

HLA B27 locus in association with Ankylosing Spondylitis in Indian Population

-an Updated Review and a single centre study

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 current study highlights the mechanism involved in the pathogenesis of AS with a focus to investigate the frequency of HLA B27 in suspected cases of AS. The patient's samples 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 thousand and four patients were tested for HLA B*27 using RT-PCR 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.

Key words: HLA-B27, RT-PCR, Ankylosing Spondylitis (AS)

INTRODUCTION

Ankylosing spondylitis (AS) belongs to seronegative spondyloarthropathies (SpA), and is characterized 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 5th or 6th 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[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 [12-16].

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 [17]. 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 Figure 1[10, 18-21].

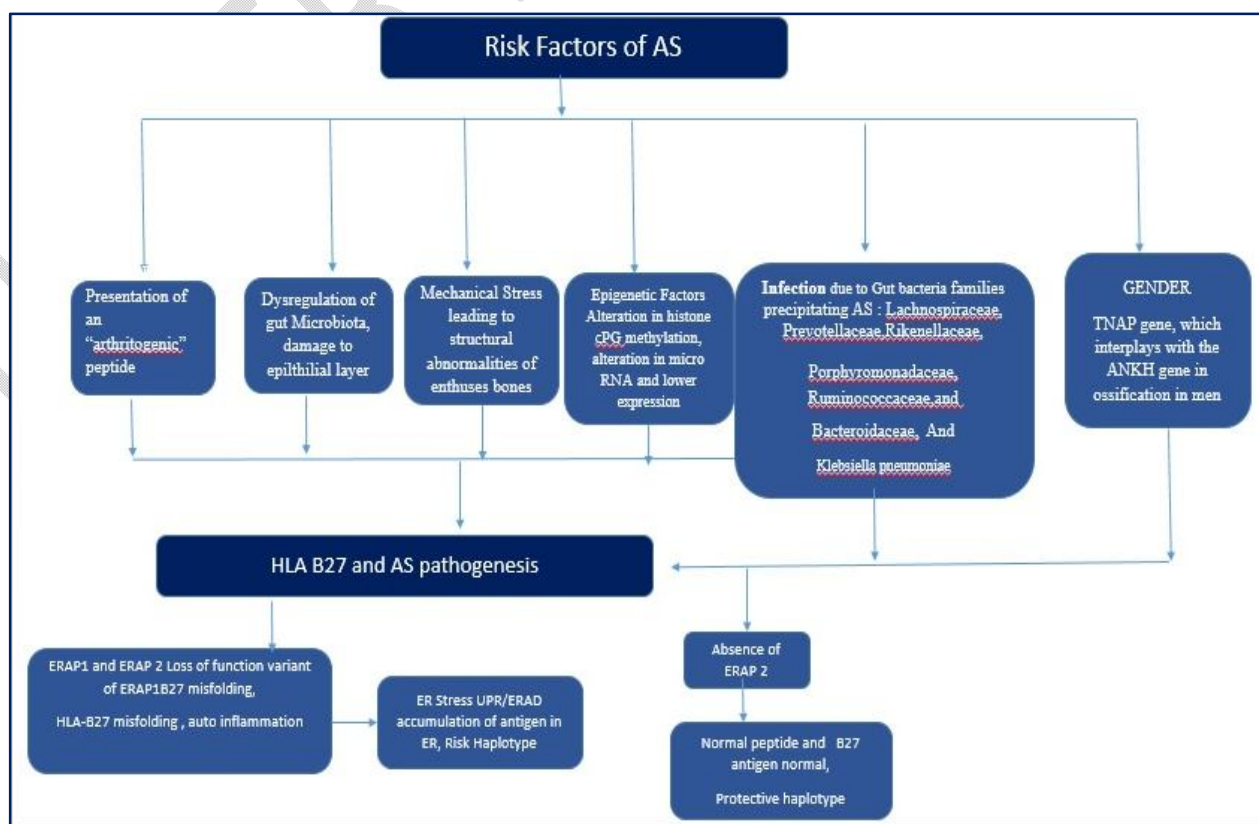
Figure 1: Flow chart showing different hypotheses showing the association with AS, (TNAP- tissue-non-specific alkaline phosphatase) HLA-B27 contributes to susceptibility to ankylosing spondylitis (AS) include the arthritogenic peptide hypothesis, the HLA-B27 misfolding and unfolded protein response (UPR)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

GWAS studies have confirmed strong genetic links with ERAP1 polymorphism in particular with HLA-B27:05 positive patients diagnosed with AS(15, 22). Therefore, HLAB27 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.

MATERIALS AND METHODS

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

testing.
testing
done
in
house



B*27
HLA
was
using

kit, including positive and negative controls of HLA-B*27. The subjects of both genders with or without family history of AS were studied for the prevalence of HLA-B*27 in the study group. We used HLA-B27 Mutation Detection -Real Time TaqMan Assay HLA-B27 Real-PCR Kit for the detection of HLA-B27 genotype. DNA extraction was performed using QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany).

It 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. 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.

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 (Figure 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.

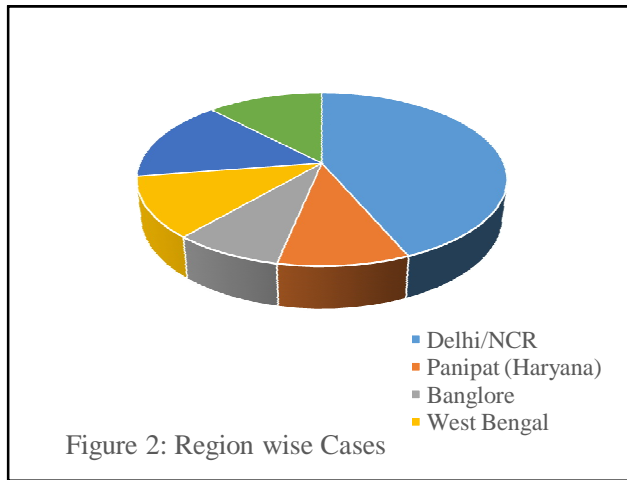
Table 1: The HLA B* 27 positive cases distributed age wise

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)
Banglore	10(0.1)
West Bengal	14 (0.11)
Dehradun	19 (0.15)
Lucknow	15(0.12)
Total	124(12.35%)



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). It shows the strongest association with suspected cases of AS. However the older age group showed more degenerative changes and more pain in joints.

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 is well established that the prevalence of HLA-B*27 varies globally in the various ethnic and racial groups. 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 males 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]). 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 Jayaprakashetal.from a tertiary care centre in India [28]. Similar results were reportedpreviously[29,30].

CONCLUSION

We report the higher frequency of HLA-B27 in males from North Indian population. Our study highlights the use of HLA-B27 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 can be helpful for early detection, predictability of disease and to avoid the disease pathogenesis.

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UNDER PEER REVIEW