# Association of HLA-B51/5 in Patients of Behcet's Disease Referred to a Tertiary Care Hospital in Pakistan

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## ABSTRACT

**Objective:** To determine the association of HLA-B51/5 in patients of Behcet's disease (BD) in local symptomatic patients.

**Study Design:** A cross-sectional study.

**Place and Duration of the Study:** Department of Immunology, Armed Forces Institute of Pathology, Rawalpindi, Pakistan, from August 2020 to July 2023.

**Methodology:** Blood samples of 100 study participants, including 50 symptomatic patients of BD and 50 disease-free individuals (healthy controls), were taken. DNA was extracted and amplified using sequence-specific primers. Electrophoresis was conducted on 2% agarose gel to separate the DNA. The presence of HLA-B51/5 alleles was determined by analysing the unique band patterns visible on the gel under the ultraviolet light. Descriptive statistics were shown as mean ± SD. Chi-square test was employed to find any significant association between HLA-B51/5 and the disease group and its severity sub-groups. A p-value of less than 0.05 was deemed significant.

**Results:** The majority of participants, 73 (73%), were females and 27 (27%) were males, with a mean age of 26.84 ± 5.31 years. HLA-B51/5 was positive in 33 of the BD patients (66.0%, <0.001) and 4 of the healthy controls (8.0%). There was no significant difference in HLA-B51 among mild, moderate, and severe BD sub-groups.

**Conclusion:** HLA-B51/5 is strongly associated with BD in local population and serves as a useful tool for diagnosis of BD. However, it is not associated with the disease severity.

**Key Words:** *Agarose gel, Behcet's disease, Electrophoresis, HLA-B51/5 allele, ICBD criteria.*

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## INTRODUCTION

The initial account of Behcet's disease (BD) was presented in 1937 by Hulusi Behcet from Istanbul.[1] BD is an enigmatic auto-inflammatory systemic vasculitis, the underlying cause of which remains unidentified. Its defining features include recurring mucocutaneous indications such as genital and oral ulceration, ocular manifestations mainly characterised by chronic relapsing uveitis, and systemic vasculitis affecting blood vessels of varying dimensions. Despite ongoing research, the exact aetiological mechanisms of BD are still uncertain, although genetic and environmental factors have been implicated.[2]

Human leucocyte antigen B51 (HLA-B51) is a frequently occurring genetic factor that is common among the Middle-Eastern, Japanese, and Turkish population.[3]

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Additionally, there are a number of other genes that have been discovered, such as heat shock proteins, tumour necrosis factor (TNF), and major histocompatibility complex class I chain-related genes.[4] The BD typically impacts individuals between the ages of 20 and 40 years, predominantly affecting young adults while also occurring less frequently in children.[5] While most cases are random, there are occasional reports of familial clusters.[6] BD has been widely associated with the HLA-B5 allele of the major histocompatibility complex, particularly with the HLA-B51 variant, which is the predominant split of the HLA-B5 broad antigen.[7] However, the strength of this genetic association varies significantly between studies, with the reported risk increases ranging from 1.3 to 16. Small sample sizes may explain these variations, although it is also plausible that the association between BD and HLA-B51/B5 could differ among various ethnic groups or clinical subtypes of BD.[8]

Conducting an assessment of the association of HLA-B51/5 in BD within the Pakistani population would be beneficial in determining its diagnostic utility for challenging cases and potentially avoiding invasive procedures. This study aimed to investigate the connection between HLA-B51/5 and specific symptoms of BD in local symptomatic Pakistani patients.

## METHODOLOGY

This cross-sectional study was performed at the Department of Immunology, Armed Forces Institute of Pathology, Rawalpindi, Pakistan, from August 2020 to July 2023 after taking formal approval from the Institutional Review Board (IRB) of Armed Forces Institute of Pathology. The sample size was calculated by WHO calculator at 95% confidence interval and 5% margin of error with prevalence of HLA-B51 as 0.06%.[9] Consecutive sampling method was used to select the samples. Informed consent was obtained from the participants before collecting the data. A total of 100 participants were included in this study. Among them, 50 patients had BD, aged between 20 and 45 years, regardless of their gender, and showed clinical symptoms indicating BD as per ICBD criteria. Fifty disease-free individuals (healthy controls) who were similar in age and gender were also included. Patients with inflammatory bowel disease, reactive arthritis, systemic lupus erythematous, and herpetic infections were excluded, as these conditions can present symptoms similar to BD.

Three millilitres (3 ml) of blood samples were obtained in ethylene diamine tetra acetic acid (EDTA) tube by puncturing the peripheral veins using a method called venipuncture. The extraction of chromosomal DNA was carried out in adherence to the guidelines given by the manufacturer (Puregene Blood Core Kit B; QIAGEN). The DNA concentration was set at 100 ng/µl. The DNA was amplified using the sequence-specific primers (SSPs) targeting HLA-B51. Subsequently, electrophoresis was conducted on a 2% agarose gel to separate the DNA. The gel was stained with the ethidium bromide for at least 30 minutes. The presence of HLA-B51/5 alleles was determined by analysing the unique band patterns visible on the gel under ultraviolet light.

The patients of BD were divided into three groups according to disease severity based on the Behcet's syndrome international study group (ISG) criteria.[10] "Mild" described cases with relatively few symptoms or mild manifestations that did not significantly impair the patient's quality of life or lead to organ damage. "Moderate" indicated more pronounced symptoms or involvement of multiple organ systems, resulting in some impairment or functional limitations. "Severe" referred to the cases with severe symptoms, extensive organ involvement, or complications that significantly impact the patient's health and well-being, possibly leading to disability or life-threatening situations.

Data were analysed using the Statistical Package for the Social Sciences (IBM SPSS Statistics for Windows version 26, IBM Corp; Armonk, USA). Mean and standard deviation were calculated for quantitative variables. Qualitative variables were recorded in terms of frequency and percentage. Differences in mean ages in the three severity groups were compared by one-way ANOVA test. Associations of HLA-B-51 with manifestations of BD were carried out using Chi-square / Fisher's exact test and odds ratio and their 95% confidence intervals were calculated. A p-value of less than 0.05 was deemed significant.

## RESULTS

A total of hundred (n = 100) participants were enrolled in this study. The majority of the participants were females [73 (73%)] while males were less common [27 (27%)]. The overall mean age of the study population was 26.84 ± 5.31 years. The mean ages of the BD group and non-disease groups were 27.76 ± 6.13 years, and 25.92 ± 4.19 years, respectively (p = 0.08).

**Table I: Demographic characteristics and HLA-B 51 status of patients with BD (n = 50) and control (n = 50) groups.**

| Parameters | Disease severity | | | p-value | Behcet's disease (BD) group (n = 50) | Control group (n = 50) | p-value |
|---|---|---|---|---|---|---|---|
| | Mild (n = 21) | Moderate (n = 19) | Severe (n = 10) | | | | |
| Gender | | | | | | | |
| Male | 5 (23.8%) | 6 (31.6%) | 1 (10.0%) | 0.43 | 12 (24%) | 15 (30%) | 0.49 |
| Female | 16 (76.2%) | 13 (68.4%) | 9 (90.0%) | | 38 (76%) | 34 (70%) | |
| Age (years) Mean ± SD | 26.57 ± 6.10 | 29.00 ± 7.48 | 27.90 ± 7.48 | 0.46 | 27.76 ± 6.13 | 25.92 ± 4.19 | 0.08 |
| HLA-B51 | | | | | | | |
| Positive | 10 (47.6%) | 13 (68.4%) | 10 (100.0%) | 0.015 | 33 (66%) | 4 (8%) | <0.001 |
| Negative | 11 (52.6%) | 6 (31.6%) | 0 (0%) | | 17 (34%) | 46 (92%) | |

**Table II: Comparison of manifestations in HLA-B51 positive and negative in BD patients (n = 50).**

| Manifestations | HLA-B51 | | Odds ratio (95% confidence interval) |
|---|---|---|---|
| | Positive (n = 36) | Negative (n = 14) | |
| Oral ulceration | 36 (100%) | 14 (100%) | - |
| Genital ulcers | 28 (77.8%) | 9 (64.3%) | 1.23 (0.77 – 1.96) |
| Erythema nodosum | 26 (72.2%) | 9 (64.3%) | 1.11 (0.74 – 1.67) |
| Folliculitis acne | 28 (77.8%) | 12 (85.7%) | 0.58 (0.11 – 3.16) |
| Eyes involvement | 16 (44.4%) | 4 (28.6%) | 2.00 (0.52 – 7.58) |
| Arthritis | 25 (69.4%) | 9 (64.3%) | 1.26 (0.34 – 4.64) |
| Vasculitis | 11 (30.6%) | 4 (28.6%) | 1.10 (0.28 – 4.28) |
| Neurological involvement | 4 (11.1%) | 2 (14.3%) | 0.75 (0.12 – 4.64) |

HLA-B51 was positive in 33 (66.0%) of the BD patients and 4 (8.0%) of the healthy controls. A strong association was observed between HLA-B51 and BD ($p < 0.001$) as shown in Table I. The gender and age were not a significant risk factor for disease severity ($p = 0.43$ and $p = 0.46$) or disease expression ($p = 0.49$ and $p = 0.08$), respectively (Table I).

Out of 50 patients with BD, 21 patients had mild disease, 19 patients had moderate, and 10 patients had severe disease. Frequency, mean age, and HLA-B51 status are given in Table I. A significant difference was observed in HLA-B51 association among different disease severity groups ($p = 0.015$) with more strong association in severe disease.

The different manifestations of BD were documented and HLA-B51 status was assessed. Oral ulcers were 100% observed in all patients with HLA-B51, followed by genital ulcers (77.8%) and follicular acne (77.8%). Table II shows the odds ratio and frequency of associations in HLA-B51 positive and negative patients having BD.

## DISCUSSION

BD is a type of systemic vasculitis that triggers auto-inflammatory responses in the body. It is identified by its symptoms affecting the mucocutaneous region, such as repeated ulcerations in the mouth and genital area, as well as ocular manifestations such as chronic relapsing uveitis. Additionally, it involves systemic vasculitis affecting large arteries, small arteries, and veins throughout the body.[11]

In this study, HLA-B51 was positive in 33 (66.0%) of the BD patients and 4 (8.0%) of the healthy controls with a significant strong association ($p < 0.001$). This study is substantiated by earlier studies. In a study by Davatchi *et al*, HLA- B51 was positive in 48.9% of the BD patients which was lower than this study.[12] Another study by Sakly *et al.* found that HLA-B51 was positive in 30% of the BD patients and 16.1% of the healthy control group.[13] This may be attributed to some sort of sampling bias in the studies. The present research also compared the HLA-B51 allele with the clinical manifestation in BD patients and a healthy control group. In the present study, most frequent clinical manifestation was oral ulceration that occurred in 36 (100%) cases, followed by genital ulcers in 28 (77.8%) cases and skin findings in 33 (91.7%) cases. Genital ulceration is a significant symptom of BD. In another study conducted by Nishiyama *et al*.,[14] they examined 83 cases of BD within families and discovered that being HLA-B51 positive increases the risk of oral ulceration while providing protection against the genital ulceration. Another study by Maldini *et al*.[15] estimated that HLA-B51/B5 is a relatively weak risk factor for genital ulceration based on 30 reports involving 1,303 patients.

This thorough analysis examined how greatly HLA-B51 influences as a risk factor of BD, showcasing one of the strongest disease-gene associations known for the complex human trait. There are a few limitations to the current study. Firstly, the authors collected the data from a single hospital in Pakistan. The present researchers could not assess the specific influence of HLA-B51 on each clinical manifestation of patients with BD, nor perform a subtype analysis of HLA-B51. So, these must be accounted for interpretation of the results of this study.

## CONCLUSION

HLA-B51 can serve as a useful alternative tool for diagnosis of BD. Failing to genotype HLA-B51 during patient evaluation can result in under-diagnosis of BD, especially in settings with limited molecular diagnostic capabilities. Additionally, the presence of HLA-B51 increases the risk of ocular lesions as well as genital ulceration in individuals with BD.

### ETHICAL APPROVAL:

Ethical approval was obtained from the Institutional Review Board of the Armed Forces Institute of Pathology, Rawalpindi, Pakistan (No: FC/IMM17-27/IRB/20/366A).

### PATIENTS' CONSENT:

Informed consent was obtained from the participants before collecting the data.

### COMPETING INTEREST:

The authors declared no conflict of interest.

### AUTHORS' CONTRIBUTION:

MA: Drafting, acquisition, analysis, and interpretation of data.
UBK: Drafting, conception, designing the study content, review, writing, and editing.
DA: Formal analysis, investigation, and resources.
MOR: Results analysis.
MH: Critical revision of content.
MZA: Data curation, review, writing, and editing.
All authors approved the final version of the manuscript to be published.

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