

HLA-DRB1 gene polymorphisms in Iranian children with Henoch-Schönlein purpura

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Background: People of all ages can suffer from Henoch-Schönlein purpura (HSP), but it is the most common vasculitis in childhood. The most important involving gene is located on chromosome 6p21.3, a region coding for human leukocyte antigens (HLAs). Among HLA genes, because of the high rate of polymorphisms, HLA-DRB1 is estimated to have a strong association with HSP. In this study, we aimed to assess the association of HLA-DRB1 alleles with HSP in Iranian children. **Materials and Methods:** This study consisted of thirty Iranian children with HSP and 35 healthy controls. Genomic DNA was extracted, and HLA typing was performed by polymerase chain reaction with sequence-specific primers technique. **Results:** The results have shown that HLA-DRB1*01 and HLA-DRB1*11 ($P = 0.002$, odds ratio [OR] = 7.579, confidence interval [CI] = 1.934–29.697 and $P = 0.039$, OR = 3.333, CI = 1.030–10.788), respectively, are the most frequent alleles associated with HSP in Iranian children population. The frequency of other alleles was not significantly different in both groups. The results also show no correlation between HLA types and disease manifestations. **Conclusion:** According to these results, there is an association between HLA-DRB1*01 and HLA-DRB1*11 gene polymorphisms and susceptibility to HSP in our study group.

Key words: Autoimmune disease, Henoch-Schönlein purpura, HLA-DRB1, polymorphism, vasculitis

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INTRODUCTION

Henoch-Schönlein purpura (HSP) is the most common childhood primary systemic small vessel vasculitis, mediated by immunoglobulin A (IgA)-immune complex depositions, with an incidence of 10–20/100,000.^[1] HSP is a multisystemic disease characterized by palpable purpura, abdominal pain, and joint and renal involvement.^[2,3] Most of children have a self-limited disease, but severe intestinal bleeding, leukocytoclasia and also in the long term severe nephrotic-nephritic syndrome are predictable.^[1,4] People in all ages can suffer from the disease, but the figure is highest in children below 10 years.^[4-6] The incidence has been reported in Asian and Caucasian more than Black population,^[1,3,4] and boys are affected more than girls in a 2:1.2 ratio.^[1,5,7] Although the etiology of HSP is not exactly

understood,^[5,8] several infective pathogens, drugs, and certain toxins can trigger the disease.^[1,3] Furthermore, genetic polymorphisms in cytokines and cell adhesion molecules involved in inflammatory responses are considered as potential causes of disease.^[1,9,10]

Among these genetic factors, human leukocyte antigen (HLA) and particularly HLA-DRB1 – encoding the most prevalent beta subunit of HLA-DR – due to its central role in the immune system by presenting peptides derived from extracellular proteins is the principal candidate for increasing incidence of many autoimmune diseases; hence, its association with HSP has been investigated and reported in the literatures.^[11-13]

Differences and similarities in the frequency of certain HLA alleles have been observed in previous reports and the results of such studies vary depending on the ethnic

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group studies.^[5,8,9] Some of these reports have shown the association of HLA-DRB1 with HSP.^[8,14-18]

To date, there have been no reports supporting a consistent relationship between HSP and HLA-DRB1 in Iranian pediatric patients. Therefore, the current study aimed to assess the association of HLA-DRB1 alleles with HSP in Iranian children.

MATERIALS AND METHODS

From September 2013 to October 2014, thirty patients with HSP who attended outpatient clinic or accepted to Mofid Children Hospital were recruited for the present study. At diagnostic time, they were between 2 and 13 years old with a mean age of 6.4 years (standard deviation [SD] = 3.06). Their disease was diagnosed by a pediatric rheumatologist, based on new classification by final European League Against Rheumatism/Paediatric Rheumatology International Trials Organisation/Paediatric Rheumatology European Society criteria for HSP and classification definition, which indicate that at least one of the following four criteria should be present: diffuse abdominal pain, any biopsy showing predominant IgA deposition, arthritis or arthralgia, and renal involvement (any hematuria ± proteinuria) in the presence of palpable purpura which was a mandatory criterion [Table 1].^[19] For control group, 35 genetically unrelated healthy adult individuals who had no history of autoimmune disease voluntarily participated in the study. The study had ethical approval from the local Ethics Committee of Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, and oral or written informed consent was obtained from all participants or their caretakers.

DNA isolation was performed by chloroform procedure^[2] from leukocytes of peripheral blood collected (2 ml) with ethylenediaminetetraacetic acid and kept at -20°C. The concentration and purity of DNA were measured by NanoDrop (Thermo Scientific, USA). We used HLA-DRB1 typing by polymerase chain reaction with sequence-specific primer (PCR-SSP).^[20] A total of nine sequence-specific oligonucleotide primers were used to identify seven

types of HLA-DRB1, and for positive control, two β-actin primers were used in every reaction. In amplification by PCR, total volume of 20 µl master mix was used for each 0.2 ml PCR tubes which involved: 2 µl PCR buffer 10X (500 mM KCl, 100 mM Tris-HCl, pH 8.8, 0.1% Tween-20, and 15 mM MgCl₂, BIORON, Germany), 0.25 µl Taq DNA polymerase (BIORON, Germany), 0.5 µl MgCl₂ 100 mM (BIORON, Germany), 0.7 µl dNTP 4 × 10 mM (Pars tous, Iran), primer (variable), genomic DNA (variable), and ddH₂O (variable). We also used prepared master mix (ampliqon Denmark Taq DNA polymerase 2X master mix red) in the total volume of 20 µl. PCR was performed with a palm cycler thermocycler (Genetix Biotech Asia (P) Ltd., India). The operation performed in four thermal cycler programs is shown in Table 2.

The PCR product resolved by electrophoresis 1.5% agarose gel (0.5X TBE for 45 min) while the gel was stained with Green Viewer before getting cold and visualized and photographed by Gel documentation (UVitec, UK) [Figure 1].

Statistical analyses

Statistically significant difference in the frequency of HLA alleles between HSP patients and healthy controls was determined by the Chi-square test. The statistically significant difference between allele frequencies for the studied polymorphisms and clinical signs was examined by Fisher's exact test using IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp; Released 2013. *P* < 0.05 was considered statistically significant, and also odds ratios (ORs) with 95% confidence intervals (CIs) were calculated.

RESULTS

Thirty pediatric patients (20 boys and 10 girls) with a mean age of 6.4 (SD = 3.06) and 35 adult controls throughout Iran were involved in investigation. The ratio of boys to girls was 2:1. All patients suffered from palpable purpura and amount of joint involvement, abdominal pain, and kidney involvement was 82.75%, 72.41%, and 10.34%, respectively. Other features such as urogenital, neurological, cardiopulmonary, and high blood pressure were seen in varying ranges of 3.44 in one case to 20.68 in six cases. A clinical scoring system consisted of the sum of three distinct scores for joint, gastrointestinal (GI), and renal involvements was used to assess disease severity. The severity of the disease was determined as low score and high score, if the clinical score was <4 and ≥4, respectively. The clinical features are shown in Table 1.^[8]

Seven alleles of HLA-DRB1 were studied among Iranian children patients. Among them, HLA-DRB1*01 and HLA-DRB1*11 were higher in patients than controls, 90%

Table 1: Demographic and main clinical characteristics of Henoch-Schönlein purpura patients

Feature	HSP (n=30)*, n (%)	HS (n=13), n (%)	LS (n=16), n (%)
Gender (male/female)	20/10	10/3	9/7
Age (year)	6.40±3.06	7.2±3.02	5.82±3.32
Purpura and/or petechiae	29 (100)	13 (100)	16 (100)
Arthralgia and/or arthritis	24 (82.75)	12 (92)	12 (75)
Gastrointestinal involvement	21 (72.41)	12 (92)	9 (56.2)
Renal involvement (proteinuria-hematuria)	3 (10.34)	3 (23)	0

*The profile of one patient was not completed. HSP=Henoch-Schönlein purpura; HS=High score; LS=Low score

Table 2: Polymerase chain reaction primers for amplification of HLA-DRB1

Alleles	Primers	Sequences (5'-3')	Fragment length	Denaturing	Annealing	Extension
HLA-DRB1						
*01	Forward	TTCTTGTCGACAGCTTAAGTTTG	261	95	62 (reduced 0.5° for 14 cycle)	72
*07	Forward	AGTTCCTGGAAAGACTCTTCT	206		55 for 19 cycle	
*04	Forward	GTTTCTGGAGCAGGTAAAC	263	94	62	72
*09	Forward	GGACGGAGCGGGTGC GGATC	222	94	62	72
*13/*14	Forward	GTTTCTGGAGTACTCTCGTC	263	94	62	72
*08	Forward	AGTACTCTACGGGTGAGTGTT	254	94	63	72
For all except *11 and *13	Reverse	CCGCTGCACTGTGAAGCTCT				
*11	Forward	ACGTTTCTGGAGTACTCTACG	179	95	59	72
*11	Reverse	CTGGCTGTCCAGTACTCCT				
*13	Forward	ACGTTTCTGGAGTACTCTACG	173	94	63	72
*13	Reverse	TGTCCAGTACTCGGCGCT				

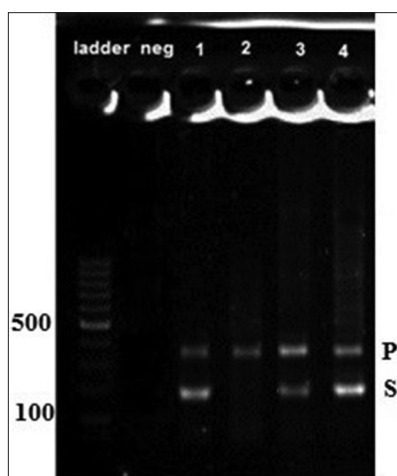


Figure 1: HLA-DRB1*11 polymerase chain reaction gel electrophoresis result. Neg = Negative control; P = Positive control; S = Specific bond

and 83% versus 54.2% and 60.0%, respectively ($P = 0.002$, OR = 7.579, CI = 1.934–29.697 and $P = 0.039$, OR = 3.333, CI = 1.030–10.788). Other allelic groups showed no significant difference between two groups. In the present study, HLA-DRB1*09 was the most common allele found in both case and control groups. The result is shown in Table 3. There was no significant difference observed between HLA-DRB1*01 and HLA-DRB1*11 alleles in patients and the severity of the disease, $P = 0.429$ and $P = 0.783$ respectively.

DISCUSSION

HSP is the most common systemic vasculitis which affects small vessels in children and rarely but with higher severity occurs in adults. It is reported that both environmental and genetic factors play a part in disease pathogenesis. Although the pathogenesis is poorly understood,^[1,10] due to inflammations in small blood vessels and capillaries, the most likely candidates are IgA1 glycosylation, polymorphism in cytokines, and cell adhesion molecules.^[1,9,21,22] Furthermore, the major histocompatibility complex (MHC) Class II genes

Table 3: Distribution of HLA-DRB1 allele frequency in Henoch-Schönlein purpura patients and control groups

Allele frequency	Control group (%)	Case group (%)	P	OR	95% CI
HLA-DRB1*01**	19 (54.3)	27 (90.0)	0.002*	7.579	1.934-29.697
HLA-DRB1*04	13 (37.1)	13 (43.3)	0.612	1.294	0.478-3.503
HLA-DRB1*07	26 (74.3)	27 (90.0)	0.104	3.115	0.758-12.802
HLA-DRB1*08	13 (37.1)	15 (50.0)	0.297	1.692	0.628-4.559
HLA-DRB1*11**	21 (60.0)	25 (83.3)	0.039*	3.333	1.030-10.788
HLA-DRB1*13	14 (40.0)	17 (56.7)	0.180	1.962	0.729-5.275
HLA-DRB1*09	32 (91.4)	29 (96.7)	0.381	2.719	0.268-27.618

OR=Odds ratio; CI=Confidence interval; * $P < 0.05$ was considered statistically significant, **The HLA-DRB1*01 and HLA-DRB1*11 alleles were significantly associated with HSP

have shown the most convincing genetic correlations with HSP and have been studied in different other autoimmune diseases such as systemic lupus erythematosus, Kawasaki, endometriosis, juvenile idiopathic arthritis (JIA), and Behçet's and Graves' disease (GD).^[13,23-29]

Identification of genetic polymorphisms associated with an increased risk of HSP would provide important information in understanding the mechanisms of disease and the ability to predict individuals who are at higher risk. The results may lead to improvements in clinical outcomes for patients including early diagnosis, design of new treatments, improvement of the long-term prognosis, especially in those with severe symptoms, and estimation of disease recurrence.^[3,8,14]

HLA typing for HLA-DRB1 alleles was done by PCR-SSP, stipulated by Olerup and Zetterquist in 1992.^[20] Several studies on the correlation between HLA-DRB1 alleles and HSP have shown similar results in different countries. HLA-DRB1*11 in India ($P = 0.006$), Turkey ($P = 0.03$), and Italy ($P = 0.007$); HLA-DRB1*01 in Italy ($P = 0.007$), Northwest Spain ($P = 0.013$), and Spanish Caucasian ($P < 0.001$); and HLA-DRB1*14 in Turkey ($P = 0.035$) have shown positive correlation with disease, whereas HLA-DRB1*07 in Italy ($P = 0.002$) and Northwest Spain ($P = 0.014$),

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HLA-DRB1*03 in Spanish Caucasian ($P < 0.01$) and HLA-DRB1*10, and HLA-DRB1*17 in Turkey ($P = 0.035$ and $P = 0.018$), respectively, have had protective role. In these studies, the role of HLA types and severity of symptoms were also investigated. In India, HLA-DRB1*11 increased in patients with kidney and GI involvement ($P = 0.004$ and $P = 0.003$), respectively, and in Turkish patients with joint involvement and nephrotic proteinuria HLA-DRB1*11 and HLA-DRB1*13 ($P = 0.025$ and $P = 0.025$), respectively, have shown a significant association. HLA-DRB1*14 strongly has been found reduced in HSP patients with joint manifestations in Turkey population ($P = 0.001$).^[8,14-18]

Our study was the first investigation on polymorphisms of HLA-DRB1 alleles in Iranian children with HSP. The results of positively susceptible alleles were consistent with previous studies. Like Italy population, we found a significant correlation between HLA-DRB1*01 and HLA-DRB1*11 with HSP. HLA-DRB1*11 is reported to have a significant association with HSP except in Northwest Spain and Spanish Caucasian. In our investigation, like what has been reported in India, and unlike other studies, we did not find any protected alleles.

Compared with other autoimmune and multifactorial disorders, HLA-DRB1*01 has shown correlation with GD among North American Caucasian, HLA-DRB1*11 with JIA and Kawasaki in Finnish and Korean population, respectively, and both of these polymorphisms among Iranian population, which shows the possibility of their role in the onset of autoimmune diseases.^[26,27,29,30]

Before concluding, we would like to mention some limitations of the present study. There is no doubt that small sample size of patients can be the major one. For more accurate result, the size of both groups should be extended in next studies among Iranian pediatrics. In addition, we did not identify suballeles which is highly recommended for future studies.

CONCLUSIONS

In the first study of correlation between HLA-DRB1 in Iranian children patient with HSP, we found out that HLA-DRB1*01 and HLA-DRB1*11 increase susceptibility to HSP, although no significant association of correlated alleles and disease severity was seen.

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Conflicts of interest

There are no conflicts of interest.

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