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Original Article

Affordable HLA-B27 Detection in Resource-Limited Settings: Evaluating Conventional PCR for Uveitis and Spondyloarthropathy in Indonesia

Rina La Distia Nora[1], Lukman Edwar[2], Made Susiyanti[3], Yulia Aziza[4], Ikhwanuliman Putera[5], Ulifna Alfiya Sifyana[6], Mei Riasanti[7], M Zakiy Waliyuddin[8], Maria Valentina Wibawa[9], Rachel Ethelind[10], Erica Widodo[11], Ratna Sitompul[12]

[1,2,3,4,6,9,10,11,12]Department of Ophthalmology, Faculty of Medicine Universitas Indonesia- Cipto Mangunkusumo Kirana Eye Hospital

[5]Department of Ophthalmology, Erasmus University Medical Center, Rotterdam, the Netherlands

[7]Indonesia Infectious Disease and Immunology Research Center (IDIRC)

[8]Indonesia Infectious Disease and Immunology Research Center (IDIRC)

Corresponding email: rina.ladistia@ui.ac.id

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ABSTRACT

Introduction: HLA-B27 is a genetic marker strongly associated with spondyloarthropathy (SpA) and acute anterior uveitis (AAU). Detection of this allele can support earlier diagnosis and targeted management. However, commercially available HLA-B27 tests are costly and often inaccessible in low- and middle-income countries, including Indonesia. Methods: A cross-sectional study was conducted involving 42 subjects: 14 with SpA, 19 with AAU, and 9 healthy controls. DNA was extracted from peripheral blood samples and analyzed using both conventional PCR (targeting exon 3 of HLA-B27) and a commercial HLA-B27 strip assay. Sensitivity, specificity, and diagnostic accuracy of conventional PCR were calculated against the commercial kit as the reference standard. Results: Conventional PCR showed high sensitivity (100%) and accuracy (85.71%) in SpA patients, indicating its potential as a reliable screening tool in this group. However, its performance in AAU patients was suboptimal, with lower sensitivity (40%) and specificity (55.56%). False positives and false negatives were observed, likely due to limitations in allele coverage by conventional primers. Conclusion: Conventional PCR is a promising, affordable alternative for HLA-B27 detection in SpA patients, particularly in resource-limited settings. However, its lower reliability in AAU cases highlights the need for careful clinical application and further optimization. Larger studies and local allele mapping are recommended to enhance diagnostic precision in diverse populations.

Introduction

Spondyloarthropathy (SpA) and acute anterior uveitis (AAU) are interrelated inflammatory conditions frequently associated with the presence of the HLA-B27 allele, a major histocompatibility complex (MHC) class I molecule involved in antigen presentation and immune regulation[1-3]. HLA-B27 is present in up to 90% of patients with ankylosing spondylitis and 50% of patients with AAU[4-6], and its presence is a valuable biomarker that supports diagnosis and guides management in both rheumatology and ophthalmology settings[7-9].

Early identification of HLA-B27 in patients presenting with inflammatory back pain or recurrent anterior uveitis can facilitate timely referral, accurate diagnosis, and appropriate treatment[2,7,10]. However, in many low- and middle-income countries, including Indonesia, access to HLA-B27 testing is often limited due to the high cost of commercial assays and the lack of insurance coverage[11]. This barrier hinders diagnostic accuracy and delays care, particularly in community and public hospital settings[11].

Commercial HLA-B27 testing methods, such as flow cytometry and allele-specific PCR kits, offer high sensitivity and broad allele coverage but require specialized equipment, trained personnel, and significant cost[12-14.] In contrast, conventional PCR is a more affordable and widely available alternative that can be adapted for basic laboratory infrastructure[5,12]. Previous studies have shown that conventional PCR can detect common HLA-B27 alleles with good concordance to commercial assays[5,12], making it a potential diagnostic solution in resource-limited settings. This study aims to evaluate the diagnostic performance of conventional PCR in detecting HLA-B27 among patients with SpA and AAU in an Indonesian clinical context.

By comparing conventional PCR with a validated commercial kit, we assess its sensitivity, specificity, and potential application as a cost-effective diagnostic tool to expand HLA-B27 testing availability in underserved populations. Addressing this gap could improve early diagnosis and management, ultimately enhancing patient outcomes.

Method

This cross-sectional diagnostic accuracy study was conducted at Dr. Cipto Mangunkusumo National General Hospital (RSCM), Jakarta, Indonesia, to evaluate the performance of conventional PCR for detecting the HLA-B27 gene in patients with spondyloarthropathy (SpA) and acute anterior uveitis (AAU), using the HLA-B27 StripAssay® (ViennaLab Diagnostics GmbH, Vienna, Austria) as the reference standard[1-2].

A total of 42 participants were enrolled: 14 with SpA, 19 with AAU, and 9 healthy controls. Diagnoses were based on ASAS and SUN criteria, and healthy controls met WHO health standards3-4. Participants under 18 years, with contraindications to venipuncture, or non-anterior uveitis were excluded. Written informed consent was obtained, and the study was approved by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia (Protocol No. KET-56/UN2.F1/ETIK/PPM.00.02/2021)[5]. Blood samples (3 mL) were collected in EDTA tubes. Genomic DNA was extracted using the Quick-DNA™ Miniprep Plus Kit (Zymo Research Corp., Irvine, California, USA) and quantified with a NanoDrop™ 2000

spectrophotometer (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA); samples with an A260/A280 ratio of 1.7–2.0 were considered adequate for further testing[6-7].

Conventional PCR targeted a 650 bp HLA-B27 fragment with a 268 bp β-globin internal control. Each 25 µL reaction contained genomic DNA, 2× PCR Master Mix (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA), primers, and nuclease-free water. Cycling conditions included denaturation at 95°C (3 min), 30 cycles of denaturation (95°C, 35 s), annealing (61.4°C, 45 s), extension (72°C, 40 s), and a final extension at 72°C (8 min). PCR products were separated on a 1.5% agarose gel stained with GelRed® (Biotium, Inc., Fremont, California, USA) and visualized under UV light. A dual band (650 bp and 268 bp) indicated positivity; absence of β-globin required repeat testing. Negative controls were included[8-9].

All samples were also tested using the HLA-B27 StripAssay®, which involves PCR amplification followed by hybridization to a lateral flow strip embedded with allele-specific probes. A visible HLA-B27 band along with an internal control band indicated a positive result[10-11].

Data were analyzed using IBM SPSS Statistics version 20.0 (IBM Corp., Armonk, New York, USA). Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were calculated using 2×2 contingency tables. Agreement was assessed by Cohen's kappa, and categorical variables were compared using Chi-square or Fisher's exact tests, with $p<0.05$ considered significant[12-13].

Results and Discussion

Results

A total of 42 participants were included in the study, comprising 14 patients with spondyloarthropathy (SpA), 19 with acute anterior uveitis (AAU), and 9 healthy controls. The demographic and clinical characteristics are presented in Table 1. In the AAU group, the mean age was 40.8 years (range 25–68), with a female predominance (57.9%). The majority of AAU patients were of Javanese ethnicity, and anterior uveitis was the most common subtype. Among SpA patients, axial spondyloarthritis, ankylosing spondylitis, and non-radiographic SpA were proportionally represented.

The overall diagnostic performance of conventional PCR for detecting HLA-B27, when compared with a commercial PCR-based strip assay, is summarized in Table 2. Across all clinical groups, the conventional PCR method demonstrated a sensitivity of 52.6%, specificity of 69.6%, and diagnostic accuracy of 61.9%. The positive predictive value (PPV) was 58.8%, and the negative predictive value (NPV) was 64.0%.

In subgroup analysis, conventional PCR showed excellent performance in the SpA group, with a sensitivity of 100%, specificity of 75%, and overall accuracy of 85.7%. All HLA-B27-positive SpA patients were correctly identified, with no false negatives. In contrast, diagnostic performance in the AAU group was limited, showing 40% sensitivity and 55.6% specificity, with multiple false positives and negatives. Among healthy controls, sensitivity was 0%, specificity was 83.3%, and diagnostic accuracy was 55.6%.

Table 1. Demographic and Clinical Characteristics of Study Participants

Characteristic	SpA (n = 14)	AAU (n = 19)	Healthy Controls (n = 9)	Total (n = 42)
Age (years), mean (range)	–	40.8 (25–68)	–	–
Sex				
Male	6 (42.9%)	8 (42.1%)	3 (33.3%)	17 (40.5%)
Female	8 (57.1%)	11 (57.9%)	6 (66.7%)	25 (59.5%)
SpA Subtype	–	–		
Axial SpA	5 (35.7%)	–	–	–
Ankylosing Spondylitis	6 (42.9%)	–	–	–
Non-radiographic SpA	3 (21.4%)	–	–	–
Type of Uveitis (AAU group)	–	–		
Anterior	–	13 (68.4%)	–	–
Anterior-intermediate	–	3 (15.8%)	–	–
Panuveitis	–	3 (15.8%)	–	–

Table 2. Diagnostic Performance of Conventional PCR for HLA-B27 Compared to Commercial Kit

Group	n	TP	TN	FP	FN	Sensitivity (%)	Specificity (%)	Accuracy (%)	PPV (%)	NPV (%)
SpA	14	6	6	2	0	100.0	75.0	85.7	75.0	100.0
AAU	19	4	5	4	6	40.0	55.6	47.4	50.0	45.5
Healthy Controls	9	0	5	1	3	0.0	83.3	55.6	0.0	62.5
Overall	42	10	16	7	9	52.6	69.6	61.9	58.8	64.0

Discussion

This study evaluated the diagnostic performance of conventional PCR for HLA-B27 detection in patients with spondyloarthropathy (SpA) and acute anterior uveitis (AAU), comparing its results to those of a commercial PCR-based assay. The findings indicate that conventional PCR is a highly sensitive and cost-effective method for detecting HLA-B27 in SpA patients, but its performance is suboptimal in AAU and healthy populations[1-2].

In the SpA subgroup, conventional PCR achieved 100% sensitivity and 85.7% accuracy, suggesting it may serve as a reliable screening tool for HLA-B27 in clinical settings where commercial assays are unavailable or unaffordable. These results align with previous studies reporting high concordance between conventional PCR and more

advanced techniques, particularly in patients with established SpA[3-4]. Importantly, the absence of false negatives in this group strengthens its utility as a diagnostic aid for this population[5-6].

However, the diagnostic utility of conventional PCR in AAU patients was limited, with only 40% sensitivity and an accuracy of 47.4%. This discrepancy may be attributed to the genetic heterogeneity of AAU patients, potential differences in associated HLA-B27 subtypes, and the presence of AAU cases not linked to SpA[7-8]. It is also possible that some HLA-B27 alleles associated with AAU are not amplified by the primers used in the conventional PCR protocol[9-10]. This underscores the need for further optimization, including the development of subtype-specific primers that align with the allele distribution in the Indonesian population[9-10].

The performance in healthy controls was similarly limited, with zero sensitivity and a diagnostic accuracy of 55.6%. While this may reflect the low expected prevalence of HLA-B27 in the general population, it also highlights the risk of false negatives if conventional PCR were used for population-level screening[11-12].

Cost remains a critical factor in diagnostic accessibility. In Indonesia, the high price of commercial kits and the lack of insurance coverage limit routine HLA-B27 testing[13-14]. Conventional PCR, with an estimated per-test cost several times lower than commercial assays, offers a practical alternative in resource-limited settings—especially for SpA cases where clinical suspicion is high[13-14].

This study has several limitations. The relatively small sample size, particularly in subgroup analyses, may limit generalizability[15-16]. Moreover, molecular confirmation of HLA-B27 subtypes by DNA sequencing- the gold standard- was not performed[17-18]. Finally, the primers used may not cover the full spectrum of HLA-B27 alleles prevalent in Indonesia, potentially affecting sensitivity[9-10].

Conclusion

In conclusion, conventional PCR represents a viable and affordable method for detecting HLA-B27 among SpA patients in resource-constrained environments[17-19]. However, its limited sensitivity in AAU and healthy populations suggests that it should be used selectively and preferably in conjunction with clinical findings. Future studies should focus on expanding the sample size, validating the assay with sequencing, and developing region-specific primers to improve diagnostic accuracy across diverse patient groups[17-19]. Implementing conventional PCR-based screening in high-suspicion SpA cases could enhance early diagnosis and reduce healthcare disparities in low-resource settings[17-19].

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