease in childhood. Chlamydophila pneumoniae (C. pneumonia) infection has been associated with bronchial hyperreactivity, new onset asthma, acute intermittent asthma, chronic asthma and asthma severity. Some studies have shown marked anti-Hsp60 seroreactivity in an exacerbation of culture-proven persistent C. pneumoniae lung infection and suggested that an allergic reaction to Hsp could produce pulmonary symptoms. **Aim of study:** This cross sectional case-control study aimed at evaluating the serological evidence of C. pneumoniae infection and its heat shock protein 60 (Hsp60) on airflow limitation and asthma severity in asthmatic children. **Methods:** We evaluated 150 asthmatic children, 84males (56%) and 66 (44%) females, their mean age was 7 ± 2.8 years, 45 with acute exacerbations representing 30% and 105 with chronic stable asthma representing 70%. They were investigated for C. pneumoniae IgG and Hsp60 after PEFR (peak expiratory flow rate) and PEFR % were done as standardized for children on dynamic spirometry **(Jaeger, Germany)** device. Patients were attending the pediatric asthma and allergy clinic as well as patients admitted inpatient in pediatric department of Benha university hospitals. Fifty age and sex-matched healthy children were included as control group in our study. **Results:** There was a highly significant correlation between C. pneumoniae positive IgG and both asthma duration and asthma grade. While there was a very highly significant correlation with peak expiratory flow rate %. Regarding Hsp60 there was a significant correlation between positive Hsp60 and asthma grade. While there was a very highly significant correlation with asthma duration and peak expiratory flow rate %. **Conclusions:** This study provides serological evidence that chronic infection with C.pneumoniae is present more in children with asthma than healthy children. Our results support positive correlation of asthma duration and severity to chronic infection with C.pneumoniae. C. pneumoniae Hsp60 has an association with the degree of airway obstruction in asthmatic children.

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Key words: childhood asthma; chlamydophila pneumoniae; Hsp60.

1. Introduction

Asthma is the most common chronic inflammatory disease in childhood. Worldwide, approximately 40% of all young children have at least one episode of asthmatic symptoms like wheezing, coughing, and dyspnea.[1] Assertions that bacterial infections may have a role in the pathogenesis of asthma, both acute and chronic, are much more controversial.[2] Of bacterial respiratory pathogens, the atypical bacteria Chlamydophila pneumoniae and Mycoplasma pneumoniae are most commonly implicated in each of these contexts. [3] C. pneumoniae infection has been associated with bronchial hyperreactivity,[4] new onset asthma, [5] acute intermittent asthma [6], chronic asthma [7] and asthma severity.[8] In 2008, a study by **Hahn and Peeling**[9] suggested that host responses to heat shock protein 60 may trigger autoimmune or inflammatory responses, resulting in immunopathologic abnormalities leading to scarring in these Chlamydia trachomatis diseases which is associated with seroreactivity against 60 KDa C. trachomatis (Hsp60). Some studies have shown marked anti-Hsp60 seroreactivity in an exacerbation of culture-proven persistent C. pneumoniae lung infection and suggested that an allergic reaction to Hsp could produce pulmonary symptoms. The highly conserved nature of these proteins raises the possibility of molecular mimicry and generation of an autoimmune response as an additional factor in immunopathogenesis. 10 A study by **Huittinen et al. (2001)**[13] showing that airflow limitation in asthma is associated with seroreactivity against the whole C. pneumoniae Hsp60 molecule raises the possibility that a similar pathologic mechanism exists for C. pneumoniae associated asthma.

Aim of the work: Objectives

- Study the serological evidence of C. pneumoniae infection in asthmatic children as well as control children.
- Find an association of C. pneumoniae infection with severity of asthma.

- Evaluate level of serum C. pneumoniae heat shock protein 60 (Hsp60), its seroreactivity and its impact on airflow limitation and asthma severity in asthmatic patients.

2. Patients and Methods:

Study population and design:

This was a case-control study of 200 children conducted in the period between July 2010 and August 2012. We evaluated 150 asthmatic children, 84males (56%) and 66 (44%) females, their mean age was 7 ± 2.8 years, 45 with acute exacerbations representing 30% and 105 with chronic stable asthma representing 70%. They were investigated for C. pneumoniae IgG and Hsp60. Patients were attending the pediatric asthma and allergy clinic as well as patients admitted inpatient in pediatric department of Benha university hospitals. Fifty age and sex-matched healthy children were included as control group in our study. Parental informed consent was obtained for every child before enrolment in the study. The pediatric Department Board ethically approved the study.

All children were subjected to the following:

Clinical assessment:

1- Full history taking and clinical examination.
2- Pulmonary function test: PEFR (peak expiratory flow rate) and PEFR % were done as standardized for children on dynamic spirometry **(Jaeger, Germany)** device, used in asthma and allergy clinic of pediatric department of Benha university hospitals.
3-Grading of asthma severity: according to ***GINA guidelines (2011).***
4- Chest X ray: to rule out other causes of chest diseases as pulmonary T.B and pneumonia.

Laboratory assessment:

1- Complete blood picture with differential white blood cells count and absolute eosinophilic count.
2- Urinalysis and stool analysis to exclude parasitic infestation.
3- Serologic analysis:

Venous blood samples were obtained for serologic evidence of previous infection by C. pneumoniae, as defined by serum IgG titer of ≥ 1:16 purified elementary bodies of C. pneumoniae by ELISA. Venous blood sample (3-5 ml) withdrawn and centrifuged to get serum. Serum samples were stored deep- frozen (-70 to -20 °C). Samples were mixed well before testing. All samples were diluted 1+100 with IgG sample diluent. 10µl sample and 1 ml IgG sample diluent were dispensed into tubes to obtain a 1+100 dilution and thoroughly mixed with a vortex. Samples are considered positive if the absorbance value is higher than 10% over the cut-off. The children with positive IgG provided a serum specimen that was tested by means of ELISA for antibodies against the whole-molecule chalmydial Hsp60 dervied from C. pneumoniae.

3. Results:

Table (1) shows comparison between cases and controls (regarding age, sex, residence, family size, family history of asthma, height, weight, body mass index, peak expiratory flow rate and peak expiratory flow rate %) with highly significant positive difference regarding family history of asthma, body mass index, PEFR and PEFR%.

Table (2) shows number and percentage of IgG positive results in cases and controls, with highly significant difference between positive asthmatics and controls.

Table (3) shows number and percentage of Hsp60 positive results in asthmatics and controls, with significant difference between positive asthmatics and controls. While, there was a highly significant difference between positive and negative children in each group.

Table (4) compares asthma exacerbation and chronic stable asthma in Chlamydophila positive and negative cases showing highly significant association between positive Chlamydophila IgG and acute exacerbation of asthma. While, there was a significant association between negative Chlamydophila IgG and chronic stable asthma.

Table (5) compares Hsp60 positive and negative results in asthma exacerbation and chronic stable asthma, showing significant association between positive serum Hsp60 and acute exacerbation of asthma and significant difference between positive Hsp60 results in the two groups of asthma.

-Table (6) shows highly significant correlation between C. pneumoniae positive IgG and both asthma duration and asthma grade. While there was a very highly significant correlation with peak expiratory flow rate %.

Table (7) shows significant correlation between positive Hsp60 and asthma grade. While there was a very highly significant correlation with asthma duration and peak expiratory flow rate %.

Table (1): Comparison of clinical data in asthmatics and controls:

Variable	Cases (No = 150)	Controls (No = 50)	test of significance		p-value
			Z	t	
Age (yrs.):					
Mean ± SD	7 ± 2.8	7.4 ± 2.9		0.86	> 0.05
Range	3.67-14.1	4.2-13.9			
Sex:					
Male	84 (56 %)	30 (60 %)	0.49		> 0.05
Female	66 (44 %)	20 (40 %)			
Residence:					
Rural	103 (68.7 %)	36 (72 %)	0.52		> 0.05
Urban	47 (31.3 %)	14 (28 %)			
Family size:					
Small (3 members)	31 (20.7%)	13 (26%)	0.78		> 0.05
Medium (4-6 members)	93 (62%)	27 (54%)	1		> 0.05
Large (≥ 7 members)	26 (17.3%)	10 (20%)	0.42		> 0.05
Family history of asthma:					
Positive	116 (77.3 %)	9 (18 %)	7.5		< 0.001**
Negative	34 (22.7 %)	41 (82 %)			
Height (cm.)**:**					
Mean ± SD	112.07 ± 15.70	116.8 ± 16.30		1.9	> 0.05
Range	92 - 145	99 - 140			
Weight (kg.**):**					
Mean ± SD	23.11 ± 6.97	24.13 ± 7.12		0.9	> 0.05
Range	15 - 41	17 - 45			
BMI (kg/m2**):**					
Mean ± SD	18.104 ± 1.98	19.185 ± 2.01		3.1	< 0.01**
Range	15.36 - 27.33	16.45 - 28.32			
PEFR (L/min.):					
Mean ± SD	176 ± 54	256.9 ± 78.54		8.1	< 0.001**
Range	90 - 300	200 - 320			
PEFR% :					
Mean ± SD	78 ± 9	96 ± 11		11.5	< 0.001**
Range	51 - 92	93 - 98			

p-value is significant if <0.05*; p-value is highly significant if < 0.01**; p-value is very highly significant if < 0.001**

Table (2): Results of Chlamydophila IgG in asthmatics and controls:

Variable	IgG positive		IgG negative		Z	P
	No	%	No	%		
Asthmatics (No = 150)	73	48.6	77	51.4	0.2	> 0.05
Controls (No = 50)	11	22	39	78	3.8	< 0.01**
Z	3.3					
P	< 0.001**					

Table (3): Results of Heat shock protein 60 in asthmatics and controls:

Variable	Hsp60 positive		Hsp60 negative		Z	P
	No	%	No	%		
Asthmatics (No = 150)	27	18	123	82	7.7	< 0.001**
Controls (No = 50)	3	6	47	94	6	< 0.001**
Z	2.05					
P	< 0.05*					

Table (4): Comparison between Chlamydophila IgG results among acute exacerbation and chronic stable asthma groups:

Variable	+ ve (73)		- ve (77)		Z	P
	No	%	No	%		
Acute exacerbation (No = 54)	35	64.8	19	35.2	2.9	< 0.01**
Chronic stable (No = 96)	38	39.6	58	60.4	1.9	< 0.05*
Z	2.9					
P	< 0.01**					

Table (5): Comparison between Hsp60 results among acute exacerbation and chronic stable asthma groups:

Variable	Hsp60 + ve (No = 27)		Hsp60 – ve (No = 46)		Z	P
	No	%	No	%		
Acute exacerbation (No=54)	18	33.3	17	31.5	2.3	< 0.05*
Chronic stable (No=96)	9	9.4	29	30.2	7.8	< 0.001**
Z	2.1					
P	< 0.05*					

Table (6): Correlations between occurrence of positive Chlamydophila IgG and asthma parameters:

Asthma parameters	(r)	p
Duration of asthma (in years)	0.2795	< 0.01**
Grade of asthma	0.25462	< 0.01**
Peak expiratory flow rate %	- 0.31132	< 0.001**

Table (7): Correlation between occurrence of positive Hsp60 and asthma parameters:

Asthma parameters	(r)	p
Duration of asthma (in years)	0.37836	< 0.001**
Grade of asthma	0.2200	< 0.05*
Peak expiratory flow rate %	- 0.54214	< 0.001**

4. Discussion:

Our work aimed at studying the role of C. pneumoniae infection in childhood asthma. On the basis of C. pneumoniae specific serology we assessed the influence of Chlamydophila infection on asthma. The number of asthmatic children with positive IgG was seventy three (48.6% of all cases); of them the cases with positive Hsp60 were twenty seven (18%). And control children with positive IgG were eleven (22% of all control children); of them the control children with positive Hsp60 were three (6%). This may suggest that C. pneumoniae infection confirmed by IgG and Hsp60 is more predominant in patients with asthma than in healthy controls. So, this difference in incidence of C. pneumoniae infection between the asthmatic and control children was statistically significant. These results agree with **Agarwal and Chander** [(12)] who found a significantly higher seroprevalence of C. pneumoniae specific IgG antibodies in patients with asthma than in similar control subjects (80% vs 59%). In our study we found that positive C. pneumoniae IgG and positive Hsp60 are associated with both chronic stable (positive IgG in 39.6% and positive Hsp60 in 9.4%) and acute exacerbation of asthma (positive IgG in 64.8% and positive Hsp60 in 33.3%) in asthmatic children .This finding is in agreement with a study done in Iran by **Torshizi and et al.,** [(13)] who concluded that positive results of C. pneumoniae were associated with both chronic stable (35%) and acute exacerbation (47.6%) of asthma in adults using cell culture. In our study we found an association between C. pneumoniae infection and duration of asthma as we found that, the mean asthma duration in C. pneumoniae IgG positive cases was 5.2 ± 4.9 years while in IgG negative cases the mean asthma duration was 3.8 ± 1.9 years (p< 0.01). These results agree with two earlier studies on adults showed that elevated IgG antibody levels to C. pneumoniae were most prominent in asthmatic patients with a long

duration of disease [14&15]. So, our findings confirm and extend these data on children by showing that incidence of IgG positivity to C. pneumoniae in long standing asthmatics is significantly higher than in recent onset asthmatics. In our study we found a significant association between positive C. pneumoniae IgG and asthma severity where we found that 30 out of 41 cases of mild persistent asthma have positive IgG ($p< 0.05$) and all cases (11 out of 11cases) of severe persistent asthma have positive IgG ($p< 0.01$), which is in agreement with several reports suggesting a relationship between severity of asthma and C. pneumoniae infection of the airways [5&6]. In our study we found that Hsp60 positive cases were 18% (27 out of 150) of asthmatics compared to 6% (3 out of 50) of controls ($p< 0.05$). So, asthmatic children were more likely to have detectable positive Hsp60 than controls. This agrees with **Yang et al.,** [16] who found that; Compared to controls, asthma patients have detectable positive Hsp60 (17.2% vs 5.1%) ($p\leq 0.001$).This finding also is in agreement with **Hahn and Peeling** [9] who reported that twenty-seven percent of asthmatic patients were C. pneumoniae Hsp60 seropositive versus eight percent of controls ($P < 0.01$). In our study, Hsp60 positive asthmatic children have lower PEFR% (67.3%) as compared to Hsp60 negative cases (97.7%). The difference was highly significant ($p< 0.001$) suggesting an association between positive Hsp60 and the severity of pulmonary obstruction. These results are in agreement with **Huittinen** [17], who reported that serum antibodies to Chlamydophila pneumoniae Hsp60 were inversely correlated with pulmonary function, as measured by FEV_1, suggesting an association with the severity of pulmonary obstruction. The association of positive C. pneumoniae Hsp60 with both asthma and the severity of pulmonary obstruction support the possibility that the Hsp60 may play a role in the pathogenesis of bronchial hyperreactivity and/or pulmonary obstruction. In our study, there was a highly significant statistical difference between Hsp60 positive and negative asthmatics regarding asthma duration as we found that, the mean asthma duration in Hsp60 positive cases was 6.4 ± 2.7 years, while in Hsp60 negative cases the mean asthma duration was 4.4 ± 2.22 years ($p< 0.01$). To our knowledge, there was no previous study discussed the correlation between Hsp60 positivity and asthma duration. In our study, controlling for age and sex, Hsp60 seropositivity was associated with lower PEFR% (67.3 ± 13.06) compared to Hsp60 seronegativity ($78.7\% \pm 6.8$) showing highly significant statistical difference ($p < 0.01$). This agrees with **Hahn and Peeling** [9] where Chlamydophila pneumoniae Hsp60 seropositivity was associated with lower forced expiratory volume in 1 second (FEV1) (66.8 ± 23.2) in asthmatic patients compared to Chlamydophila pneumoniae Hsp60 seronegativity (74.1 ± 19.4) showing significant statistical difference ($P < 0.05$). In our study, we found a significant difference between Hsp60 positivity in asthma exacerbated group and chronic stable group (33.3% vs 9.4%) ($p < 0.05$). Also, there was a highly significant positive difference between asthma exacerbated group and healthy control children (33.3% vs 6%) ($p < 0.01$). But, there was no significant difference between chronic stable asthmatics and healthy control children (9.4% vs 6%) ($p > 0.05$). In other studies, Hsp60 was shown to either inhibit or exacerbate asthma through different mechanisms. However, the deleterious effects of Hsp60 on asthma likely surpassed any beneficial inhibitory effects [18].

Conclusion:

This study provides serological evidence that chronic infection with C.pneumoniae is present more in children with asthma than healthy children.

Our results support positive correlation of asthma duration and severity to chronic infection with C.pneumoniae. C.pneumoniae Hsp60 has an association with the degree of airway obstruction in asthmatic children.

Recommendations:

We recommend further multicenter studies on a larger scale of asthmatic children to explain the role of C. pneumoniae and its Hsp60 in asthma because this protein can be expected to be developed into a new treatment target for asthma.

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