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Frequency of HLA DQβ1*0201 and DQβ1*0301 Alleles and Total Serum IgE in Patients with Bronchial Asthma: A Pilot Study from Pakistan

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ABSTRACT

In Pakistan about 3.7% of the population is suffering from asthma, a chronic inflammatory disorder of airways. Asthma has wide spectrum of predisposing factors including environment and genetics. Many studies have been performed to determine association of asthma with serum IgE and major histocompatibility complex (MHC) alleles but conflicting results were reported. Therefore, present study was designed to determine frequency of HLA-DQβ1*0201 and DQβ1*0301 alleles in patients with bronchial asthma.

This case control study included 85 asthmatic patients and 85 healthy controls. HLA-DQβ1*0201 and DQβ1*0301 alleles were detected by allele specific PCR and serum IgE was determined by ELISA.

Median and inter-quartile range (IQR) of total IgE level were more increased in asthma patients (585.7 IU/mL and 247.2-848.1 IU/mL) compared to healthy controls (65.1 IU/mL and 28.1-181.3 IU/mL) ($p<0.001$). Frequency of HLA-DQβ1*0201 and -DQβ1*0301 alleles was more in healthy controls (32% and 38%, $p=0.616$) as compared to bronchial asthma patients (28% and 26%, $p= 0.09$). There was a significant association of IgE levels and HLA-DQβ1*0201 allele. Patients positive for HLA-DQβ1*0201 allele had low level of serum IgE 357.2 IU/mL (153.9-634.3 IU/mL) compared to the patients negative for this HLA allele i.e. 642.9 IU/mL (289.8-1299.5IU/mL) ($p=0.005$), whereas, HLA-DQβ1*0301 allele was not associated with total serum IgE level ($p=0.865$).

Our findings show that HLA-DQβ1*0201 and -DQβ1*0301 alleles were not associated with asthma; however, HLA-DQβ1*0201 allele was associated with low levels of total serum IgE in the study population.

Keywords: Asthma; HLA-DQβ1; Immunoglobulin E; Human leukocyte antigen (HLA)

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INTRODUCTION

Prevalence of asthma varies widely from 1% to 18% worldwide, with 3.75% affected population in Pakistan.[1] Asthma is a complex multifaceted disease

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with various predisposing factors[2] in which genetics of the individual plays a vital role. Asthma is categorized into extrinsic asthma induced by environmental allergen such as cockroach, fecal fragments of house dust-mite, pollens,[3] and intrinsic asthma induced by non-immune mechanisms that include aspirin ingestion, respiratory infection, cold, stress, and exercise.[4]

Human leukocyte antigens (HLA) are part of the major histocompatibility complex (MHC) with more than 200 genes. There are three types of MHC gene products; MHC class-I, class-II, and class-III.[5] Out of three MHC genes, class II genes are highly polymorphic which determines the particular peptide that they present to CD4+ T cells, thus regulates immune response.[6]

HLA haplotype influence susceptibility to asthma and atopic diseases in 36% to 79% of patients.[7-10] An increased frequency of HLA-DQβ1*06 allele has been reported in Iranian patients with asthma.[11] Percentage of HLA DRβ1*1501-DQβ1*0602-DPβ1*0501 haplotype was documented as 19% and 3.1% in Korean patients with toluene diisocyanate (TDI)-induced occupational asthma and in healthy controls respectively.[12] Decreased allelic percentage of HLA-DQβ1*03 in patients with Alternaria-sensitive 'moderate-severe' asthma as compared to healthy controls was reported.[13] There was no association between DRβ1 and DQβ1 in Slovak bronchial asthma patients.[14] A high frequency of DRβ1*01, DRβ1*03, DQβ1*0201, DQβ1*0302, and DQα1*0501 alleles and low frequency of DRβ1*04 and DQβ1*0603/8 alleles was suggested in Indian asthmatics as compared to healthy controls.[10] Common HLA haplotypes in different asthma phenotypes were HLA-DRB1 in allergic asthma, HLA-DQB1 in occupational asthma and HLA-DPB1 in aspirin-sensitive asthma.[15]

Since there is limited data on HLA association with asthma in Pakistani population, this study was designed to determine frequency of HLA-DQβ1*0201 and DQβ1*0301 alleles in bronchial asthmatic patients who had history of allergy and to compare frequency of these alleles with non-asthmatic controls.

MATERIALS AND METHODS

Sample and data collection

This study is a case-control study that included 85 asthmatic patients and 85 non-asthmatics as controls. The study was performed in the Department of Immunology, University of Health Sciences Lahore (UHS) Pakistan. It was approved by the 'Ethical Review Committee' and 'Advanced Studies Research Board' (No. UHS/ERC-4/11-09-2013) of UHS Lahore. Clinically diagnosed asthmatic patients of 18-40 years of either sex with the history of allergy (validated modified questionnaire of European Community Respiratory Health Survey) were recruited from the Asthma Clinic, Gulab Devi Hospital Lahore after the approval of Ethical review committee. Patients with diabetes and autoimmune disorders were excluded. Normal subjects of 18-40 years of either gender with no history of atopy, asthma, and allergic diseases were randomly selected as control group. Informed written consent was obtained from all the study participants.

Complete blood count (CBC) was performed with Sysmax 1000X-I (Japan) and total serum IgE by enzyme-linked immunosorbent assay (ELISA) Kit (BioCheck, Inc. California, USA) according to the manufacturer's instructions.

HLA typing:

DNA extraction was performed using standard phenol-chloroform method. HLA DQβ1*0201 and DQβ1*0301 alleles were determined by sequence specific primers[17] (Table 1). Optimization of polymerase chain reaction (PCR) was made by positive samples of HLA-DQβ1*0201 and –DQβ1*0301 alleles (Courtesy Dr. Shagufta Khaliq, Department of human genetics, UHS, Lahore, Pakistan). PCR amplifications were carried out in 10 µL reaction mixtures consisting of 2 µL of diluted DNA (25ng/µL), 10× PCR buffer [10 mM Tris-HCl (pH 8.8 at 25°C), 50 mM KCl, 0.08% (v/v) Nonidet P40], 1.5 mM MgCl2, 0.4 µL of mixture containing 100 µM of each dNTP, 5nM of each primer and 1 U of Taq DNA polymerase (Vivantis, USA). Each reaction set was followed by HLA-DQβ1 alleles PCR with positive and negative controls. Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) gene as internal quality control was also used in each set of experiment. Temperature profile consisted of an initial denaturation at 95˚C for 4 minutes, 30 cycles of denaturation at 95˚C for 30 seconds, annealing at 58^{o}C for HLA-DQβ1*0201 and at 56˚C for DQβ1*0301 for 30 seconds, and extension at 72^{0}C for 45 seconds, followed by 10 minutes final extension step at 72^{0}C. After the completion of reaction, 5µL of the PCR reaction product was mixed with 1µL of 6x loading dye (Vivantis, USA) and

Table 1. Sequences of gene primers used for the amplification of HLA alleles and *GAPDH* gene

Sr.no	Allele	Primer	Product size
1.	HLA-DQβ1*0201-F	5'GTGCGTCTTGTGAGCAGAAG3'	206bp
	HLA-DQβ1*0201-R	5'TGCAAGGTCGTGCGGAGCT3'	
2.	HLA-DQβ1*0301-F	5' GCCGCTGGGGCCGCCTGA3'	123bp
	HLA-DQβ1*0301-R	5' TGCAAGGTCGTGCGGAGCT3'	
3.	GAPDH-F	ACCACAGTCCATGCCATCAC	453bp
	GAPDH-R	TCCACCACCCTGTTGCTGTA	

loaded on to 1.8 % Tris-acetate EDTA (TAE)agarose gel. Five[5] µL of ladder (50bp size marker) (Invitrogen, USA) was also loaded as standard marker. Gel was electrophoresed at 80 V for 40 minutes or till it covered 2/3 of the distance from wells, then observed under ultra violet (U.V) light using GelDoc system (BioRad, USA).

Statistical Analysis

Data were entered and analyzed using IBM SPSS statistics for windows version 22.0 (Armonk, NY: IBM Corp., USA). Mean ± SD were reported for quantitative variables where data was normally distributed. Median and interquartile range (IQR) were reported for quantitative variables where data was not normally distributed; while frequency and percentage for qualitative variables. Kolmogorov-Smirnov and Shapiro-Wilk test were applied to determine normality of data, student *t-test* and Mann-Whitney test to observe group mean differences where data were normally distributed and where data were not normally distributed respectively. A *p* value of < 0.05 was considered as statistically significant. False discovery rate (FDR) was calculated for multiple comparisons by Benjamini and Hochberg method and *p*-values <0.025 were deemed significant after Benjamini and Hochberg adjustment.[17]

RESULTS

Among 85 bronchial asthma patients there were 23 (13.53 %) males and 62 (36.47%) females, 85 healthy controls comprised of 31 (18.24%) males and 54 (31.76%) females. In comparison the gender difference was not significant between patients and controls (*p*=0.18). Mean ±SD of age of the patients was higher (28.7±7.6 years) as compared to controls (27.9±4.6 years) but this difference was not statistically significant (*p*=0.38).

Median (IQR) of total leukocyte count (TLC), eosinophil percentage, eosinophil absolute count, basophil percentage and basophils absolute count of asthma patients were significantly higher compared to controls (*p* <0.001). Mean ± SD of Hemoglobin (Hb) and lymphocyte percentage of controls were higher compared to asthma patients which was statistically significant; however after FDR it was not significant (*p*=0.04 and 0.03 respectively). The difference of percentage of neutrophil and monocyte between asthma patients and healthy controls was not significant, *p*=0.877 and 0.442 respectively (Table 2).

Family history of asthma was present among 42 (49.41%) of asthmatic patients, while 19 (11%) of healthy controls. Among asthmatic patients, 24 (28.2%) had HLA-DQβ1*0201 and 22 (25.9%) had DQβ1*0301 allele while in controls, 27 (31.8%) had HLADQβ*0201 and 32 (37.6%) had DQβ1*0301 allele (Figure 1 and 2). In comparison the difference between two groups was not statistically significant (*p*=0.616 and *p*=0.09 respectively).

The median and IQR of total IgE level was statistically significant higher in asthma patients 585.7IU/mL (247.2-848.1IU/mL) as compared to healthy controls 65.1IU/mL (28.1-181.3IU/mL) with (*p* <0.001) (Table 3). Total serum IgE was significantly higher in HLA-DQβ1*0201 negative patients 642.9 IU/mL (289.8-1299.5IU/mL) comparing to HLA-DQβ1*0201 positive patients 357.2 IU/mL (153.9-634.3 IU/mL) (*p*=0.005), whereas there was no significant difference of total IgE serum levels in HLA-DQβ1*0301 negative patients 598.5 IU/mL (236.4-932.6 IU/mL) in relation to HLA-DQβ1*0301 positive patients 517.7 IU/mL (277.3-736 IU/mL) (*p*=0.865) (Table 4).

Table 2. Comparison of complete blood count (CBC) indices between patients of asthma and controls

Parameter	Asthma Patients (n=85)	Controls (n=85)	*p* value
Hb (g/dl) mean±SD	13.4±1.4	13.9±1.8	0.04
TLC×10^9/L Median(IQR)	9.9(7.4-12.5)	7.4(6.1-8.2)	<0.001*
Neutrophil % Median(IQR)	57.8(46.6-67.2)	57.1(54.4-61.4)	0.877
Lymphocyte% mean±SD	29.4 ± 8.3	37.8±5.1	0.03
Monocyte % Median(IQR)	8.9(7.3-10.7)	8.3(6.9-9.6)	0.442
Eosinophil % Median (IQR)	7.9(5.4-12.1)	2.3 (1.3-3.7)	<0.001*
Eosinophil Abs (×10^9/L) Median (IQR)	0.7(0.5-0.9)	0.15(0.08-0.3)	<0.001*
Basophil % Median (IQR)	0.5(0.3-0.9)	0.2(0.1-0.4)	<0.001*
Basophil Abs. (× 10^9/L) Median (IQR)	0.03(0.01-0.04)	0.02(0.01-0.03)	<0.001*

Abs: Absolute Count, %: percentage, IQR: interquartile range, Hb: Hemoglobin, TLC: Total leukocyte count, **p*-values less than 0.025 were deemed significant after Benjamini and Hochberg adjustment.

Table 3. Comparison of HLA-DQβ1 alleles and total serum IgE concentration between asthma patients and controls

Parameter	Asthma patients (n=85)	Controls (n=85)	*p* value	Odd Ratio (95% CI)
HLA-DQβ1*0201	24 (28.2 %)	27 (31.8 %)	0.616	0.787 (0.40-1.53)
HLA-DQβ1*0301	22(25.9%)	32(37.6%)	0.099	0.562(0.29-1.09)
Total IgE Conc. (IU/mL) Median(IQR)	585.7(247.2-848.1)	65.1(28.1-181.3)	<0.001*	----------

CI: Confidence interval, HLA-DQβ1: Human leukocyte antigen-DQ gene beta one,

Conc.: Concentration, IgE: Immunoglobulin E, IU/mL: International Units per milliliter

**p* value<0.05 statistically significant

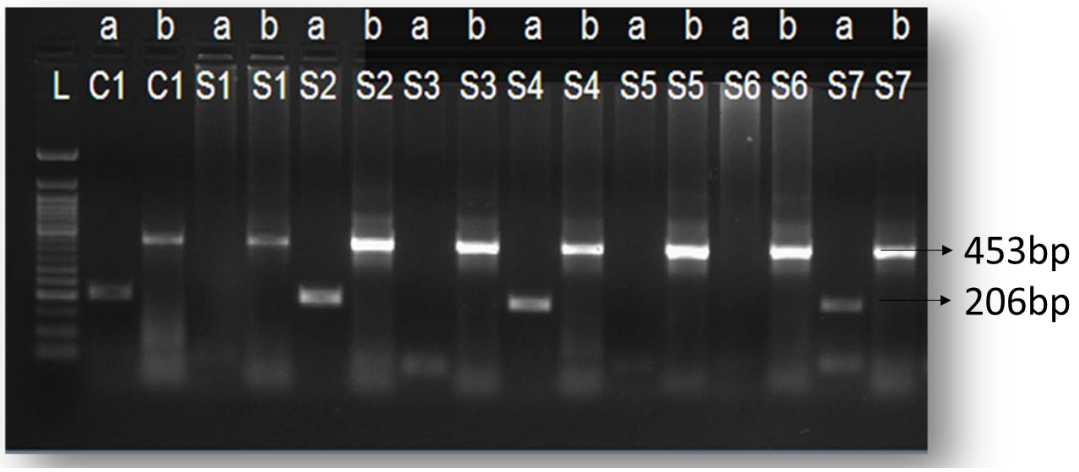

Figure 1. Amplification of HLA-DQβ1*0201 allele and glyceraldehyde 3-phosphate dehydrogenase *(GAPDH)* as internal control through PCR to study the frequency of HLA-DQβ1*0201 allele in asthma patients and healthy controls. Amplified products were run on 1.8% TAE agarose gel for 60 min. the samples loaded on the gel are as follows; lane 1 (L): 50 bp DNA marker, Lane 2-3: amplified positive control DNA samples of HLA-DQβ1*0201 with 'a' showing amplified HLA-DQβ1*0201 fragment of 206bp and 'b' showing *GAPDH* amplified product of 453bp, Lane 4-17: DNA samples of different asthma patients S1-S4 and healthy controls S5-S7 where 'a' showing the presence or absence of HLA-DQβ1*0201 allele as amplified fragment of 206bp and 'b' showing the amplified fragment of *GAPDH* as an internal control.

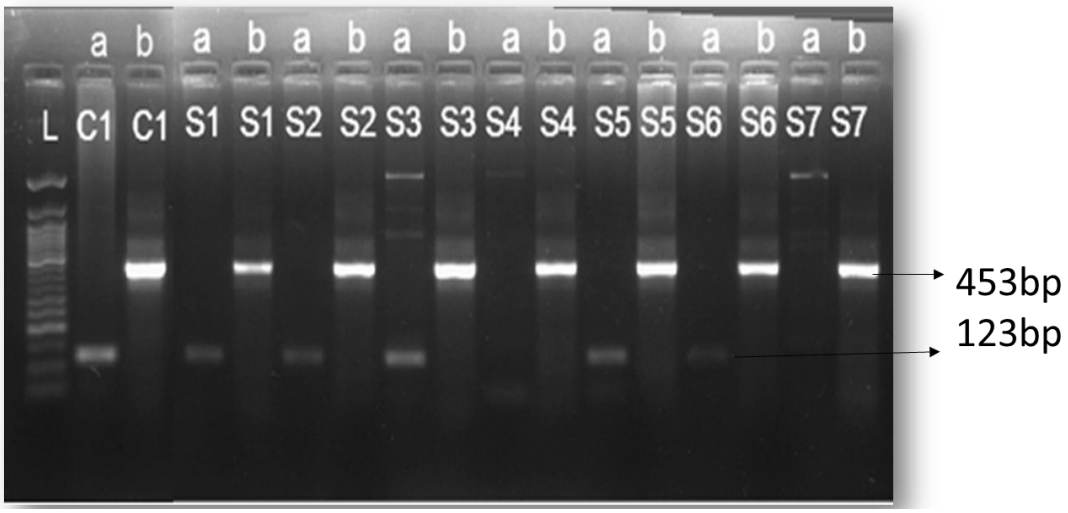

Figure 2. Amplification of HLA-DQβ1*0301 allele and glyceraldehyde 3-phosphate dehydrogenase (*GAPDH)* as internal control through PCR to study the frequency of HLA-DQβ1*0301 allele in asthma patients and healthy controls. Amplified products were run on 1.8% TAE agarose gel for 60 min. the samples loaded on the gel are as follows; lane 1 (L): 50 bp DNA marker, Lane 2-3: amplified positive control DNA samples of HLA-DQβ1*0301 with 'a' showing amplified HLA-DQβ1*0301 fragment of 123bp and 'b' showing *GAPDH* amplified product of 453bp, Lane 4-17: DNA samples of different asthma patients (S1-S4) and healthy controls (S5-S7) where 'a' showing the presence or absence of HLA-DQβ1*0301 allele as amplified fragment of 123bp and 'b' showing the amplified fragment of *GAPDH* as an internal control.

Table 4. Comparison of total serum IgE concentration in asthma patients with and without HLA-DQ*β1* alleles

Parameter	Asthma Patients (n=85) Total IgE Conc.(IU/mL) Median (Interquartile range)	*p* value	Odds Ratio (95% CI)
HLA-DQβ1*0201 Positive	357.2(153.9-643.3)	0.005*	2.89 (0.96-8.72)
HLA-DQβ1*0201 Negative	642.9(289.8-1229.5)		
HLA-DQβ1*0301 Positive	517.7(277.3-736)	0.865	0.86 (0.25-2.96)
HLA- DQβ*0301 Negative	598.5(236.4-932.6)		

Conc.: Concentration, HLA-DQ*β1*: Human leukocyte antigen-DQ gene beta one, IgE: Immunoglobulin E, IU/mL: International Units per milliliter, **p* value<0.05 statistically significant

DISCUSSION

In the current study, percentages of HLA-DQβ1*0201 and HLA-DQB1*0301 alleles were less in asthma patients compared to healthy controls but this difference was not statistically significant. This finding indicates that HLA-DQβ1*0201 and DQβ1*0301 alleles were neither a risk nor a protective factor for bronchial asthma. In contrast to our results, Madore et al have reported that DQβ1*0601 allele and allele DQβ1*0201 are associated with susceptibility and protection against peanut allergic asthma respecitvely.[18] A Korean study described percentage of HLA-DQβ1*0301 allele in aspirin-induced asthma patients as 13.2% and in healthy controls as 15.1% (p >0.05).[19] HLA DQ β1*0201 was more common in healthy

controls comparing to Indian pediatric asthma patients, which was not however statistically significant.[20] Frequencies of HLA-DQβ1*0201 and DQβ1*0301 alleles in Iranian pediatric allergic asthma patients were suggested as 18% and 22% and in healthy controls as 19% and 23% (p=1 and 0.9) respectively.[11] There were equal frequency of HLA-DQβ1*02 allele in Korean TDI-induced occupational asthma patients and healthy controls.[12] The current study confirmed the findings of the above mentioned studies, reporting that there was a high frequencies of HLA-DQB1*0201 and DQβ1*0301 in healthy controls.

In contrast to the current study, there are a number of studies, which have reported high frequencies of different HLA alleles in asthma patients. Gao et al reported increased frequency of DQβ1*0201 in Chinese asthmatics sensitive to common allergens ($p < 0.01$) and an increased frequency of DQβ1*0301 in controls ($p < 0.05$).[21] Kim et al observed increased percentage of DQβ1*02 allele in aspirin-induced Korean asthma patients ($p> 0.05$).[19] Another study reported insignificant increased frequency of HLA-DQβ*0301 allele in TDI-induced Korean occupational asthma patients in relation to healthy controls ($p> 0.05$).[12] The discrepancy between different studies and the current study could be due to asthma against specific antigen and the difference in target alleles.

In the current study, total serum IgE was higher in asthma patients, which is due to allergy as bronchial asthma patients with the history of allergy were also included in the study. The results are in agreement with the study of Sandeep et al who suggested raised IgE in bronchial asthma patients [22] and An et al who documented high level of IgE in pediatric asthmatics with sickle cell disease.[23] Johansson et al also reported increased serum level of total IgE in severe asthmatics due to common allergens.[24] Demirjian et al also reported increased total serum IgE in atopic asthma patients (based on history of allergy).[25] Raised serum IgE has been detected in parasitic and non-parasitic infections, atopic diseases, inflammatory diseases, hematologic malignancies, cutaneous diseases, cystic fibrosis, nephritic syndrome, and primary immunodeficiency diseases.[26,27]

In the current study, total serum IgE of HLA-DQβ1*0201 negative patients was significantly high compared to HLA-DQβ1*0201 positive patients whereas total serum IgE in HLA-DQβ1*0301 negative patients was not significantly high as compared to HLA-DQβ1*0301 positive patients. The results regarding frequency of HLA-DQβ1*0201 is in agreement with Movahedi et al., who suggested increased serum total IgE in DQβ1*0201 negative Iranian asthma patients.[11] However, is not in accordance with Movahedi et al regarding DQβ1*0301 the present study, as they reported an association of DQβ1*0301 with high serum level of total IgE in asthmatics.[11] This difference may be attributed to genetic variability of the studied subjects in two populations. Parapanissiou et al reported DQB1*0301-4 as susceptible alleles for raised level of total serum IgE in bronchial asthma of Greek children (p=0.0006).[28] Moffatt et al reported significant association of asthma, HLA-DQ, and HLA-DRβ1 with total IgE.[29] Our finding regarding frequency of DQβ1*0301 is in agreement with Lama et al who also documented no association of high total serum IgE with DQβ1*0301.[10]

On the basis of this study, we conclude that there was no significant difference in the frequency of HLA-DQβ1*0201 and HLA-DQβ1*0301 alleles in asthmatics and controls suggesting that these alleles may not be associated with asthma in our population. But a significant association of HLA-DQβ1*0201 allele, with low total serum level of IgE was observed. The major limitation of the current study is smaller sample size and analysis of only two HLA alleles in asthmatic patients. It is therefore suggested to conduct a study analyzing all HLA alleles on a larger samples size to establish any association of HLA allele with the susceptibility of asthma.

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