HLA-B27 Frequency and its Association with Ankylosing Spondylitis in Indian Population:A Multi-city Analysis from a Single-center 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 ankylosing spondylitis is charachterized by inflmmation of spine, backpain, stiffness of lower back and hips, extrarticular organs such as eyes, and cardiovascular system.In severe casesn 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 the frequency of HLA B27 in suspected cases of AS. The patient's sample were recruited from rheumatology clinics of India from age group of 13-69 years. Patients with suspectedcases 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 diagnosticgenetic marker for detection of AS.

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

INTRODUCTION

Ankylosing spondylitis (AS) belongs to seronegative spondyloarthropathies (SpA), and ischarachterized 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 ocuur 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[2],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 ben found in Yemeni population (17%).

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 heritabilityand 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) havingAS[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 75subtypes (B*27:01 to B*27:62) studied so far[8]. HLA B2705 and HLA B2704 are the commonestsubtypes reportedin 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].

Althugh 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].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 figure1.

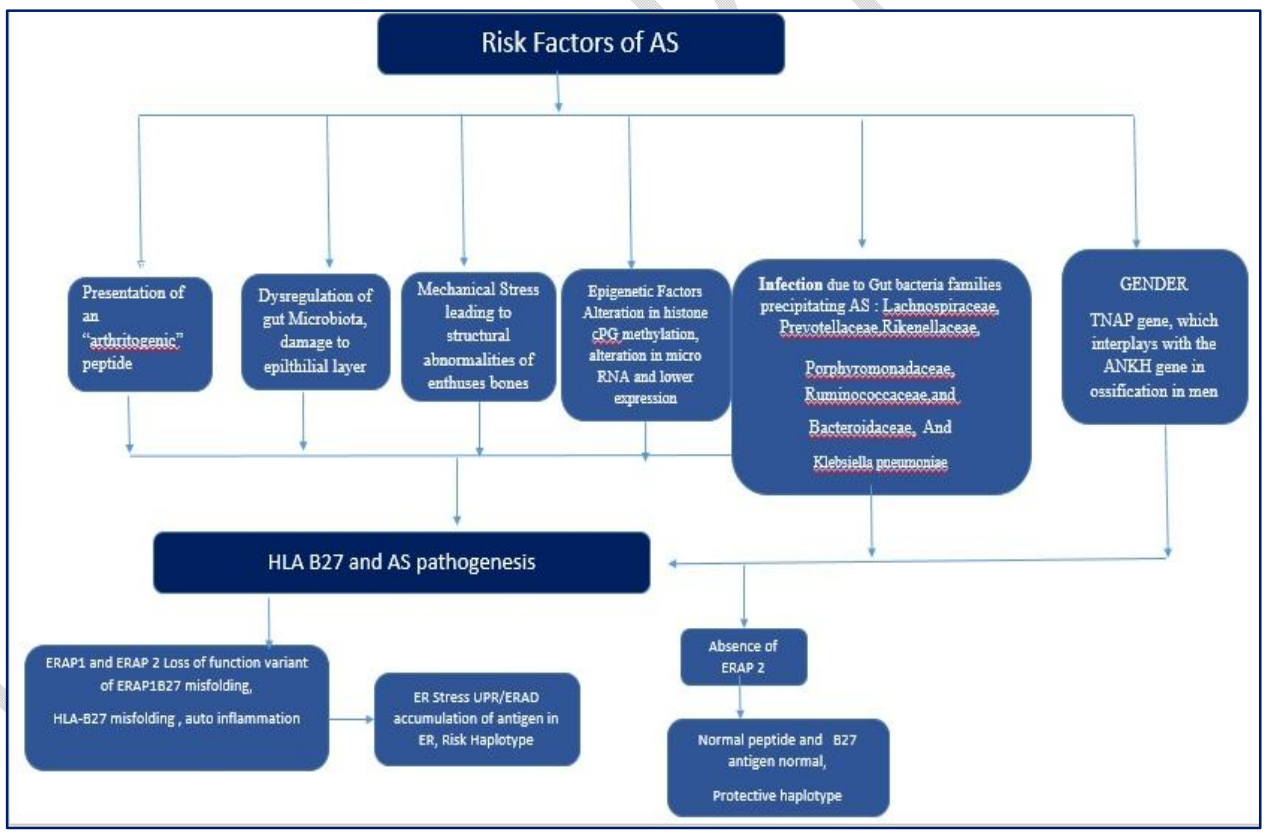
Three major hypotheses are (i) the abnormal peptide processing and presentation contribute towards the pathogenesis of AS via interaction between HLA B27 and endoplasmic reticulum amino peptidase (ERAP) 1 (ii) misfolding of HLA B27 gene and altered coonfiguarion of peptide in the endoplasmic reticulum (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].

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 withsusceptibility 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 Clinicianfrom the rheumatology clinics during the study period with symptoms of inflammatory lower back pain for more than 3 monthswere included, with their consent. One thousands and andtwenty four wereincluded in the study who were tested in our department for HLA-



B*27 testing.

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 arthritogenicpeptide hypothesis, the HLA-B27 misfolding and unfolded protein response (UPR) hypothesis, and the HLA-B27 free heavy-chain and surface homodimer formation.

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 vacutainer 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 in house kit,includingpositive and negative controls of HLA-B*27 and stringent quality control was maintained throughout the whole proces. We used HLA-B27 Mutation Detection -Real Time TaqMan AssayHLA-B27 Real-PCR Kit for the detection of HLA-B27 genotype.The kit contains reagents and enzymes for the specific amplification of prcise 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, annealing 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.

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. The statiscal analysis was done using Student t test and significalnt p value less than 0.05 was considered.

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Table 1: The HLA B* 27 poistive cases distributed agewise

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

Table2: The HLA B* 27 poistive cases distributed regionwise

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%)

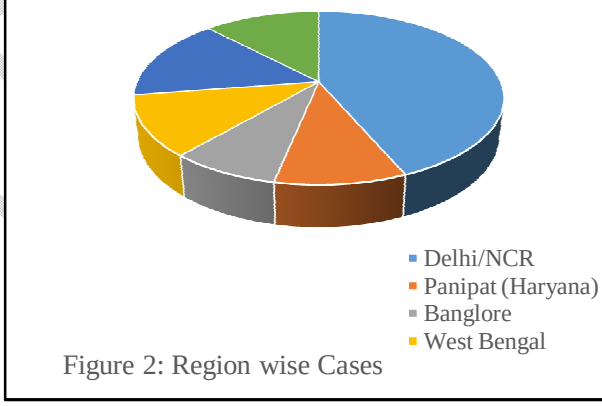

Figure 2: Region wise Cases

The clinical manifestation were found to be more prevalent in men belonging to the age group of 31-45 years and 46-70yrsin 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) subjectswas lesser than in male (n=98) ($p<0.01$). It shows the strongest association with suspected cases of AS. However the older agegroup showed moredegnerative changes and more pain in joints as indicated by other supportive investigations (data not presented).

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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 previouslyreported withan increased B27 antigen frequency among the North Indian groups (>5%) compared to the South Indian groups (<5%) [12]. It is a well established that the prevalence of HLA-B*27varies globaly 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 sponyloarthritis, 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 themand 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 Jayaprakashetal.from a tertiary care centre in India [29]. Similar results were repotedpreviously[29,30,31].

Conflict of Interest: None

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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, predictibility of disease and to avoid the disease pathogenesis.

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.

Ethical Approval:

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

Consent

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

Disclaimer (Artificial intelligence)

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc. have been used during the writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

Details of the AI usage are given below:

1. No we havenot used any such AI technologies or App and purely prepared manually by the authors.

2.

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