Content available at: https://www.ipinnovative.com/open-access-journals

Panacea Journal of Medical Sciences

Journal homepage: https://pjms.in/

Original Research Article

Demographic and immunological status of Seronegative HLAB27 patients in Central India -A prospective study

Sadhna Sodani[1], Debjani Taraphdar[2]*, Ranjana Hawaldar[3]

[1]Sampurna Sodani Diagnostic Clinic, Indore, Madhya Pradesh, India
[2]Dept. of Molecular Biology, Sampurna Sodani Diagnostic Clinic, Indore, Madhya Pradesh, India
[3]Dept. of Pathology, Sampurna Sodani Diagnostic Clinic, Indore, Madhya Pradesh, India

Abstract

Introduction: HLA B27 is a class I major histocompatibility antigen, which can cause susceptibility to several diseases, including ankylosing spondylitis, reactive arthritis (Reiter's syndrome), inflammatory bowel disease, and psoriasis.
Aim and Objective: The present study aims to analyse the demographic and immunological status of seronegative HLAB27 patients in Central India.
Material and Methods: A cross-sectional study was conducted to measure the prevalence of HLAB27 positive cases in Central India among seronegative spondyloarthritis (SSA) patients. HLAB27 was analysed by Real time PCR. Demographic and inflammatory marker status were compared with HLAB27-status.
Result: A total of 688 cases of SSA were included in this study from June 2023 to February 2024. Among these, 103 were positive (15%) for HLA B27 on the basis of Real time PCR analysis. Males had a higher prevalence than females in the HLA-B27 positive group (62.1% vs. 37.9%). Among referred 66 juvenile SSA cases, 27 were positive for HLAB27 by PCR (41%) with the highest positivity rate observed among juvenile male patients (55.5%). Both ESR and CRP were found to be higher among juvenile cases.
Conclusions: HLA-B27 by PCR can be an indicator for seronegative SpA cases and is recommended to both suspected adults and children to minimise the multiple medical visits and diagnoses.

Keywords: HLA B27, Seronegative spondylo arthritis, Real time PCR, Central India

Received: 13-07-2024; **Accepted:** 16-12-2024; **Available Online**: 19-08-2025

1. Introduction

Seronegative spondyloarthritis (SSA) includes ankylosing spondylitis (AS), reactive arthritis (ReA), psoriatic arthritis (PsA), undifferentiated spondyloarthritis and enteropathy related arthritis (EA).[1] Spondyloarthritis (SpA) is a family of inflammatory rheumatic diseases that are not associated with rheumatoid factor (RF), the marker of adult rheumatoid arthritis. The prevalence of SpA ranges from 0.2 to 1.61% in the general population worldwide. The human leukocyte antigen (HLA-B27) is a class I antigen of the major histocompatibility complex, and it is strongly associated with SpA.[1] The presence of the HLA-B27 allele differ from geographic area, data sources, and the study population.[2] It is reported in 2-12% of the general population worldwide. There is a strong association between HLAB27 positivity and the severity of immune-mediated inflammatory disease.[3] The association of HLAB27 within the broader group of seronegative SpA varies significantly, ranging between 50% and less than 95%. Among ankylosing spondylitis (AS) patients, HLA-B27 is present in 90% cases worldwide, and it varies between ethnic populations.[4,5]

Ankylosing Spondylitis causes inflammation of bones in the spine. The major clinical symptoms include back pain, stiffness of the lower spine, and progressive spinal rigidity accompanied by inflammation of the hips, shoulders, peripheral joints and fingers/toes.[6] In India, the first report on HLA-B27 in AS was in 1977, reported from PGIMER, Chandigarh, with a 94% positivity rate.[7] The frequency of

*Corresponding author: Debjani Taraphdar
Email: taraphdar.debjani@gmail.com

https://doi.org/10.18231/pjms.v.15.i.2.369-372

HLA-B27 and its subtypes in the Indian population is ~6%. The frequency of HLA-B27 is more than 90% in ankylosing spondylitis (AS), compared to other subtypes of the "SpA" family.[7] HLA-B27 positive AS patients were found to have a significantly shortened life expectancy.[8] Increased mortality is significant among women with HLA-B27-positive AS. The diagnosis of juvenile spondyloarthritis among SSA is less considered in young children who present with leg and back pain.[9,10] Immunological markers may rise in some of the cases but are mostly inconclusive in radiographs.[11] There is no specific diagnosis of ankylosing spondylitis, but the presence of the HLA-B27 gene in most people with the disease provides more information to clinicians when making a diagnosis and determining the extent of the disease.

The present study aims to analyse the demographic and immunological status of seronegative HLAB27 patients in Central India. In this study, we studied the prevalence of HLA-B27 cases by detecting HLA-B27 gene by Real time PCR. Investigations were emphasised on juvenile cases also that have not been reported from Central India till date. The inflammatory markers erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were also correlated.

2. Materials and Methods

2.1. Study subjects

In this study, we tested seronegative spondyloarthritis (SSA) patients referred from rheumatology outpatient clinics in Indore, Madhya Pradesh for the status of HLA-B27. A total of 688 cases of SSA were included in this study during the period of eight months from June 2023 to February 2024. In this study the blood sample was collected in a sterile EDTA tube and clot activator tube.

2.2. Data collection

Clinical data were collected from medical records. Data consisted of the clinical history of the disease, including demographics.

2.3. Procedure

Detection of HLA B27 through Real time PCR was performed in the Molecular Biology department of our center. Nucleic acid extraction from blood was used as a sample for this test. Extraction was performed by TRUPCR Blood DNA Extraction kit (Cat. No. 3B205, 3B Black Bio Biotech India Ltd.) as per the manufacturer's instructions. Polymerase chain reaction (PCR) was performed using HLA B-27 Real Time PCR Kit (TruPCR, 3B Blackbio) on Real time PCR Instrument, Quantstudio 5 (Thermofisher, USA), and target amplification of the allelic gene region. The PCR detection system includes an endogenous internal control in order to avoid false negative results.

The Real time PCR was programmed with an initial denaturation at 94ºC for 10 min for 1 cycle and 37 cycles for denaturation at 94ºC for 15 s. Further, annealing, extension, and fluorescence were measured at 60ºC for 60 s for 37 cycles.

Rheumatoid factor (RA) was determined by the nephelometry method at Agappe, Mispa i2 from serum specimens. C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were measured as inflammatory markers. Serum CRP was measured by the immunoturbidimetric method on Atellica Solution (Siemens, Germany) according to the manufacturer's instructions. ESR was measured by Roller 20 (Alifax) instrument.

2.4. Statistical analysis

Percentage and frequency were calculated for categorical variables.

3. Results

In eight months, a total of 688 SSA cases including 66 cases of juvenile chronic arthritis were analysed. Out of 688 cases, 103 were positive (15%) for HLA-B27 (**Table 1**). Gender-wise distribution showed that 64 patients were male (62.1%) with a median age of 31 years (range 11-73 years) and 39 were female (37.9%) with a median age of 46 years (range 14-72 years). Male: female ratio was approximately 1.6:1.

A total of 66 SSA cases below 18 years old were referred for HLA-B27 PCR tests. Of them, 27 were positive for HLA-B27 by PCR. The positivity rate among them was high (41%). Out of 27 HLAB27 JSpa patients, 55.5% male patients were positive (25/45), whereas the positivity rate among females was low, i.e., 9.5% (02/19). In this study, the male to female ratio was 1.6:1.

Immunological findings are presented in **Table 2** for HLA-B27 positive patients. The mean level of total leucocyte count (TLC) did not differ in HLA-B27 positive and negative cases. All the patients were negative to RF. The correlation of HLA-B27 positivity with ESR showed ESR positivity was higher (91%) among the patients less than 18 years of age as compared to the adults (59%). CRP level was also higher in juvenile patients as compared to adults (75% vs 61.9%).

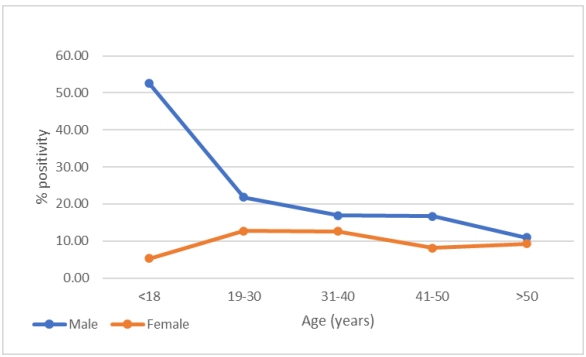

Figure 1: Percent positivity of HLAB27 patients. Highest positivity rate was observed among male juvenile cases.

Table 1: Demographic data of HLA B27 positive patients. Patients were categorized based on age and gender.

Characteristics	Male (n=344)			Female (n=435)			Male: Female: Ratio
	Positive cases	Total tested	Percent positive cases	Positive cases	Total tested	Percent positive cases	
Age (years)							
<18	25	45	**55.56**	02	21	9.52	21:1
19-30	13	63	20.63	07	62	11.29	1.7:1
31-40	14	92	15.22	11	98	11.22	1.3:1
41-50	12	69	17.39	07	97	7.22	1.4:1
>50	10	76	13.16	15	160	9.38	0.5:1
Total	74	344	21.51	42	435	8.97	1.6:1

Table 2: Immunological status of HLAB27 positive patients.

	Increased ESR patients (mm in 1st hr)	Percentage	Mean value± S D B27-positive	Increased CRP patients (mg/L)	Percentage	Mean ± SD B2 7-positive
Juvenile (<18 years of age)	10 (n=11)	91%	48.17 ± 29.39	06 (n=08)	75%	50.55 ±34.53
Adult (>18 years of age)	25 (n=42)	59%	36.36 ± 17.85	26 (n=42)	61.9%	31.56 ± 40.72
Total	35 (n=53)	66%		34 (n=50)	39%	

4. Discussion

HLA-B27 is a major histocompatibility complex (MHC) class I molecule, which is expressed on most nucleated human cells and platelets. The strong association has been reported between HLA-B27 antigen and ankylosing spondylitis (AS) and a few other rheumatic disorders (Reiter's syndrome, acute anterior uveitis, and inflammatory bowel disease).[12] HLA-B27 testing is routinely used to screen for AS patients due to the unavailability of specific tests.[13]

The first report from India on HLA-B27 in AS was in 1977 from PGIMER, Chandigarh, with a reported 94% positivity rate. More than 100 subtypes of HLA-B27 have been characterised, with varying associations with spondyloarthritis. The prevalence of HLA-B27 varies in different countries. The estimated prevalence in the United States is six to eight percent. In New Zealand, Caucasian controls (9.2%) were similar to the prevalence of 9.5% previously reported in a dataset of 5,926 UK controls.[6] The prevalence of HLA-B27 in India was ~6% in earlier reports.[7] In our study, the estimated prevalence of HLAB27 in seronegative rheumatic patients is 14% (89/622). SpA is a common rheumatic disease in the country and "slipped disc" and "tuberculosis' are the two most common misdiagnoses.

In our study, we observed higher percent positivity due to awareness among Orthopedic Physicians in Madhya Pradesh. Increasing awareness and recognition among female SpA reported a significantly lower proportion (3:1 male to female) of peripheral arthritis among Indian axial SpA (axSpA) patients in contrast with earlier reports in the 1980s and 1990s with a higher proportion 18:1.[7] In our study, the male to female ratio in HLA-B27 positive cases is 1.6:1. In our previous published study in the year 2016, maximum patients (54.8%) were in the 21-40 years age group with an M:F ratio of 1.65:1.[14] The result was in concordance with other studies from India;[3,15,16] male members showed a preponderance as compared to females in HLA B27 positivity. We also found that people in the age group of 19-30 years were the most affected among adults. Similar observations were also reported by Kankonkar et al., 21-39 years were mostly affected.[17] According to the Spondylitis Association of America, people typically develop AS between 17 and 45 years of age; however, an average diagnostic delay of 6.7 years was also reported in patients with AS and axial spondyloarthritis (axSpA).

Juvenile spondyloarthritis (disease onset ≤18 years of age) patients present with clinical symptoms that show overlap with the adult spectrum, with some differences.[18] Positivity of HLA-B27 in childhood SSA was 71.4 percent (50/70) in our cases. Gender-wise analysis showed that 76.7 percent (46/60) males and 20.0 percent (02/10) of the female patients were HLA-B27 positive among them. JspA with male preponderance was reported in earlier reports also.[17]

AS disease activity score (ASDAS) combines patient-reported assessments with CRP or ESR.[20] In our study, none of the HLA-B27 cases were positive for rheumatoid factor; however, ESR and CRP were raised in HLA-B27 positive cases in all age groups, which is also correlated with other studies.[21]

5. Conclusion

HLAB27 diagnostic delay can adversely impact the health status of patients with SpA. Our study concludes that HLA-B27 by PCR should be done in suspected children because it can indicate early cases of juvenile spondyloarthropathy when radiological changes are not present. If not considered, it could lead to multiple medical visits and diagnoses. Many patients with this allele may not develop an HLA-B27 associated syndrome but could develop a greater-than-average risk of having certain autoimmune disorders, which could be taken care of after the diagnosis.

6. Source of Funding

None.

7. Conflict of Interest

None.

Reference

1. Kavadichanda CG, Geng J, Bulusu SN, Negi VS, Raghavan M. Spondyloarthritis and the Human Leukocyte Antigen (HLA)-B*27 Connection. *Front Immunol*. 2021;8(12):601518.
2. Londono J, Santos AM, Peña P, Calvo E, Espinosa LR, Reveille JD, et al. Analysis of HLA-B15 and HLA-B27 in spondyloarthritis with peripheral and axial clinical patterns. *BMJ Open*. 2015;5:e009092.
3. Kumar S, Doss RSA, Rebekah G, Mathews NS, Danda D, Mathew J, et al. Prevalence of HLA-B*27 subtypes in the Tamil population of India with Ankylosing spondylitis and its correlation with clinical features. *Hum Immunol*. 202;82(6):404-8.
4. Vollenhoven RF, Chapter 42 - Evaluation and Differential Diagnosis of Polyarthritis, Editor(s). Firestein GS, Budd RC, Gabriel SE, McInnes IB, O'Dell JR, Kelley and Firestein's Textbook of Rheumatology (Tenth Edition), Elsevier, 2017:615–24.
5. Oral D, Erdal GG, Tekeş S, Yücel İ, Em S. Prevalence of HLA B27 in Patients Diagnosed with Ankylosing Spondylitis (AS) in Diyarbakır, Southeastern Region of Turkey. *Niger J Clin Pract*. 2024;27(1):29–34.
6. Zhu W, He X, Cheng K, Zhang L, Chen D, Wang X, et al. Ankylosing spondylitis: etiology, pathogenesis, and treatments. Bone Res. 2019;7:22.
7. Malaviya, Anand N. Spondyloarthritis in India. *Indian J Rheumatol*. 2020;15(1):S2–5.
8. Li Z, Khan MK, van der Linden SM, et al. HLA-B27, axial spondyloarthritis and survival. *Ann Rheum Dis*. 2023;82(12):1558–67.
9. Hartman GH, Renaud DL, Sundaram M, Reed AM. Spondyloarthropathy presenting at a young age: case report and review. *Skeletal Radiol*. 2007;36(2):161-4.
10. Weiss PF, Colbert RA. Juvenile Spondyloarthritis, A Distinct Form of Juvenile Arthritis. *Pediatr Clin N Am*. 2018 65(4):675–90.
11. Sonkar GK, Usha, Singh S. Is HLA-B27 a useful test in the diagnosis of juvenile spondyloarthropathies?. *Singapore Med J*. 2008;49(10):795–9.
12. Erin M. Scanlon, Thomas G. Mason, 78 - Seronegative Inflammatory Arthritis, Editor(s). Bernard F. Morrey, Joaquin Sanchez-Sotelo, Mark E. Morrey, Morrey's the Elbow and its Disorders (Fifth Edition), Elsevier, 2018:742–6.
13. Sharma N, Sharma V, Masood T, Nautiyal SC, Sailwal S, Singh RK, et al. Usage of Conventional PCR Technology for the Detection of HLA-B27 Allele: A Significant Molecular Marker of Ankylosing Spondylitis. *Indian J Clin Biochem*. 2013;28(2):189–92.
14. Hawaldar R, Sodani S. Prevalence of HLAB27 in Patients of Central Madhya Pradesh. *Indian J Pathol: Res Pract*. 2016;5(2):177–81
15. Aggarwal R, Malaviya AN. Clinical characteristics of patients with ankylosing spondylitis in India. *Clin Rheumatol*. 2009;28(10):1199–205.
16. Chavan H, Samant R, Deshpande A, Mankeshwar R. Correlation of HLA B27 subtypes with clinical features of ankylosing spondylitis. *Int J Rheum Dis*. 2011;14(4):369–74.
17. Smith JA, Burgos-Vargas R. Outcomes in Juvenile-Onset Spondyloarthritis. *Front Med (Lausanne)*. 2021;8:680916.
18. Kankonkar SR, Raikar SC, Joshi SV, Tijoriwala SJ. Association of HLA B27 antigen in Indian patients of ankylosing spondylitis and other autoimmune diseases. *J Assoc Physicians India*. 1998;46(4):345–50.
19. Resende GG, Saad CGS, de Oliveira DCM, Filho JSSB, Sampaio-Barros PD, Pinheiro MM, et al. HLA-B27 positivity in a large miscegenated population of 5,389,143 healthy blood marrow donors in Brazil. *Adv Rheumatol*. 2023;63:16.
20. Wiąk-Walerowicz K, Wielosz E. Comparison of Ankylosing Spondylitis Disease Activity Score and Bath Ankylosing Spondylitis Disease Activity Index tools in assessment of axial spondyloarthritis activity. *Reumatologia*. 2024;62(1):64–9.
21. Kumar S, Haque SS, Sinha MK, Agrawal R, Singh A, Kumar U, et al. Clinical Correlation between HLA-B27 and Inflammatory Marker in Case of Ankylosing Spondylitis (AS). *MOJ Immunol*. 2016; 4(2):00122.

Cite this article: Sodani S, Taraphdar D, Hawaldar R. Demographic and immunological status of Seronegative HLAB27 patients in Central India -A prospective study. *Panacea J Med Sci*. 2025;15(2):369-372.