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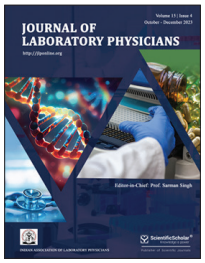
Journal of Laboratory Physicians

Article in Press



Original Article

Clinico-immunological profile of seronegative axial spondyloarthritis with special emphasis on the role of human leukocyte antigen B27

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Received: 26 August 2024
Accepted: 05 February 2025
EPub Ahead of Print: 28 March 2025
Published:

DOI
10.25259/JLP_196_2024

Quick Response Code:

ABSTRACT

Objectives: Human leukocyte antigen B27 (HLA-B27) is an allele of the major histocompatibility complex Class I molecules and is considered a causative genetic factor in seronegative spondyloarthritis. Seronegative spondyloarthritis (SpA) is a group of immune-mediated arthritis involving but not confined to the axial skeleton. The entities have overlapping yet different presentations. To understand the phenotypic implications of the B27 genotype in the disease process, we have compared the phenotype of HLA-B27 positive (H+) versus HLA-B27 negative (H−) spondyloarthritis patients.

Materials and Methods: A retrospective observational study including 218 patients with features of axial SpA was included in the study. Polymerase chain reaction-sequence-specific primer method was performed for the genotyping of B27.

Statistical analysis: Two groups of H+ and H− patients were compared using the Chi-square test.

Results: 116 H+ and 102 H− patients with features of spondyloarthritis were analyzed. Younger age, sacroiliitis, multiple joint pain, morning stiffness, uveitis, and higher disease activity (raised C-reactive protein) were significantly associated with H+ compared to H− groups. Family history of SpA and related features was present in both groups, but it was slightly more common in H+ than H−.

Conclusions: The present study is one of the very few Indian studies elaborating on the impact of H+ genotype in the phenotype of axial seronegative SpA. HLA-B27 has a causative genetic association with seronegative SpA. However, H− patients also had a positive family history in 35% of patients in the present study, thus pointing toward other genetic associations apart from HLA B 27.

Keywords: Ankylosing, Human leukocyte antigen B27, Seronegative, Spondylarthropathies, Spondylitis

INTRODUCTION

Seronegative spondyloarthritis (SpA) is characterized by inflammatory arthritis with overlapping features and is negative for all serological markers. The worldwide prevalence of SpA ranges from 0.2% to 1.6%.[1] B27 association is with various classes of seronegative SpA, the majority including ankylosing spondylitis (AS) (75–90%). Other clinical entities under seronegative spondyloarthritis are also associated with B27 including 75-90 % of undifferentiated SpA, 20-50% in psoriatic arthritis (PsA), 30-60% of reactive arthritis (ReA) and 10-40% enteropathy

related arthritis (EA).[1] All the subtypes of SpA share the majority of clinical characteristics with only a few defining features that may differentiate between them. The main clinical features include back pain, axial/peripheral arthritis, and inflammatory bowel disease.

AS is the prototype and most common SpA with a very strong H+ association. Human leukocyte antigen B27 (HLA-B27) is now a diagnostic criterion in the Assessment in Spondyloarthritis International Society classification criteria (ASAS) for SpA.[1] The prevalence of + in radiographic axial SpA ranges from 71% to 85% in various studies.[2] The frequency of H+ in the control/healthy population ranged from 2% to 3.5% in various Indian studies.[3,4]

However, HLA-B27 is not an essential criterion for diagnosis of SpA and very few studies are available comparing the differences between disease phenotype in H+ and H− groups. Even fewer studies are available in the eastern part of North India, where limiting factors include the lower socio-economic status of a relatively large fraction of the population and the lack of advanced diagnostic facilities for genetic studies.

This study aimed to describe the features of SpA in this region of India and also to study the clinical and immunological differences between H+ and H− patient groups.

MATERIALS AND METHODS

This is a retrospective observational and cross-sectional study conducted during the period between 2020 and 2022 in the University Grants Commission Advanced Immunodiagnostic Training and Research Center. Two hundred and eighteen patients with features of axial SpA were diagnosed by the physician/rheumatologist according to ASAS/European Spondyloarthropathy study group criteria in our outpatient department and were recruited in the study.[5,6] Exclusion criteria included unavailability of clinical or HLA-B27 data. Detailed clinical history, family history, and radiological details were noted, other laboratory and immunological test details were recorded, and HLA-B27 was done by polymerase chain reaction (PCR)-sequence-specific primer method (BAG Diagnostics).

HLA-B27 allele-specific primers were added to purify deoxyribonucleic acid (DNA), and amplification was achieved only if the primers completely matched the target allele. The mismatched primers, due to the absence of HLA-B27, did not generate amplicons. Gel electrophoresis was done to separate and analyze the PCR products. Internal control band was seen in all cases and controls to indicate the integrity of the PCR reaction. In HLA-B27 positive cases, two bands were observed, the band of B27 had a length of 420bp and/or 85 bp. In HLA-B27 negative patients, only a band of internal control was observed.

Routine investigations and quantitative C-reactive protein (CRP) were performed (Immage 800, Beckman Coulter, USA). Immunological tests in the form of serum antinuclear antibody (ANA), double-stranded DNA (dsDNA), anti-Sjögren's syndrome-related antigen A (anti-SSA), anti-Sjögren's syndrome-related antigen B (anti-SSB), anti-Smith, anti-centromere, anti-Jo-1, anti-RNP, and anti-Scl-70 were done. ANA was done by indirect immunofluorescence and dsDNA by enzyme immunoassay. Specific markers were tested by dot blot immunoassay from AESKU diagnostic, Germany.

Ethical approval was taken from the Institutional Ethics Committee. To statistically compare between H+ and H− groups, the Chi-square test was done using the Statistical Package for the Social Sciences software version 21.

RESULTS

A total of 116 H+ and 102 H− patients were included in the present study. The male-female ratio was more skewed toward males (6:1) in H+ than H− (2:1). H+ patients were younger with a mean age of 28 years compared to mean age of 33 years in H−. Ten patients were younger than 16 years of age, out of which seven were H+ (70% of juvenile SpA) and three were H−(30% of juvenile SpA).

The clinical and radiological features of sacroiliitis were much more significant in H+ (83%) versus H− (20%) [Table 1].

Multiple joint pain was significantly more associated with H+ (57%) rather than H−(41%). Morning stiffness was also much more common in H+ (95%) than H− (62%) and this association was statistically significant.

Uveitis was significantly associated with HLA positive SpA (24%) when compared to HLA negative SpA (6%).

Other features such as spondylitis, skin lesions, photosensitivity, oral ulcers, gastritis, uveitis, and urinary tract infection (UTI) were more common in H+ than in H−, but this was not statistically significant. Few patients of H+ had a history of infections such as tuberculosis (six patients) and Human immunodeficiency virus (HIV) (one patient) while H− had no such history of infections. Genetic associations in the form of a positive family history of SpA and related features were observed in both groups but it was slightly more common in H+ than H− group.

Other autoantibodies were also sometimes associated with SpA such as ANA (seen in one HLA-B27 negative patient) and RA factor (seen in two patients each in both HLA groups). Anti-cyclic citrullinated peptide (Anti-CCP) was positive in 3% of HLA-B27 negative SpA.

In the laboratory investigations performed, raised CRP titers were seen more commonly in H+ (83%) versus H− (20%) and this association was significant ($P < 0.05$).

Table 1: Clinical and immunological features of HLA-B27 positive and negative SpA.

	HLA-B27 Positive (*n*=116) (%)	HLA-B27 Negative (*n*=102) (%)	*P*-value	χ^2
Age	3–60 years (mean 28 years)	14–70 years (mean 33 years)	**0.02**	9.9
Gender (M: F)	6:1	2:1		
Sacroiliitis	96 (83)	21 (20)	**<0.001**	**84.36**
Spondylitis	39 (34)	25 (24)	0.18	2.17
IBP	68 (59)	63 (61)	0.63	0.22
Multiple joints	66 (57)	42 (41)	**0.02**	**5.36**
Morning stiffness	110 (95)	64 (62)	**<0.001**	**34.67**
Skin lesion	17 (15)	8 (8)	0.11	2.48
Photosensitivity	31 (27)	24 (24)	0.58	0.29
Oral ulcers	19 (16)	10 (10)	0.15	2.03
Gastritis	46 (40)	33 (32)	0.26	1.25
Uveitis	28 (24)	6 (6)	**<0.001**	**13.74**
UTI	20 (17)	12 (12)	0.25	1.29
Trauma	23 (20)	20 (20)	0.96	0.001
Family history	43 (37)	36 (35)	0.78	0.07
HIV	1 (1)	0		
History of TB	6 (5)	0		
CRP positive	**17 (15)**	**5 (5)**	**0.01**	**5.69**
Anti-CCP positive	0	3 (3)		
ANA	0	1 (1)		
RA factor	2 (1.7)	2 (2)		
Duration	0.5 months to 20 years (mean 3.6 years)	0.3 months to 16 years (mean 2.3 years)		

HLA-B27: Human leukocyte antigen B27, SpA: Seronegative spondyloarthritis, IBP: Inflammatory back pain, UTI: Urinary tract infection, ANA: Antinuclear antibody, CRP: C-reactive protein, Anti CCP: Anti-cyclic citrullinated peptide, RA factor: Rheumatoid factor, M: Male, F: Female, HIV: Human immunodeficiency virus. A *P*-value less than 0.05 is considered statistically significant and highlighted in bold.

DISCUSSION

The present study showed male predominance, but in H+ group, the male: female ratio was 5.8:1, and in H− group the male: female ratio was 2:1. Similar findings with greater male predominance in HLA-B27 positive SpA were seen in other studies by Zhang *et al.* and Yang *et al.*[2,7,8] However, a study by Arévalo *et al.* showed male-female ratio of 3:1 with no difference between the two groups.[9] Indian studies also showed male predominance in both groups.[10] Aggarwal *et al.* also showed a male-female ratio of 5:1 similar to the present study[11] [Table 2].

The present study showed no significant difference in the family history of SpA in both groups. Other studies also showed an association of family history in both groups but stronger with HLAB27.[2,7,8] Study by Aggarwal *et al.* showed positive family history in 53% of patients.[11] In HLAB27 negative patients, there may be other genetic associations leading to familial transmission. Parasannanavar *et al.* reported a study of HLA-B27 negative patients of SpA with significantly increased HLA B*07 positivity and decreased HLA B*40.[12]

Our study showed H+ patients to be significantly younger than H− group. A similar result was reported by other studies also.[2,7-9] An Indian study by Shougrakpam *et al.* showed slightly younger age in the HLA-B27 positive group (32 vs. 35 years).[10]

Radiological features of sacroiliitis were significantly associated with H+ compared to H− patients. A study done by Chung *et al.* and Yang *et al.*[7,8] showed likewise results and increased predominance of sacroiliitis in H+ patients; however, Zhang *et al.* found no difference in musculoskeletal disease in the two groups.[2,7,8] Shougrakpam *et al.* showed significantly more axial skeleton involvement in HLA-B27 positive patients (86%).[10]

Multiple joints involvement including peripheral joints and morning stiffness was significantly correlated with H+ in the present study (57% vs. 41% in H−). Similarly Yang *et al.* also reported increased peripheral arthritis (approx. 40%), hip joint involvement and lumbar spine involvement (when compared to thoracic or cervical) in H+.[8] A study by Arévalo *et al.* and Chung *et al.* showed contradictory

Table 2: Comparison of HLA-B27 positive and negative SpA in Indian and western literature.

Study	Male predominance in HLA-B27 positive	Family history	Age	Axial disease expression	Peripheral arthritis	Disease duration	uveitis	Disease activity/ CRP
Zhang *et al.*,[2] China	Yes (75% males)	More in HLA+ve	Younger in HLA+ve (33 y)	No difference	No difference in prevalence (35%)	Longer in HLA + (5 y)	More in HLA + (10.5%)	Raised in HLA +
Chung *et al.*,[7] 2011	Yes (51% males)	More in HLA+ve (30%)	Younger in HLA+ve (32y)	-	Higher prevalence in HLA -ve	No difference	More in HLA + (9.5%)	No significant difference
Yang *et al.*,[8] China	Yes (80% males)	More in HLA+ve (22.5%)	No difference (26y)	Spinal involvement is more in HLA +	Higher prevalence in HLA+ve	-	No difference (9.5%)	-
Arévalo *et al.*,[9] Spain	No (75% males)	More in HLA+ve (22%)	Younger in HLA+ve (26 y)	No difference	Higher prevalence in HLA -ve (15.4% vs 21.8%)	No difference	No significant difference (22%)	No difference
Shougrakpam *et al.*,[10] India	Yes (74% males)	-	Younger in HLA+ve (32y)	Significantly more in HLA +	No difference	Longer in HLA +		Severe in HLA +
Aggarwal *et al.*,[11] India	Yes (84% males)	Present in 50% of patients	24 y	87%	66%	9 years	25.7%	-
Present study	Yes (85% males)	Present in 37% of patients	Younger in HLA+ve (28y)	Significantly more in HLA + (83%)	Significantly more in HLA + (57%)	3.6 years	Significantly more in HLA + (24%)	Significantly elevated in HLA + (15%)

HLA-B27: Human leukocyte antigen B27, SpA: Seronegative spondyloarthritis, CRP: C-reactive protein

results with a higher prevalence of peripheral arthritis in HLA negative group.[7,9] Zhang *et al.* showed no difference in joint involvement between the two groups. The present study shows a greater proportion of patients with multiple joint involvement compared to other Western studies showing 30%-50% of patients with peripheral arthritis.[2] However, another Indian study showed that 62% of patients with peripheral joint involvement, although the difference was not significant.[10] Aggarwal *et al.* reported an even higher proportion of peripheral arthritis (66%).[11] It is possible, that patients in India are showing more frequent peripheral joint involvement including hip joint and knee joint.

Uveitis was more common in H+ (24%) and this association was also significant in the present study. Similarly, other studies also showed a significant association of H+ with uveitis. The prevalence of uveitis ranged from 10% to 38.5% in different studies and a meta-analysis showed pooled prevalence of 25.8%.[2,13]

Other features such as spondylitis, skin lesions, photosensitivity, oral ulcers, gastritis, and UTI were more common in H+ than in H– but the association was not statistically significant. A study by Zhang *et al.* shows the association of psoriasis with absence of HLA-B27. Furthermore, the absence of HLA-B27 would understandably lead to a delay in diagnosis as in this study.[2] Chung *et al.* reported a significant association of extra-articular manifestations with H– SpA.[7]

Many studies have found H+ to be significantly more associated with family history when compared to H– patients.[2] However, the present study showed slightly more patients with a family history in H+ than H– group; however, this was not statistically significant. Other studies showed lesser familial inheritance (approx. 22.5% in Yang *et al.*, 11% in a study by Zhang *et al.*) in H– patients than in the present study (35%).[2,8] This finding of increased familial clustering in the Indian population will have to be verified by larger studies.

Other autoantibodies such as anti-CCP (seen in 3% in H–), rheumatoid factor (2% in both groups), and ANA (1% in HLA-B27 negative patients) were seen. Minor levels of autoantibodies are also seen in healthy populations and this finding could be incidental. Still, the possibility of co-

existence of other autoimmune diseases along with SpA cannot be ruled out in these patients. A study from India also reported the presence of autoantibodies in SpA patients.[14]

Many Indian and Western studies have noted higher disease activity and higher CRP titers in H+ patients like the present study.[10] Mishra and Singai reported 42% CRP positivity in their study.[3]

Limitation of this study is that follow-up is not available, so the difference between the two groups in prognosis and response to treatment is not evaluated.

CONCLUSIONS

The present study is one of the very few Indian studies elaborating on the impact of HLA-B27 in the phenotype of axial SpA. The significant differences in the two groups of SpA based on HLA-B27 positivity include predominance of male gender, younger age, radiological features of sacroiliitis, morning stiffness, involvement of multiple joints, uveitis, and raised CRP in H+. Furthermore, H− patients also had a positive family history in 35% of patients, thus pointing toward other genetic associations apart from HLA-B27. More studies on larger sample sizes and follow-up are needed to confirm these findings as well as to search for other susceptibility genes.

As the spectrum of manifestations of SpA is quite variable ranging from musculoskeletal to extra-articular manifestations like uveitis, these patients present frequently in orthopedics for backache, and the role of the orthopedician is to keep the clinical features of seronegative SpA in mind, know what tests to perform and refer to a rheumatologist if diagnosed.

Author contributions: NC, BN, MY, SK, JS, SY: Concept, design, definition of content, selection of cases; NC, BN, MY, SK: Investigation and statistics. All authors contributed equally to the writing of the manuscript.

Ethical approval: The study approved by the Institutional Ethics Committee at Institute of Medical Sciences, Banaras Hindu University, number Dean/2022/EC/3549 dated 20th October 2022.
Declaration of patient consent: The authors certify that they have obtained all appropriate patient consent.
Financial support and sponsorship: Institute of Eminence seed grant given by Banaras Hindu University.
Conflicts of interest: There are no conflicts of interest.
Use of artificial intelligence (AI)-assisted technology for manuscript preparation: The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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How to cite this article: Chaudhary N, Naik B, Yadav M, Kumar S, Singh J, Yadav S. Clinico-immunological profile of seronegative axial spondyloarthritis with special emphasis on the role of human leukocyte antigen B27. J Lab Physicians. doi: 10.25259/JLP_196_2024