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Association of HLA DRB1 Alleles with Asthma in Pakistani Population of Punjab Region

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SBB did all the experiments and manuscript preparation. MIM and MUH help in providing asthma patients and healthy Controls. RFK and MSA review the manuscript. ZA designed this study. ZA and SBB supervised all the experiments and review the manuscript.

Key words
IgE, Family history, Gender, HLA DRB1* 07, HLA DRB1* 11, HLA DRB1* 12, HLA DRB1* 13

ABSTRACT

Asthma is a heterogenous disease with different endotypes. Among the various causative agents of asthma, allergens and genetic susceptibility play a key role. HLA is a complex region, and most of the GWAS studies on asthma are limited to simple gene markers. This study aimed to determine the association of HLA DRB1 alleles with atopic asthma in the Pakistani population. Blood samples of 568 subjects were collected from Punjab, Pakistan. HLA typing was performed by using sequence-specific primers (SSP). Total immunoglobulin E (IgE) was estimated by ELISA. Graph pad Prism 8 was used for statistical analysis. HLA DRB1* 0701-02 and HLA DRB1* 1301-04 showed a positive association with atopic asthma having an odd's ratio (OR) of 2.173 and 3.564 respectively. While HLA DRB1* 1101-04 and HLA DRB1* 1201-02 showed a negative association having OR of 0.4853 and 0.4299 respectively. HLA DRB1* 0701-02 had a positive association with family history (p = <0.0001*, OR= 3.641, 95% CI= 1.852-7.257) and female gender (p= 0.0003). Total IgE level was high in asthma patients (Mean = 508.7 IU/ml). In conclusion, this study suggested the association of atopic asthma with HLA DRB1 alleles in the Pakistani population of the Punjab region. Our study reveals that two HLA DRB1 alleles have a positive, and two alleles have a negative association with atopic asthma in the Pakistani population. This finding emphasized that further large-scale studies are needed in the Pakistani population to determine the association of atopic asthma with genetic susceptibility.

INTRODUCTION

Asthma, a complex chronic inflammatory disease of the airways is a major cause of morbidity, disability, and health resource utilization for those who are affected. Asthma is characterized by wheezing, coughing, recurring episodes of airflow obstruction, and shortness of breath (Kumar *et al*., 2009). Causative agents of asthma can be allergic (pollens, molds, cockroach residues, furred animals, dust mites, medicines, and food) or non-allergic (exercise, tobacco smoke, cold air, and infections) (Kim and Mazza, 2011). Asthma is a complex obstructive disease of the respiratory system triggered by the interaction of genetic susceptibility and environmental factors. Genome wide association studies (GWAS) identified various chromosomal regions having susceptible genes for asthma, like 2q, 3p, 5q, 6p21, and 12q23, 17q21 (Howard *et al*., 2000; Shi *et al*., 2022).

Despite the better recognition of asthma, its worldwide prevalence is increasing day by day. Exacerbations and day-to-day symptoms of asthma have increased by almost 30% in the past 20 years (Ho, 2010). Global Initiative for Asthma (GINA) reported that 300 million people worldwide are suffering from asthma and that count would increase to 400 million people by 2025 (Nunes *et al*., 2017). Owing to its early onset, asthma is one of the most common respiratory diseases that affect children and young adults. Almost 250,000 people annually die due to asthma. This death rate can be reduced with a better understanding and management of asthma

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(World Health Organization, 2007). All age groups are affected by asthma, ranging from 1% in Vietnam to 21% in Australia (Lai *et al*., 2006). The prevalence of asthma varies widely from country to country, but the disparity is narrowing due to rising prevalence in low- and middle-income countries due to increasing urbanization, and plateauing in high-income countries (World Allergy Organization, 2011).

Variations in the human leukocyte antigens (HLA) region encoding the major histocompatibility complex (MHC), at the chromosomal position 6p21 are associated with different phenotypes of asthma. Studies showed that the HLA class II genes such as HLA-DQ, HLA-DR, and HLA-DP are specifically associated with Th2-associated asthma (Marsh *et al*., 1981). In some Chinese studies association of HLA class I have also been found with bronchial asthma, e.g., subjects carrying HLA-B5 are more likely to develop asthma in their childhood than those carrying HLA-B17 (Huang *et al*., 1981). In some other studies, an association of HLA-DR and HLA-DQ has been found with certain foods (cow milk allergen) and aeroallergens (mites, molds, pollens, etc.) (Southampion, 1999; Kauppinen *et al*., 2012). The studies conducted on some Asian populations depicted HLA DRB1 07 as a risk factor for asthma, as one of them conducted on the Iraqi population showed a high frequency of HLA DRB1 070101 in asthma patients as compared to healthy controls (Mehdi *et al*., 2018). Similarly in the Korean population Significantly high frequency of HLA DRB1 07 was found in citrus red mites induced asthma patients (Cho *et al*., 2000). HLA DRB1 11 and HLA DRB1 12 represented contrasting findings. Two different studies conducted on the Pakistani population show different results, in one study high frequency of HLA DRB1 11 was found in asthma patients as compared to healthy controls (Javed *et al*., 2015) while in another study frequency of HLA DRB1 11 and 12 was low in aero allergen patients as compared to healthy controls (Hashmi *et al*., 2017). Although the sample size was small in both studies. While in another study conducted in Korea, an association of HLA DRB1 was found with allergic dermatitis patients (Park *et al*., 2012).

The disparity has been observed in alleles of the HLA region of different populations in association with asthma and other atopic diseases, but limited studies are available in the Pakistani population. So, in the Pakistani population, there is an immense need to find the association of HLA alleles with atopic asthma. The current study aims to determine the association of five HLA DRB1 alleles with atopic asthma in the Punjab region of Pakistan.

MATERIALS AND METHODS

Subjects

It is a case control study. The subjects included 306 asthmatics who visited the OPD of the Pulmonology department at Lahore General Hospital (LGH), Lahore, Pakistan; and 262 gender-matched healthy controls with no symptoms of allergy and asthma. Patient sampling was performed from December 2017 to May 2019. Subjects ranging from 13 to 70 years old, both males and females, were randomly enrolled in the study. 5ml of blood was collected from each subject in two different collection tubes for IgE estimation and DNA extraction. Ethical approval was obtained from the ethical board of Lahore General Hospital to conduct this study. Lab work was performed at the Institute of Microbiology and Molecular Genetics, University of the Punjab, Lahore, Punjab, Pakistan.

Clinical evaluation

Asthma patients were enrolled by the physician as per GINA guidelines (Reddel *et al*., 2015). The patient must have 2 of the following symptoms: (1) wheezing, (2) cough, (3) dyspnea, and (4) chest tightness. Detailed patient history and oral and written consent were also taken for conducting this study. Secly, Bronchodilator reversibility should be >12% improved than the baseline forced expiratory volume in 1st sec (FEV1) after the bronchodilator use. Asthma patients with the following complications were excluded from the study: (1) pregnant at the time of enrollment or intending to get pregnant during the 3 months treatment period, (2) prior diagnosis of chronic obstructive pulmonary disease (COPD), (3) prior diagnosis of congestive heart failure, and (4) smear-positive pulmonary tuberculosis cases.

Total IgE level quantification

Total IgE level of all subjects quantified by solid phase enzyme linked immunosorbent assay (ELISA). Bioshock, Inc, USA kit was used for this purpose (Mohammed *et al*., 2015). A cut-off value of 150 IU/ml was considered for non-sensitized subjects while allergen-sensitized asthma patients had higher IgE values. 20 µl of standards, controls, and subjects blood serum were added to the wells of the microtiter plate. After that 100 µl of zero buffer was added to all the selected wells and thoroughly mixed for 10 sec. Then, the microtiter plate was incubated for 30 min at 18-25 °C. After incubation, the microtiter plate was emptied by flicking inverted in the sink and rinsed with washing buffer 5 times. After rinsing all remaining water droplets were removed by striking against the absorbent paper towel. Later 150 µl of enzyme conjugate reagent was added to all the wells and thoroughly mixed for 10

sec and incubated for 30 min under the same conditions. Then rinsing and drying steps were repeated as described above. TMB reagent (100 µl) was added to all the wells and incubated in dark for 20 min at 25 °C after smooth mixing. Finally, 100 µl of stop solution was added to stop the reaction and thoroughly mixed for 30 sec or until the blue color appeared in the wells. For the quantitative measurement, optical density was measured at 450 nm by using a plate reader (BioTek Synergy Htx multi-mode reader). A standard curve was plotted to determine the unknown IgE values of all the samples.

DNA extraction and HLA typing

DNA extraction was performed by the kit method using a Gene All Biotechnology Co. Ltd. kit from Seoul, South Korea. DNA was extracted from whole blood according to the manufacturer's instructions. The quality of DNA was determined by gel electrophoresis and quantification was performed on a Colibri Micro volume Spectrophotometer (Titertek Berthold).

HLA typing was done for 5 HLA DRB1 alleles (DRB1*0701-02, DRB1*1001, DRB1*1101-04, DRB1*1201-02, and DRB1*1301-04) by PCR, using SSP Primers (Olerup and Zetterquist, 1992). GAPDH was used as an internal control (Paulukat *et al*., 2001). All the primer sequences of HLA DRB1 and GAPDH are described in Table I. Thermo scientific Dream Taq™ green PCR master mix (2X) was used for all the PCR reactions and gradient PCR was performed for optimization. For a 25 µl of reaction mixture 12.5 µl of TaqMan green reaction mix, 1 µl of each primer (reverse and forward), 2 µl of DNA, and 8.5 µl of PCR H_2O were used. Reaction conditions were as follow: initial denaturation at 94°C for 5 min; 35 cycles with denaturation at 94°C for 30 sec, and annealing time was 50 sec with different annealing temperature for different alleles (Table I), and elongation at 72 °C for 30 sec, and final elongation was performed at 72 °C for 8 min. The efficacy of PCR amplification was determined by resolving 7µl of PCR reaction mixture by gel electrophoresis on a 2% agarose gel. Amplicon sizes range from 174bp to 517bp.

Statistical analysis

Frequencies of HLA alleles in asthmatic patients and healthy controls were compared by Chi-square test or Fisher's exact two-tailed test. A p-value of less than 0.05 was considered significant. Clinical data among different groups were also compared by using the Chi-square test or Fisher's exact test for categorical variables. Odd's ratios were also calculated and 95% of confidential interval (CI) was used. All these analyses were performed by using Graph pad Prism 8.

RESULTS

Clinical data

This study was conducted on 568 people, among them, 306 were asthma patients and 262 were healthy controls. Demographic and clinical data are presented in Table II. Out of 306 asthmatic patients, 107 (34.96%) were male and 199 (65.03) were female, while healthy controls included 118 (45.04%) males and 144 (54.96%) females. The mean age of the asthmatic patient was 36.96 (14.27) and the healthy control was 28.66 (7.522). 52.3% of asthma patients reported a positive family history of asthma or any other allergic disease (allergic rhinitis or eczema).

Table I. Primer sequences used for HLA DRB1 sequence-specific and GAPDH gene amplification.

S. No.	Alleles	Primer sequence	Annealing temp (°C)	Product size
1	DRB1*0701-02	F 5′ CCTGTGGCAGGGTAAGTATA 3'	62	232bp
		R 5′ CCCGTAGTTGTGTCTGCACAC 3'		
2	DRB1*1001	F 5′ CGGTTGCTGGAAAGACGCG 3'	62	204bp
		R 5′ CTGCACTGTGAAGCTCTCAC 3'		
3	DRB1*1101-04	F 5′ GTTTCTTGGAGTACTCTACGTC 3'	63	176bp
		R 5′ CTGGCTGTTCCAGTACTCCT 3'		
4	DRB1*1201-02	F 5′ AGTACTCTACGGGTGAGTGTT 3'	63	248bp
		R 5′ CACTGTGAAGCTCTCCACAG 3'		
5	DRB1*1301-04	F 5′ CCCGCTCGTCTTCCAGGAT 3'	60	130bp
		R 5′ GTTTCTTGGAGTACTCTACGTC 3'		
6	GAPDH (Internal Control)	F 5′ ACCACAGTCCATGCCATCAC3'	65	576bp
		R 5′TCCACCACCCTGTTGCTGTA3'		

Table II. Demographic and clinical data of asthma patients and healthy controls.

Characteristics	Asthma patients n=306	Healthy controls n= 262	P value	Odd's ratio CI (95%)
Gender				
Male	107 (34.96%)	118 (45.04%)	.0160	1.524
Female	199 (65.03%)	144 (54.96%)		(1.084-2.127)
Age				
Mean (SD)	36.96 (14.27)	28.66 (7.522)	<0.0001	NA
Median	35.5	26		
Residential status				
Urban	238 (77.8%)	206 (78.62%)	NA	NA
Rural	68 (22.2%)	56 (21.38%)		
Family history				
Yes	160 (52.3%)	NA	NA	NA
No	146 (47.7%)			
BMI (Kg/m²)				
Mean (SD)	25.29 (5.552)	24.02 (6.109)	0.0014	NA
Median	24.63	23.08		
BMI range				
<18.5	30 (9.8%)	53 (20.23%)	NA	NA
18.5 - 24.9	125 (40.85%)	109 (41.6%)		
25 - 29.9	88 (28.76%)	63 (24.05%)		
>30	63(20.59%)	37 (14.1%)		
% FEV1 of Pred.				
Mean (SD)	63.73 (6.006)	74.67 (2.715)	<0.0001	NA
Median	63	74		
Δ FEV1 (%)				
Mean (SD)	13.89 (4.148)	NA	NA	NA
Median	13			
IgE (IU/ml)				
Mean (SD)	508.7 (325.7)	73.61(43.17)	<0.0001	NA
Median	505.6	67.38		

NS, Non-Significant; NA, Not Applicable.

The mean body mass index (BMI) of asthmatic patients was 25.29 while most of the patients were overweight or obese (88 (28.76%) asthmatics had 25-29.9 Kg/m² while 63 (20.59%) asthmatics had >30 Kg/m²). The mean BMI of the healthy controls was 24.02 (6.109). The mean FEV1 of asthma patients was 63.73 (6.006) while for healthy controls it was 74.67 (2.715) as shown in Table II. Change in FEV1 (ΔFEV1) from baseline of asthmatic patients was 13.89% (4.148). The mean IgE value of asthmatic patients was 508.7 (325.7) IU/ml while for healthy control it was 73.61(43.17) IU/ml.

HLA DRB1 alleles association with asthma

HLA typing of all HLA DRB1 alleles was performed by using SSP-PCR as shown in Figure 1. Compared with the healthy controls, frequencies of HLA DRB1*0701-02 and HLA DRB1*1301-04 were higher in the asthmatic patients while the frequencies of HLA DRB1*1101-04 and

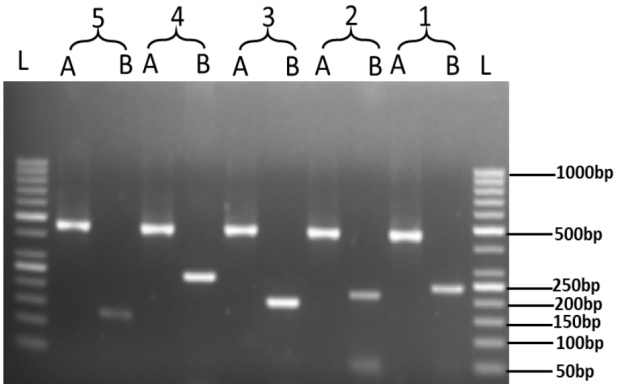

Fig. 1. HLA Typing using SSP Primers: L, 50 base pair ladder, A: GAPDH (internal Control), B: different HLA DRB1 alleles. Lane 1: HLA DRB1* 0701-02, Lane 2: HLA DRB1* 1001, Lane 3: HLA DRB1* 1101-04, Lane 4: HLA DRB1* 1201-02, Lane 5: HLA DRB1* 1301-04.

Table III. Association of HLA DRB1 alleles with asthma patients and healthy control.

Parameters	Healthy controls (n=262)		Asthma patients (n=306)		P value	Odd's ratio CI (95%)
	+ve	-ve	+ve	-ve		
DRB1*0701-02	24 (9.16%)	238 (90.84%)	55 (17.97%)	251 (82.03%)	0.0033*	2.173 (1.305-3.68)
DRB1*1001	23 (8.72%)	239 (91.22%)	25 (8.17%)	281 (91.83%)	0.8800	0.9245 (0.5061-1.636)
DRB1*1101-04	70 (26.72%)	192 (73.28%)	46 (15.03%)	260 (84.97%)	0.0008*	0.4853 (0.318-0.7382)
DRB1*1201-02	40 (15.27%)	222 (84.73%)	22 (7.19%)	284 (92.81%)	0.0028*	0.4299 (0.2463-0.7534)
DRB1*1301-04	31 (11.83%)	231 (88.17%)	99 (32.35%)	207 (67.65%)	0.0001*	3.564 (2.312-5.598)

Table IV. Association of the asthma patient's family history with HLA DRB 1 alleles.

Family history	DRB1*0701-02		DRB1*1001		DRB1*1101-04		DRB1*1201-02		DRB1*1301-04	
	+ve (%)	-ve (%)	+ve (%)	-ve (%)	+ve (%)	-ve (%)	+ve (%)	-ve (%)	+ve (%)	-ve (%)
Yes	42 (76.36)	118 (47.01)	6 (24.00)	154 (54.80)	12 (26.09)	148 (56.92)	6 (27.27)	154 (54.23)	45 (45.45)	115 (55.56)
No	13 (23.6)	133 (52.99)	19 (76.00)	127 (45.20)	34 (73.91)	112 (43.08)	16 (72.73)	130 (45.77)	54 (54.55)	92 (44.44)
P value	<0.0001*		0.0033*		0.0002*		0.0247*		0.1121	
Odd's ratio CI (95%)	3.641 (1.852-7.257)		0.2604 (0.1055-0.6365)		0.2671 (0.137-0.5341)		0.3166 (0.1248-0.821)		0.6667 (0.4082-1.077)	

HLA DRB1*1201-02 were lower in asthmatics (Table III). HLA DRB1* 1001 did not show any significant difference among the asthmatic patients and healthy controls. Out of 568 total enrolled subjects, 55 (17.97%) were positive for HLA DRB1*0701-02 in asthmatics while 24 (9.16%) were positive among the healthy controls. They showed a significant p-value of 0.0033 and OR of 2.173 with 95% confidence intervals (CI) ranging from 1.305-3.68, which is considered a positive factor associated with asthma disease. In the case of HLA DRB1*1101-04, 46 (15.03%) were positive in asthmatics and 70 (26.72%) were positive in healthy controls with a significant p-value of 0.0008. The OR calculated for HLA DRB1*1101-04 was 0.4853 with a 95% CI ranging from 0.318-0.7382, which is considered a negatively associated factor with asthma disease. HLA DRB1*1201-02 also showed a negative association with asthma. 22 (7.19%) asthmatics were positive for HLA DRB1*1201-02 while 40 (15.27%) were positive in healthy controls with a significant p-value of 0.0028. OR also show a negative association i.e., 0.4299 with a 95% CI of 0.2463-0.7534. Comparing the frequencies of HLA DRB1*1301-04, asthma patients appeared with higher frequency i.e., 99 (32.35%) than healthy controls i-e 31(11.83%). They showed a significant p-value of 0.0001, OR of 3.564, and 95% CI of 2.312-5.598, which is considered a positive factor associated with asthma. The overall highest frequency had been shown by HLA DRB1* 1301-04, and the lowest frequency had been shown by HLA DRB1*1001 (Table III).

Association of family history of asthma patients with HLA DRB1 alleles

Family history association of asthma patients with HLA DRB1 alleles was also determined. HLA DRB1* 0701-02 showed a positive association with the family history while all other alleles showed a negative association with a significant p-value and OR except HLA DRB1*1301-04 (Table IV). Out of 55 positive HLA DRB1*0701-02 asthmatics, 42 (76.36%) had a family history of asthma or any other allergic disease while

13(23.6%) had no family history. A significant p-value of <0.0001with OR of 3.641 and 95% CI ranging from 1.852-7.257 was found, which indicated family history as a positive factor. HLA DRB1*1001 showed more positive cases among asthmatics with no family history. So, 19 (76.00%) asthmatics belong to the group with no positive family history while 6 (24.00%) asthmatics had a positive family history. A significant p-value of 0.003 was found with OR of 0.2604 and 95% CI of 0.1055-0.6365. The same trend was observed in HLA DRB1* 1101-04 and HLA DRB1*1201-02. Out of 46 positive HLA DRB1* 1101-04 cases, 34 (73.91%) had no family history while 12 (26.09%) had a positive family history. p-value was found significant i-e 0.0002 and OR of 0.2671 with 95% CI ranging from 0.137-0.5341. In the case of HLA DRB1* 1201-02, 16 (72.73%) patients showed no family history while 6 (27.27%) showed a positive family history with a significant p-value of 0.0247. OR (0.3166) also indicated a negative association with family history with 95% CI ranging from 0.1248-0.821.

Gender association of asthma patients with HLA DRB1

Gender association of asthmatic patients with HLA DRB1 alleles was also revealed. In all of them higher frequency was observed in female asthmatic patients as compared to males (Table V). In HLA DRB1* 0701-02, 8 (7.48%) out of all the male asthmatic patients were positive while in the case of females 47 (23.62) out of all the female patients were positive. A significant p-value of 0.0003 and OR of 0.2613 with a 95% CI of 0.1237-0.5771 was found, which indicated that females are more prone to asthma. In HLA DRB1* 1201-02, compared to the asthmatic males (3(2.80%) high frequency of asthmatic females (19(9.55%) was observed. A significant p-value of 0.0354 and OR of 0.2733 with a 95% CI of 0.0839-0.9537 was found. So, HLA DRB1* 0701-DRB1* 0702 and HLA DRB1*1201-DRB1*1202 showed a significantly higher number of asthmatic females and a positive association with these alleles.

Association of HLA DRB1 alleles with atopic and non-atopic asthma patients

The association of HLA DRB1 alleles with asthma patients was also determined. Cases were divided into two groups: non-atopic with IgE less than 150 IU/ml and atopic with IgE more than 150 IU/ml. Out of all asthma patients, there were 49 non-atopic patients while 257 were atopic asthma patients. Compared with all the HLA DRB1 alleles, HLA DRB1*0701-02 showed a maximum number (13) of positive asthma cases associated with non-atopic asthmatics. While HLA DRB1* 1001, HLA DRB1*1101-04, HLA DRB1*1201-02, and HLA DRB1* 1301-04 represented 2, 7, 2, and 13 cases of non-atopic asthma patients respectively, and 23, 39, 20, and 86 atopic asthma patients, respectively (Fig. 2).

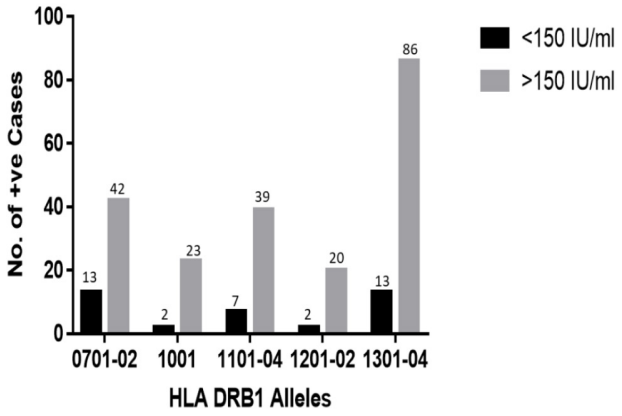

Fig. 2. Association of HLA DRB1 allele frequency with non-atopic (<150 IU/ml) asthma patients and atopic (>150 IU/ml) asthma patients.

Table V. Association of HLA DRB1 Alleles with gender.

Gender	DRB1*0701-03		DRB1*1001		DRB1*1101-04		DRB1*1201-02		DRB1*1301-04	
	+ve (%)	-ve (%)	+ve (%)	-ve (%)	+ve (%)	-ve (%)	+ve (%)	-ve (%)	+ve (%)	-ve (%)
Male	8 (7.48)	99 (92.52)	6 (5.61)	101 (94.39)	12 (11.21)	95 (88.79)	3 (2.80)	104 (97.20)	28 (26.17)	79 (73.83)
Female	47 (23.62)	152 (76.38)	19 (9.55)	180 (90.45)	34 (17.09)	165 (82.91)	19 (9.55)	180 (90.45)	71 (35.68)	128 (64.32)
P value	0.0003*		0.2783		0.1841		0.0354*		0.0971	
Odd's ratio CI (95%)	0.2613 (0.1237-0.5771)		0.5628 (0.2267- 1.38)		0.613 (0.3125-1.231)		0.2733 (0.0839-0.8537)		0.639 (0.3785-1.059)	

DISCUSSION

In the current study, the distribution of 5 HLA DRB1 alleles among asthmatic patients from Punjab, Pakistan was investigated. More than 90% of the patients belong to the central Punjab region of Pakistan. 84% of asthma cases were suffering from atopic asthma. Out of the 5 HLA DRB1 alleles, 2 were found more frequently in the asthmatic patients as compared to controls while 2 were observed to play a protective role. The results were statistically significant.

In this study, HLA DRB1* 0701-02 and HLA DRB1* 1301-04 were more frequent in the asthmatic patients as compared to the healthy control group. Frequency of HLA- DRB1* 0701- 02 in asthma patients (17.97%) was almost double as compared to healthy controls (9.16%) (p-value = 0.0033, OR = 2.173, 95% CI = 1.305-3.68) and the frequency of HLA DRB1* 1301-02 in asthma patients (32.35%) was almost triple as compared to the healthy controls (11.83%) (p-value =0.0001, OR = 3.564, 95%CI = 2.312-5.598). HLA DRB1* 1301-04 is also the most frequent among all the HLA DRB1 alleles, investigated in this study. In another study conducted in Baghdad, Iraq by Batool Mutar Mahdi (Mahdi *et al*., 2018) and colleagues in 2018, a high frequency of HLA DRB1* 070101 was found in asthma patients (35%) as compared to healthy controls (7.5%). In another study conducted in Kufa, Iraq on adult asthmatics a substantially high frequency of HLA DRB1* 07 was observed in asthmatics (31%) as compared to healthy controls (2.4%) (Eidan *et al*., 2019). The same trend was observed in a study conducted on the European population, where HLA-DRB1* 0701 was determined as a risk factor in asparaginase allergies (Fernandez *et al*., 2014). In another study conducted on, Korean adults suffering from citrus red mite (*Panonychus citri*) sensitized asthma, the high allelic frequency of HLA DRB1* 07 was determined as compared to healthy controls i-e 17.6% vs 4.1% (Cho *et al*., 2000). A study was conducted on moderate to severe asthma patients who were sensitive to molds and a high frequency of HLA DRB1* 13 was observed in these children (Knutsen *et al*., 2010). So, the studies conducted on HLA Class II exhibited a high frequency of HLA DRB1* 07 and HLA DRB1* 13 in different populations, and this study was also in compliance with previous studies.

HLA DRB1*1101-04 and HLA DRB1* 1201-02 appeared as negatively associated alleles with asthma, in this study. HLA DRB1* 1101-04 was positive in 15.03% asthmatics and 26.72% controls (p = 0.0008, OR = 0.4853, 95% CI = 0.318-0.7382), While HLA DRB1* 1201-02 was positive in 7.19% asthma patients and 15.27% controls (p =0.0028, OR = 0.4299, 95% CI =0.2463-0.7534). Overall, the frequency of HLA DRB1* 1101-04 was higher than HLA DRB1* 1201-02. A study conducted by our research group in 2017, among Allergic patients and healthy controls in the Pakistani population showed the same trend. The frequency of HLA DRB1*11 in Allergic patients was 13.6% vs 40% in healthy controls which shows a significant difference (p = <0.0001). In the same study, the frequency of HLA DRB1* 12 was also low in allergic patients as compared to healthy control (20.0% vs 27.5%), but this difference was not statistically significant (Hashmi *et al*., 2017). A study on rheumatoid arthritis patients in the Southeastern Anatolia region of Turkey showed a low frequency of HLA DRB1*11 in patients as compared to healthy controls (14.6% vs 24.4%, p = 0.02) (Batmaz *et al*., 2013). In contrast to this, a positive association of HLA DRB1* 11 was found in atopic dermatitis (AD) patients in Korean children as compared to controls (12.4% vs 1.8%, Corrected p = 0.048) (Park *et al* 2012). In another study conducted in Pakistan; a high frequency of HLA DRB1* 12 alleles was found in asthmatic patients (42.0%) as compared to control (20.0%). The difference was significant (p = 0.037), but the sample size was small (50 asthmatics vs 30 control) (Javed *et al*., 2015). This study was also in contrast to our result, but our sample size was bigger.

Association of family history with HLA DRB1 alleles was also determined. HLA DRB1*0701-02 was positively associated with family history while HLA DRB1* 1001, HLA DRB1* 1101-04, and HLA DRB1* 1201-02 were negatively associated with family history (Table IV). In the latter case, higher frequencies were observed in asthma cases with no family history. A study conducted on childhood asthma predicted that there is no influence of family history on the development of asthma (Juhn *et al*., 2007). But a study conducted on allergic patients of the Pakistani population represented a positive association of family history with HLA DRB1*11 and HLA DRB1* 12. Although the association was not statistically significant (Hashmi *et al*., 2017).

The influence of gender differences on asthma onset and its exacerbations had been reported in various studies. Accelerated asthma frequencies had been reported in females more than males, after adolescence. Researchers reported varied reasons for this difference, among them top of the list were hormonal differences and different environmental factors (Almqvist *et al*., 2008; Miyasaka et., 2022; Senna *et al*., 2021). In the present study, higher asthma frequency was observed in females. When the association of HLA DRB1 alleles was determined with gender in asthmatics, higher frequencies were found in females in all alleles, but a statistically significant association was determined with HLA DRB1* 0701-02

and HLA DRB1* 1201-02. In HLA DRB1* 0701-02, 7.48% males and 23.62% females, out of total asthmatics were positive (p= 0.0003, OR= 0.2613, 95% CI= 0.1237-0.5771). While in HLA DRB1* 1201-02, 2.80% males and 9.55% females were positive (p= 0.0354, OR= 0.2733, 95% CI= 0.0839-0.8537). However, a statistically significant association of gender with HLA DRB1 alleles was first time determined in this study.

Many studies conducted on serum IgE levels of asthma showed that most of the patients represented elevated levels of IgE (Ahmed and Ad'hiah, 2022; Shaban *et al*., 2021). In the present study, out of 306 asthma patients, 49 were non-atopic (IgE = <150IU/ml) while 257 were atopic (IgE = >150IU/ml). So, this study is also following the previous studies. HLA DRB1 alleles association with IgE was also determined. In HLA DRB1* 0701-02, 26.53% of cases were associated with low IgE levels while 16.34% of cases were associated with high IgE levels. While in HLA DRB1* 1001, HLA DRB1* 1201-02, and HLA DRB1* 1301-04 higher frequencies were observed in atopic asthma patient's vs non-atopic asthma patients i-e 8.94% vs 4.08%, 7.78% vs 4.08%, and 33.46% vs 26.53%, respectively. In HLA DRB1* 1101-04, no difference was observed in atopic vs non-atopic patients i-e 15.17% vs 14.28%. A study conducted in Iran showed the association of HLA DRB1* 11 with high IgE levels and HLA DRB1* 07 and HLA DRB1* 13 with low IgE levels (Masoud *et al*., 2008). Another study conducted in Barcelona; Spain also showed the association of HLA DRB1* 13 with lower IgE levels (Soriano *et al*., 1997). The results regarding HLA DRB1*13 contrast with our study. This may be due to the difference in populations and large-scale studies can further resolve this issue.

CONCLUSION

This study was conducted on the Pakistani population to determine the association of atopic asthma with 5 different HLA DRB1 alleles. HLA DRB1* 0701-02 and 1301-04 have a positive association with asthma while HLA DRB1* 1101-04 and 1201-02 have a negative association. The Association of HLA DRB1 alleles with family history, gender, and IgE was also determined. The limitation of this study is the sample size and there is also an immense need to study more alleles regarding HLA in the Pakistani Population. In the future, the study should be conducted on a large sample size with more HLA alleles to better understand the genetic susceptibility of the Pakistani population.

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Ethics statement

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Statement of conflict of interest

The authors have declared no conflict of interest.

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