Prevalence of HLA-B27 in Turkish Patients with Ankylosing Spondylitis and Non-radiographic Axial Spondyloarthritis

Ankilozan Spondilitli ve Non-radyografik Aksiyal Spondiloartritli Türk Hastalarda HLA-B27 Prevalansı

ABSTRACT Objective: Human leukocyte antigen (HLA)-B27 is a genetic risk factor associated with ankylosing spondylitis (AS) and non-radiographic axial spondyloarthritis (nr-axSpA). Although there are studies regarding the HLA-B27 prevalence in patients with AS in Turkey, there is insufficient information on the HLA-B27 prevalence in patients with nr-axSpA. We aimed to evaluate the prevalence of HLA-B27 positivity among patients with AS and nr-axSpA, and its association with disease activity. Material and Methods: A cross-sectional study was conducted on 860 participants (762 with AS and 98 with nr-axSpA), fulfilling the modified New York criteria and/or Assessment of Spondyloarthritis International Society (ASAS) classification criteria. Disease activity was assessed through the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and Ankylosing Spondylitis Disease Activity Score with C-reactive protein (ASDAS-CRP). Results: HLA-B27 was positive in 69.3% of all patients, in 70.1% of patients with AS, and in 63.3% of patients with nr-axSpA. There was a significant difference in HLA-B27 positivity among the AS patients with a higher ASDAS and higher BASDAI score (respectively; p<0.001, p=0.002), while there was no relation between the HLA-B27 positivity and disease activity in patients with nr-axSpA (p=0.6, p=0.5, respectively). Conclusion: The prevalence of HLA-B27 is relatively low among patients with AS and nr-axSpA in Turkey, which might be attributed to the geographic location of Turkey leads a multi-ethnic society. Moreover, HLA-B27 is associated with higher disease activity in AS patients, but there is no such relationship in nr-axSpA patients.

Keywords: HLA-B27 antigen; spondylitis, ankylosing; prevalence

ÖZET Amaç: İnsan lökosit antijeni (HLA)-B27, ankilozan spondilit (AS) ve non-radyografik aksiyal spondiloartrit (nr-axSpA) ile ilişkili genetik bir risk faktörüdür. TürkİYE’de AS’nin, nr-axSpA hastaları arasında HLA-B27 prevalansı ile ilgili çalışmaların olması rağmen nr-axSpA hastalarında HLA-B27 prevalansı hakkında yeteri bir bilgi bulunmamakta. Bu çalışmada, AS ve nr-axSpA hastalarında HLA-B27 pozitifinin görülme sıklığı ve hastalık aktivitesi ile ilişkisinin değerlendirilmesi amaçlanmıştır. Gereç ve Yöntemler: Çalışmaya, modifiye New York ve veya ASAS (Ulusal Spondilo Artrit Değerlendirme Derneği) kriterlerini karşılayan 762 AS ve 98 nr-axSpA olmak üzere toplam 860 hasta dahil edilmiştir. Hastalık aktivitesi Bath Ankilozan Spondilit Hastalık Aktivite Indeks (BASDAI) ve Creakit protein içeren Ankilozan Spondilit Hastalık Aktivite Skoru (ASDAS-CRP) ile değerlendirilmiştir. Bulgular: HLA-B27 tüm hastaların %69,3’üne, AS’nin hastaların %70,1’ine ve nr-axSpA’nın hastaların %63,3’üne pozitifi. AS hastalarında daha yüksek ASDAS ve BASDAI skorları ile HLA-B27 pozitifliği arasında anlamli bir ilişki bulunmamış, (sarsılsı: p<0,001, p=0,002), nr-axSpA hastalarında HLA-B27 pozitifliği ile hastalık aktivitesi arasında anlamli bir ilişki saptanmadı (sarsılsı: p=0,6, p=0,5). Sonuç: HLA-B27 prevalansı, TürkİYE’deki AS ve nr-axSpA hastalarında nispeten düşük olup, bu durum TürkİYE’nin coğrafı konumuna ve farklı etnik yapıları içermesine atfedilebilir. Ayrıca HLA-B27 pozitifliği AS hastalarında daha yüksek hastalık aktivitesi ile ilişkili olup, nr-axSpA hastalarında böyle bir ilişkiye yoktur.

Anahtar Kelimeler: HLA-B27 antijeni, spondilit, ankilozan; prevalans
is the most important genetic risk factor for AS and nr-axSpA. About 100 sub-alleles of HLA-B27 have been described up to the present.

Prevalence studies and animal models suggest that HLA-B27 is involved in the pathogenesis of both AS and nr-axSpA. Despite several genetic studies concerning the mechanism and activation process of HLA-B27, the real triggering act of this gene is still unknown. There are several hypotheses for the role of HLA-B27 in the pathogenesis of AS and nr-axSpA. The most accepted one is the abnormal processing of antigenic peptides and endoplasmic reticulum stress resulting from the tendency of HLA-B27 to misfold and form homo-dimers.

The frequency of the HLA-B27 also varies in terms of ethnicity and geography. A decrease in HLA-B27 prevalence is observed in the Northern Hemisphere from north to south and from west to east. It is assumed that these differences are due to the negative selection of malaria, and in regions where malaria is endemic, the prevalence of HLA-B27 is low and vice versa. The prevalence of HLA-B27 in the normal population is significantly lower in the Middle Eastern and Arab countries than in Western countries.

Although there are studies regarding the HLA-B27 prevalence in patients with AS in Turkey, there is insufficient information on the HLA-B27 prevalence in patients with nr-axSpA. Therefore, in this study, we aimed to evaluate; (1) the prevalence of HLA-B27 positivity among patients with AS and nr-axSpA, and (2) its association with disease activity, which was evaluated through the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and Ankylosing Spondylitis Disease Activity Score with C-reactive protein (ASDAS-CRP).

MATERIAL AND METHODS
DESIGN
A cross-sectional study was planned on AS and nr-axSpA patients, who applied to rheumatology and physical therapy and rehabilitation outpatient clinic. Ankara University Clinical Research Ethics Committee approved the study approval and written informed consent of participants were obtained prior to the performance of any study procedures, which was in accordance with the Helsinki Declaration.

PARTICIPANTS
A total of 860 patients, 98 with nr-axSpA and 762 with AS, were included in the study. Patients, who fulfilled the modified New York criteria and/or the Assessment of Spondyloarthritis International Society (ASAS) classification criteria, were enrolled starting from April 2014 to June 2018. Patients, who had already a diagnosis and had been followed up in the outpatient clinic, were included in this study during their routine control. Patients, who had a new diagnosis during the study period, were excluded in the study since it might be a factor that would disturb the comparison of ongoing treatment efficacy.

DEMOGRAPHIC VARIABLES
Age and gender were used for analysis.

CLINICAL FEATURES
Disease duration, time on antitumor necrosis factor (TNF) and nonsteroidal antiinflammatory drugs (NSAIDs) treatment, time from disease onset to anti-TNF treatment, time from diagnosis to anti-TNF treatment were recorded.

LABORATORY MEASUREMENTS
All assays were performed in the same biochemical laboratory. Blood samples of all patients were obtained at the time of enrollment of the study. A total of 10 cc blood was collected from the participants, who had no HLA-B27 results during their follow-up, after 12 hours of fasting. Blood samples were centrifuged at 4,000 repetitions per minute for 10 minutes. Also, as an acute phase reactant, CRP was studied at the time of enrollment.

DISEASE ACTIVITY
Disease activity was assessed through BASDAI and ASDAS-CRP. Thresholds were determined as follows: for BASDAI inactive disease (<4) and active disease (≥4); for ASDAS-CRP inactive disease (<1.3), moderate disease activity (1.3-2.1), high disease activity (2.1-3.5) and very high disease activity (>3.5).
STATISTICAL ANALYSIS

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 21 software (IBM SPSS Statistics for Windows, Version 21.0., Armonk, New York, USA). The variables were investigated using visual (histograms and probability plots) and analytical methods (Kolmogorov-Smirnov test) to determine normal or non-normal distributions. Descriptive analyses were presented using means, standard deviations and frequencies. The Student’s t-test was used to compare age, disease duration, time on anti-TNF and NSAIDs treatment, time from disease onset to anti-TNF treatment, time from diagnosis to anti-TNF treatment. The Chi-square test was used to compare the gender and HLA-B27 positivity among groups. A 5% Type-I error level was used to infer statistical significance.

RESULTS

DEMOGRAPHIC AND CLINICAL FEATURES OF THE PATIENTS

Demographic and clinical features of the patients are given in Table 1. Disease duration, time on anti-TNF treatment, time on NSAIDs, time from disease onset to anti-TNF treatment and time from diagnosis to anti-TNF treatment were lower in nr-axSpA group compared to AS (p<0.001 for all comparisons). HLA-B27 was positive in 596 patients (69.3%), while there was no difference between the groups in terms of HLA-B27 positivity (AS: 70.1%, nr-axSpA: %63.3, p=0.2).

ANKYLOSING Spondylitis Disease Activity Score with C-Reactive Protein and HLA-B27 Positivity

Mean ASDAS-CRP and BASDAI scores were higher in the AS group when compared to nr-axSpA group (p<0.001 for both) (Table 1). Table 2 shows the HLA-B27 positivity of the patients according to the baseline ASDAS-CRP values. There was a significant difference in HLA-B27 positivity among the AS patients with a higher ASDAS-CRP (p<0.001). However, no significant difference was observed in HLA-B27 positivity among the nr-axSpA group (p=0.6).

BATH ANKYLosing Spondylitis Disease Activity Index and HLA-B27 Positivity

Table 3 gives information about the HLA-B27 positivity of the patients according to the baseline BASDAI scores. There was a significant difference in HLA-B27 positivity among the AS patients with a higher BASDAI score (p=0.002). However, no significant difference was observed in HLA-B27 positivity among the nr-axSpA group (p=0.5).

![Table 1: Demographic and clinical features of the patients.](image-url)
DISCUSSION

The discovery of a potential association between HLA-B27 and AS produces a series of clinical studies describing the geographical prevalence and its potential relationship with the disease activity.\(^2,4,6,10\) In this study, we presented the prevalence of HLA-B27 positivity among patients with AS and nr-axSpA, and its association with disease activity. Although HLA-B27 positivity among the Turkish patients diagnosed with AS has been investigated before, this is the first study evaluating the HLA-B27 positivity among the patients with AS and nr-axSpA in Turkey.\(^11-14\) The fact that nr-axSpA patients were included in our analysis is the strength of our study. However, it is known that the different subtypes (HLA B2705, B2701, B2703, B2707, B2708, B2710, B2713, B2714, B2715, B2719 and B2725) and its distribution differ in terms of geography.\(^15\) Therefore, the absence of HLA-B27 subtyping might be seen as a limitation of our study.

We found the HLA-B27 positivity 69.3% in all patients, 70.1% in patients with AS, and 63.3% in patients with nr-axSpA. Günal et al. compared the clinical features of AS and the frequencies of HLA-B27 and its alleles in patients from Turkey with other series.\(^11\) Similarly to our results, HLA-B27 was found to be positive in 70% of patients. The authors concluded that the lower frequency of HLA-B27 in their study than the other studies with Caucasians may be attributed to different genetic and/or environmental factors in Turkey. This was also valid in our study.

The geographic location is accepted as one of the determining factors for the prevalence of HLA-B27 positivity. There were different results when the countries close to Turkey were examined. Kassimos et al. investigated the HLA-B27 antigen in young Greek males with AS, and they found that the 90% of the patients had HLA-B27 antigen.\(^16\) Fallahi et al. assessed the prevalence of HLA-B27 positivity in AS patients in Iran. Similarly with our findings, they

**TABLE 2:** HLA-B27 positivity among the patients with ankylosing spondylitis and non-radiographic axial spondyloarthritis according to the baseline ankylosing spondylitis disease activity score with C-reactive protein.

|       | N=860 | HLA-B27 | ASDAS-CRP | ASDAS-CRP | ASDAS-CRP | ASDAS-CRP |
|-------|-------|---------|-----------|-----------|-----------|-----------|
|       |       |         | <1.3      | 1.3-2.1   | 2.1-3.5   | >3.5      |
|       |       |         |           |           |           |           |
| AS    |       |         | 41        | 68        | 214       | 211       |
| (n=762)|      |         | (7.7)     | (12.7)    | (40.1)    | (39.5)    |
|       |       |         |           |           |           |           |
| Nr-axSpA |     |         | 16        | 62        | 104       | 46        |
| (n=228)|      |         | (7.0)     | (27.2)    | (45.6)    | (20.2)    |

**TABLE 3:** HLA-B27 positivity among the patients with ankylosing spondylitis and non-radiographic axial spondyloarthritis according to the baseline Bath ankylosing spondylitis disease activity index scores.

|       | n=860 | HLA-B27 | Moderate | Severe |
|-------|-------|---------|----------|--------|
|       |       |         | (>4)     | (<4)   |
| AS    |       |         |          |        |
| (n=762)|      |         | 182      | 352    |
|       |       |         | (34.1)   | (65.9) |
|       |       |         | 105      | 122    |
|       |       |         | (46.1)   | (53.9) |
| Nr-axSpA |     |         | 32       | 30     |
| (n=98) |      |         | (51.6)   | (48.4) |
|       |       |         | 21       | 15     |
|       |       |         | (58.3)   | (41.7) |

Data are presented as number (percent).
AS: Ankylosing spondylitis; ASDAS: Ankylosing spondylitis disease activity score; CRP: C-reactive protein; HLA: Human leukocyte antigen; nr-axSpA: Non-radiographic axial spondyloarthritis.
found that 74.8% of AS patients was \textit{HLA-B27} positive.\textsuperscript{17} Having also a different ethnicity and environmental factors in Iran, like Turkey, might have given this result. In a recent study, Ziade et al. calculated the prevalence \textit{HLA-B27} in aSpA in Lebanon.\textsuperscript{18} With the agreement of our findings, they found that the low prevalence of \textit{HLA-B27} (41.1%) in aSpA.

We found a significant difference in \textit{HLA-B27} positivity among the AS patients with a higher ASDAS and higher BASDAI score (p<0.001, p=0.002, respectively), while there was no relation between the \textit{HLA-B27} positivity and disease activity in patients with nr-axSpA. Popescu et al. examined the relationship between functional and activity scores in AS.\textsuperscript{19} Similarly to our results, the authors found that \textit{HLA-B27} positive patients had a median BASDAI 5 times higher than \textit{HLA-B27} negative patients (p=0.033).\textsuperscript{19} Fallahi et al. searched the potential relationship between disease severity and the \textit{HLA-B27} positivity.\textsuperscript{17} They found the severity markers toward higher values in the \textit{HLA-B27} positive group with no significant difference.

As mentioned above, one of the interesting findings of our study was that the \textit{HLA-B27} positivity had no relation with disease activity in the nr-axSpA group. Although treatment modalities, such as anti-TNF, were used in both AS and nr-axSpA groups at similar rates, this result in the nr-axSpA group might be explained with the shorter disease duration and earlier use of anti-TNF treatment. The fact that the basal ASDAS-CRP and BASDAI scores were significantly higher in the AS group compared to the nr-axSpA group is another indicator that could explain this finding. Similarly to our findings, in a recent study, Lubrano et al. found that one of the most important indicators of achieving low disease activity following the onset of anti-TNF therapy in patients with axial spondyloarthritis (AS + nr-axSpA) was short disease duration.\textsuperscript{20} The reason we could not explain this situation with the use of NSAIDs is that we did not differentiate patients based on continuous and/or intermittent NSAID use. On the other hand, the cross-sectional nature of our study is the biggest limitation in terms of the generalizability of these results.

Although it is not one of the main objectives of our study, an important point is noteworthy when we consider the above-mentioned paragraph. In the last decade, both the common use of the sacroiliac magnetic resonance imaging system and the introduction of new axial spondyloarthritis classification criteria developed by ASAS, which enables diagnosis of nr-axSpA patients, have led not only to early diagnosis but also to effective treatments to start earlier.

\section*{CONCLUSION}

Consequently, the frequency of \textit{HLA-B27} positivity in patients with AS and nr-axSpA is low, similar to the other studies conducted in our geography. Moreover, \textit{HLA-B27} is associated with higher disease activity in AS patients, but there is no such relationship in nr-axSpA patients.

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\section*{Conflict of Interest}

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.
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