Genetic Association of HLA-A*26, -A*31, and -B*51 with Behcet's Disease in Saudi Patients

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ABSTRACT

BACKGROUND: HLA-B*51 has been universally associated with Behcet’s disease (BD) susceptibility, while different alleles of HLA-A have also been identified as independent BD susceptibility loci in various ethnic populations. The objective of this study was to investigate associations of HLA-A and -B alleles with BD in Saudi patients.

MATERIALS AND METHODS: Genotyping for HLA-A and HLA-B was performed using HLA genotyping kit (Lab type® SSO) in 120 Saudi subjects, including 60 BD patients and 60 matched healthy controls.

RESULTS: Our results revealed that frequencies of HLA-A*26, -A*31, and -B*51 were significantly higher in BD patients than in controls, suggesting that HLA-A*26, -A*31, and -B*51 are associated with BD. The frequency of HLA-B*51 was significantly lower in BD patients than in controls. Stratification of genotyping results into active and nonactive forms of BD revealed that the frequency of HLA-A*31 was significantly higher in the nonactive form than in the active form of BD, while there was no significant difference in the distribution of other alleles between the two forms of BD.

CONCLUSION: This study suggests that HLA-A*26, -A*31, and -B*51 are associated with susceptibility risk to BD, while HLA-B*51 may be protective in Saudi patients. However, larger scale studies are needed to confirm these findings.

KEYWORDS: Behcet’s disease, HLA, genotyping, genetics, Saudis

INTRODUCTION

Behcet's disease (BD) is a chronic autoimmune/inflammatory disorder characterized by recurrent orogenital ulcers, cutaneous inflammation, and uveitis. In addition to its typical mucocutaneous and ocular manifestations, BD targets the musculoskeletal, vascular, nervous, and gastrointestinal systems.1–3 The prevalence of BD is geographically influenced, and it is more prevalent in countries along the silk route, particularly in the East Asia4,5 and the Middle East.6–11 Its prevalence is highest in Turkey, followed by Egypt, Morocco, Iraq, Saudi Arabia, Japan, Iran, Korea, and China.12,13 Although the specific etiology of BD remains elusive, extensive studies have suggested that autoimmunity, genetic factors, and environmental factors are involved in its pathogenesis.1,3,14,15

Like many autoimmune disorders, BD has significant genetic associations with particular alleles of the class I and II human major histocompatibility complex (MHC), and studies of these associations have led to significant insights into the molecular underpinnings of these disorders.16–18 The human leukocyte antigen (HLA) region on chromosome 6p21.31 contains multiple genes encoding highly variable antigen-presenting proteins and plays a key role in antigen presentation and activation of T cells.19 HLA protein, HLA-B*51, encoded by HLA-B is the strongest known genetic risk factor for BD. Associations between BD and other factors within the MHC have also been reported, although the strong regional linkage disequilibrium complicates their confident disentanglement from HLA-B*51. Single nucleotide polymorphism mapping with logistic regression of the MHC identified the HLA-B/MICA region and the region between HLA-F and HLA-A as independently associated with BD.16,20–24 Genetic association studies on Saudi BD patients are scanty.13,25,26 The Saudi population being a closed and isolated society with a high rate of consanguinity (inbreeding) represents a valuable resource for studying such genetic associations, and the present study was aimed at investigating the association of HLA-A and B genetic variants with BD in Saudi patients.

MATERIALS AND METHODS

In the present study, we recruited 60 BD Saudi patients (aged 20–64 years) and an equal number of healthy controls, matched for age (20–60 years), sex, and ethnicity (Saudi) from
Prince Sultan Military Medical City, Riyadh, Saudi Arabia, for genetic analysis of HLA alleles. The exclusion and inclusion criteria were followed strictly for the selection of patients and controls. Subjects with any other inflammatory/autoimmune diseases were excluded from this study. A questionnaire was filled for each subject to collect past medical history, drug in use, and relevant lifestyle-related questions. None of the subjects had any malignancies or other chronic illnesses in the past. This study was approved by the Research and Ethical Committee of Prince Sultan Military Medical City, Riyadh, Saudi Arabia, and the written informed consent was obtained from each subject before participation. This research work complied with the principles of the Declaration of Helsinki. The diagnosis of BD was made based on the criteria of the International Study Group for BD. We evaluated the clinical features such as oral ulcers, genital ulcer, ocular inflammation, musculoskeletal, cutaneous, gastrointestinal lesions, nervous, pulmonary, cardiovascular manifestations, and vascular lesions. The active and nonactive forms of BD were determined at the time of study after the assessment of clinical parameters. A detailed information along with demographic characteristics are mentioned in our recently published article.

Peripheral blood (3 mL) from healthy controls and patients was drawn in EDTA-containing vials, and genomic DNA was extracted using the QIAamp® DNA mini Kit (Qiagen) according to the manufacturer’s protocol. The purity of DNA was determined at 260/280 nm using a Nano-Drop spectrophotometer (Thermo Fisher Scientific). Only DNA samples having a 260–280 nm absorbance ratio between 1.7 and 2.0 and a final concentration of 20–30 ng/μL were considered appropriate.

HLA genotyping. HLA genotyping was performed by the reverse sequence-specific oligonucleotide polymerase chain reaction (PCR) technique using genotyping kits Lab type® SSO (One Lambda) as per the manufacturer’s protocol. The regions of DNA exons 2 and 3 for the loci A and B were amplified. The allele-specific biotinylated primer accompanying the kits was used for the amplification of DNA. The PCR amplification was programmed at 96 °C for three minutes followed by five cycles of 96 °C for 20 seconds, 60 °C for 20 seconds, and 72 °C for 20 seconds; 30 cycles of 96 °C for 10 seconds, 60 °C for 15 seconds, and 72 °C for 20 seconds and extension at 72 °C for 10 minutes. The amplified product was also run on 5% agarose gel (Pulsed Field certified Agarose; Bio-Rad Laboratories) to check the amplification of specific exon of each locus. The remaining PCR product was then hybridized with oligonucleotide probes sequence-specific conjugates with fluorescent microspheres. The hybridized products were analyzed by using flow analyzer running LAB Scan 100™ xPONENT (One Lambda) and fluorescence intensity in each microsphere was identified. HLA Fusion™ program (One Lambda) was used for HLA data analysis.

Statistical analysis. Frequencies of various alleles of HLA polymorphism were compared between BD patients and controls and analyzed by Fisher’s exact test and P values ≤0.05 were considered significant. The significance of the differences in distribution of alleles was calculated after Bonferroni correction to minimize error due to multiple comparison tests. The binary logistic regression analysis was also performed for each of homozygous (two alleles) and heterozygous (one allele) HLA-A or HLA-B alleles contributing independently to BD. Chi-square test was used to calculate % confidence interval (CI). Genetic data were also expressed as an odd ratio interpreted as relative risk (RR) according to the method of Woolf as outlined by Schallreuter et al. RR indicates the number of times the risk of disease is higher in terms of allele in BD patients than in controls. The RR was calculated for all the subjects using the following formula:

\[ RR = \frac{a \times d}{b \times c} \]

where \( a \) is the number of patients with expression of allele, \( b \) the number of patients without expression of allele, \( c \) the number of controls with expression of allele, and \( d \) the number of controls without expression of allele.

Etiologic Fraction (EF) indicates the hypothetical genetic component of the disease. The values 0.0–0.99 are of significance. EF was calculated for positive association only where RR > 1 using the following formula:

\[ EF = \frac{(RR - 1)f}{RR}, \text{ where } f = \frac{a}{a + c} \]

Preventive fraction (PF) indicates the hypothetical protective effect of one specific allele/genotype against the disease. PF was calculated for negative association only where RR < 1 using the following formula. Values <1.0 indicate the protective effect of the allele against the manifestation of disease.

\[ PF = \frac{(1 - RR)f}{RR(1 - f) + f}, \text{ where } f = \frac{a}{a + c} \]

Results
Clinically, all BD patients (100%) had oral ulcers, 80.32% genital ulcer, 70.49% ocular, 67.21% musculoskeletal, 60.65% cutaneous, 36.06% gastrointestinal, and 22.95% patients had nervous system involvement. The results of genotyping for HLA-A and HLA-B in BD patients and controls are summarized in Tables 1–5. The frequency of HLA-A*02 (38.33%) was the highest, followed by that of HLA-A*26, HLA-A*31, HLA-A*68 (10.83% each), HLA-A*23, HLA-A*24 (5.83% each), HLA-A*01, HLA-A*30, HLA-A*32 (3.33% each), HLA-A*03, HLA-A*33 (2.5% each), HLA-A*11, HLA-A*29, and HLA-A*69 (0.83% each). Comparison of allele frequencies between the BD patients and controls indicated that the frequencies of...
Table 1. Comparison of allele frequencies of HLA-A in patients with Behcet’s disease and controls.

| HLA-A ALLELES | BD (60) N (FREQ. %) | CONTROLS (60) N (FREQ. %) | P-VALUE | ODDS RATIO (95% CI) | EF/PF |
|---------------|---------------------|----------------------------|----------|---------------------|-------|
| HLA-A*01     | 4 (3.33)            | 10 (8.33)                  | 0.166    | 0.379 (0.115–1.245) | 0.317 |
| HLA-A*02     | 46 (38.33)          | 35 (29.16)                 | 0.136    | 1.509 (0.880–2.587) | 0.188*|
| HLA-A*03     | 3 (2.50)            | 8 (6.66)                   | 0.137    | 0.359 (0.092–1.387) | 0.327 |
| HLA-A*11     | 1 (0.83)            | 5 (4.16)                   | 0.121    | 0.193 (0.022–1.679) | 0.407 |
| HLA-A*23     | 7 (5.83)            | 11 (9.16)                  | 0.341    | 0.613 (0.229–1.641) | 0.196 |
| HLA-A*24     | 7 (5.83)            | 11 (9.16)                  | 0.341    | 0.613 (0.229–1.641) | 0.196 |
| HLA-A*25     | 0 (0)               | 1 (0.83)                   | 0.499    | –                   | –     |
| HLA-A*26     | 13 (10.83)          | 4 (3.33)                   | 0.041b   | 3.523 (1.114–11.139) | 0.546*|
| HLA-A*29     | 1 (0.83)            | 3 (2.50)                   | 0.371    | 0.327 (0.033–3.196) | 0.339 |
| HLA-A*30     | 4 (3.33)            | 11 (9.16)                  | 0.068    | 0.341 (0.105–1.105) | 0.339 |
| HLA-A*31     | 13 (10.83)          | 2 (1.67)                   | 0.005c   | 7.168 (1.581–32.498) | 0.702*|
| HLA-A*32     | 4 (3.33)            | 4 (3.33)                   | 1.00     | 1.00 (0.244–4.094) | –     |
| HLA-A*33     | 3 (2.50)            | 1 (0.83)                   | 0.371    | 3.051 (0.312–29.758) | 0.504*|
| HLA-A*68     | 13 (10.83)          | 13 (10.83)                 | 1.00     | 1.00 (0.443–2.257) | –     |
| HLA-A*69     | 1 (0.83)            | 0 (0)                      | 0.499    | –                   | –     |
| HLA-A*74     | 0 (0)               | 1 (0.83)                   | 0.499    | –                   | –     |

Notes: *Data for EF. **Statistically significant using Fisher’s exact test. **P = 0.08 Bonferroni corrected. ***P = 0.656 Bonferroni corrected.

Abbreviations: N, number of allele; BD, Behcet’s disease; EF, etiological fraction; PF, preventive fraction.

Table 2. Comparison of allele frequencies of HLA-A in active and nonactive Behcet’s disease.

| HLA-A ALLELES | ACTIVE (34) N (FREQ. %) | NON ACTIVE (26) N (FREQ. %) | P-VALUE | ODDS RATIO (95% CI) | EF/PF |
|---------------|-------------------------|-----------------------------|----------|---------------------|-------|
| HLA-A*01     | 3 (4.11)                | 1 (1.92)                    | 0.632    | 2.353 (0.237–23.307) | 0.430*|
| HLA-A*02     | 31 (45.59)              | 15 (28.85)                  | 0.087    | 2.066 (0.960–4.448) | 0.346*|
| HLA-A*03     | 2 (2.94)                | 1 (1.92)                    | 1.00     | 1.545 (0.136–17.522) | 0.231*|
| HLA-A*11     | 0 (0)                   | 1 (1.92)                    | 0.433    | –                   | –     |
| HLA-A*23     | 4 (5.88)                | 3 (5.77)                    | 1.00     | 1.021 (0.218–4.773) | 0.113*|
| HLA-A*24     | 3 (4.41)                | 4 (7.70)                    | 0.465    | 0.553 (0.118–2.590) | 0.385 |
| HLA-A*26     | 9 (13.24)               | 4 (7.70)                    | 0.388    | 1.830 (0.530–6.312) | 0.313*|
| HLA-A*29     | 0 (0)                   | 1 (1.92)                    | 0.433    | –                   | –     |
| HLA-A*30     | 2 (2.94)                | 2 (3.85)                    | 1.00     | 0.757 (0.103–5.565) | 0.114 |
| HLA-A*31     | 3 (4.41)                | 10 (19.23)                  | 0.015a   | 0.193 (0.050–0.745) | 0.489 |
| HLA-A*32     | 1 (1.47)                | 3 (5.77)                    | 0.312    | 0.243 (0.246–2.414) | 0.437 |
| HLA-A*33     | 1 (1.47)                | 2 (3.85)                    | 0.578    | 0.373 (0.032–4.231) | 0.355 |
| HLA-A*68     | 8 (11.76)               | 5 (9.61)                    | 0.774    | 1.253 (0.384–4.082) | 0.068 |
| HLA-A*69     | 1 (1.47)                | 0 (0)                       | 0.999    | –                   | –     |

Notes: *Data for EF. **Statistically significant using Fisher’s exact test.

Abbreviations: N, number of alleles; EF, etiological fraction; PF, preventive fraction.

allele HLA-A*026 and HLA-A*31 were significantly higher in BD patients than in controls (P = 0.041, OR = 3.523, 95% CI = 1.11–11.139, EF = 0.546, and P = 0.005, OR = 7.168, 95% CI = 1.581–32.498, EF = 0.702, respectively). However, after applying Bonferroni correction, the P values are not significant (P = 0.08 and P = 0.656, Table 1). An increased frequency of HLA-A*02 was also found in BD patients as compared to controls (38.33% vs. 29.16%), but the difference was not statistically significant (P = 0.172, Table 1). When the data were grouped on the basis of the active and nonactive forms of BD, the frequency of HLA-A*31 allele was significantly higher in the nonactive form than in the active form of BD (P = 0.015), while the frequency of HLA-A*26 did not differ significantly in two groups (Table 2).

The frequency of HLA-B*51 was significantly higher in BD patients than in controls (P = 0.0001, OR = 3.631,
The frequency distribution of homozygous/heterozygous alleles of HLA-A*26, HLA-A*31, and HLA-B*51 in BD and controls is shown in Table 5. The binary logistic regression analysis performed for each of the homozygous and heterozygous HLA alleles indicated that HLA-B*51 allele, both in homozygous (two alleles) and heterozygous (one allele) conditions, is significantly associated with susceptibility to BD in Saudi patients (P = 0.0001 and P = 0.010, respectively). On the other hand, HLA-A*26 is associated in heterozygous (one allele) conditions with BD, while upon stratification of HLA-A*31 into heterozygous and homozygous conditions, the association lost significance (P = 0.089).

### Discussion

The significantly higher frequency of HLA-A*26 in BD cases than in controls suggested that HLA-A*26 is associated with susceptibility to BD in Saudi patients. The binary logistic regression analysis (Table 5) also indicated that HLA-A*26 is associated in heterozygous (one allele) conditions with BD in Saudi patients. The HLA-A gene has been genotyped in BD patients with different ethnicities, and HLA-A*26 was reported to be associated with BD in Taiwan, Greece, and Japan.\(^{30-32}\) HLA-A*26 has been associated with the ocular manifestation, an outcome of BD indicating its contribution to the risk of BD.\(^{31,32}\) Itoh et al.\(^{33}\) found weak association of HLA-A*26 with BD and suggested some secondary influence on the onset of BD. In addition, an association between the HLA-A*26:01 subtype and BD has been reported in Japanese and Korean.\(^{16,22}\) HLA-A*26:01 not only has been reported to be a primary susceptibility allele of BD in Japan,\(^{22}\) but a recent study also found that the frequency of HLA-A*26:01 was significantly increased in BD patients with uveitis, particularly in the HLA-B*51 negative subset.\(^{32}\) Our results also suggested...
HLA association in Behcet’s disease

**Table 4. Comparison of allele frequencies of HLA-B in active and nonactive Behcet’s disease.**

| HLA-B ALLELES | ACTIVE (34) N (FREQ. %) | NON ACTIVE (26) N (FREQ. %) | P-VALUE | ODDS RATIO (95% CI) | EF*PF |
|---------------|--------------------------|-----------------------------|---------|---------------------|-------|
| HLA-B*07      | 2 (2.95)                 | 2 (3.85)                    | 1.00    | 0.757 (0.103–0.565) | 0.114 |
| HLA-B*08      | 8 (11.76)                | 4 (7.70)                    | 0.549   | 1.601 (0.454–5.634) | 0.238*|
| HLA-B*14      | 1 (1.47)                 | 1 (1.92)                    | 1.00    | 0.761 (0.046–12.463) | –     |
| HLA-B*15      | 1 (1.47)                 | 2 (3.85)                    | 0.578   | 0.373 (0.032–4.231) | 0.355 |
| HLA-B*18      | 0                        | 2 (3.85)                    | 0.185   | –                   | –     |
| HLA-B*27      | 1 (1.47)                 | 0                            | 0.999   | –                   | –     |
| HLA-B*35      | 3 (4.41)                 | 4 (7.70)                    | 0.465   | 0.553 (0.118–2.590) | 0.254 |
| HLA-B*38      | 0                        | 1 (1.92)                    | 1.00    | 0.761 (0.046–12.463) | –     |
| HLA-B*39      | 1 (1.47)                 | 0                            | 0.999   | –                   | –     |
| HLA-B*41      | 1 (1.47)                 | 0                            | 0.999   | –                   | –     |
| HLA-B*42      | 0                        | 1 (1.92)                    | 0.433   | –                   | –     |
| HLA-B*44      | 0                        | 1 (1.92)                    | 0.433   | –                   | –     |
| HLA-B*49      | 1 (1.47)                 | 0                            | 0.999   | –                   | –     |
| HLA-B*50      | 6 (8.82)                 | 7 (13.5)                    | 0.555   | 0.622 (0.195–1.976) | 0.218 |
| HLA-B*51      | 39 (57.35)               | 25 (48.07)                  | 0.358   | 1.452 (0.702–3.001) | 0.189*|
| HLA-B*52      | 0                        | 1 (1.92)                    | 0.433   | –                   | –     |
| HLA-B*53      | 1 (1.47)                 | 0                            | 0.999   | –                   | –     |
| HLA-B*57      | 2 (2.95)                 | 0                            | 0.504   | –                   | –     |
| HLA-B*58      | 1 (1.47)                 | 0                            | 0.999   | –                   | –     |

**Note:** *Data for EF.

**Abbreviations:** N, number of alleles; EF, etiological fraction; PF, preventive fraction.

that allele **HLA-A*31** is associated (OR = 7.168, EF = 0.702) with the risk of BD. After applying Bonferroni correction, the P value is not significant (P = 0.08) possibly due to the small sample size. As this is the first study where **HLA-A*31** is found to be associated with BD susceptibility risk, these results remain to be replicated in other cohorts. However, when genotypic data were stratified on the basis of active and nonactive forms of BD, we found that the frequency of **HLA-A*31** was significantly (P = 0.015) higher in the inactive form of BD than in the active form.

In general, several earlier reports are consistent with the present study and HLA-A gene has been suggested to constitute a second independent susceptibility locus.20–22 Kang et al.16 showed that certain HLA-A alleles are responsible for the unique clinical features of BD. Due to the small sample size, we could not assess any relationship between particular

**Table 5. HLA allele frequencies and their association with Behcet’s disease.**

| ALLELE      | BD (60) N (FREQ. %) | CONTROLS (60) N (FREQ. %) | ODDS RATIO (95% CI) | P-VALUE |
|-------------|---------------------|----------------------------|---------------------|---------|
| HLA-B*51 (hh) | 14 (23.33)         | 34 (56.67)                 | Ref                 | –       |
| HLA-B*51 (HH) | 17 (28.33)         | 2 (3.33)                   | 20.64 (4.20–101.41) | 0.0001* |
| HLA-B*51 (Hh) | 29 (48.34)         | 24 (40.00)                 | 2.93 (1.28–6.69)   | 0.010*  |
| HLA-A*26 (hh) | 47 (78.0/33)       | 56 (93.33)                 | Ref                 | –       |
| HLA-A*26 (HH) | 0                  | 0                          | –                   | –       |
| HLA-A*26 (Hh) | 13 (21.67)         | 4 (6.67)                   | 3.87 (1.18–12.67)  | 0.025*  |
| HLA-A*31 (hh) | 50 (83.33)         | 58 (96.67)                 | Ref                 | –       |
| HLA-A*31 (HH) | 3 (5.00)           | 0                          | –                   | –       |
| HLA-A*31 (Hh) | 7 (11.67)          | 2 (3.33)                   | 4.06 (0.81–20.44)  | 0.089   |

**Notes:** The binary logistic regression analysis was performed for each of homozygous and heterozygous HLA alleles contributing independently to Behcet’s disease. *Statistically significant.

**Abbreviations:** HH, homozygous; Hh, heterozygous; hh, no allele; N, number of subjects.
HLA-A type and clinical feature of the patient. The small sample size might be one of the limitations of this study. Nevertheless, we believe that the results of our study are unlikely to be affected by systematic errors such as population stratification, because the source of our controls and cases represents the same Saudi population. On the contrary, some reports indicated that HLA-A alleles are not associated with increased risk of BD in Palestine, Jordan, Iran, Ireland, Italy, and Turkey.14–38

The significantly higher frequency of HLA-B*51 in Saudi BD patients than in controls with P = 0.0001, OR = 3.631, and EF = 0.521 together with Bonferroni corrected P = 0.0022 indicated that HLA-B*51 is strongly associated with BD susceptibility. Earlier, Yabuki et al.26 studied 13 Saudi BD patients and reported significantly increased frequency of HLA-B51 in BD patients as compared to controls. Several studies across the globe in different ethnicities have shown strong evidences for HLA-B*51 susceptibility to increased risk of BD.17,18,37–40 HLA-B*51 alone increases the risk of BD up to 40%–80% in different ethnicities and known as universal risk factor for BD.18,31,41 The present finding of HLA-B*51 and increased risk of BD in Saudis (RR = 3.93) are corroborated with earlier reports from different ethnic populations: RR = 3.51 and P = 0.065 for the Japanese population,17 P = 1.35 × 10−73 and OR = 5.15 for the Chinese Han population,40 P = 0.0003 and OR = 2.39 for the Korean population, RR = 3.51 for the Iranian population,18 OR = 6.24 for the Turkish population,38 P = 4.11 × 10−41 and OR = 4.63 for the Sardinia population,19 OR = 5.15, P = 1.35 × 10−73 for the Spanish population,17 and many more. However, HLA-B*51 alone is neither necessary nor sufficient to determine BD, and several HLA-A and -B alleles may independently contribute to the risk of BD.17,24,42

On the other hand, the frequency of HLA-B*15 was significantly lower in Saudi BD patients than in controls, suggesting that HLA-B*15 may be protective against BD in Saudis. Contrary to our result, HLA-B*15 has been associated with BD in some populations.24,42,43 Ombrello et al.24 indicated that HLA-B*51, -A*03, -B*15, -B*27, -B*49, -B*57, and -A*26 each contributed independently to BD risk in Turkish population. Piga and Mathieu42 in a meta-analysis reported that besides HLA-B*57 being primarily associated, HLA-A*26, HLA-B*15, and HLA-B*5701 are also independently associated with BD and suggested for further studies to clarify the functional relevance of the different genes found to be associated with disease susceptibility and the potential interactions between genes located within and outside the MHC region. Our study supports that besides HLA-B*51 being primarily associated, HLA-A alleles are also independently associated with susceptibility to BD.

Conclusion
It is concluded that HLA-A*26, -A*31, and -B*51 are associated with BD in Saudi patients, while HLA-B*15 may be protective. However, further studies on population genetics with larger sample size are required to strengthen these findings.

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Author Contributions
Conceived and designed the experiment: FA. Analyzed the data: SA, M.Albalawi. Wrote the first draft of the manuscript: MM. Contributed to the final writing of the manuscript: MArfin. Agree with manuscript results and conclusions: AA, FA. Jointly developed the structure and arguments for the paper: MArfin, MM. Made critical revisions and approved final version: AA, MArfin. All authors reviewed and approved of the final manuscript.

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