Seronegative spondarthritis and human leucocyte antigen association

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ABSTRACT

The human leucocyte antigen (HLA) B27 has been associated with seronegative spondarthritis universally, but the associations varies (19-94%) in different Indian population groups. It is possible that this variation is due to patient selection bias. Here, we use strict clinical, radiological and serological criteria to select 79 patients with seronegative spondarthritis (SSA) and compare them with 110 healthy, normal individuals from the same ethnic background. The standard National Institute of Health (NIH) microlymphocytotoxicity assay is used to evaluate HLA B27 status in both groups. Significant association between HLA B27 and AS disease was found (odds ratio [OR]: 72.22; $P<0.0001$; confidence interval [CI]=1.5-3.8) when compared to the control group. Furthermore, HLA B5 was decreased when compared to the control group (OR: 0.39; $P<0.01$). Those in the 20-40 age group were more vulnerable, with a male preponderance (7:1) over females. These findings confirm the strong association of the HLA B27 allele with various types of spondarthritis and suggest that allele detection would help in the diagnosis of AS where clinical presentation is unclear and in identifying family members at risk.

KEY WORDS: Association. HLA-B27 antigen. Serologic Tests. Spondylitis, ankylosing.

Introduction

Demonstration of major histocompatibility complex (MHC)-linked immune response and/or immune suppressive genes[1,2] has resulted in various disease association studies at population level to seek an association with human leucocyte antigens (HLA).[3] Currently, it is believed that some HLA alleles show genetic linkage disequilibrium with certain disease-related genes and regulate the immune response, and the association of HLA haplotypes in autoimmune diseases supports this hypothesis.

The tendency for autoimmune diseases to run in families is well documented[4] and the association of ankylosing spondylitis (AS) with HLA B27 is consistent with a dominant or additive mode of inheritance that predicts a predisposition to disease.

Seronegative spondarthritis (SSA) comprises disease entities such as AS, Reiter's syndrome (RS), psoriatic arthritis (PSA), uveitis, colitic arthropathy and plantar fasciitis. AS is a chronic inflammatory disease that begins primarily in the sacroiliac joints and goes on to involve the spine and other large joints. In addition, it is associated with systemic symptoms such as pulmonary infiltration, uveitis and aortic regurgitation. Prevalence among Caucasians is estimated at 5-100/1000 adults. The frequency of HLA B27 in AS patients is 88-90%, compared with 4-8% in controls; however, different races show different rates of association,[3] and the Indian population is well known for its genetic diversity.[5]

An association between HLA B27 and AS was first reported in 1973,[6] and that with other members of the SSA group was confirmed later;[7-9] however, in reality many patients present with mild sacroiliaitis or with pauciarticular arthritis without clear-cut clinical and radiological features of AS or other well-defined SSA. In the study reported here, the patients are from this ill-defined group.

The proportion of HLA B27-positive individuals who develop clinical symptoms and signs of the disease has been estimated at 1-7%; however, thus far most studies have been performed in Western countries and these show that HLA B27 frequency varies with race. Extensive study of the Indian population has not been performed but scattered reports have been published[4,8-11] and report wide discrepancy in seropositivity (19 – 94%).

The aim of the present study is to examine the HLA association in patients with SSA in Mumbai, India, using strict clinical, radiological and serological criteria to define any association.

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Materials and methods

Selection of patients and controls

A total of 79 sporadic patients, referred to the hospital between 1996 and 1999, were selected using strict criteria for SSA. A detailed questionnaire was used to obtain patient histories and other population-specific details. Age ranged from 20 (±2) to 40 (±2) years, with a 7:1 male to female ratio. Presentation is summarised in Table 1.

Originally, the patients had been evaluated fully (radiology, immunology and haematology) in orthopaedic

Table 1. Joint involvement among HLA B27-positive seronegative spondarthritis patients in Mumbai

Joints	Number positive (%)
Type of joint involved	
Polyarticular	33 (42)
Pauciarticular	5 (6)
Monoarticular	8 (10)
Order of joint involved	
Spine	12 (15)
Knee	5 (6)
Ankle	2 (3)
Other joints involved	
Temperomandibular	1 (1)
Foot joint	7 (9)
Interphalangeal	6 (8)

or rheumatology clinics, and for the purposes of this study were re-evaluated by one of us (KG).

Patients with SSA presented, either singly or in combination, with sacroiliaitis with spondylitis; pauciarticular arthritis; persistent pain and tenderness in the tendoachilles or heel; or pauciarticular arthritis associated with a recent history (three months previously) of dysentery, together with high ESR (>40 mm/h) and negative results for rheumatoid factor, antinuclear antibody (ANA) and double-stranded DNA (dsDNA). The disease lasted for six months and responded to non-steroidal anti-inflammatory drugs (NSAIDS) before the diagnosis was made. Some showed systemic symptoms such as weight loss, low-grade fever, urethritis and uveitis, and one patient presented with psoriasis. HLA typing was performed only if the symptoms had persisted for at least six months.

Age- and sex-matched healthy individuals belonging to the same economic status and ethnic background comprised the control group, and were selected from a population survey conducted during the study period.

HLA typing

Heparinised blood (5-10 mL, containing 20 iu preservative-free heparin/mL) was drawn freshly from each individual and the lymphocytes were separated by Ficoll-Hypaque density gradient centrifugation.[12] HLA A and B phenotypes were determined for both patients and controls using the standard two-stage NIH microlymphocytotoxicity assay.[13] A minimum of two antisera – commercial (Biotest; Pelfreez) and indigenous [14] in origin – were used for each specificity.

Statistical analysis

Phenotype frequencies, odds ratio (OR), probability value, X^2 test with Yates' correction[15,16] and confidence intervals (CI)[17] were estimated using our database and computer programmes. As each individual was tested for several HLA alleles and the same data used to compare frequency, it was possible that one of the alleles could have deviated significantly by chance. To overcome this error the Bonferoni inequality method[18] was used in which the P value was multiplied by the number of alleles compared.

Table 2. HLA association in ankylosing spondylitis patients from Mumbai

HLA	Patients (%PF) $n=79$	Controls (%PF) $n=110$	Odds ratio (OR)
A1	26.60	17.80	1.66
A2	30.40	29.70	1.03
A3	15.20	16.80	0.89
A9	45.60	39.60	1.27
A10	3.80	13.90	0.28
A11	16.50	23.80	0.64
A19	39.20	38.60	1.03
A24	3.80	20.80	0.17
A28	12.70	11.90	1.08
A30	1.30	1.00	1.28
B5	13.90	29.70	0.39
B7	17.70	18.80	0.94
B8	2.50	3.00	0.91
B12	17.70	17.80	1.00
B13	10.10	9.90	1.04
B15	7.60	16.80	0.43
B16	1.30	4.00	0.41
B17	7.60	12.90	0.58
B18	1.30	6.90	0.24
B21	2.50	4.00	0.70
B22	1.30	6.90	0.24
B27	51.90	1.00	72.22*
B35	22.80	24.80	0.90
B37	10.10	5.90	1.75
B40	19.00	26.70	0.65
B44	8.90	9.90	0.90
B49	2.50	1.00	2.16**
B51	1.30	12.90	0.13
B53	8.90	3.00	2.91**
B55	1.30	4.00	0.41
B62	1.30	7.90	0.21
B63	1.30	1.00	1.28

%PF: percentage phenotype frequency

Confidence interval: 1.5 – 3.8

* $P < 0.001$

** $P < 0.05$

Results

The phenotype frequencies of HLA A and HLA B antigens in SSA patients were compared with the controls (Table 2), and significant increase was observed in HLA B27 frequency (52% *vs* 1%, $P<0.0001$, OR = 72.2, CI = 1.5 – 3.8). HLA A10, B5, B18 and B22 were found to be decreased in the patient group compared with the control group (A10: 4% *vs* 14%, OR=0.28, $P<0.05$; B5: 14% *vs* 30%, OR=0.39, $P<0.01$; B18 and B22: 1% *vs* 7%, OR=0.24, $P<0.05$). However, these differences for HLA A10, B18 and B22 did not remain significant after the P value correction was applied. Table 3

Table 3. Comparison of the HLA B27 association in Indian patients

Population	Patient number studied	Allele	%PF	Reference
North Indian	17	B27	94	10
North Indian	25	B27	92	11
Maharastrian	40	B27	83	4
Gujarathi	108	B27	30	9
Maharastrian	76	B27	19	9
Asian Indian	62	B*2705	52	8
Marathi Hindus	79	B27	52	Present study

% PF: percentage phenotype frequency.

compares the percentage phenotype frequency data obtained in the present study with the HLA B27 associations reported in other Indian populations.

Discussion

Heterogeneity in HLA antigen association with a particular disease in different populations may be due to linkage of a susceptible gene or genes with different HLA alleles. It is also possible that the putative disease susceptibility gene lies in the other HLA regions[19,20] or is due to synergism and epistasis between HLA antigens.[21] It was estimated that the population prevalence of AS is 0.25 to 2.2 per 1000,[3] but more recent studies have shown that the percentage prevalence is much higher.[9,22]

The present study shows a high percentage phenotype frequency and corroborates the findings of a number of earlier studies.[6,9,23,24] However, contrary to the findings of van der Linder,[25] the present study showed a male preponderance among the patients. The disease is clinically active and more severe in the 20 – 40 age group, as observed by Achuthan *et al*.[26]

HLA B27 has shown extremely high correlation with clinically well defined AS patients. However, this association persists with other well-defined members of the spondylitis family, generally known as seronegative spondarthritis, but to a much lesser degree. In this study, we selected 79 patients in whom the major problem was sacroiliaitis, pauciarticular arthritis, plantar fasciitis, spondylitis with reduced chest expansion, or reduced expansion of the lumbar vertebra with raised ESR.

Most patients did not present with full-blown AS, hence the term SSA was used. We believe that the majority of patients attending rheumatology clinics present in this way, rather than with clear-cut clinical and radiological features of AS. We ruled out other autoimmune diseases by careful clinical examination, application of a range of tests for autoimmunity, and follow up for six months prior to HLA study. Our findings agree with those of many other investigators, having shown a high association between HLA B27 antigen and SSA. Hence, testing for the presence of HLA B27 antigen could be useful in the diagnosis of AS.

Recently developed molecular technologies employing the polymerase chain reaction and allele specific primers have made it possible to split HLA B27 into 11 allele subtypes (i.e. HLA B*2701 – 2723).[27] Molecular subtyping of HLA B27 among the patients studied may provide further help in characterising the B27 allele subtypes present in this region of India, as would the identification of relevant amino acid residues in the peptide binding groove and/or T-cell receptor binding portion of the HLA B27 subtype. □

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