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Original article

ASSOCIATION OF HLA-B AND HLA-DRB1 LOCI AND INFLAMMATORY CYTOKINES WITH THE CLINICAL PRESENTATION OF PATIENTS WITH JUVENILE IDIOPATHIC ARTHRITIS

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Abstract

Juvenile idiopathic arthritis (JIA) is the most common rheumatic chronic disease in childhood presented with various clinical symptoms of arthritis in children younger than 16 years.The aim of this study was to evaluate the correlation between HLA-B, HLA-DRB1 and inflammatory cytokines with the clinical presentation of juvenile idiopathic arthritis.

In this study, we analyzed 35 patients with juvenile idiopathic arthritis diagnosed at the University Clinic for Pediatric Diseases in Skopje, North Macedonia in the period from 2018 - 2024. All patients fulfilled the inclusion criteria for entering the study, that is, the ILAR reevaluated criteria from 2001 for diagnosis of juvenile idiopathic arthritis. Patients were genotyped for HLA-B and HLA-DRB1 loci. Concentrations of IL-1, IL-6 and TNF-alpha were determined in patients' serum at two time points, before starting the treatment and 6 months after therapy. These analyses were performed at the Institute of Immunobiology and Human Genetics, Faculty of Medicine in Skopje, North Macedonia.

The most frequent HLA-B allelic groups in our patients were HLA-B*27, B*35 and B*18, whilst in HLA-DRB1 locus the most frequent were DRB1*11, *01, *04 and *16. Increased risk for development of JIA was detected for HLA-B27 (p=0.001959, OR=3.39), B*15 (p=0.000058, OR=7.29) and DRB1*08 (p=0.02758, OR=4.54). Cytokine levels in patients decreased after therapy.

Conclusion: HLA typing is important in JIA patients for detection of different subtypes and detection of the concentration of proinflammatory cytokines helps in the therapy management of these patients.

Keywords: juvenile idiopathic arthritis, HLA-B, HLA-DRB1, inflammatory cytokines, IL-1, IL-6, TNF-alpha, biological therapy

Introduction

Juvenile idiopathic arthritis (JIA) is the most common chronic rheumatic disease in childhood [1]. JIA unifies all forms of chronic childhood arthritis, affecting not only joints, but extra-articular structures including eyes, skin and internal organs, leading to disability and even associated fatality. The International Ligue Against Reumatism (ILAR) stratifies subtypes of

autoimmune inflammatory disorders, defined by the number of joints affected, the presence of systemic symptoms and detection of rheumatoid factor (RF). According to this classification, juvenile idiopathic arthritis is divided into the following subgroups: oligoarticular (persistent or extended), polyarticular (RF negative or RF positive), systemic juvenile idiopathic arthritis, psoriatic arthritis, enthesitis-related arthritis and non-defined arthritis[1,2]. Patients with JIA are mostly female, two years and older at the time of diagnosis, although males are affected too.

Oligoarticular JIA is characterized by inflammation of up to four joints, predominantly affecting the joints of the lower extremities such as knee and ankle, with high frequency of positivity to anti-nuclear antibody (ANA) and high risk of chronic uveitis[2,3].

Polyarticular JIA affects five or more large/small joints and is hallmarked by injury to the metacarpophalangeal joints and wrists [4]. Enthesitis-related arthritis (ERA) resembles oligoarthritic form, affecting the joints of the lower limb in association with enthesitis. Due to its association with lower limb and sacroiliac joints, enthesitis, uveitis and presence of HLA-B27, Ravelli *et al*. have suggested ERA to be a disease belonging to the group of spondyloarthropathies[1,2]. Psoriatic arthritis often proceeds to oligoarthritis or RF-negative polyarthritis and involves more commonly the small joints accompanied by dactylitis, psoriatic rash and/or nail pitting[1,3,5].

Standing apart from other subtypes, systemic juvenile idiopathic arthritis (sJIA) manifests not only with widespread joint arthritis, but also with significant range of systemic inflammation symptoms: spiking fever, generalized lymphadenopathy, migratory salmon-pink rash, serositis (pericarditis is most common, pleuritis and peritonitis), hepatosplenomegaly, macrophage activation syndrome (MAS). Approximately 10% of sJIA patients present systemic symptoms with associated macrophage activation syndrome (MAS), a potentially life-threatening condition with histopathological features that include accumulation of terminally-differentiated macrophages with high hemophagocytic activity[2,6-8].

The etiology of juvenile idiopathic arthritis is unknown. Environmental factors, including infectious agents, vaccinations, antibiotics, vitamin D deficiency, stress and trauma have been proposed as risk factors. Infectious viruses (Epstein-Barr virus, Parvovirus B, Rubivirus, Hepatitis B virus) and bacteria (*Salmonella spp*., *Shigella spp*, *Campilobacter spp*., *S. pyogenes*, *M. pneumoniae*, *Chlamydophila pneumonia*) have been reported as causal factors provoking JIA[2,9]. The main hallmark of JIA is joint inflammation with tissue destruction[10]. Initiation of JIA pathophysiological cascade includes abnormal activation of T-cells, B-cells, natural killer (NK) cells, dendritic cells (DC), macrophages and neutrophils and the production of proinflammatory mediators that cause joint destruction and systemic complications[2]. Several studies have documented genetic associations to JIA[2,11-15]. Genetic linkage depends on the subtype and may be divided into two groups: HLA genes and non-HLA related genes. HLA-class II genes have mostly been associated with predisposing JIA. DRB1*04 is the predisposing allelic group for systemic arthritis in Northen Europe, DRB1*04, *01 and *03 for polyarticular JIA and DRB1*08, *11, *13, *02, A*02 (early onset) and B*27 (late onset) oligoarticular JIA[11]. Association with HLA class II and the presence of ANA suggests that an adaptive immune response is predominant in the pathogenesis of the oligoarticular JIA. However, activated neutrophils with altered phenotype and dysfunction, as well as impaired synovial monocytes and macrophages with reduced capacity to phagocytose, have recently been identified in synovial fluid of patients with oligoarticular JIA[2,16,17]. Abundant amounts of proinflammatory cytokines are released in the tissue including IL-1 beta, IL-6 and TNF-alpha. TNF-alpha is responsible for synovitis and inflammatory events; IL-1 is responsible for joint destruction, and IL-6 is responsible for systemic symptoms like fever and rash.

The treatment of juvenile idiopathic arthritis starts at the time of diagnosis with nonsteroidal anti-inflammatory drugs (NSAIDs) like ibuprofen 30-40/mg/kg per day in duration of 4 to 6 weeks, to reduce morning stiffness of the joints, followed by disease-

modifying anti-rheumatic drugs (DRAMDs), most often methotrexate and/or corticosteroid intra-articular injection. With the blocking of prostaglandin production via inhibition of cyclooxygenase-1 and cyclooxygenase-2, NSAIDs obtain both analgesic and anti-inflammatory effects. Local corticosteroid joint injections are effective in synovitis and may be a first-line treatment for oligoarthritis alone or in addition to DMARDs[22]. Systemic administration of high dose corticosteroids provides good short-term effect, especially in systemic juvenile idiopathic arthritis (sJIA) patients, but has no influence on the long-term disease outcome. Moreover, its prolonged administration is associated with severe side effects including osteoporosis, growth suppression, immunosuppression and metabolic effects[18].

The American College of Rheumatology (ACR) recommends early use of DMARDs, specifically MTX [20,21], leflunomide and/or sulfasalazine. Methotrexate is considered to be the first choice of DMARD for oligo and pJIA when NSAIDs and intraarticular steroids are insufficient [19,20,21]. There is recommendation for starting biological therapy in children who don't respond to conventional therapy like IL-6 inhibitors (Tocilizumab) and IL-1 inhibitor (Anakinra), for systemic forms of juvenile idiopathic arthritis. TNF inhibitors (Adalimumab, Infliximab, Etanercept), together with Methotrexate therapy are recommended [20-24]. The aim of our study was to determine the HLA-B and HLA-DRB1 loci and their correlation with the clinical presentation in our patients, as well as to determine the concentration of pro-inflammatory cytokines IL-1, IL-6 and TNF-alpha in patients with juvenile idiopathic arthritis before starting the treatment and 6 months after receiving therapy.

Material and methods

Study group

In this study, we analyzed 35 patients with JIA diagnosed at the University Clinic for Pediatric Diseases in Skopje, North Macedonia in the period from 2018 until 2024. All patients fulfilled the inclusion criteria for entering the study, that is, the ILAR reevaluated criteria from 2001 for diagnosis of juvenile idiopathic arthritis. Exclusion criteria for entering the study were patients older than 16, patients with septic arthritis, rheumatic fever and reactive arthritis.

Methods

HLA-B and HLA-DRB1 loci were genotyped at the Institute of Immunobiology and Human Genetics, Faculty of Medicine in Skopje, North Macedonia with SSO method from One Lambda, USA according to the manufacturer's instructions. The concentration of IL-1, IL-6 and TNF-alpha were determined in the serum of patients at two time points, before starting the treatment and 6 months after therapy, with magnetic beads technology using multiplex kits, R&D systems, USA. The fluorescence was measured using Luminex 200 instrument.

Statistical analysis

The genotype and phenotype frequency were calculated with counting. Differences in HLA between the group of patients with JIA and the control group (previously published data) [25] were calculated using χ^2 test and Fisher's exact test when values were less than 5. Level of significance was established at $p<0.05$ and when statistical significance was reached, odds ratio (OR) was calculated to measure the magnitude of the association. The Statistica 10, SPSS 20.0 was used for statistical analyses of cytokine concentrations.

Results

We analyzed 35 patients with JIA in our study, 23 (65.7%) were female and 12 (34.3%) were male, with statistical significance (p=0.0086) (Table 1). The mean age of our patients was 9.8 (3.0-15.0) years. Females were younger at the time of the diagnosis compared to males (p=0.040). According to their nationality, most of the patients were Macedonians (71.4%) and

all, except one, live in the cities in North Macedonia. 57.1% of all patients live in Skopje, which is in line with the fact that almost one third of the population in North Macedonia lives in the capitol. We observed four forms of JIA in our patients. The most common was the polyarticular form of the disease, found in 17 patients (48.6%). Monoarticular form was present in 9 (25.7%) patients, oligoarticular form in 6 (17.1%) and only 3 (8.6%) patients had systemic form of the disease.

Table 1. Demographic data of patients in the study

Gender	***Number***	***%***	***p value***
Male	12	34.3	
Female	23	65.7	
Total	35	100	
			0.0086
Age	***Mean (years)***	***SD***	
Total	9.8 (3.0 – 15.0)	3.399703	
Male	11.4 (5.0 – 15.0)	3.260182	
Female	9.0 (3.0 – 14.0)	3.233349	
			0.040
Nationality	***Number***	***%***	
Macedonians	25	71.4	
Albanians	5	14.3	
Bosnian	1	2.9	
Roma	4	11.4	
Total	35	100	
			0.0000
Place of living			
Town	34	97.1	
Village	1	2.9	
Total	35	100	
Town			
Skopje	20	57.1	
Kumanovo	3	8.6	
Prilep	2	5.7	
Strumica	2	5.7	
Makedonski Brod	1	2.9	
Shtip	1	2.9	
Bitola	1	2.9	
Veles	1	2.9	
Valandovo	1	2.9	
Ohrid	1	2.9	
Kavadarci	1	2.9	
Village Lazhani around Prilep	1	2.9	
Total	35	100	
Forms of JIA			
Polyarticular	17	48.6	
oligoarticular	6	17.1	
monoarticular	9	25.7	
systemic	3	8.6	
Total	35	100	
			0.0474

We identified 18 HLA-B allele groups in our cohort of patients. The most frequent HLA-B loci were HLA-B*27 and HLA-B*35 with 14.29% and HLA-B18 with 11.42%. Eleven HLA-DRB1 allele groups were detected, and only four of them had frequencies above 10% (Table 2). The most frequent was HLA-DRB1*35 with 35.71%, followed by HLA-DRB1*01 (12.87%), HLA-DRB1*04 (11.43%) and HLA-DRB1*16 (11.43%). Statistically significant difference between patients with JIA and the control group was reached for three allele groups: B*27 (p=0.001959, OR=3.39), B*15 (p=0.000058, OR=7.29) and DRB1*08 (p=0.02758, OR=4.54).

Table 2. Frequency of HLA-B and HLA-DRB1 allele groups in patients with JIA

HLA allele group	Genotype (freq. %)	Phenotype (N)[$]	p value (OR)	HLA allele group	Genotype (freq. %)	Phenotype (N)[$]	p value
B*27	14.29%	9	0.001959 (3.39)	DRB1*11	35.71%	20	0.20663
B*35	14.29%	10	0.862023	DRB1*01	12.87%	8	0.15451
B*18	11.42%	7	0.357912	DRB1*04	11.43%	7	0.44591
B*44	10%	7	0.525	DRB1*16	11.43%	8	0.66833
B*51	10%	7	0.338804	DRB1*08	5.71%	4	0.02758 (4.54)
B*15	8.57%	6	0.000058 (7.29)	DRB1*15	5.71%	4	0.6388
B*40	5.72%	4	0.7648	DRB1*14	4.28%	3	1
B*38	4.29%	3	0.7211	DRB1*03	4.28%	3	0.6096
B*08	2.85%	2	0.4110	DRB1*07	2.86%	2	0.2116
B*14	2.85%	2	0.6994	DRB1*12	2.86%	2	0.2784
B*49	2.85%	2	0.665	DRB1*13	2.86%	2	0.1491
B*52	2.85%	2	0.6347				
B*55	2.85%	2	0.6347				
B*07	1.43%	1	0.1081				
B*13	1.43%	1	0.7113				
B*37	1.43%	1	1				
B*39	1.43%	1	1				
B*41	1.43%	1	1				

[$]Homozygotes are calculated as 1.

The presence of different allele groups in all four forms of JIA are presented in Table 3. The most frequent alleles in polyarticular JIA were HLA-B*27 and HLA-DRB1*11 and in oligoarticular JIA, B*35, B*15 and DRB1*11. A small number of patients had monoarticular or systemic form of JIA, so the significance level was not calculated.

Table 3. HLA allele frequencies in JIA subgroups

HLA allele group	Polyarticular JIA (N[$])	Oligoarticular JIA (N[$])	Monoarticular JIA (N[$])	Systemic JIA (N[$])
B*27	6	1	2	/
B*35	4	3	2	1
B*18	3	1	2	1
B*44	5	/	2	/
B*51	4	/	2	1
B*15	1	3	/	2
B*40	2	/	2	/
B*38	/	1	2	/
B*08	/	/	2	/
B*14	2	/	/	/
B*49	1	/	/	1
B*52	2	/	/	/
B*55	/	1	1	/

B*07	/	/	1	/
B*13	1	/	/	/
B*37	1	/	/	/
B*39	1	/	/	/
B*41	1	/	/	/
DRB1*11	11	5	3	1
DRB1*01	4	/	3	1
DRB1*04	3	/	4	/
DRB1*16	3	2	2	1
DRB1*08	2	/	2	/
DRB1*15	3	/	1	/
DRB1*14	2	/	1	/
DRB1*03	1	1	1	/
DRB1*07	/	/	1	1
DRB1*12	1	/	/	1
DRB1*13	1	1	/	/

[$]Homozygotes are calculated as 1.

Table 4 shows the levels of IL-1beta, IL-6 and TNF-alpha in patients with different forms of JIA, at the moment of diagnosis and 6 months after starting the treatment. These differences were not statistically significant. The movement of the cytokine levels at the two time points is illustrated in Table 5.

Table 4. Concentration of IL-1 beta, IL-6 and TNF-alpha in different forms of JIA before therapy and 6 months after therapy

Forms of JIA	Concentration of IL-1 beta before therapy			Concentration of IL-1 beta six months after therapy			p value
	N	Mean(pg/ml)	SD	N	Mean(pg/ml)	SD control	
polyarticular	17	9.5 (1.9031-21.8451)	7.137777	13	10.4 (1.984-15.2287)	5.565828	p=0.38627
oligoarticular	6	8.0 (1.9031-13.5834)	6.033935	5	6.8 (0.88-15.2287)	7.028990	p=0.89273
monoarticular	9	7.1 (0.8829-15.2287)	6.236090	7	7.6 (1.438-15.2287)	6.687641	p=0.75315
systemic	3	10.0 (1.9031-16.0528)	7.278944	2	14.4 (13.5834-15.2287)	1.163403	p=0.65472
total	35	8.6 (0.8829-21.8451)	6.540887	27	9.3 (0.88-15.2287)	6.071004	
	Concentration of IL-6 before therapy			Concentration of IL-6 six months after therapy			
polyarticular	17	5.4 (0.4206-56.3134)	13.42329	13	2.2 (0.4206-12.9828)	3.281689	p=0.32699
oligoarticular	6	2.5 (0.4206-6.8619)	2.34327	5	1.2 (0.4206-1.7592)	0.483819	p=0.06789
monoarticular	9	1.0 (0.4206-1.54)	0.47106	7	1.2 (0.4206-1.7592)	0.401041	p=0.77150
systemic	3	22.0 (1.3194-63.4557)	35.86013	2	1.0 (1.0267-1.0267)	0	p=0.17971
total	35	5.2 (0.4206-63.4557)	13.86764	27	1.7 (0.4206-12.9828)	2.312026	
	Concentration of TNF-alpha before therapy			Concentration of TNF-alpha six months after therapy			
polyarticular	17	3.6 (0.7133-8.1415)	2.426059	13	4.5 (0.0098-15.2287)	4.074915	p=0.75315
oligoarticular	6	4.0 (0.7133-9.2193)	3.529921	5	2.0 (0.0009-5.0342)	2.295093	p=0.06789

monoarticular	9	4.1 (0.0009-17.1985)	5.354181	7	3.1 (0.0009-5.7063)	2.440684	p=0.61209
systemic	3	3.6 (1.6153-5.0342)	1.759903	2	3.7 (3.4106-4.0494)	0.451700	p=0.17971
total	35	3.8 (0.0009-17.1985)	3.405745	27	3.6 (0.0009-15.2287)	3.286556	

Table 5. Percentage of patients with increased, normal, decreased or missing data for concentration of cytokines

Cytokine	First time point		After 6 months	
IL 1	N	%	N	%
increased	18	51.4	15	42.9
normal	17	48.6	9	25.7
decreased			4	11.4
missing			7	20.0
IL6				
increased	3	8.6	1	2.9
normal	32	91.4	24	68.6
decreased			2	5.7
missing			8	22.9
TNF alfa				
increased	1	2.9		
normal	34	97.1	26	74.3
decreased			1	2.9
missing			8	22.9

Discussion

In our study we analyzed 35 patients diagnosed with juvenile idiopathic arthritis. Most of our patients were females (65.7%), which is in concordance with previously published data[1,2], and female patients were younger at the time of diagnosis. JIA is a clinically heterogeneous disease with different clinical aspects among children [4]. Seventeen of our JIA patients had polyarticular form of the disease, 9 had monoarticular, 6 had oligoarticular and 3 had systemic form of the disease. Genetic factors may contribute to better understanding of the etiology and pathogenesis of the different forms of JIA.

Several studies have shown that the major histocompatibility complex (MHC) is the most important genetic risk factor for JIA [13]. In our group of patients with JIA, the most frequent HLA-B allele groups were HLA-B*27, B*35 and B*18. When we compared the frequencies with the healthy population [25], statistically significant differences were found for two allele groups HLA-B*27 (p=0.001959, OR=3.39) and HLA-B*15 (p=0.000058, OR=7.29) that increased the risk of JIA. The most frequent allelic groups in HLA-DRB1 locus were DRB1*11, *01, *04 and *16, but statistical significance for an increased risk of JIA was found only for DRB1*08 (p=0.02758, OR=4.54). In a meta-analysis, an increased risk of JIA was found for HLA-B*27, HLA-DRB1*08 and HLA-DRB1*11 alleles, while HLA-DRB1*04 showed a protective role for JIA[2,11]. We divided the frequencies among different forms of JIA, but the numbers were too small to reach statistical significance. In the meta-analysis, oligoarticular JIA was associated with HLA-DRB1*08, HLA-DRB1*11 and HLA-DPB1*01:02 alleles, while an increased risk for RF negative polyarticular JIA was found for alleles DRB1*08, DRB1*11 and DPB1*01:03. An increased risk of RF positive JIA polyarthritis was found for DRB1*04 and DRB1*01 alleles[2,13]. Hersh and Prahalad in their review[13] considered together RF-negative oligoarticular and polyarticular JIA due to its phenotypic similarity, with the exception of the number of affected joints. HLA-DRB1*08 was shared as a common predisposing factor, as has been confirmed by our results.

At the time of diagnosis, half of the patients (51.4%) had increased levels of IL-1 beta. After treatment, we observed decrease in the level in 11.4% of patients. Most of the patients (91.4%) had normal IL-6 levels at the time of diagnosis. We detected only three patients with increased concentration, two of them had decrease in the concentration after 6 months. Most of the JIA patients (97.1%) had normal TNF-alpha levels. The concentration was increased in one patient and decreased to normal values after 6 months.

In a two-year prospective study, Spirchez M *et al.*[26] evaluated the relation of IL-1 alpha, IL-6 and TNF-alpha in children with juvenile idiopathic arthritis with disease activity and severity. They compared the results of 40 JIA patients with 18 healthy children. They observed higher IL-6 levels ofin patients with active disease compared to patients with inactive disease. In addition, in every JIA group, there was a statistically significant difference between the values in active disease and when in remission. They concluded that IL-6 can serve as a biomarker for disease activity and severity.

Rooney M, David J, *et al.*[27] in their study evaluated 5 children with systemic juvenile arthritis. IL6 levels were high in the patients with systemic form and correlated with disease activity. No such correlation with disease activity was found in the polyarticular group. Systemic JIA patients were followed in their febrile phase of the illness when IL6 levels increased and fell with the fever.

In our study, the number of patients was low, so the associations for some loci were weak. Due to the smaller population size, it is difficult to have larger cohort, but the results can be used in different meta-analysis in order to discover JIA susceptibility to certain subtypes across ethnic groups depending on the presence of specific alleles that either predispose or protect the individual.

Conclusion

In our study, we found three allelic groups in HLAB and -DRB1 loci to be associated with JIA. HLA-B*27, HLA-B*15 and HLA-DRB1*08 convey a higher risk of development of different forms of JIA. The concentration of proinflammatory cytokines is important for selecting the best therapeutical strategy for JIA patients.

Conflict of interest statement. None declared

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