Original Article

Study the Association between Interleukin-4 Polymorphism and Specific *Chlamydia Pneumoniae* Immunoglobulin E in Asthmatic Children

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Abstract

Background: Asthma is a chronic disorder caused by complex interactions between genetic and environmental influences. **Objective:** Investigate the possible correlation between interleukin-4 *(*IL-4) gene polymorphisms and development of specific *Chlamydia pneumoniae* immunoglobulin (Ig) E levels. **Materials and Methods:** A total of 87 children, including 57 males and 30 females with asthma with ages between 1 and 16 years, attended the Respiratory Clinic at Karbala Pediatric Hospital, with a nonasthmatic children group which have the same age and gender. Restriction fragment length polymerase chain reaction was performed to determine IL-4 C-589T genetic polymorphisms. Total IgE level, *C. pneumoniae* IgG, and *C. pneumoniae* IgE antibodies were measured using the commercial quantitative enzyme-linked immunosorbent assay kits. **Results:** *C. pneumoniae* IgG and IgE antibodies were significantly increased in patients as compared with controls ($P < 0.001$ and $P = 0.024$, respectively). The CT genotype of IL-4 C-589T polymorphism was associated with asthmatic children ($P < 0.01$). A high *C. pneumoniae* IgE levels were found to be associated with CC genotypes ($P = 0.01$). On the other hand, there were no significant differences in serum *C. pneumoniae* IgG levels depending on different IL-4 C589T genotypes ($P = 0.662$). **Conclusion:** The development of *C. pneumoniae* IgE antibody in asthmatic children did not depend on IL-4 polymorphism.

Keywords: Asthma, *Chlamydia pneumonia*, immunoglobulin E, polymorphism

Introduction

Asthma is a condition that is likely caused by complex interactions between multiple genetic and environmental influences.[1] Risk factors are associated with asthma mortality and morbidity such as exposure to environmental triggers, low-income households, chronic stress, child psychological problems, parental stress, obesity, physical inactivity, and unhealthy diets.[2] Respiratory tract viruses have emerged as the most frequent triggers for exacerbations in children and adults.[3,4] Further, infections with atypical bacteria appear to play a role in the induction and exacerbation of asthma in children and adults.[5] The role of bacterial infection in asthma is varied in that it may contribute to the initial development of the clinical onset of asthma or exacerbate established asthma.[6] Bacterial organisms can increase inflammation and airway hyperresponsiveness in a patient with known asthma.[7]

Chlamydia pneumoniae and *Mycoplasma pneumoniae* are responsible for chronic inflammation and have been implicated in the pathogenesis of asthma when the host immune system fails to eradicate the bacteria.[8] Repeated infections with *C. pneumoniae* may induce Th1-type and Th2-dominant cytokine responses in the airways of neonatal mice. More significantly, this infection elicits pathogen-specific immunoglobulin (Ig) E production, which differentially affects the development of key features of allergic airway disease and asthma phenotype in the adult.[9-11] The specific IgE generated and sustained by *C. pneumoniae* chronic infection may be one of several mechanisms that contribute to asthma pathogenesis.[12] In addition, several gene polymorphisms have been associated

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with susceptibility to asthma and allergy.[13] Some genes may influence the development of asthma, whereas others modify asthma severity or the patient's response to therapy.[14] Several studies suggested that the 589T allele in rs2243250 is associated with increased serum or plasma interleukin-4 *(*IL-4) levels and is linked to total serum IgE levels.

The genetic variation may differ in a participants response to environmental exposure. Unfortunately, no previous studies locally or internationally detected the influence of IL-4 polymorphism on the immune response to *C. pneumoniae* infection. Thus, this research was done to notice, if IL-4 polymorphism can alter asthmatic patients' response to *C. pneumoniae* infections. This work aimed to investigate the possible correlation between IL-4 gene polymorphisms and development of specific *C. pneumoniae* IgE levels.

Materials and Methods

Study design and sample size

It is a case–control study that involved 87 asthmatic children (57 males and 30 females) attending the asthma Clinic at Karbala Teaching Hospital for Children in the period extending from January 2022 to May 2022 and 87 nonasthmatic children (46 males and 39 females). The nonasthmatic children (control) had the age and sex of asthmatic children randomly selected from the local community. All asthmatic children had the European Respiratory Society/American Thoracic Society criteria for asthma.[15] All asthmatic and nonasthmatic participants underwent a total serum IgE test and excluded asthmatic patients with a total IgE concentration of < 100 IU/ml from the study. The participants ages in this study ranged from 1 to 16 years.

Ethical approval

The study was approved by the Research Ethics Committee of Babylon University, a Medical College in Babylon, Iraq (2022-05/12).

Patient consent form

The participants who revealed readiness to participate in this study were supplied with written informed consent and verbal information regarding the aim of the study.

Sample collection and processing

Sera were collected from each participant; sera were used to determine the total serum IgE levels for all samples by Combiwash Max-Planck-Ring 21 automated immunoassay analyzer (Human, Germany) using AccuBind total IgE enzyme-linked immunosorbent assay (ELISA) kit (LOT NO.25K1D1). IgG antibody plasma levels against the *C. pneumoniae* (Human *C. pneumoniae* IgG, Cpn IgG ELISA Kit, LOT NO.20220426, SUNLONG, China) and IgE antibody plasma levels against the *C. pneumoniae* (Human *C. pneumoniae* IgE, Cpn IgE ELISA Kit, LOT NO.20220426, SUNLONG, China) were measured to all participants using commercial quantitative ELISA kits in an automated instrument (Combiwash Max-Planck-Ring 21).

Genotyping

Genotyping of interleukin-4 C-589T polymorphism

The genomic DNA was extracted from the nucleated cells of study groups under the aseptic condition and according to the protocol of FavorPrep™ blood/cultured cells genomic DNA, Favorgen, Taiwan. The extracted DNA was safely stored at − 20°C for later use.

The nucleotide sequences of the forward and reverse primer used for polymerase chain reaction (PCR) are 5-TGG GTA AGG ACC TTA TGG ACC-3 and 5-GGT GGC ATC TTG GAA ACT GT-3, respectively.[16] A PCR mixture included 1 µl of 10 pmol of each specific primer, 12.5 µl of Green Master Mix (Go Taq®, Promega), 6.5 µl of nuclease-free water, and 4 µl of DNA.

The amplification was reformed by including the reaction mix for 35 cycles in a thermocycler. Each cycle consisted of denaturation of DNA at 94°C for 15 s, followed by annealing at 60°C for 30 s, and extension at 72°C for 30 s with an initial delay for 6 min at 94°C at the beginning of the first cycle and 5 min delay at 72°C at the end of the past cycle.

Amplification products were digested with appropriate restriction enzymes (BslF I, Sibenzyme) at optimal temperatures (37°C) for 2 h. The reaction mixture included: 7.5 µl of PCR solution, 1 µl of enzyme buffer, 0.1 µl of Bovine serum albumin (BSA), and 1 U of the restriction enzyme. The reaction products were separated on 1.5% agarose gels with ethidium bromide at 70 V for 150 min and visualized in ultraviolet light. IL-4 restriction enzyme products were distinguished when the DNA band of the sample separated into two bands with a length of 120 bp and 78 bp for the CC genotype, three bands with lengths of 198, 120, and 78 bp for the CT genotype, whereas only one band with a length 198 bp in TT genotype (DNA band was not separated).

Statistical analysis

Data were introduced into a specific Software Statistical Package for the Social Sciences (SPSS) version 21 (San Diego, California, USA) for Windows (GraphPad Software, San Diego, California, USA) for statistical analysis. The results were expressed as mean ± standard deviation comparisons between the two means were performed using the *t*-test. $P < 0.05$ indicates statistical significance and is highly significant if the $P < 0.001$. The Chi-square test is used to compare the two categorical variables. Genotypes of IL-4 C-590T were presented as percentage frequencies, and one-tailed assessed significant differences between their distributions in asthmatic patients and controls's exact probability (*P*). In addition, the odds ratio was also estimated to define the association between a genotype with the disease. Direct gene counting methods calculated allele frequencies of genes.

Results

There was no significant difference ($P > 0.05$) in age and sex between asthmatic patients and healthy controls. On the other hand, there was a highly significant difference ($P < 0.001$)

in total serum IgE levels and *C. pneumoniae* IgG ng/L between asthmatic and healthy children, as shown in Table 1. Further, according to the *t*-test, there were statistically significant changes in *C. pneumoniae* IgE between the two groups ($P = 0.024$).

The statistical analysis, as shown in Table 2, found that the homozygous genotype CC recorded higher frequency (82.75%) in healthy children than in asthmatic patients (64.4%), with a highly significant difference ($P = 0.006$). While the genotype heterozygous CT frequency was significant in patients (34.5%) compared to control (17.24%). The allele frequency for allele C was 81.61% in patients compared with control 91.38%, whereas for the allele T was 18.39% in patients compared with control 8.6% with a highly significant difference ($P = 0.009$).

The study showed that a high *C. pneumoniae* IgE levels were found to be associated with CC genotypes ($P = 0.01$). On the other hand, there were no significant differences in serum *C. pneumoniae* IgG levels depending on different IL-4 C589T genotypes ($P = 0.662$), as shown in Table 3.

Discussion

Asthma is a complex gene–environment interactions.[17] Infections with *C. pneumoniae* appear to play a role in the induction and exacerbation of asthma.[5] One possible interpretation of this induction and exacerbation of asthma is *C. pneumoniae*-specific IgE antibody production. This antibody may be induced by 5 chlamydial antigens (lipopolysaccharide antigen, Crp A, heat shock protein 60, putative outer membrane protein, and 250 kDa).[18] Since there was no evidence of how these chlamydial antigens induce IgE production, whereas there was a clear association between IL-4 polymorphism and increased IgE production in asthmatic patients,[19] thus we performed this study to detect if there is any association between IL-4 polymorphism and induction of *C. pneumoniae*-specific antibody.

This is the first study in Iraq and the world that detect the correlation between IL-4 C589T polymorphism and the presence of *C. pneumoniae* IgG and IgE antibodies in asthmatic children.

The result of this study is consistent with previous studies, which detect specific *C. pneumoniae* antibodies in asthmatic children.[20,21] In addition, the result is consistent with meta-analysis studies, which revealed that IL-4 gene - 589C/T polymorphism was a susceptibility risk for asthma.[22-26]

Our study showed a significant specific *C. pneumoniae* IgE antibody production in asthmatic children compared to healthy children, and this result is also consistent with previous studies.[12] Thus, the main question is what risk factor causes

Table 1: Demographic and clinical parameters of the study participants ($n=87$)

Parameters	Asthmatic patients	Healthy controls	Test	*P*
Age (year), mean±SD	7.833±3.652	7.520±3.658	*t*-test=0.568	0.285
Sex, *n* (%)				
Male	57 (65.52)	46 (52.87)	χ^2=2.326	0.127
Female	30 (34.48)	39 (44.82)		
Total IgE (IU/mL), mean±SD	398.88±227.156	49.34±66.831	*t*-test=12.388	0.000**
C. pneumoniae IgG (ng/L)	24.89±16.65	10.93±6.75	*t*-test=7.252	0.000**
C. pneumoniae IgE (ng/L)	8.037±4.645	6.52±3.062	*t*-test=1.995	0.024*

*Significance level at $P \leq 0.05$, The significant level was intended as **$P<0.0001$. *C. pneumonia: Chlamydia pneumonia*, IgE: Immunoglobulin E, IgG: Immunoglobulin G, SD: Standard deviation

Table 2: Frequencies of genotypes and alleles in the study participants

IL-4 C-589T	Asthmatic patients, *n* (%)	Healthy control, *n* (%)	OR^	*P*	(95% CI)
CC	56 (64.4)	72 (82.75)	0.376	0.006**	0.185–0.764
CT	30 (34.5)	15 (17.24)	2.526	0.01*	1.241–5.141
TT	1 (1.1)	0	3.041	0.49	0.122–75.867
C allele	142 (81.61)	159 (91.38)	0.419	0.009**	0.217–0.805
T allele	32 (18.39)	15 (8.6)			

*Significance level at $P \leq 0.05$, **Highly significance level at $P \leq 0.01$, ^OR with 95% CI. OR: Odds ratio, CI: Confidence interval

Table 3: Association between immunological parameters and genotype of IL-4 C-589T gene in asthmatic children

Parameters (ng/L), mean±SD	IL-4 C-589T genotypes		*t*-test	*P*
	CC ($n=53$; 60.9%)	CT ($n=29$; 33.3%)		
C. pneumoniae IgE	8.555±4.889	6.459±2.403	2.649	0.01*
C. pneumoniae IgG	22.895±10.839	21.834±10.068	0.439	0.662

*Significant difference at $P \leq 0.05$. *C. pneumonia*: *Chlamydia pneumonia*, SD: Standard deviation, IgE: Immunoglobulin E, IgG: Immunoglobulin G

the class switch of *C. pneumoniae* IgG to IgE in asthmatic children.

Naïve human B-cells are stimulated by IL-4 and anti-CD40 to generate IgE.[27] IL-4 concentration regulates the isotype switching of IgE.[28] Several studies suggested that the - 589T allele in rs2243250 is associated with increased serum or plasma IL-4 levels and is linked to total serum IgE levels. The influence and importance of IL-4 C-589T polymorphism is a single nucleotide polymorphism located within cytokine gene promoter regulatory sequences;[16] the promoter mutation is known to cause functionally essential consequences for gene expression.[29] Depending on this, we thought the polymorphism in IL-4 may increase the production of specific *C. pneumoniae* IgE in asthmatic children.

Indeed, asthmatic patients who have the IL-4 - 589T allele showed lower specific *C. pneumoniae* IgE levels compared with asthmatic children with the C allele. The *C. pneumoniae* IgE antibody was at a higher level in the CC genotype [Table 3]. Since the CC genotype was in higher frequency in nonasthmatic children [Table 2]; therefore, the development of *C. pneumoniae* IgE antibody in asthmatic children did not depend on IL-4 polymorphism.

The other molecular mechanisms and genetic polymorphisms may influence IL-4 levels and IgE class switching.[30] Unfortunately, this study estimates only the correlation between IL-4 C589T polymorphism and specific *C. pneumoniae* IgE production. Thus, another study is required to detect the cause of *C. pneumoniae* IgE production in asthmatic children.

Conclusion

In this study, the IgE-mediated asthma in Iraqi children has been associated with the CT IL-4 C589T genotype and the *C. pneumoniae* infections. Indeed, the associated between polymorphism and bacterial infection with asthma is independent, since the development of *C. pneumoniae* IgE antibody in asthmatic children did not depend on IL-4 polymorphism.

Limitation of the study

The present study has some limitations that must be pointed out. First, the study included children attending the asthma clinic at Karbala Teaching Hospital for Children without considering other provinces in other parts of Iraq. Second, the analysis detected IL-4 polymorphism at the C589T site, which may have contributed to the inconsistent understanding of the effect of polymorphism in other locations. Thus, further studies in the same patients and controls are recommended to help understand the combined effect of different single-nucleotide polymorphisms in other sites.

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Conflicts of interest

There are no conflicts of interest.

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