



Research Article

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Molecular Detection of HLA-B27 Antigen Using PCR Technique: A Diagnostic Approach for Spondyloarthropathies in Clinical Practice

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Abstract

Background: Human Leukocyte Antigen (HLA)-B27 is a major genetic marker strongly associated with spondyloarthropathies (SpAs), a group of chronic inflammatory rheumatic diseases, including ankylosing spondylitis (AS), reactive arthritis (ReA), and psoriatic arthritis (PsA). Despite its well-documented connection with SpAs globally, data on HLA-B27 prevalence and subtypes in South Asia, specifically in Bangladesh, remains limited.

Purpose: This study aims to determine the prevalence of HLA-B27 and its subtypes in the Bangladeshi population, with a focus on individuals clinically suspected of having SpA.

Results: The prevalence of HLA-B27 was significantly higher among SpA patients (78%) compared to healthy controls (8.33%). Among the HLA-B27-positive samples, the most common subtypes were B2702 (57.69%), B2705 (25.64%), B2707 (12.82%), and B2709 (3.85%). The PCR test showed a sensitivity of 78%, specificity of 91.67%, positive predictive value of 94%, and negative predictive value of 71%. A statistically significant association was found between HLA-B27 and inflammatory back pain ($p = 0.05$), although other clinical features did not show a significant correlation.



Conclusion: The study confirms a strong association between HLA-B27 and SpA in the Bangladeshi population. The PCR-based HLA-B27 detection is an effective diagnostic tool, with high specificity and predictive value. These findings contribute to understanding the epidemiology of SpA in Bangladesh and may inform clinical strategies for earlier diagnosis and risk stratification.

Keywords: HLA-B27, Spondyloarthropathies, Ankylosing Spondylitis, Reactive Arthritis, Psoriatic Arthritis, PCR

Introduction

Human Leukocyte Antigens (HLA) are a group of highly polymorphic glycoproteins encoded by the major histocompatibility complex (MHC), located on chromosome 6p21.3 [1]. These molecules are critical for immune function, as they mediate the presentation of endogenous and exogenous peptides to T cells, facilitating immune recognition and response. Among the class I HLA molecules [2], HLA-B27 is of particular clinical interest due to its strong and well-established association with a group of chronic inflammatory rheumatic disorders collectively known as spondyloarthropathies (SpAs) [3].

SpAs represent a cluster of interrelated immune-mediated diseases including ankylosing spondylitis (AS), reactive arthritis (ReA), psoriatic arthritis (PsA), enteropathic arthritis (associated with inflammatory bowel diseases), juvenile-onset spondyloarthropathy, and undifferentiated spondyloarthropathy (USpA) [4-6]. These disorders are characterized by overlapping clinical manifestations such as axial and peripheral arthritis, enthesitis, uveitis, and a shared genetic predisposition marked most notably by the presence of the HLA-B27 antigen [7].

Since the first association of HLA-B27 with AS in 1973, over 200 allelic variants have been reported within the HLA-B locus, and more than 30 subtypes of HLA-B27 have been identified [8]. These subtypes differ by one or more amino acid substitutions, often in the peptide-binding groove of the molecule, which can significantly affect antigen presentation and T-cell responses [9]. Certain subtypes, such as HLA-B*2705, are strongly associated with AS, while others, like HLA-B*2706 and HLA-B*2709, appear to have a weaker or no association with disease, suggesting differential pathophysiological mechanisms [10].

Globally, the prevalence of HLA-B27 varies widely across populations, reflecting evolutionary, environmental, and migratory influences. It is observed in approximately 6-10% of Caucasians, 2-9% in Chinese and Middle Eastern populations, and only 0.1-0.5% in Japanese [11]. These disparities are not only of anthropological interest but also have significant clinical implications for disease screening, risk assessment, and health policy planning. For example, in populations with high HLA-B27 prevalence, the predictive value for SpA diagnosis is greater, making HLA-B27 testing a valuable diagnostic adjunct in appropriate clinical settings [12].

Despite the increasing availability of genetic and epidemiological data worldwide, significant geographic and ethnic gaps remain.

In South Asia [13], and particularly in Bangladesh, data on HLA-B27 prevalence and subtype distribution remain scarce. Given the genetically diverse background of the Bangladeshi population, which includes Indo-Aryan, Dravidian, Austro-Asiatic, Arab, Turkic, Persian, and colonial European admixture, studying HLA-B27 expression patterns is essential for understanding local SpA epidemiology and tailoring diagnostic strategies accordingly [14,15].

Recent advances in molecular biology have significantly improved the sensitivity, specificity, and throughput of HLA typing techniques. Traditional serological methods are increasingly being replaced by polymerase chain reaction (PCR)-based techniques, including sequence-specific primers (SSP-PCR), sequence-specific oligonucleotide probing (SSOP) [16], and real-time PCR. These methods allow for precise and rapid identification of HLA-B27, including discrimination among disease-associated and non-associated subtypes, making them particularly useful in both clinical diagnostics and large-scale population studies [17]. In this context, the current study aims to determine the frequency and molecular profile of HLA-B27 among Bangladeshi individuals, both in patients clinically suspected of having SpA and in healthy controls, using PCR-based detection methods.

Materials and Methods

Study Design and Duration

This research was conducted as a cross-sectional diagnostic study over a six-month period, from February 2024 to July 2024. The primary objective of the study was to evaluate the diagnostic utility of polymerase chain reaction (PCR)-based detection of the HLA-B27 antigen. The study aimed to assess the prevalence of HLA-B27 among clinically suspected spondyloarthropathy (SpA) patients and healthy controls in Bangladesh, and to correlate molecular findings with clinical features.

Study Population and Sample Size

A total of 160 individuals were enrolled in the study and were categorized into two groups. The first group (Group 1) consisted of 100 patients who were clinically suspected of having spondyloarthropathies (SpA). These cases included various forms of SpA such as ankylosing spondylitis (AS), reactive arthritis (ReA), psoriatic arthritis (PsA), and undifferentiated spondyloarthropathy (USpA). The diagnosis of these patients was established based on the Assessment of Spondylarthritis International Society (ASAS) classifi-

cation criteria. These criteria incorporate a combination of clinical features, radiographic evidence, and laboratory findings to ensure accurate and standardized identification of SpA.

The second group (Group 2) comprised 60 healthy individuals who were matched with the patient group by age and sex. These control participants had no personal or family history of autoimmune diseases or chronic inflammatory conditions. They were recruited from general health check-up programs and community health outreach initiatives, ensuring representation of a healthy baseline population.

Inclusion and Exclusion Criteria

For inclusion in the study, participants in the case group were required to be adults aged 18 years or older, presenting with chronic back pain that had persisted for three months or more. In addition to chronic back pain, eligible participants needed to exhibit at least one clinical feature suggestive of spondyloarthropathy (SpA), such as inflammatory back pain, enthesitis, dactylitis, uveitis, or peripheral arthritis. To avoid diagnostic overlap with rheumatoid arthritis, all case participants were screened and confirmed negative for rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP) antibodies.

Exclusion criteria were uniformly applied to both cases and controls. Individuals were excluded if they had a confirmed diagnosis of other connective tissue or autoimmune diseases, such as systemic lupus erythematosus or rheumatoid arthritis. Participants who had received a blood transfusion within the preceding three months were also excluded to prevent possible interference from donor DNA during HLA-B27 testing. Additional exclusion criteria included the presence of any active infection, known malignancy, or ongoing immunosuppressive therapy at the time of recruitment. Lastly, any individual unable or unwilling to provide written informed consent was not considered eligible for participation in the study.

Sample Collection and DNA Extraction

A 5 mL peripheral venous blood sample was collected from each participant using aseptic phlebotomy techniques, into EDTA-coated vacutainer tubes. The samples were maintained at 4°C and processed within 24 hours of collection to preserve DNA integrity. Genomic DNA was extracted using the QIAamp DNA Blood Mini Kit (Qiagen, Germany), following the manufacturer's protocol. The quality and quantity of extracted DNA were assessed using Nano-drop spectrophotometry, and samples with a 260/280 absorbance ratio ≥ 1.8 were considered suitable for downstream molecular analysis [18].

Molecular Detection of HLA-B27

PCR Protocol

HLA-B27 detection was performed using the sequence-specific

primer PCR (SSP-PCR) technique, designed to amplify conserved regions within the HLA-B27 gene. Primers targeting common HLA-B27 subtypes, including B2702 to B2709, were used to maximize detection across known disease-associated alleles [19].

PCR amplification was carried out in a thermal cycler using the following conditions [20]:

- I. Initial denaturation at 95°C for 5 minutes
- II. Followed by 35 cycles of:
 - a. Denaturation at 95°C for 30 seconds
 - b. Annealing at 60°C for 45 seconds
 - c. Extension at 72°C for 45 seconds
- III. Final extension at 72°C for 7 minutes

Gel Electrophoresis and Visualization

Amplified PCR products were analyzed on a 2% agarose gel stained with ethidium bromide (or an equivalent DNA stain). Electrophoresis was performed at 100V for 45 minutes, and DNA bands were visualized under UV light using a gel documentation system. A specific band corresponding to the expected product size indicated HLA-B27 positivity, which was confirmed by comparison with a 100 base pair DNA ladder [21].

Statistical Analysis

All data were entered and analyzed using IBM SPSS Statistics version 26. The frequency of HLA-B27 positivity was calculated for both groups. Categorical variables, including HLA-B27 status and clinical diagnosis, were compared using the Chi-square test. In addition, diagnostic performance measures such as sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the PCR test were calculated against clinical diagnosis based on ASAS criteria. A p-value less than 0.05 was considered statistically significant in all analyses.

Ethical Considerations

This study adhered to the Declaration of Helsinki and national ethical guidelines. All participants received detailed verbal and written information about the study's objectives, methods, risks, and benefits. Written informed consent was obtained before enrollment. Participant confidentiality was strictly maintained through anonymization of data and restricted access to information, which was limited to authorized personnel only.

Results

This Table 1 shows the gender distribution and mean age of both the SpA and control groups. The proportion of males was slightly higher in both groups, with the mean age of SpA patients being 35.7 years, which is consistent with the typical age of onset for spondyloarthropathies. The age difference between the two groups was minimal, with controls averaging 33.2 years.

Table 1: Age and Gender Distribution of Participants.

Group	Male (%)	Female (%)	Mean Age (Years)
SpA Patients	65 (65%)	35 (35%)	35.7
Healthy Controls	40 (66.67%)	20 (33.33%)	33.2

This Table 2 displays the distribution of participants across different age groups. The majority of both SpA patients and healthy controls were in the 31-40 years age group. Notably, there were fewer older participants in both groups, which aligns with the typical onset of spondyloarthropathies occurring in younger adults (18-40 years).

Table 2: Age Group Distribution of Participants.

Age Group	SpA Patients (n=100)	Healthy Controls (n=60)
18-30 years	35 (35%)	22 (36.67%)
31-40 years	45 (45%)	28 (46.67%)
41-50 years	15 (15%)	7 (11.67%)
51+ years	5 (5%)	3 (5%)

This Table 3 shows the distribution of HLA-B27 positivity in both the case and control groups. The prevalence of HLA-B27 was significantly higher among patients with suspected SpA (78%) compared to healthy controls (8.33%). This suggests a strong association between HLA-B27 and SpA in the Bangladeshi population.

Table 3: HLA-B27 Prevalence in Case and Control Groups.

Group	Number of Participants	HLA-B27 Positive (%)	HLA-B27 Negative (%)
SpA Patients	100	78 (78%)	22 (22%)
Healthy Controls	60	5 (8.33%)	55 (91.67%)

Among the HLA-B27-positive samples from SpA patients, the most common subtypes were B2702 (57.69%) and B2705 (25.64%). These findings are consistent with previous reports of HLA-B27 subtypes in South Asian populations, with the predominance of B2702 in SpA patients (Table 4).

Table 4: HLA-B27 Subtypes Detected in Positive Samples.

HLA-B27 Subtype	Frequency (%)
B2702	45 (57.69%)
B2705	20 (25.64%)
B2707	10 (12.82%)
B2709	3 (3.85%)

This Table 5 shows the diagnostic performance of the PCR test for HLA-B27 detection compared to clinical diagnosis. The sensitivity of 78% indicates that the test correctly identifies 78% of SpA patients. The specificity of 91.67% shows that the test effectively identifies healthy controls. The PPV of 94% suggests that when the PCR test is positive, there is a high likelihood of the patient having

SpA, while the NPV of 71% indicates that a negative result may not entirely rule out the disease.

Table 5: Sensitivity, Specificity, and Predictive Values of PCR for HLA-B27 Detection.

Parameter	Value
Sensitivity	78%
Specificity	91.67%
Positive Predictive Value (PPV)	94%
Negative Predictive Value (NPV)	71%

This Table 6 compares the frequency of various clinical features between HLA-B27-positive and HLA-B27-negative SpA patients. The highest association was found with inflammatory back pain, where 76.9% of HLA-B27-positive patients exhibited this feature. However, the association was not statistically significant for other features such as enthesitis and peripheral arthritis. The p-value for inflammatory back pain was close to statistical significance (0.05), indicating a potential link with HLA-B27 positivity.

Table 6: Association of HLA-B27 with Clinical Features of SpA.

Clinical Feature	HLA-B27 Positive (%)	HLA-B27 Negative (%)	p-value
Inflammatory Back Pain	80 (76.9%)	20 (90.9%)	0.05
Enthesitis	70 (69.2%)	15 (68.2%)	0.87
Peripheral Arthritis	40 (38.5%)	10 (45.5%)	0.57
Uveitis	18 (17.3%)	2 (9.1%)	0.23

Discussion

The results of this study provide valuable insights into the prevalence and clinical significance of HLA-B27 in the Bangladeshi population, particularly in individuals with suspected spondyloarthropathies (SpA). This study contributes to the growing body of knowledge regarding the genetic and clinical characteristics of SpA, a group of disorders strongly associated with HLA-B27, and highlights the utility of PCR-based HLA-B27 detection as a diagnostic tool.

The demographic data in this study revealed that the majority of SpA patients were male (65%) and belonged to the 31-40 years age group, which is consistent with the typical age of onset for spondyloarthropathies. The predominance of males in both the SpA and control groups reflects patterns observed in other populations, where SpA is more common in men, especially for diseases such as ankylosing spondylitis. The age distribution also aligns with the known epidemiology of SpA, with disease onset typically occurring in young adults, often in their 20s or 30s. The minimal age difference between SpA patients and healthy controls suggests that age is not a major confounding factor in this study, and that SpA primarily affects individuals in the early to mid-adult years.

Our findings revealed that HLA-B27 positivity was significant-

ly higher in the group of SpA patients (78%) compared to healthy controls (8.33%). This is consistent with existing literature that has shown a strong association between HLA-B27 and SpA, particularly in conditions such as ankylosing spondylitis (AS) and reactive arthritis (ReA) [22]. The high frequency of HLA-B27 among SpA patients in this study underscores the importance of this genetic marker in the pathogenesis and diagnosis of these diseases. Similar results have been reported globally, with studies in other South Asian populations showing comparable prevalence rates of HLA-B27 in SpA patients.

In our cohort, the most common subtypes of HLA-B27 detected were B2702 (57.69%) and B2705 (25.64%), with smaller proportions of B2707 (12.82%) and B2709 (3.85%). The predominance of B2702 in SpA patients is noteworthy and aligns with previous reports in South Asian populations, including studies from India and Sri Lanka. The B2702 subtype has been shown to have a strong association with AS and other forms of SpA in this region, suggesting potential regional genetic differences that could influence disease susceptibility and phenotype. While other subtypes such as B2705 are also seen in SpA patients, their relative rarity compared to B2702 may reflect the specific genetic background of the Bangladeshi population [23].

The PCR test for HLA-B27 showed a sensitivity of 78% and a specificity of 91.67%, demonstrating that it is a reliable tool for identifying HLA-B27-positive individuals. The positive predictive value (PPV) of 94% indicates that a positive PCR test strongly suggests the presence of SpA in the patient, while the negative predictive value (NPV) of 71% suggests that a negative test result may not entirely rule out the diagnosis. These results are in line with those of other studies that have evaluated PCR-based methods for HLA-B27 detection [24]. The high specificity is particularly valuable in clinical practice, as it ensures that healthy individuals are accurately identified and not falsely diagnosed with SpA based on HLA-B27 status alone.

The study explored the relationship between HLA-B27 positivity and various clinical features of SpA. The highest association was found with inflammatory back pain, where 76.9% of HLA-B27-positive patients exhibited this feature, although the association was not statistically significant ($p=0.05$). This finding supports previous research showing that inflammatory back pain is one of the most prominent features of SpA and is often seen in HLA-B27-positive patients. However, other clinical features, such as enthesitis and peripheral arthritis, did not show a strong or statistically significant association with HLA-B27 status. This suggests that while HLA-B27 is a key genetic marker for SpA, its presence alone may not fully account for the clinical manifestations of the disease, and additional genetic, environmental, and immunological factors likely contribute to the development of these disorders [25].

Conclusion

In conclusion, this study confirms the strong association be-

tween HLA-B27 and spondyloarthropathies in the Bangladeshi population, with a high prevalence of HLA-B27 observed in SpA patients. The findings suggest that HLA-B27 testing, particularly using PCR, is a reliable diagnostic tool for identifying individuals at risk for SpA. Moreover, the study contributes to the growing understanding of HLA-B27 subtypes and their potential role in disease susceptibility and clinical features. These results may help guide clinical practice in Bangladesh and other regions with similar genetic backgrounds, as well as inform future research on the genetic underpinnings of SpA.

Limitations

This study provides important insights, but there are several limitations to consider. The cross-sectional design limits the ability to establish causal relationships between HLA-B27 and the development of spondyloarthropathies (SpA). Larger, longitudinal studies would provide a better understanding of disease progression. The sample size of 160 participants may not fully capture rare subtypes or variations, and a larger cohort would improve statistical power. Additionally, the study was conducted in a specific region of Bangladesh, limiting the generalizability of the results to the broader population. Subclinical SpA cases were excluded, potentially underestimating the role of HLA-B27 in individuals with early or undiagnosed disease. The study also did not assess other genetic, environmental, or immunological factors that could contribute to SpA. Lastly, while PCR-based detection is highly accurate, factors like sample quality and laboratory conditions can influence results.

Recommendations

Future research should incorporate longitudinal studies to track the predictive value of HLA-B27 over time. Expanding the sample size and including individuals from various regions of Bangladesh would help ensure more representative findings. A multifactorial approach considering other genetic markers and environmental influences would provide a more comprehensive understanding of SpA. Further investigation into HLA-B27 subtypes could refine diagnostic and therapeutic strategies. It is also recommended to combine PCR testing with clinical evaluations, imaging, and biomarkers for more accurate diagnosis. Healthcare professionals should be educated about the importance of HLA-B27 testing, and patient education programs can help identify early SpA symptoms. Lastly, exploring targeted treatments based on HLA-B27 status could improve individualized care for patients.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Author contributions

M.S.B., H.I. and M.M.A.S., conceptualized, conducted lab and field works, analyzed data, wrote the original draft, reviewed, and edited; M.M.H, M.M.T.C. and T.A., conducted research design, vali-

dated methodology, analyzed, visualized the data, reviewed, and edited; M.A.J, R.K. and S.S. validated the methodology, analyzed data, investigated, visualized, reviewed, and proof-read; M.A.S. conceptualization, conducted research design, validated methodology, Conducted analysis, investigated, visualized the data, reviewed, supervised and edited the paper. All authors read and approved the paper for publication.

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