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Detection ofHLA-B27 by Truenat Real-Time PCR in Suspected Ankylosing Spondylitis Patients - A Hospital-Based Study

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Abstract

Background and Aim: Ankylosing spondylitis (AS) is a chronic inflammatory spondyloarthropathy strongly associated with the HLA-B27 genetic marker. Conventional detection methods are often costly or technically demanding in low-resource settings. Truenat RT-PCR, a compact, chip-based molecular platform, offers rapid and accessible diagnostic capability. The present study determined the practical diagnostic utility of Truenat RT-PCR in a resource-constrained environment and evaluated the prevalence of HLA-B27 in clinically suspected AS patients at a tertiary care hospital in Uttar Pradesh.

Materials and methods: A retrospective observational study was conducted at the Department of Microbiology, Hind Institute of Medical Sciences (HIMS), Barabanki, Uttar Pradesh, from January 2023 to December 2024. The hospital primarily serves a semi-urban and rural population.185 patients presenting with features suggestive of Ankylosing spondylitis were included. Genomic DNA extracted from peripheral blood samples was analyzed for HLA-B27 using Truenat RT-PCR. Demographic data, OPD/IPD distribution, and positivity rates were assessed.

Results: Among the 185 patients, ages ranged from 15–45 years, with a mean age of 25.6 years among HLA-B27-positive patients. Males were predominated (65%), and 59 patients (31.9%) tested positive for HLA-B27, with slightly higher positivity among males than females (33.1% vs. 28.3%). HLA-B27 positivity was higher

among OPD patients (39.2%) than IPD patients (27.9%). There was no significant difference in age between HLA-B27-positive and negative groups (p=0.111); however, CRP levels were significantly higher in HLA-B27-positive patients (12.1 ± 9.6 vs. 5.6 ± 4.9 mg/L; p<0.001), indicating greater inflammatory activity.

Conclusion: The study found 31.9% HLA-B27 positivity, with higher CRP levels among positive patients. Truenat RT-PCR was a rapid and practical detection method, particularly suitable for resource-limited settings. Further validation is recommended.

Keywords: HLA-B27, Truenat, Real - Time PCR, Ankylosing Spondylitis

Introduction

Ankylosing spondylitis (AS), a relatively common form of spondyloarthritis (SpA), primarily impacts the axial musculoskeletal system. The primary causes of the disease were sacroili it is and spondylitis, which resulted in the formation of syndesmophytes, which ultimately led to ankylosis and the loss of spinal mobility.[1]

Patients with AS have also been diagnosed with asymmetrical peripheral oligoarthritis, inflammatory back pain, enteropathy, and peripheral arthritis. Extra skeletal manifestations, including psoriasis, chronic inflammatory bowel disease, cardiovascular or pulmonary complications, and anterior uve it is, may also accompany the disease.[2] The prevalence of AS is low, affecting 0.1 to 1.0% of general populations, and the incidence of the disease ranges from 0.5 to 14 per 100,000 individuals per year.[3] AS is an autoimmune disease in which the risk of developing the disease is genetically established to be over 90%.[4,5] In 1973, the discovery of a remarkably high association between AS and HLA - B27 confirmed a significant genetic predisposition. Despite the fact that HLA - B27 is present in over 90% of patients in the majority of ethnic groups that present Ankylosing spondylitis, a proportion of AS cases have been reported that do not involve HLA-B27.[6]

A gene complex that encodes the major his to compatibility complex proteins in humans is known as the human leukocyte antigen system. The immune system is regulated by these cell-surface proteins. While HLA molecules are most renowned for their involvement in transplantation, specific diseases are associated with specific HLA molecules. T cells are presented with antigenic peptides (derived from self and non self antigens) by HLA-B27 (subtypes B * 2701-2759), an MHC class I surface antigen encoded by the B locus in the MHC on the short (p) arm of chromosome no. 6. MHC class I molecules are glycoprotein's that are expressed on the surface of the majority of nucleated human cells and platelets.[7]AS and a few other rheumatic disorders (Reiter's syndrome, acute anterior uveitis, and inflammatory bowel disease) are strongly associated with the presence of the HLA-B27 antigen. The HLA-B27 surface antigen is expressed in 90% of AS patients, whereas it is only present in 8% of healthy individuals. Consequently, HLA-B27 testing is frequently employed to diagnose AS.[1]

The risk of developing AS is significantly higher in HLA-B27-positive relatives than in HLA-B27-positive cases. Approximately 100 subtypes of HLA-B27 have been identified. The "parent" HLA-B27 molecule is HLA-B*2705, which is the most prevalent subtype and is present in nearly every population worldwide. The other subtypes found in AS patients are B*2702, B*2704, and B*2707, which have evolved from the parent molecule.[8]The amino acid composition of these

subtypes varies, which affects the molecule's peptide-binding properties.[9]

In order to identify the HLA-B27 allele, a variety of methods have been devised, such as serology and polymerase chain reaction (PCR), which includes the standard PCR with sequence-specific primers (SSP). Nevertheless, PCR necessitates post - PCR manual procedures and is a time-consuming process.

At present, the rapid detection and amplification of PCR products has been facilitated by the use of a double-stranded DNA binding SYBR Green reagent in real-time PCR or qualitative PCR (qPCR).[10]

Several of these limitations are addressed by Truenat RT-PCR, a rapid, microchip-based molecular diagnostic platform that was developed in India. It is battery-operated, portable, and user-friendly, and it generates outcomes within approximately one hour.

Despite its extensive use in the diagnosis of infectious diseases, its application to genetic markers, such as HLA-B27, is relatively new and poorly documented.

The present investigation examined the practical diagnostic utility of Truenat RT-PCR in a resource-constrained environment and evaluated the prevalence of HLA-B27 in clinically suspected AS patients at a tertiary care hospital in Uttar Pradesh.

Materials and Methods

Study Design and Setting

A retrospective observational study was conducted at the Department of Microbiology, Hind Institute of Medical Sciences (HIMS), Barabanki, Uttar Pradesh, from January 2023 to December 2024.

The hospital primarily serves a semi-urban and rural population.

Study Population

185 patients presenting with features suggestive of Ankylosing spondylitis were included.

Inclusion Criteria

- Patients aged ≥10 years
- Chronic inflammatory back pain (>3 months).
- Reduced spinal or sacroiliac mobility.

Exclusion Criteria

- Hemolyzed, insufficient, or contaminated blood samples.
- Inadequate DNA yield.
- Patients with confirmed AS already under long-term management.

Sample Collection

Peripheral venous blood (3–5 mL) was collected in EDTA vials under aseptic conditions and transported promptly to the molecular laboratory.

DNA Extraction

DNA extraction was performed using the Truenat sample preparation kit:

1. Cell lysis
2. DNA binding to the filter membrane
3. Washing to remove inhibitors
4. Elution of purified DNA

Extracted DNA was used immediately for amplification.

Truenat Real-Time PCR Assay

A total of 6 µL of extracted DNA was loaded onto the microchip test card, which was then placed into the Truenat micro-PCR device.

Amplification and detection were performed automatically within the device, while internal quality controls ensured the validity and reliability of the test. The final results were displayed as either “Detected” or “Not Detected.

Figure 1: Truelab Duo real-time PCR analyzer used for HLA-B27 detection

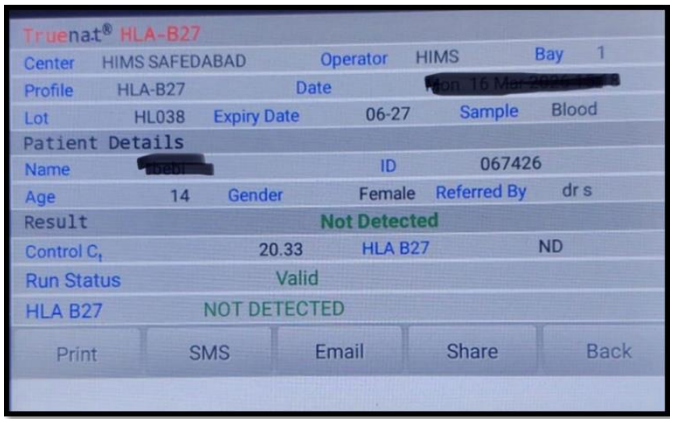

Figure 2: Truenat HLA-B27 assay showing a negative result.

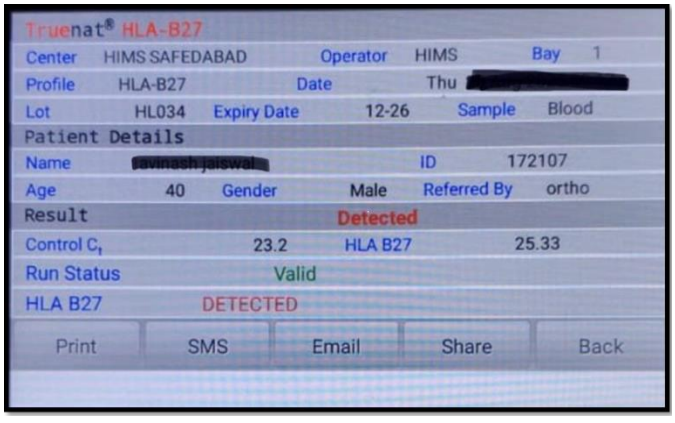

Figure 3: Truenat HLA-B27 assay showing positive result

Statistical Analysis

Data were compiled in Microsoft Excel. Descriptive statistics were used to calculate proportions, percentages, and mean values.

Results

In the present study, the patients were aged between 15 and 45 years, with a mean age of 25.6 years among those who tested positive.

The demographic profile shows that males constituted the majority of the study population (65%), whereas females accounted for 35%. Thus, the study population demonstrated a male predominance (figure 1).

A total of 59 patients (31.9%) tested positive for HLA.33.1% of males tested positive, compared with 28.3% of females. Thus, the positivity rate was slightly higher among males than females (figure 2).

Among the 185 patients, 150 were from the IPD and OPD departments, comprising 107 OPD patients and 43 IPD patients. Among the OPD patients, 42 out of 107 (39.2%) tested positive, whereas among the IPD patients, 12 out of 43 (27.9%) tested positive. Thus, the positivity rate was higher among OPD patients compared with IPD patients (figure 3).

There was no statistically significant difference in age between the HLA-B27-positive and HLA-B27-negative groups ($p = 0.111$). Patients who were HLA-B27 positive had significantly higher CRP levels (12.1 ± 9.6 mg/L) compared with those who were HLA-B27 negative (5.6 ± 4.9 mg/L).

This difference was highly statistically significant ($p < 0.001$), indicating greater inflammatory activity in the HLA-B27-positive group (Table 1)

Chart 1: distribution of patients according to gender

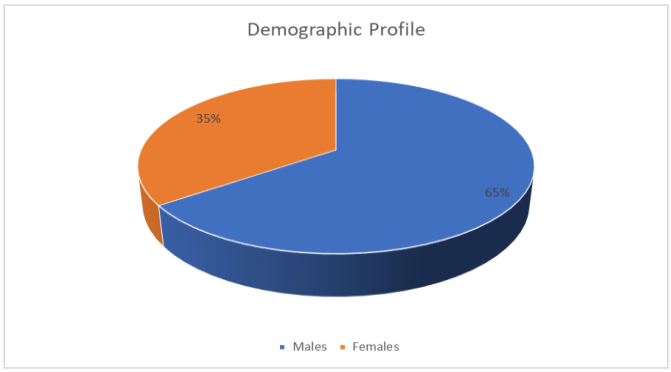

Chart 2: Gender Distribution of HLA-B27 Positive Cases

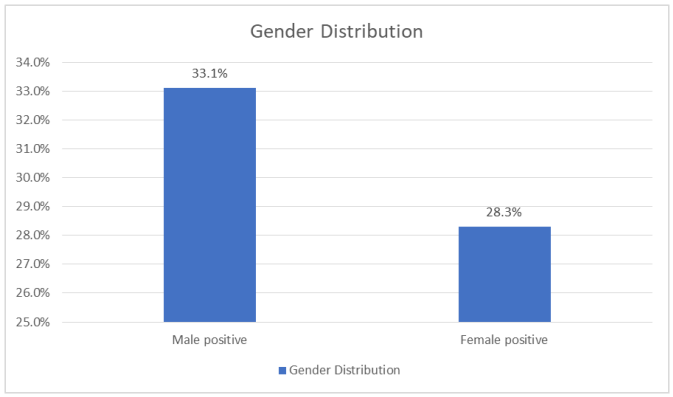

Chart 3: OPD vs IPD HLA-B27 Positive Cases

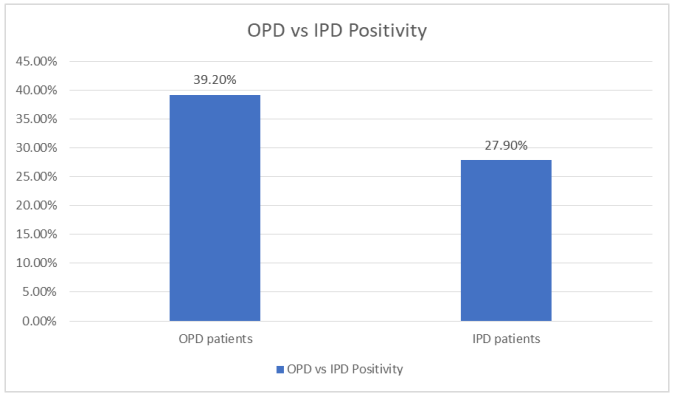

Table 1: CRP and age in between HLA-B27-positive B27-negative

	HLA-B27 Positivity (n=59)	HLA-B27 negative (n=126)	P value
Age (years)	25.54±8.7	28.58±11.5	0.111
CRP (mg/L)	12.1±9.6	5.6±4.9	<0.001

Discussion

The primary method employed for HLA Class I analysis was serological typing. Nevertheless, the field of His to compatibility and Immunogenetics has experienced an exponential increase in the application of DNA technology over the past twenty years as a result of the emergence of molecular biology. DNA-based typing is primarily concerned with the identification of genetic variations and may reveal differences that are of minimal biological significance. There are numerous constraints associated with serological tests, such as the unavailability of specific antisera for all HLA-B27 alleles due to the increased number of known HLA-B27 alleles, the requirement to conduct the test within six hours of blood collection, the requirement to use a minimum of five milliliters of blood, the difficulty of confirming homozygosity, and the difficulty of performing the test in immune-suppressed patients.[11]

In our study, the patients were aged between 15 and 45 years, with a mean age of 25.6 years among those who tested positive. Al-Mukhtar S et al[11] reported the age of patients in their study was ranged from 16-35 years with a mean age of 28.2 ± 3.5 years.

The demographic profile in our study showed that males constituted the majority of the study population (65%), whereas females accounted for 35%. Thus, the study population demonstrated a male predominance. In a comparable investigation, Priyadharshini N et al[1] discovered that the age of the participants ranged from 12 to 60 years, with a mean age of 36.5 years. They also reported a predominance of males in their research.

The condition was more frequently observed among males, as indicated by the similarity in gender distribution between the two studies. Nevertheless, the discrepancy in the mean age of the participants may be

attributed to the age distribution of the participants, the study population, and the sample characteristics. In general, the results of the current study were in agreement with those of Priyathersini N et al[1].

In our study, out of 185 patients, a total of 59 patients (31.9%) tested positive for HLA. 33.1% of males tested positive, compared with 28.3% of females. Thus, the positivity rate was slightly higher among males than females. Al Mukhtar S et al[11] employed the real-time PCR method to detect HLA-B27 in suspected AS Iraqi patients. They identified 20% of the patients as "B27 positive," which was consistent with the results obtained using the SSO method. The positivity rate was lower than that of our study.

According to Priyathersini N et al[1], the overall positivity rate for HLA-B27 was 21.9%, with a significantly higher prevalence of HLA-B27 among male patients than females. Numerous studies have demonstrated that the number of patients in AS was three to four times greater than that of women, and the mean age at diagnosis was substantially lower in males.

The stronger association between HLA-B27-positive disease and radiographic AS in men may partially explain the greater frequency of HLA-B27 positivity among males. Nevertheless, the diagnosis of Ankylosing spondylitis cannot be established solely by HLA-B27 positivity, and the prevalence of the condition varies significantly based on the clinical characteristics of the study population and ethnicity.[12]

In the present study, Among the 185 patients, 150 were from the IPD and OPD departments, comprising 107 OPD patients and 43 IPD patients. Among the OPD patients, 42 out of 107 (39.2%) tested positive, whereas among the IPD patients, 12 out of 43 (27.9%) tested positive. Thus, the positivity rate was higher among OPD patients compared with IPD patients.

This discovery may be indicative of disparities in the clinical characteristics and referral patterns of the two patient populations. The OPD may have evaluated patients who primarily presented with musculoskeletal symptoms indicative of axial spondyloarthritis at an earlier stage of the disease, whereas the IPD may have represented a more heterogeneous group with more severe or complex conditions. HLA-B27 testing is typically employed as an adjunctive investigation in patients with clinical suspicion of axial spondyloarthritis, rather than as a standalone diagnostic test. Consequently, the observed disparity in positivity rates may be attributable to variations in the clinical indications for testing between patients with OPD and those with IPD.[1]

According to Priyathersini et al[1], HLA-B27 positivity was detected in approximately 22% of patients with suspected Ankylosing spondylitis. They also discovered that males and younger patients were more likely to be positive. In the same vein, a recent hospital-based study revealed that 33.8% of patients with spondyloarthritis were positive for HLA-B27. This positivity was independently associated with male sex and a younger age range of 15–30 years.[13] These results indicate that the observed positivity rate can be significantly influenced by the clinical profile of the population being tested.

CRP is one of the acute phase reactants that causes an increase in blood levels in response to inflammation. In general, elevated levels of CRP are indicative of underlying inflammation, such as that caused by trauma or infection. In contrast, patients who are treated with antibiotics or who have infections caused by low

virulence organisms may exhibit deceptively low CRP levels. The reference range for a normal RA level was less than 20. It was determined that CRP values exceeding 1.0 mg/dL were aberrant.[1]The age difference between the HLA-B27-positive and HLA-B27-negative groups was not statistically significant in our study ($p = 0.111$). Compared to patients who were HLA-B27 negative (5.6 ± 4.9 mg/L), those who were HLA-B27 positive had significantly higher CRP levels (12.1 ± 9.6 mg/L). This discrepancy was highly statistically significant ($p < 0.001$).

This discovery is consistent with prior research that has shown that CRP is a critical indicator of disease activity and systemic inflammation in ankylosing spondylitis (AS). Nashel et al. reported that CRP levels were substantially elevated in patients with active AS, thereby corroborating its usefulness as an indicator of inflammatory activity.[14] In the same vein, a comprehensive investigation of 851 patients with active axial AS discovered that elevated CRP was prevalent and was linked to functional impairment and disease activity.[15]

Therefore, the significantly elevated CRP observed in the HLA-B27-positive group in the present study may suggest a greater inflammatory burden in these patients. However, CRP should be interpreted in conjunction with clinical findings and imaging, rather than being regarded as a standalone marker of HLA-B27-associated disease.

Diagnostic Utility of Truenat RT-PCR

Truenat RT-PCR offered several advantages, including a rapid turnaround time of less than one hour, minimal requirement for technical expertise, and portability, making it suitable for use in peripheral and semi-urban laboratories. The system also incorporated integrated quality controls, ensuring reliable and valid results. Its relatively low operational requirements and cost-effectiveness further supported its utility in resource-limited and semi-urban hospital settings.

Limitation

The study had a few limitations. Its retrospective, single-center design limited clinical correlation and generalizability. HLA-B27 testing was not compared with flow cytometry or PCR-SSP, and MRI findings were not included, limiting correlation with radiological features.

Conclusion

The current study revealed that 31.9% of clinically suspected Ankylosing spondylitis patients were positive for HLA-B27, with a slight male predominance. The CRP levels of HLA-B27-positive patients were significantly elevated, suggesting a higher level of inflammatory activity. Truenat RT-PCR offers a practical, portable, and rapid method for HLA-B27 detection, which has the potential to be beneficial in resource-limited healthcare settings and semi-urban areas. Nevertheless, these findings require validation thru additional multicentric studies that employ comparative molecular methodologies, larger sample sizes, and MRI correlation.

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