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HLA-B27 Frequency and its Association with Ankylosing Spondylitis in Indian Population: A Multi-City Analysis from a Single-Center Study

Vandana Sharma [a++*], Sanjay Kumar [a], Rahul Katara [a], Shivani Sharma [a], Vipin Kumar [a], Namrata Pandey [a], Satish Kumar Singh [a], Shashikant Verma [a], Sachin Kumar [a] and Ranvijay Prasad [a]

[a] Core Diagnostics, Gurugram, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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[++] *Consultant Transplant Scientist, HLA & Molecular department;*
**Corresponding author: E-mail: vandana.sharma@corediagnostics.in, vandana.gene@gmail.com;*

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ABSTRACT

The human leukocyte antigen HLA-B*27 is a Class 1 antigen of the major histocompatibility complex (MHC) with B locus and has been established in association with pathogenesis of Ankylosing Spondylitis (AS) since 1973. AS is a multifactorial disease which occurs due to interaction between genes, environment, mechanical stress, microbiota and infection. The ankylosing spondylitis is characterized by inflammation of spine, backpain, stiffness of lower back and hips, extrarticular organs such as eyes, and cardiovascular system. In severe cases this may cause the fusion of vertebrae. The current study highlights the mechanism involved in the pathogenesis of AS with a focus to investigate the frequency of HLA B*27 in suspected cases of AS. The patients were recruited from rheumatology clinics of India from age group of 13-69 years. Patients with suspected cases of AS who met the clinical criteria for AS were tested for HLA-B*27. One thousands and four patients were tested for HLA B*27 using RTPCR method. Out of 1004 subjects, 124 (12.35%) were positive for HLA-B*27. Among these, Male/Female ratio was 2.7. Majority of subjects were from North India. The current study highlights the HLA B27 positivity in association with pathogenesis of AS and indicates that HLA B27 serves as a rapid prognostic and diagnostic genetic marker for detection of AS.

Keywords: HLA-B27; RT-PCR; Ankylosing Spondylitis (AS).

1. INTRODUCTION

Ankylosing spondylitis (AS) belongs to seronegative spondyloarthropathies (SpA), and is charachterized by inflammation of joints, the sacroiliac joints, axial skeleton, and less frequently, peripheral joints, other extra-articular organs such as the eyes, skin, and the cardiovascular system [1]. The chronic inflammation involves the attachment of tendons, ligaments and joint capsules to bone results in alterations in joint architecture and joint fusions [2]. These symptoms appear in second or third decade of life, not evident in early ages of life and degenerative changes likely to occur in people older or in 5^{th} or 6^{th} decade of life. Epidemiological studies have suggested that the prevalence of AS in a White population is 0.1% to 0.2% , in Chinese population 0.24%, 0.2% in Asians and about 0.86% in Caucasians, United Arab Emirates (UAE) 0.5%, Saudi Arabia 2.6%, Kuwait 4%, Iraq 2.1%, Lebanon 1.4%, Tunisia 3.2%, and Syria 1.4% and a higher frequency has been found in Yemeni population (17%)[2].

HLA-B*27 gene is encoded by an allele of the major histocompatibility complex (MHC) class I HLA-B region, located on the short arm of chromosome 6 [3]. HLA B27 may cause around 20.1% of AS heritability and has strong genetic component associated, displayed by strong familial aggregation studies [4]. AS is inherited in families with an increased risk in siblings of patients with 82-fold higher than the disease prevalence [5]. Twin studies have reported even more than 90% of AS susceptibility is genetic while the environmental trigger likely to contribute [5].

An individual's chances of developing HLA B27 positivity and AS during a lifetime is only 1%–2% increases up to 20% with a first-degree relative (FDR) having AS [5]. The recurrence risk of AS in different degrees of relatives (independent of HLA B27) was found to be: 63% in monozygotic twins, 8.2% in FDR, 1.0% in second-degree relatives and 0.7% in third-degree relatives [6]. AS has been observed more common among young men with a Male/Female ratio of roughly 2 to 1 [7]. HLA B27 is a polymorphic gene and has around 75 subtypes (B*27:01 to B*27:62) studied so far[8]. HLA B2705 and HLA B2704 are the commonest subtypes reported in the South Indian population [9-11]. Several studies have documented the frequency of the antigen (B*27) in diverse Indian population and in diagnosis of seronegative Spondyloarthritis [12-17].

Although a number of hypothesis have been suggested but the exact cause of AS is still not clear. AS can occur as a result of altered immune response, triggered by a complex intricate of genetics, ethnicity, and environmental factors [18]. The main risk factors of AS include family history of disease, gender, immunological and microbial infections, altered gut microbiota and others have been presented in the Fig. 1.

Three major hypotheses are (i) the abnormal peptide processing and presentation contribute towards the pathogenesis of AS via interaction between HLA B27 and endoplasmic reticulum

amino peptidase (ERAP) 1 (ii) misfolding of HLA B27 gene and altered configuration of peptide in the endoplasmic reticulum (ER) triggers ER stress and the unfolded protein response (UPR) may occur (iii) The arthritogenic peptides from microbes presented by HLA-B27 to stimulate CD8+T cells which subsequently interact with HLA-B27-bound self-peptides, presented in Fig. 1 [10, 19-22].

GWAS studies have confirmed strong genetic links of ERAP1 polymorphism in particular with HLA-B27:05 positive patients diagnosed with AS (15, 22). Therefore, HLA B27 plays an important role in the pathogenesis of AS disease. This study was designed to identify the HLA B27 positive patients and to rule out the disease association with susceptibility to AS from Indian population.

2. MATERIALS AND METHODS

The study was done in the HLA department Core Diagnostics, Gurugram from March 2022 to March 2024. All the AS patients diagnosed as well as suspected (Majority of cases,93%) with symptoms were identified by Clinician from the rheumatology clinics during the study period. The patients with symptoms of inflammatory lower back pain for more than 3 months were included, with their consent. One thousands and four were included in the study who were tested in our department for HLA-B*27 testing.

The subjects of both genders with or without family history of AS were recruited for the prevalence of HLA-B*27 in the study group.

The whole blood sample (2ml) was collected in EDTA vaccutainer with written informed consent form of patient. The blood sample was subjected to DNA extraction using QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany) and stored at -20°C. The quality and quantity of DNA was checked using qubit and further subjected to RTPCR. The patients were selected and confirmed by the clinicians. HLA testing was done using inhouse kit, including positive and negative controls of HLA-B*27 and stringent quality control was maintained throughout the whole process. We used HLA-B27 Mutation Detection -Real Time TaqMan Assay HLA-B27

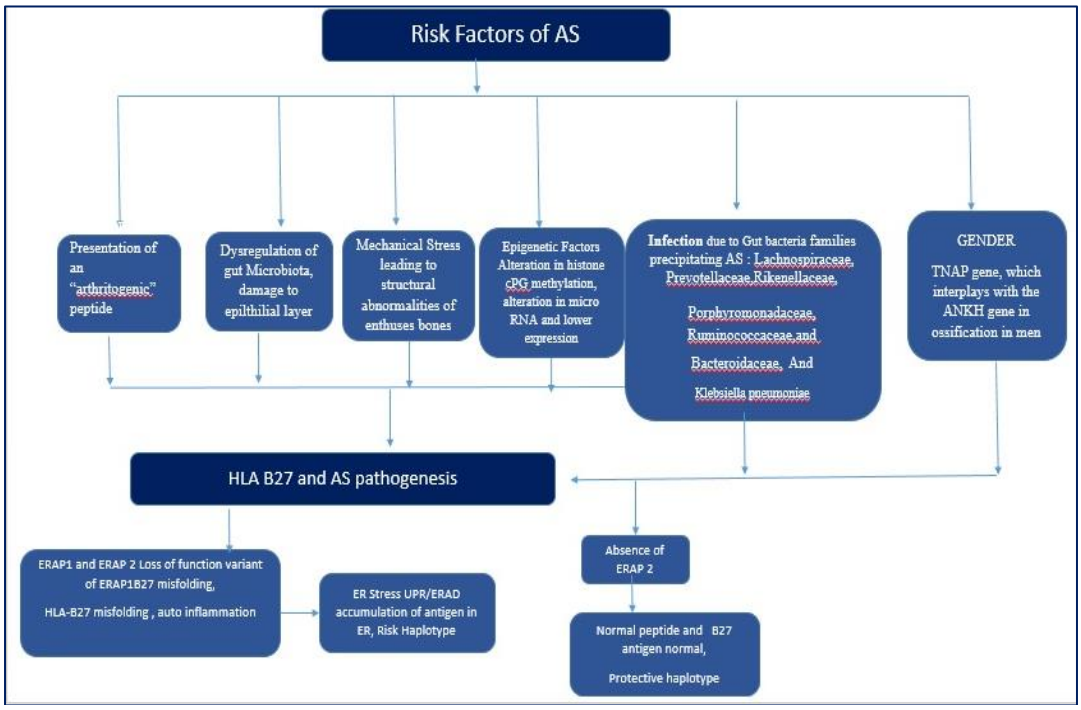

Fig. 1. Flow chart showing different hypotheses showing the association with AS, (TNAP-tissue-non-specific alkaline phosphatase) HLA-B27 contributes to susceptibility to ankylosing spondylitis (AS) include the arthritogenic peptide hypothesis, the HLA-B27 misfolding and unfolded protein response (UPR) hypothesis, and the HLA-B27 free heavy-chain and surface homodimer formation

RTPCR Kit for the detection of HLA-B27 genotype. The kit contains reagents and enzymes for the specific amplification of precise region of the HLA-B27 of human genome, and for the direct detection of the specific amplicon in FAM channel. The results were confirmed on the agarose gel. In addition, it contains an internal control amplification system to identify possible PCR inhibition. External positive control (HLA-B27 Positive Template) was included. TaqMan master Mix by, primer and probes by Eurofins and Molecular Biology Grade Water by MolBio HIMEDIA. The limit of detection -upto 10% and analytical sensitivity -2.5ng/µl. The primers and probe set for detection of both normal and mutant allele has been provided in the kit. The RTPCR was performed using Qiagen Rotor Gene Q Thermocycler. The reaction conditions of polymerase chain reaction amplification consists of the total number of cycles to be run and the temperature and duration of each step in the cycle. An initial denaturation step at 95 °C for 3min 1 cycle and 45 cycles of denaturation 95 °C for 15 sec, followed by annealing annealing at 60 °C for 45sec. The HLA-B27 alleles showed 141 bp PCR product representative of positive samples with CT value was observed. The target FAM channel was considered for amplified product and for internal control VIC was taken into consideration.

3. RESULTS

We observed the prevalence of HLA-B27 among patients with majority of suspected as well as confirmed cases of AS living in North India 54/124 positive cases (Fig. 2). The frequency of HLA-B27 among the positive patients in our study was found to be 12.35% among positive patients across India (Table 1 and Table 2). Data is described in terms of number of cases and percentages as appropriate. The statistical analysis was done using Student t test and p value less than 0.05 was considered significant.

Table 1. The HLA B* 27 positive cases distributed age wise

Age Group (years)	Positive
13-30	31(25%)
31-45	39(31.4%)
46-70	54(43.5)

Table 2. The HLA B* 27 positive cases distributed region wise

Region	Numbers (%)
Delhi/NCR	54 (43.5%)
Panipat (Haryana)	12(0.1)
Bangalore	10(0.1)
West Bengal	14 (0.11)
Dehradun	19 (0.15)
Lucknow	15(0.12)
Total	124(12.35%)

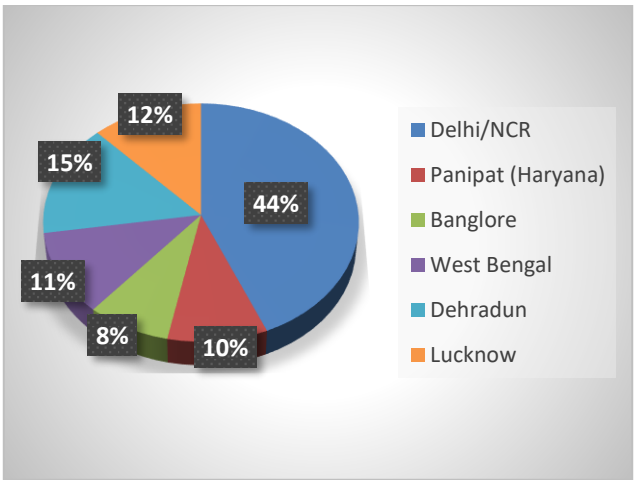

Fig. 2. Region wise Cases

The clinical manifestation were found to be more prevalent in men belonging to the age group of 31-45 years and 46-70yrs in suspected cases of AS. HLA B27 positivity in relation to suspected AS disease progression was found to be more in men as compared to women and children and in the second and third decade of life (32% and 26%). In our study, analysis by gender and age brings out that the AS among females (n=26) subjects was lesser than in male (n=98) ($p<0.01$). It shows the strongest association with suspected cases of AS. However the older age group showed more degenerative changes and more pain in joints as indicated by other supportive investigations (data not presented).

4. DISCUSSION

We observed 43.5% cases from North India with the male to female ratio of 2.7 to 1. Our results are in concordance with previous studies previously reported with an increased B27 antigen frequency among the North Indian groups (>5%) compared to the South Indian groups (<5%) [12]. It has been well-established previously that the prevalence of HLA-B*27 varies globally in the various ethnic and racial group. South Indian patients with AS showed a predominant association with B*27:05 and B*27:04[9]. The most common AS-associated B27 subtypes observed in Asian Indians are B*27:05, B*27:02, B*27:04, and B*27:07. The frequency of positive cases was found to be 12.35% in our population. Previously, among Indian patients with Spondyloarthritis, HLA-B27 has been reported to be 6% to 11.3% [23,24]. The AS and HLA B27 positivity was more among male as compared to females with a ratio of 2.7[6,10].

High prevalence in male subjects can be due to disease predominance in them and may be lower clinical presentation of Indian females at health care centers for the testing because of various socioeconomic reasons [25, 26].

As observed in previous studies the disease manifested in the second or third decade of life, and has a higher prevalence in men than women (ranges from 2.6:1 – 5:1 [27,28]. A higher number of men 64% were found to be HLA-B27 positive among the SpA patients when compared to women 36% in a study by Jayaprakash et al. from a tertiary care center in India [29]. Similar results were reported previously [29,30,31].

5. CONCLUSION

We report the higher frequency of HLA-B27 in males from North Indian population. Our study highlights the use of HLA-B*27 RTPCR as rapid genetic marker for early detection of suspected cases of AS prior to its clinical manifestation to stratify the at risk population and to plan the further healthy intervention to avoid manifestation/progression of disease. This should be replicated on large sample size from all the regions of India with detection of allele subtypes among positive patients. HLA-B27 as a routine periodical investigation in yearly screening or should be included in Health Checkup for early detection as it is cost effective and can be helpful for early detection, predictability of disease and to avoid the disease pathogenesis.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

CONSENT

As per international standards or university standards, patient(s) written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standards or university standards written ethical approval has been collected and preserved by the author(s).

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COMPETING INTERESTS

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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