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Effect of HLA-B*27 and its Subtypes on Clinical Manifestations and Severity of Ankylosing Spondylitis in Iranian Patients

Sasan Fallahi[1, 2], Mahdi Mahmoudi[2], Mohammad Hossein Nicknam[3], Farhad Gharibdoost[2], Elham Farhadi[4], Azad Saei[5], Keramat Nourijelyani[6], Noushin Ahmadzadeh[2], and Ahmad Reza Jamshidi[2]

[1] Internal Medicine Division, Baharloo Hospital, Tehran University of Medical Sciences, Tehran, Iran
[2] Rheumatology Research Center, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran
[3] Molecular Immunology Research Center, Tehran University of Medical Sciences, Tehran, Iran
[4] Department of Haematology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[5] Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[6] Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

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ABSTRACT

The aim of this study was to assess the role of HLA-B*27 and it's subtypes in determining severity and clinical manifestations of ankylosing spondylitis (AS).

A total of 163 AS patients were assessed for clinical manifestations and severity using structured questionnaires. HLA-B*27 screening and B*27 sub-typing were performed by PCR.

One hundred twenty two patients (74.8%) were B*27 positive. The male to female ratio, peripheral arthritis, steroid use, intense dorsal kyphosis and decrease of cervical slope had a significantly higher frequency in B*27 positive patients compared to B*27 negative ones (p=0.01, 0.001, 0.01, 0.04 and 0.04, respectively). However, the age of diagnosis was significantly lower in B*27 positive patients (p=0.005). Trend in uveitis and some severity markers including: BASMI and ASQoL were toward higher values in B*27 positive group with no significant difference. After controlling confounding variables, significant relationship was found only between B*27 and BASMI (p=0.01). B*27 subtypes in patients were included B*2705: 48.4%, B*2702: 42.6%, B*2704: 5.7% and B*2707: 3.3%. No significant differences were seen for severity markers and clinical manifestations between subtypes; although trend toward lower values of severity markers, less intense dorsal kyphosis and less decrease of cervical slope were observed in B*2704 and B*2707 versus other polymorphisms.

Clinical features and severity of AS is influenced by HLA-B*27. Trend toward higher severity markers in B*2705 and B*2702 versus other polymorphisms might be subject of interest for evaluation in other ethnicities with concentration to other novel susceptibility genes co-inherited in each B*27 subtype.

Keywords: Ankylosing Spondylitis; Clinical manifestations; HLA-B*27; HLA-B*2705

Corresponding Author: Ahmad Reza Jamshidi, MD; Rheumatology Research Center, Tehran University of Medical Sciences, Shariati Hospital, Kargar street, Tehran, Iran, Postal code: 1411713135, Tel/Fax: (+98 21) 8802 6956, E-mail: jamshida@sina.tums.ac.ir

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INTRODUCTION

Ankylosing spondylitis (AS) is a chronic rheumatic disease which belongs to the group of spondyloarthropathies. Spine is mainly affected which causes pain and stiffness with initial erosion and later bony fusion of involved joints in axial skeleton. In addition, peripheral arthritis, uveitis, Inflammatory Bowel Disease (IBD), psoriasis, pulmonary fibrosis, aortic regurgitation, amyloidosis, IgA nephropathy and urolithiasis are other manifestations of AS. These clinical features may lead to lifelong physical impairments, functional disabilities, depression and detrimental impact on socioeconomic status and quality of life.[1-5]

Genetic factors and specifically Human leukocyte antigen ,HLA-B*27 are well established as major predisposing factors to AS. This Major Histocompatibility Complex MHC gene is one of the most important factors that play a major role in evaluation, pathogenesis and prognosis of AS. The proportion of HLA-B27 in predisposing an individual to AS is about 20-40%.[6] In most populations, the prevalence of AS is closely related to the frequency of HLA-B*27 and it's subtypes which have been associated with AS (B*2701, B*2702, B*2703, B*2704, B*2705, B*2707, B*2708, B*2710, B*2713, B*2714, B*2719 and B*2725).[7, 8] More than 70 subtypes for HLA-B*27 have been known since 1973.[9] Some of the most common B*27 subtypes associated with AS include: B*2705 and B*2702 in northern and southern America, Europe and Middle East (Iran, Turkey and Syria), B*2704 and B*2705 in Far East Asia (China and Japan), B*2706 and B*2704 in South East Asia (Malaysia) and B*2705 and B*2704 in Korea and India.[10-19] B*2702 is also, the predominant subtype in Lebanese, Semitic and Greek Cypriot populations.[13]

There are several physical, psychosocial and occupational damages related to AS. Therefore, studies on different factors that are related to disease severity help clinicians to predict the need for aggressive therapy. Studies during the last 20 years (for instance, Brown et al. study) revealed that severity and clinical manifestations of AS is mainly controlled by genetic factors.[20] Brown et al. observed no linkage on MHC genes. However, in their study a linkage was observed with BASDAI on chromosomes 11q, 13p, 16p, 18p and 20q and with BASFI on chromosome 2q. They also observed a linkage with age of symptom onset on chromosome 11q. The effect of B*27 on severity and clinical manifestations was not assessed because their survey was performed only on B27 positive patients.[20] HLA-B*27 has been established to play a major role in susceptibility to AS but little has been known about the genetic control of disease severity. Few studies were found throughout the world about the effect of HLA-B*27 and its subtypes on severity of AS.[13, 17, 21-24] Genetic heterogeneity among different races and populations may also, influence the relationship of HLA-B*27 and B*27 subtypes on disease severity. Therefore, the aim of current study was to evaluate the correlation of HLA-B*27 and its subtypes with disease severity and detailed clinical manifestations in Iranian patients with AS.

PATIENTS AND METHODS

Patients

One hundred sixty three consecutive AS patients of 16 years of age and older were recruited into the cross sectional study from three centers: Iran Rheumatology Center, Iranian AS association and rheumatology clinic of Shariati Hospital, Tehran University of Medical Sciences. HLA-B27 screening and sub-typing were performed in Rheumatology Research Center Laboratory in Shariati hospital. All patients fulfilled the modified New York 1984 criteria for AS.[25] They were evaluated with structured questionnaires and physical examination by a rheumatologist. Clinical data included age, sex, ethnicity, BMI, smoking, educational level, age at disease onset, age of diagnosis, duration of disease, peripheral arthritis, enthesitis, changing the curvature of cervical, dorsal and lumbar spine from normal state, primary involved joint, sacroiliitis grading by pelvic radiography, assessment for uveitis, psoriasis, IBD, other extra-articular manifestations and drug treatments such as non steroidal anti-inflammatory drugs (NSAIDs), disease modifying anti-rheumatic dugs (DMARDs) such as sulfasalazine (SSZ), Methotrexate (MTX) and biologic drugs such as infliximab and/or etanercept. Disease severity was assessed by severity markers including the Bath AS Disease Activity Index (BASDAI),[26] Bath AS Functional Index (BASFI),[27] Bath AS Metrology Index ($BASMI_{10}$),[28,29] Bath AS Global Score (BAS-G)[30]and AS Quality Of Life (ASQoL).[31] Validity and reliability of Iranian versions of these questionnaires were assessed and defined with acceptable values in a

previous study.[32] The patients gave informed consent in accordance with Helsinki declaration before enrolling and the project was approved by ethics committee of Tehran University of Medical Sciences.

HLA-Typing

Genomic DNA was extracted from whole blood samples by standard phenol-chloroform-proteinase-K method. The presence of HLA-B*27 and its subtypes were detected by polymerase chain reaction with sequence specific primer (PCR-SSP). HLA-B27 screening and sub-typing was performed by Olerup SSP™ HLA*B27 kit (Olerup SSP AB, Hasselstigen, Sweden). The PCR amplifications were carried out in an ABI2720 thermal cycler (Applied Biosystems, USA). PCR products were visualized in 2% agarose gel under UV illumination (UVitec) following ethidium bromide staining and were documented by photography.

Statistical Analysis

Positive and negative B*27 patients and also patients with different B*27 subtypes were compared with respect to categorical variables such as arthritis, enthesitis, extra-articular manifestations and other clinical manifestations using Chi-square test and where needed, Fisher's exact test. Continuous variables such as severity markers were compared between B*27 positive and B*27 negative groups using Mann-Whitney U-test. Kruskal-Wallis test was used to evaluate the significant differences of continuous variables between different HLA-B*27 subtypes. For assessing the correlation of HLA-B*27 on severity markers and controlling the effect of confounding variables, multiple linear regressions test was used. The multiple linear regressions test was also used to evaluate the correlation of B*27 subtypes on each severity marker concurrent with controlling probable confounding variables. Given that there were more than two subtypes for HLA-B*27, dummy variable was constructed with this manner: B*2705 was considered as reference point and dummy variable was constructed for B*2702. B*2704 and B*2707 positive patients were combined and constituted another group due to small number of the patients with these two alleles (totally eleven individuals). A dummy variable was also constructed for B*2704 / B*2707 group. All statistical analyses were performed using SPSS for windows 18. Statistical tests were two sided and significant level with $p< 0.05$ was considered significant.

RESULTS

Out of 163 patients, there were 129 males (79.1%) and 34 (20.9%) females. The median age was 37 years (range 18-65). HLA-B*27 was positive in 122 individuals (74.8%). Five hundred twenty eight healthy controls (with no autoimmune diseases and no family history of AS) were also evaluated for HLA-B*27 status and its subtypes. Among controls, eighteen individuals (3.4%) were B*27 positive. The frequencies of HLA-B*27 between patient and normal population was compared and tested using the chi-squared test. There was a significant difference in HLA-B*27 frequency between the patients and normal population ($p < 0.001$).

Comparing Clinical Features between Positive and Negative HLA-B*27 Patients

Different features of the patients including: age, sex, age of diagnosis, age at disease onset, disease duration, family history of AS, history of any other autoimmune diseases, history of urogenital and gastrointestinal infections at disease onset, ethnicity, smoking, BMI and educational level were compared between positive and negative B*27 patients. Significant differences were observed between two groups only for gender, age of diagnosis and history of other autoimmune diseases. Male gender were 83.6% in B*27 positive group compared with 65.9% in B*27 negative group (p=0.01). Median age of diagnosis was 29 years (range 10-64) in B*27 positive group compared with 33 years (range 17-57) in B*27 negative group (p=0.005). Other autoimmune diseases (diabetes mellitus and thyroid autoimmune disease) were 4.9% in B*27 positive group compared with 24.4% in B*27 negative group (p<0.001).

The most common primary involved joint was hip (39.8%). Significant differences were not seen between positive and negative B*27 patients for hip involvement as a primary involved joint (38.3% versus 43.9%, p=0.52). There were also no significant differences between positive and negative B*27 patients for enthesitis (68% versus 43.9%, p=0.58), decrease of lumbar lordosis (61.5% versus 58.5%, p=0.73) and sacroiliitis grading (p=0.72). However, significant differences were observed between B*27 positive and B*27 negative groups for peripheral

arthritis (58.5% versus 29.3%, respectively, p=0.001), increase in dorsal kyphosis (59.8% versus 41.5%, respectively, p=0.04) and decrease of cervical slope (54.9% versus 36.6%, respectively, p=0.04).

The most common and the rarest location for enthesitis included lumbar spine (49.1%) and knee (1.2%), respectively. Other regions of enthesitis included dorsal spine (44.2%), hip (20.9%), cervical spine (16%), chest wall (15.3%), grater trochanter (8.6%) and heel (6.7%). Enthesitis regions in positive and negative B27 individuals were not significantly different (p>0.05). The most common location for peripheral joint involvement (at the time of study) was coxofemoral joint (38% of the total affected individuals). Other regions of peripheral arthritis were shoulder (14.7%), ankle (9.8%), knee (8.6%), wrist (3.1%), elbow (3.1%) and metatarsophalangeal joints (1.3%). A significant difference between positive and negative B*27 patients was observed only for coxofemoral joint involvement (42.6% versus 24.4%, respectively, p=0.03).

Extra-articular manifestations among all affected individuals included uveitis (14.1%), IBD (6.7%), psoriasis (4.3%), urolithiasis (11.7%), hematuria and proteinuria not caused by urolithiasis (0.6%), aortic regurgitation (0.6%), pulmonary fibrosis (1.2%) and recurrent oral aphtous (36.3%). Comparison between positive and negative B*27 patients did not show significant differences for extra-articular morbidities (p>0.05). However, a trend in uveitis and IBD were toward the more frequency in B*27 positive patients compared to B*27 negative patients (15.6% versus 9.8%, p=0.73 and 7.4% versus 4.9%, p=0.35, respectively).

Comparison of severity indices and chest expansion between positive and negative B*27 patients were summarized in table 1. Drug treatments were compared between B*27 positive and negative patients. Treatments included NSAIDs, MTX, SSZ, oral and/or parenteral corticosteroids equivalent to at least 5mg/day Prednisolone at the time of clinical exam and at least one of the biologic drugs (infliximab and/or ethanercept) at anytime during the disease progress. The number of DMARDs was also compared between B*27 positive and negative patients (Table 1). The multiple linear regressions model showed a significant relationship between HLA-B*27 and BASMI (regression coefficient [R] 0.76, 95% confidence interval [CI95%] 0.17-1.35, p=0.01) (Table 2). But relationships were not significant between HLA-B*27 and other severity indices (p>0.05) (Table 3).

Table 1. Comparison of severity markers and drug treatments between positive and negative B*27 patients with AS

Severity marker		B27 positive	B27 negative	Total	*P*-value
BASDAI*		2.6, 4.85, 6.3 0.2, 10	2.85, 4.2, 6.4 0.6, 8.5	2.7, 4.6, 6.3 0.2, 10	0.81
BASFI*		1.4, 3.95, 6.25 0, 10	1.6, 3.7, 6.45 0.2, 8.5	1.4, 3.9, 6.4 0, 10	0.81
BASMI*		2.6, 3.7, 5.8 0.6, 8.6	1.9, 3.6, 5 0.6, 8.4	2.4, 3.6, 5.6 0.6, 8.6	0.21
ASQoL*		3.75, 8, 13 0, 18	3, 7, 11.5 0, 18	3, 8, 13 0, 18	0.34
BAS-G*		3, 5.5, 7.5 0, 10	3, 4.5, 7.25 0, 10	3, 5, 7.5 0, 10	0.36
Etanercept or Infliximab		13 (10.7%)	6 (14.6%)	19 (11.7%)	0.57
Corticosteroid		54 (44.3%)	9 (22%)	63 (38.7%)	0.01
NSAIDs		115 (94.3%)	39 (95.1%)	154 (94.5%)	1.00
Sulfasalazine		91 (74.6%)	28 (68.3%)	119 (73%)	0.43
Methotrexate		48 (39.3%)	11 (26.8%)	59 (36.2%)	0.14
Number of DMARDs	0	24 (19.7%)	12 (29.3%)	36 (22.1%)	0.34
	1	57 (46.7%)	19 (46.3%)	76 (46.6%)	
	2	41 (33.6%)	10 (24.4%)	51 (31.3%)	
Chest expansion* (cm)		3, 4, 6 0, 14	3, 4, 5 1.5, 9	3, 4, 5.5 0, 14	0.98

Presented values for continuous variables are 25th percentile, median, 75th percentile, minimum and maximum, respectively.

Table 2. Regression analysis for relationship of HLA-B*27 and some other variables with BASMI

Variable	Regression coefficient	Standard error	Confidence interval 95%	*P*-value
HLA-B27	**0.76**	0.30	0.17 to 1.35	0.01
Age	0.09	0.02	0.05 to 0.13	< 0.001
Sex	0.78	0.31	0.17 to 1.40	0.01
Age of diagnosis	0.05	0.03	-0.003 to 0.1	0.07
Age at disease onset	-0.09	0.02	-0.14 to -0.05	< 0.001
Non steroidal anti-inflammatory drug	0.97	0.57	-0.16 to 2.10	0.09
Sulfasalazine	-0.73	0.30	-1.33 to -0.14	0.02
Corticosteroid	0.26	0.30	-0.34 to 0.85	0.39
Methotrexate	-0.15	0.30	-0.74 to 0.44	0.62
At least one biologic drug (Ethanercept or Infliximab)	0.24	0.40	-0.54 to 1.02	0.55

Table 3. Regression analysis for relationship between HLA-B*27 and each severity index after controlling other variables* in patients with AS

Disease severity index	Regression coefficient	Standard error	Confidence interval 95%	*P*-value
BASMI	0.76	0.3	0.17 to 1.35	0.01
BASDAI	0.34	0.44	-0.53 to 1.20	0.47
BASFI	0.43	0.51	-0.57 to 1.42	0.40
ASQoL	1.8	0.99	-0.16 to 3.76	0.07
BAS-G	0.61	0.5	-0.37 to 1.59	0.22

* Other variables included age, sex, age of diagnosis, age at disease onset and different drug treatments.

HLA-B*27 Subtypes

The results of sub-typing were as follows: 48.4% B*2705, 42.6% B*2702, 5.7% B*2704 and 3.3% B*2707. Among B*27 positive patients, 83.6% were male and 16.4% were female. The median age was 36 years (range 18-65). Sub-typing for eighteen B*27 positive healthy controls showed these results: nine individuals (50%) B*2705, one individual (5.6%) B*2702, seven individuals (38.9%) B*2707 and one individual (5.6%) B*2703. The B*2704 was not observed in healthy controls. Frequencies of the HLA-B*27 subtypes between the patients and the normal population were compared and tested separately for each subtype using the Fisher's exact test. The frequency of B*2702 and B*2707 was significantly different between patients and normal population (p=0.002 and p<0.001, respectively). However, frequency of B*2705 and also B*2704 was not significantly different between the patients and healthy individuals (p>0.05).

Comparing Clinical Features between Different B*27 Subtypes

Among the features studied (the same features compared between positive and negative B*27 patients), only family history of AS had borderline significant difference between B*27 subtypes (34.6% in B*2705, 37.3% in B*2702 versus no individual in B*2704 / B*2707 group, p=0.05).

Articular and peri-articular features including: primary involved joint, enthesitis, peripheral arthritis, decreasing lumbar and cervical lordosis, increasing dorsal kyphosis and sacroiliitis grading were compared among B*27 subtypes. No significant differences were observed among subtypes (Table 4). Extra-articular manifestations (uveitis, IBD, psoriasis, pulmonary fibrosis, aortic regurgitation, renal disease and oral aphtous) were also compared among B*27 subtypes but no significant differences were found for these manifestations (p>0.05).

Table 4. Articular and peri-articular characteristics of HLA-B*27 positive patients with AS in different B*27 subtypes

Characteristics		B2702	B2705	B2704, B2707	Total	*P*-value
Primary involved	Cervical spine	0	2 (3.4%)	1 (9.1%)	3 (2.5%)	0.31
article	Dorsal spine	10 (19.6%)	10 (17.2%)	5 (45.5%)	25 (20.8%)	
	Hip	19 (37.3%)	25 (43.1%)	2 (18.2%)	46 (38.3%)	
	Knee	5 (9.8%)	5 (8.6%)	0	10 (8.3%)	
	Ankle	5 (9.8%)	1 (7.7%)	1 (9.1%)	7 (5.8%)	
	Lumbar spine	9 (17.6%)	11 (19%)	2 (18.2%)	22 (18.3%)	
	Others	3 (5.9%)	4 (6.9%)	0	7 (5.8%)	
Peripheral arthritis		28 (53.8%)	37 (62.7%)	6 (54.6%)	71 (58.2%)	0.61
Enthesitis		36 (69.2%)	41 (69.5%)	6 (54.5%)	83 (68%)	0.60
sacroiliitis grading	2	14 (26.9%)	18 (30.5%)	6 (54.5%)	38 (31.1%)	0.51
	3	25 (48.1%)	29 (49.2%)	3 (27.3%)	57 (46.7%)	
	4	13 (25%)	12 (20.3%)	2 (18.2%)	27 (22.1%)	
Decrease of lumbar lordosis		33 (63.5%)	36 (61%)	6 (54.5%)	75 (61.5%)	0.85
Increase of dorsal kyphosis		33 (63.5%)	35(59.3%)	5 (45.5%)	73 (59.8%)	0.53
Decrease of cervical slope		32 (61.5%)	31 (52.5%)	4 (36.4%)	67 (54.9%)	0.27

Table 5. Comparison of severity markers and drug treatments between different B*27 subtypes in HLA-B*27 positive patients with AS

Severity marker		B2702	B2705	B2704/ B2707	Total	*P*-value
BASFI*		1.425, 3.8, 6.15	1.5, 4.5, 6.6	0.3, 3.1, 5.2	1.4, 3.95, 6.25	0.35
		0, 10	0, 9.8	0.1, 5.6	0, 10	
BASDAI*		2.8, 4.85, 6.28	2.6, 5.1, 6.4	2.6, 3.8, 5.6	2.6, 4.85, 6.3	0.62
		0.2, 10	0.6, 9.4	1, 7.2	0.2, 10	
ASQoL*		3.25, 8, 11.75	4, 9, 14	2, 6, 8	3.75, 8, 13	0.24
		0, 18	0, 18	1, 14	0, 18	
BASMI*		2.6, 4.10, 5.8	2.6, 3.6, 5.8	2.2, 3.4, 3.8	2.6, 3.7, 5.8	0.46
		1, 8.6	1.4, 8.4	0.6, 6.8	0.6, 8.6	
BAS-G*		3.13, 5.5, 7.5	3, 5.5, 7.5	2.5, 4, 5	3, 5.5, 7.5	0.16
		0, 10	0, 10	0.5, 7.5	0, 10	
Steroid		26 (50%)	23 (39%)	5 (45.5%)	54 (44.3%)	0.50
Nonsteroidal anti-inflammatory Drugs		48 (92.3%)	57 (96.6%)	10 (90.9%)	115 (94.3%)	0.40
Sulfasalazine		39 (75%)	43 (72.9%)	9 (81.8%)	91 (74.6%)	0.81
MTX		20 (38.5%)	26 (44.1%)	2 (18.2%)	48 (39.3%)	0.26
Number of DMARDs	0	8 (15.4%)	14 (23.7%)	2 (18.2%)	48 (39.3%)	0.18
	1	29 (55.8%)	21 (35.6%)	7 (63.6%)	57 (46.7%)	
	2	15 (28.8%)	24 (40.7%)	2 (18.2%)	41 (33.6%)	
At least one biologic drug		6 (11.5%)	6 (10.2%)	1 (9.1%)	13 (10.7%)	0.95
Chest expansion* (cm)		3, 4, 6	3, 4, 5	2, 4, 7	3, 4, 6	0.70
		0.5, 9	0, 7	2, 14	0, 14	

* Presented values for continuous variables are 25th percentile, median, 75th percentile, minimum and maximum, respectively.

Comparing severity indices, chest expansion and drug treatments among patients with different B*27 subtypes were summarized and compared (Table 5). After entering different variables including: age, gender, age of diagnosis, age at disease onset, different drug treatments and B*27 subtypes in multiple linear

regressions model, significant relationship was not found between B*27 subtypes and severity indices (p> 0.05).

DISCUSSION

The results demonstrated that disease severity and clinical manifestations of AS are controlled by HLA-B*27 status to some extent. More rapidly evolution of disease in B*27 positive versus B*27 negative patients might be the reason for lower age of diagnosis in former group. In our study, we found an equal disease duration in positive and negative B*27 patients and the more severe changes in dorsal and cervical spine posture in B*27 positive patients compared with B*27 negative ones. These findings demonstrated that B*27 positive patients might have more rapidly progressive disease. Also, more frequent peripheral arthritis in B*27 positive patients was due to the higher frequency of hip involvement in this group (42.6% vs. 24.4%). Trend toward higher scores was noted for BASMI and ASQoL in B*27 positive patients compared with B*27 negative ones without significant differences between the two groups (Table 1). After controlling probable confounding variables, we found that HLA-B*27 had a significant relationship with only BASMI and not with BASDAI, BASFI, BAS-G and ASQoL (Table 3). We also noted that besides the HLA-B*27 status, age, sex and age at disease onset might be the meaningful indices amongst several features for evaluation of disease severity (BASMI). These results could provide helpful clue for individualized treatment in AS patients (Table 2). More use of steroid in B27 positive versus B27 negative patients may indicate that the disease is more severe in the former group (Table 1). Amongst the extra-articular manifestations, only uveitis and IBD and also enthesitis had tendency to be more frequent in the B*27 positive group versus the B*27 negative group (15.6% versus 9.8% and 7.4% versus 4.9% and 68% versus 43.9%, respectively) but cardiac, pulmonary, kidney disease and psoriasis were similar in the two groups.

The majority of our findings were compatible with results of other studies: In a meta-analysis, Linssen showed an earlier disease onset, more severe spinal disease, more frequent peripheral arthritis and anterior uveitis but less frequent psoriasis and IBD in B*27 positive AS patients versus B*27 negative ones.[33] Feldtkeller et al. reported a significantly younger average age at disease onset and also a trend toward more frequent uveitis in B27* positive patients versus B*27 negative ones.[34] Khan et al. showed no differences in extra-articular features between positive and negative B*27 patients except the more frequent uveitis in the former group.[35] Brown et al. found no significant difference for BASSI (Bath AS severity index) between positive and negative B27 patients in UK (0.057 versus 0.0413, respectively).[21] In Finland, Jaakkola et al. noted an earlier age of symptom onset and earlier age of diagnosis in B*27 positive patients versus B*27 negative ones. They also, showed B*27 positive cases were more likely to have anterior uveitis but significant associations were not seen between the B*27 and other clinical features.[24] Results of Wu et al. in China revealed a significantly younger average age at disease onset in B*27 positive versus B*27 negative patients (21.1±6.2 versus 28±7.9 years, respectively). Other clinical features including: family history, the location of first symptom, peripheral arthritis and extra-articular manifestations were not statistically different between the two groups. However, peripheral arthritis and extra-articular manifestations had tendency to be more frequent in B*27 positive than B27 negative cases (43/93 versus 1/5 and 14/93 versus 0, respectively).[36] In Mexico, Vargas-Alarcon et al. revealed by an investigation on spondyloarthropahties that the age at disease onset, sex and disease severity appeared to be influenced by HLA-B*27. On the other hand, B*27 positive patients with spondyloarthropathy were mostly young men who had more severe and active disease (measured by BASDAI, BASFI, chest expansion and Schober's test) compared with B*27 negative patients.[22] In UK, Freeston et al. illustrated that there were trend toward higher BASDAI, BASFI and ASQoL scores, more steroid, NSAID and biologic drug use and more frequent ocular, pulmonary and cardiac manifestations in B*27 positive patients compared with B*27 negative patients. Conversely, gastrointestinal, dermatological and genitourinary features had tendency to be more frequent in B*27 negative patients (although, statistically significant differences were not seen between positive and negative B*27 patients).[23]

Another major result of our study was defining the B*27 subtypes in patients with AS. The two most common B*27 subtypes were B*2705 and then B*2702. This result was partially compatible with the studies conducted before in Middle East (Turkey, Syria).[12, 14, 16] The two less frequent B*27 alleles in our

patients were B*2704 (7 patients) and B*2707 (4 patients). There was borderline significant difference in family history of AS between patients with different B27 polymorphisms (37.3% in B2705 and 34.6% in B2702 versus no one in B2704 and B2707 alleles). Therefore, the two latter subtypes in our region were probably found only in patients with sporadic AS. Contrary to this result is the survey of Wu et al in China in which positive family history was reported, 40% in B*2715, 20.7% in B*2704 and 8% in B*2705.[36] This discrepancy might be due to differences between the B*27 polymorphisms in Middle East and Far East Asia which predispose individuals to AS. In our study, there was not any significant differences between B*27 subtypes for the recorded clinical manifestations. Also, No significant correlation between B*27 subtypes and BASDAI, BASFI, ASQOL, BAS-G and BASMI were found, even after controlling confounding variables. However, increase in dorsal kyphosis and decrease of cervical slope had tendency to be less frequent and severity markers had tendency toward lower scores in patients with B27*04/B*2707 versus patients with B*2702 and B*2705 alleles. A trend toward less disease severity in patients with B27*04 or B*2707 polymorphisms may be justified to some extent by comparing the B*27 subtypes of our AS patients with the B*27 subtypes distributions in Iranian healthy individuals. Limited investigations are available considering clinical correlations of B*27 subtypes. Park et al. study on Korean patients with AS revealed no correlation between B*27 subtypes (B*2704 or B*2705) and clinical features or disease severity (uveitis, peripheral arthritis, BASDAI scores).[17] In their study, Wu et al. noted that the average onset age of disease in patients with the B*2704 was significantly lower than B*2705 ones (20.45 ± 4.5 versus 26.67 ± 9.95, respectively). They also found that peri-articular involvement was more common in B*2704 positive patients compared with B*2705 ones but without significant differences (83.72% versus 11.63%).[36] Another report by Chavan et al. showed no significant difference between two major subtypes (B*2705 and B*2704) in Indian AS population for clinical features. However, a trend in AS-associated uveitis was found in B*2704 positive patients compared with B*2705 positive ones (34.78% versus 16.36%, respectively).[13]

One of the advantages of our study was the larger number of B*27 negative patients in comparison with Freeston, Wu and Jaakkola surveys. This increased the ability of our survey to demonstrate differences between positive and negative B*27 patients. Another advantage was to evaluate the disease severity not only using the subjective structured questionnaires (BASDAI, BASFI, BAS-G and ASQoL) but also using the objective questionnaires (BASMI). Although, radiographic sacroiliitis grading was reported in our patients, the systematic radiographic disease severity indices such as BASRI (Bath AS radiologic index) were not used. This was a limitation in our survey.

Current study demonstrated the relationship of HLA-B*27 status with clinical features and severity of disease (confirmed by BASMI in our study). Even though, this relationship is at least partly dissimilar in different ethnic and geographic populations. Correlations between B*27 subtypes with several clinical features such as uveitis, peri-articular disorders and age at disease onset were not significant in our survey. But a trend toward lower markers of activity, better functional status, better quality of life and better spinal and hip mobility (measured by BASMI) in patients with the B*2704 or B*2707 versus patients with the B*2705 or B*2702 alleles, indicated probably a milder disease in the former group of Iranian AS patients. This result was somewhat different from Wu[36] and Chavan studies.[13] Genetic factors responsible for clinical features and disease severity of AS patients may be at least partly dissimilar between different races. That might be the cause of disparities between results of this study with Wu and Chavan studies in China and India, respectively. This could be a hint of interest for future investigations in different ethnic groups of AS patients and in each B*27 subtype, separately.

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