Original Article

EVALUATION OF 278 HLA-B27 POSITIVE PATIENTS SUSPECTED OF SERONEGATIVE SPONDYLOARTHROPATHIES

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ABSTRACT

Objective: To determine HLA-B27 prevalence in patients suspected of Seronegative spondyloarthropathy referred to the Transplantation Department of Blood Transfusion Organization, and to evaluate clinical findings among HLA-B27 positive patients.

***Methodology*:** One thousand six hundred ten patients having clinical manifestation of seronegative SpAs were screened for HLA typing by serological methods from January 1997 to June 2002 at Transplantation Department of Blood Transfusion Organization, Ahwaz, Iran. Serologic-based HLA typing using Antigen-specific sera to determine a person's HLA type was performed. Among these patients, individuals found HLA-B27 positive were investigated regarding clinical findings, age, and sex distribution**.**

Results: In this study the frequency of HLA-B27 antigen was 17.26% (278 cases). The minimum age in males was 10 years and the maximum age in female was 70 years. Median age with seronegative SpAs findings (34.2% including 28.42% females, 71.57% males) was 20-30 years. Based on our results, the most frequent clinical manifestation, was peripheral joints arthritis (58.7%; 34.35% females, 65.65 % males). There were no association between any of the major clinical manifestations and age or sex distribution.

Conclusions: These findings confirm the strong association of the HLA B27 allele with various types of spondyloarthritis and suggests that HLA typing would help in the diagnosis of seronagative SpAs, specially ankylosing spondylitis with indeterminate clinical presentation and also in identifying at risk family members.

KEY WORDS: Seronegative, Spondyloarthropathies (SNSA), HLA-B27 antigen.

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INTRODUCTION

Seronegative spondyloarthropathies (SNSA) include a group of diseases with arthritis that are negative for rheumatoid factor. The borders of the disease are sometimes obscure, and SNSA has its own classification criteria. The investigation on the prevalence of HLA-B27 will be important for the understanding of SNSA pathogenesis.[1]

The association of HLA-B27 with ankylosing spondylitis was first described in 1973,[2] and is among the strongest described for a HLA locus. The recent demonstration that HLA-B27 can interact with a number of different immunoreceptors on different cell types has opened up promising new avenues of research

into clarifying its role in the pathogenesis of spondyloarthropathy.[3]

The finding that the natural role of HLA molecules is peptide binding and presentation to T cells led to the suggestion that the spondyloarthropathies result from the ability of HLA-B27 to bind a unique set of peptides. This 'arthritogenic' peptide hypothesis proposes that disease results from an HLA-B27-restricted cytotoxic T-cell response to a peptide or peptides found only in joint and other affected tissues. Such a peptide could be bound and presented by all disease-associated HLA-B27 subtypes, but not by other class I molecules. Pathogenic T cells might be primed in the joint or at other sites such as the genital or gut mucosa. A modification of this original hypothesis could entail a breakdown of self-tolerance by initial HLA-B27-restricted presentation of a peptide or peptides derived from one of the triggering pathogens.[3-6]

If the disease association of HLA-B27 is indeed a consequence of its physiological role in peptide presentation, HLA-B27-restricted cytotoxic T lymphocytes (CTL), specific for self-epitopes or bacterial epitopes, should be demonstrable in the involved joints of patients with spondyloarthropathies[3,7] (Figure-1).

Possession of the human leukocyte antigen (HLA) class 1 allele HLA-B27 is strongly associated with development of the spondyloarthritidis, a group of related diseases including ankylosing spondylitis (AS), Reiter's Syndrome, reactive arthritis, psoriatic arthritis, Crohn's disease, and ulcerative colitis.[8-10] These conditions share clinical features including arthritis of the spine and large joints, and involvement of the skin, eye, genital mucosa, heart, aortic valve, and kidney.[11] Seronegative spondylarthritis are frequently characterized by extra-articular manifestations. They are frequently in recurrent uveitis. Between the cutaneous manifestations should be mentioned erythema nodosum, typical of inflammatory bowel diseases, and keratoderma blenorrhagicum, in the Reiter's syndrome. Cardiac complications in ankylosing spondylitis include aortic valve regurgitation and arrhythmia and, more rarely, mitral valvulopathy, cardiomyopathy and pericarditis. Pulmonary involvement in AS includes ventilatory restrictive syndrome and fibro-bullous disease of the apex. Vertebral osteoporosis is a very important extra-articular manifestation because of the possibility of spontaneous fractures of the vertebrae. Central neurological manifestations include medullary compression from cervical sub-luxation while the most important peripheral involvements are lumbar stenosis and the cauda equina syndrome. Type AA amyloidosis is a rare late complication of the AS, possible cause of death especially in patients with aggressive disease. Kidney complications can be observed as consequences of prolonged anti-inflammatory therapy, but the most frequent renal complications are amyloidosis and mesangial IgA segmental and focal glomerulonephritis[8] (Table-I).

In one report, HLA-B27 and associate antigens incidence were studied in 620 cases of seronegative spondiloarthropathies (SNS) and 262 controls of a Venezuelan mestizo popula-

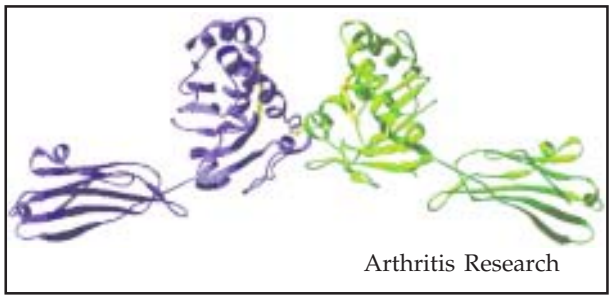

Fig-1: Hypothetical molecular model of the HLA-B27 heavy chain homodimer structure. The alpha 1, 2, and 3 domains of two HLA-B27 molecules are shown in ribbon form, bound peptide shown. Orientation: cell surface at bottom of picture (*Arthritis Res* 2002,4(Suppl 3):S153-S158).

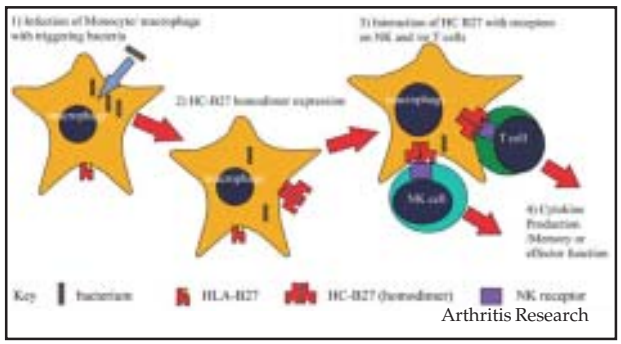

Fig-2: Hypothetical model for the role of HLA-B27 homodimers in the pathogenesis of spondyloarthritis. NK, natural killer (*Arthritis Res* 2002,4(Suppl 3):S153-S158).

tion from Zulla state between 1985 and 1995. The incidence of HLA-B27 was 20.96% of all cases of SNS. It was increased in patients with ankilosing spondylitis (AS) 33.33% and Reiter's syndrome (RS) 30%, but not in uveitis (Uv) 20% an psoriatic arthropathy (PsA) 0%. The incidence in the control group was 4.2%.[9]

In another report, the most frequent symptom at diagnosis of SNSA was inflammatory low back pain, followed by asymmetric oligoarthralgia and Achilles tendonitis and/or plantar fasciitis Systemic complications were revealed as eye and skin involvement. Human leukocyte antigen (HLA) typing showed various patterns among patients, in which HLA-B27 was positive in 40% of patients with ankylosing spondylitis.[12]

PATIENTS AND METHODS

Study Population: The target population in present study included all patients suspected of SNSAs referred to Transplantation Department of Blood Transfusion Organization for HLA typing, from January 1997 to June 2002.
HLA Typing: Serologic-based HLA typing using Antigen-specific sera was used to determine a patient's HLA type. The complement mediated microcytotoxicity test was performed using commercial kit including antigen-coated microplates (Biotest, Germany), and the test was done according to manufacturer's instruction. This was a prospective descriptive-analytic research study and our method for sampling was census. Each of HLA-B27 positive patients were evaluated for age and sex distribution and at least one of the major clinical features of NSAs.
Data Analysis: Collected data were analyzed with SPSS version 10.0 and Pearson's chi square test was used.

RESULTS

A five-year prospective study was carried out to obtain information of 1610 patients suspected to SNSAs. (Table-I.) Two hundred seventy eight patients were found to be HLA-B27 positive. Among 278 HLA-B27 positive patients, 102 (36.7%) cases were females and 176(63.3%) were males. Mean age was 29.82 years (SD=10.75). Mean age of females 30.65 years (SD=11.89) vs. mean age of males 29.33 years (SD=10.03). The youngest male and female were 10 and 13 years old whereas the oldest male and female were 65 and 70 years old respectively. Age distribution of patients was not normal distribution.

Table-I: Common features of SNSAs

Absence of
1. Rheumatoid factor
2. Subcutaneous ('rheumatoid') nodules

Clinical manifestations
1. Sacroilitis/Spondylitis
2. Inflammatory peripheral arthritis (often asymmetrical)

*Extra-articular manifestation**
3. Ocular inflammation (such as conjunctivitis,acute uveitis)
4. Alteration of skin (psoriasiform skin or nail lesion,erythema nodosum)
5. Buccal laceration of mouth,small ,and large intestine
6. Enthesopathy
7. Thrombophelebitis
8. Pyoderma gangraenosum

Familial aggregation
Association with HLA-B27

For data analysis, patients were divided into six 10 year-age groups. The maximum number of patients were in 20-30 years group (34.2%) 27 patients (28.46%) were females and 68 (71.56%) were male patients (Table–II). On the basis of at least one major clinical manifestation of SNSAs, patients were classified into 6 groups including Sacroilitis/Spondylitis, Inflammatory peripheral arthritis, Ocular inflammation, Alteration of skin, Buccal alceration, and Enthesopathy (Table-III).

In this study, Inflammatory peripheral arthritis was the commonest 163 cases (58.7%); including 56(34.35%) female and 107(65.64%) males. Alteration of skin were least encountered 6 cases (2.15%) including 3(50%) female and 3(50%) male. Other major clinical mani-

Table-II: Frequency of age groups on basis of sex

Sex /age group	*10-20 (%)*	*21-30 (%)*	*31-40 (%)*	*41-50 (%)*	*51-60 (%)*	*61-70 (%)*
Female	18 (36)	27 (28.42)	34 (41.46)	19 (50)	2(25)	2(40)
Male	32 (64)	68 (71.57)	48 (58.53)	19 (50)	6 (75)	3(60)

festations of SNSAs are presented in Table-III. The association of clinical manifestations was investigated in different age groups among the patients (Table-IV). No significant association was found between age and sex groups and each of the major clinical manifestations.

DISCUSSION

Seronegative spondyloarthropathies are a group of disorders characterized by inflammation of the spine, sacroiliac joints, and peripheral arthritis along with various characteristic extra-articular features. Their pathogenesis and immunogenetics have not yet been fully elucidated. Ankylosing Spondylitis (AS) is probably the best studied of these diseases. It has now been 35 years since the association of human leukocyte antigen (HLA) B27 and AS has been demonstrated.[13] Since then, a plethora of association studies and linkage studies unequivocally demonstrate that genetic determinants within or near the major histocompatible complex (MHC) are critical to the etiology of AS.[13]

Our study demonstrates that prevalence of male patients with at least one major clinical manifestation of SNSAs is greater than female patients, although there were no significant association between sex distribution of patients and at least one major clinical manifestation of SNSAs.

In population surveys, 1 to 6% of adults inheriting HLA-B27 have been found to have AS.[8] In contrast in families with AS, the prevalence is 10 to 30% among adult first – degree relatives inheriting HLA-B27. The concordance rate in identical twins is approximately 65%. It is currently believed that susceptibility to AS is determined almost entirely by genetic factors, with HLA-B27 comprising about one-third of the genetic component.[14]

Table-III: Frequency of major clinical manifestation on the basis of sex

Clinical Feature	Sex	
	Female (%)	*Male (%)*
1. Sacroilitis/Spondylitis	22 (39.28)	34(66.71)
2. Inflammatory peripheral arthritis	56(34.35)	107(65.64)
3. Ocular inflammation	8(44.45)	10(55.56)
4. Alteration of skin	3(50)	3(50)
5. Buccal ulceration	5(33.34)	10(66.67)
6. Enthesopathy	8(40)	12(60)

HLA-B27 and associate antigens incidence were studied by Rivera S. and colleagues in 620 cases of seronegative spondiloarthropathies (SNS) and 262 controls of a Venezuelan mestizo population from Zulla state between 1985 and 1995. The incidence of HLA-B27 was 20.96% of all cases of SNS. It was increased in patients with ankilosing spondylitis (AS) 33.33% and Relter's syndrome (RS) 30%, but not in uveitis (Uv) 20% and psoriatic arthropathy (PsA) 0%.[15]

Luukkain RK and colleagues investigated sacroiliitis in patients with seronegative Oligoarthritis. Thirty consecutive patients with seronegative oligoarthritis and no other signs of spondylarthropathy were included. Sacroiliac (SI) joints were investigated by both radiography and magnetic resonance imaging. HLA B27 antigen was studied and family history was reexamined in 2006. Five patients had sacroiliitis. Additionally, 15 patients had HLA B27 antigen or family history of either psoriasis or ankylosing spondylitis. Their conclusion was that during the first decade of seronegative oligoarthritis, HLA B27 antigen, family history, and especially imaging of SI joints reveal in two thirds of the patients the spondylarthritic nature of their disease.[16]

In the 35 years since the initial reports of the association of HLA-B27 with ankylosing

Table-IV: Frequency of major clinical manifestation on the basis of age group

Clinical feature /Age group	*10-20 yrs (%)*	*21-30 yrs (%)*	*31-40yrs (%)*	*41-50yrs (%)*	*51-60yrs (%)*	*61-70yrs (%)*
Sacroilitis/Spondylitis	7(12.5)	21(37.5)	18(32.14)	7(12.5)	2(3.75)	1(1.75)
Inflammatory peripheral arthritis	34(2.85)	53(32.51)	50(30.67)	19(11.65)	5(3.06)	2(1.22)
Ocular inflammation	4(22.23)	3(16.67)	4(22.23)	7(38.89)	0	0
Alteration of skin	1(16.67)	3(50)	0	1(16.67)	0	1(16.67)
Buccal ulceration	2(13.34)	7(46.67)	3(20)	2(13.34)	1(6.67)	0
Enthesopathy	2(16)	8(40)	7(35)	2(15)	0	1(5)

spondylitis (AS) and subsequently with Reiter's syndrome, psoriatic spondylitis, and the spondylitis of inflammatory bowel disease, the association of HLA-B27 with the seronegative spondyloarthropathies has remained one of the best examples of a disease association with a hereditary marker. HLA-B27 has been recognized as representative of a spectrum of diseases, ranging from the majority of HLA-B27-positive individuals who have no disease at all, through those with isolated eye or skin involvement, to those with critical eye, heart, and peripheral joint compromise of full-blown AS.

In the present study the frequency of HLA-B27 antigen was found to be 17.26%. The maximum number of patients with seronegative SpAs (34.2% including 28.42% females, 71.57%males) were found between 21-30 years olds group. Most frequent clinical manifestations, was peripheral joints arthritis (58.7% including 34.35% females, 65.64 % males). Inflammatory peripheral arthritis being the most frequent (58.7%) patients 56(34.35%) female and 107(65.64%) male. Skin alteration were the least frequent 6(2.15%) patients including 3(50%) female and 3(50%) male. The frequency of Sacroilitis/Spondylitis was 56(20.14%) patients including 22 (39.28%) female and 34 (60.71%) male.

Shankarkumar and colleagues in their study described that patients in the 20-40 age group were more vulnerable. Their findings confirm the strong association of the HLA B27 allele with various types of spondyloarthritis and suggest that allele detection would help in the diagnosis of AS where clinical presentation is unclear and in identifying the family members at risk [17]. In our study, both inflammatory peripheral arthritis and Sacroilitis/Spondylitis were most frequent in 20-40 years old group. In conclusion, our findings confirm the strong association of the HLA B27 allele with various types of seronagative SpAs. No association between each of major clinical manifestations with age and sex distribution. We suggest that HLA Typing would help in the diagnosis of seronagative SpAs specially AS where clinical presentation is unclear and in identifying the family members at risk.

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