Int J Hum Genet, 7(3): 235-239 (2007)
PRINT: ISSN 0972-3757 ONLINE: 2456-6360
DOI: 10.31901/24566330.2007/07.03.03

HLA Association in Seronegative Spondyloarthritis Patients From Mumbai, India

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KEYWORDS Seronegative spondarthritis; HLA B27; caste system; India

ABSTRACT Seronegative spondyloarthropathies (SSA) are a group of inflammatory disorder, which clinically involves axial skeleton, sacroiliac and shoulder joints. These patients often complain of low back pain with prominent morning stiffness or nocturnal pain. The association of HLA-B27 allele and its novel subtypes with spondyloarthropathy remains one of the best examples of a disease association with a hereditary marker. The study population comprises of 6180 suspected SSA patients who were referred to the HLA Laboratory of Sir Hurkisandas Nurrotumdas. Hospital, Mumbai, India, from September 1985 to May 2005, for their HLA antigen profile. These were compared with 5000 ethnically matched control group whose HLA typing was performed during the same period by the standard NIH microlymphocytotoxicity assay using commercially available sera, defining a single HLA specificity. Our results revealed that HLA-B27 (8.49% vs. 2.37%) is significantly associated with SSA when compared to controls. Among the B7 CREG alleles such as HLA-B7, HLA-B40, HLA-B22 and HLA B42, the HLA-B7 (10.14% vs. 2.42%) and HLA B-40 (10.28% vs. 5.03%) allele frequencies were significantly increased among the HLA-B27 negative patients when compared to the HLA-B27 positive patients. HLA B27 allele distribution among the different caste and population groups revealed that maximum allele frequencies were observed among the Gujarathi and Maharastrians with distinctive variations among the caste groups studied and reported from India. Our results confirm the HLA B27 association in SSA patients, but the strength of this association varies considerably between racial and ethnic groups. Further, HLA-B7 and HLA B-40 alleles may also be associated with HLA-B27 negative patients with clinical symptoms of spondyloarthropathy.

INTRODUCTION

The first human leukocyte antigen haplotype association with inflammatory disease was discovered in 1973 (Brewerten et al. 1973) correlating HLA-B27 with Ankylosing Spondylitis. This remains one of the strongest known associations of disease with HLA-B27. Since then, more than 100 disease associations have been made, including many ocular diseases and systemic diseases with specific ocular manifestations. These also include Ankylosing Spondylitis, Reiter's Syndrome, Uveitis, inflammatory bowel disease like Colitic Arthropathy and Plantar fascitis and Psoriatic Arthritis (Schlosstein et al. 1993).

Seronegative spondyloarthropathies (SSA) are a group of inflammatory disorder, which clinically involves axial skeleton, sacroiliac and shoulder joints. These patients often complain of low back pain with prominent morning stiffness or nocturnal pain. The association of HLA-B27 allele and its novel subtypes with spondyloarthropathy remains one of the best examples of a disease association with a hereditary marker. However, the prevalence of HLA-B27 varies among populations and ethnic groups worldwide (Khan 1995).

HLA-B27 associations with seronegative spondyloarthritis in different Indian populations vary from 18 – 94% as compared to 1.4 – 8% of the general population. Indian population comprises of a range of genetic diversity (Lopez-Laurea et al. 1995). The proportion of HLA-B27 positive patients who correlate clinically are 1 – 5%. Earlier reports have shown wide discrepancy in the seropositivity among the Indian population. Hence the study was undertaken to compare the HLA association in seronegative spondylarthritis patients from Mumbai, India.

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MATERIALS AND METHODS

A total of 6180 sporadic patients referred with strict criteria for seronegative spondarthritis for HLA typing during Sep 1985 – May 2005 were included. A detailed questionnaire regarding the patient history and other population specific details were recorded. The mean average age group of the patient were 20 ± 2 to 40 ± 2 with a 7: 1 male to female ratio. The clinical presentation of these cases has been summarized (Table 1). These patients were evaluated in orthopedic and rheumatology clinics. They had radiology of affected joints, sacroiliac joints, Lumbar spines, full blood count with ESR, Rheumatoid factor, and autoimmune parameters like ANA, DS DNA, and Urine for protienuria. Seronegative spondarthritis patients (SSA) are patients who presented with singly or in combination (i) Sacroeliatis with spondilytis, (ii) Pauciarticular arthritis, (iii) persistent pain and tenderness in the tendoachillis or heel, (iv) Pauciarticular arthritis associated with history of dysentery 3 months back, along with high ESR > 40mm/hr and negative for rheumatoid factor, ANA, ds DNA. The disease lasted for 6 months and responded to NSAIDS before the diagnosis was made. Some of the patients had systemic symptoms like weightloss, low-grade fever and some of them had urethritis Uveitis and three patients had Psoriasis. The present study encompasses this group of patients with seronegative spondarthritis including clinically and radiologically well-defined Ankylosing spondylitis. The HLA on these patients were done only if the symptoms persisted for at least 6 months. Five thousand age and sex matched healthy individuals belonging to the same economic status and ethnic background during the same period comprised the controls for this study. The HLA B27 allele was determined on all the patients and controls using the standard two-stage NIH micro lymphocytotoxicity assay. A minimum of two to three antisera was used for each specificity. The antisera were of commercial (Biotest; Pelfreez) in origin. The phenotype frequencies, odds ratio, probability value, chi-square with Yates correction etiological fraction and preventive fraction were estimated as described earlier (Shankarkumar 2003). Since each individual is tested for several HLA alleles and the same data used for comparing the frequency; it is possible that one of the alleles will by chance deviate significantly. To overcome this error, the P value is corrected by use of Bonferoni inequality method i.e. by multiplying it with the number of alleles compared.

Table 1: Involvement of Joint among HLA B27 positive ankylosing spondylitis patients

Joints	*Percentage*
Type of Joint Involved	
Polyarticular	72.4
Pauciarticular	14.1
Monoarticular	18.1
Order of Joint Involved	
Spine	17.3
Knee	16.1
Ankle	12.59
Other Joints Involved	
Temperomandibular	6.2
Foot joint	18.8
Interphalengeal	13.38

RESULTS

It was interesting to observe that in majority of the patients Polyarticular joint (72.4%) was involved followed by monoarticular (18.1%) and pauciarticular joint (14.1%). Among the B27 positive patients the spine (17.3%) was affected in maximum patients followed by the knee joint (16.1%) and the ankle joints (12.59%). The other joint involved were the foot joint (18.8%), the intrphalengeal joint (13.38%) and the temperomandibular joint (6.2%) Table 1. HLA-B27 allele associations among 6180 patients as compared to control group of 5000 has been shown in Table 2, where OR = 3.682, EF = 0.116

Table 2: HLA Association in SSA patients

HLA	*Patients n=6180 n+*	*Patients n=6180 %AF*	*Controls n=5000 n+*	*Controls n=5000 %AF*	*OR*	*EF*	*P value*
B7	462	3.73	634	6.34	0.556		3.54E-20
B40	733	5.93	1416	14.16	0.34		7.00E-107
B22	55	0.44	205	0.02	0.21		4.21E-29
B42	10	0.08	12	0.001	0.673		3.53E-01
B27	1050	8.49	237	2.37	3.682	0.116	1.51E-90

and P value is 1.51E-90. The analysis of HLA-B27 related CREG alleles among HLA-B27 positive patients and HLA-B27 negative patients have been represented in Table 3. It was observed that HLA-B7 (OR - 4.988, EF - 0.15, p value- 3.15E-67) and HLA-B40 (OR - 2.31, EF - 0.113 and p value -9.56E-22) were significantly increased among the HLA-B27 positive patients.

The Table 4 represents the HLA-B27 allele distribution among the different caste and population of the total 1050 HLA-B27 positive patients compared to normal individuals reported from India (Shankarkumar 2003). The maximum 27.04% and 28.38% percentage phenotype frequencies were among the Maharashtrian and Gujarathi populations respectively, further it was

Table 3: HLA B7 CREG alleles among B27 Positive v/s B27 Negative patients

HLA	*Patients n=1050*	*Patients*	*Controls n=5130*	*Controls*	*OR*	*EF*	*P value*
	n+	*%AF*	*n+*	*%AF*			
B7	213	10.14	249	2.42	4.988	0.15	3.15E-67
B22	21	1	34	0.3	3.058	0.013	2.63E-05
B40	216	10.28	517	5.03	2.31	0.113	9.56E-22
B42	4	0.1	6	0.5	3.265	0.002	5.20E-02

Table.4: HLA B27 allele distribution among different Indian populations.

Population	*Place*	*State*	*Region studied*	*Samples*	*%PF*	*AS pts* N=1050*	*%PF*
North India (Populations)							
N.Indian Hindus	Delhi	Delhi	North India	400	6.00		
N.Indian Hindus	Delhi	Delhi	North India	289	5.20		
N.Indian Hindus	Lucknow	U.Pradesh	North India	59	19.60		
Marathi Hindus	Mumbai	Maharastra	Western India	392	4.10	284	27.04
Gujarathi Hindus	Mumbai	Maharastra	Western India	414	6.30	298	28.38
Jains	Mumbai	Gujarat	Western India	161	3.70	74	7.04
Parsi	Mumbai	Maharastra	Western India	67	0.00	1	0.09
Christians	Mumbai	Maharastra	Western India	31	29.00		
Muslims	Mumbai	Maharastra	Western India	105	9.50		
Sindhi	Mumbai	Punjab	North India			29	2.76
Punjabi	Mumbai	Punjab	North India			26	2.47
Sikh	Mumbai	Punjab	North India			3	0.28
Caste Group							
Nadars	Madurai	Tamil Nadu	South India	101	2.00		
Kallars	Madurai	Tamil Nadu	South India	36	0.00		
Naidus	Madurai	Tamil Nadu	South India	57	1.80		
Iyers	Madurai	Tamil Nadu	South India	74	1.40		
Gurkhas	Siliguri	Bengal	Eastern India	50	8.00		
Bhargavas	Lucknow	U.Pradesh	North India	100	0.00		
Chaturvedis	Lucknow	U.Pradesh	North India	100	0.00		
Agarwal	Lucknow	U.Pradesh	North India			14	1.33
Bhaiya	Lucknow	U.Pradesh	North India			99	9.42
Brahmins	Mumbai	Maharastra	Western India	55	5.50	99	9.42
CKP	Mumbai	Maharastra	Western India	50	10.00	19	1.80
Kunbi	Mumbai	Maharastra	Western India	26	11.50	37	3.52
Mahars	Mumbai	Maharastra	Western India	32	0.00		
Maratha	Mumbai	Maharastra	Western India	289	11.70	97	9.23
Pathare Prabu	Mumbai	Maharastra	Western India			4	0.38
Koli	Mumbai	Maharastra	Western India			11	1.04
Agri	Mumbai	Maharastra	Western India			17	1.61
Kutchi	Mumbai	Gujarat	Western India			63	6.00
Vaisnhnav	Mumbai	Gujarat	Western India			39	3.71
Patels	Nadiad	Gujarat	Western India	112	2.70	37	3.52
V Prajapathi	Surat	Gujarat	Western India	50	10.00		
Brahmins	Mumbai	Gujarat	Western India			56	5.33
Rajput	Mumbai	Gujarat	Western India			29	2.76

* Present study

observed that the B27 allele frequency distribution among the various caste groups differed within each group.

DISCUSSION

Our findings in the present study corroborate other studies, which show a high percentage phenotype frequency (Brewerten et al. 1973; Cavalli-Saforza and Menozi 1993; Shankarkumar et al. 2002; Kankonkar et al. 1998). The findings of van der Linder et al. (1984) show a male preponderance over female patients as observed in our study. Further the disease is clinically active and more severe in the age group of 20-40 years as observed by Achuthan et al. (1990). The prevalence rate in the present study supports the observation that has been reported by Chopra et al. (1988), who found a rate of 8.3% in healthy Indians in Pune (West India), but higher than the 1.7% found in Indians an another study from Mumbai by Bale et al. (1980).

Our studies revealed that HLA-B7 and HLA-B40 alleles were strongly associated among the HLA-B27 negative group of patients, which confirm studies worldwide. HLA-B27 allele diversity in Indians relating to the ethnic origin and caste system has been analyzed and increased prevalence has been reported from North India when compared to South India (Shankarkumar 2003). The B27 antigen frequency distribution among the Indian population, caste groups and tribal groups revealed an increased prevalence of HLA B27 allele among North Indian caste/population groups (2.7% - 29%) over the South Indian caste/population groups (0.9% - 2.1%). Furthermore, it has been observed that Western Indian caste/population groups from Maharashtra and Gujarat state had a differential frequency of B27 (1.4% - 15%).

Population-specific distribution of HLA alleles is necessary both in population genetics and in HLA disease association studies (Bodmer 1987). Anthropological studies show that the distribution of HLA alleles differ from one ethnic group to another and new alleles may yet be discovered in the Indian population (Rozemuller et al. 2002). The various Indian caste groups differ in their origin, migration and settlement. Genetic data analysis suggest that the inhabitants of the western part of the Eurasian steppes, originally settled by Caucasoid people speaking Indo-European languages who migrated in various directions are believed to be the earlier settlers in Indian subcontinent. Migration has a linear effect on HLA-B27 antigen frequency and Neolithic demic diffusion and could be the cause of nationwide genetic gradients. Extensive typing of the Indian population will be necessary to resolve the evolutionary implications of HLA-B27 subtypes and their population demography and relative disease association in the Indian context.

Further, it is well established that HLA-B27 subtypes differ in their ethnic distribution, perhaps as a result of genetic and geographical origin; however, the B*2705 subtype is present in almost all the populations studied worldwide, and is over-represented in the circumpolar and sub arctic regions of Eurasia and North America. Recently, novel HLA-B27 allele subtypes, such as B*2714 (Shankarkumar et al. 2003) and B*2718 (Chhaya 2005) have been reported to be associated with Ankylosing Spondylarthritis from western India. Further Molecular analysis of these HLA-B27 positive individuals along with HLA-B27 related CREG alleles like B7, B40, B22 and B42 will enlighten the allelic diversity and its evolutionary implications on the disease association.

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