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Detection of Human Leukocyte Antigen (HLA) B27 among Patients of Seronegative Spondyloarthropathies Referred to a Tertiary Care Center

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A B S T R A C T

Human leukocyte antigen B27 (HLA-B27), a class I molecules of the major histocompatibility complex has a strong disease association with different types of spondarthropathies (SpA). The strength of this disease association varies markedly among racial and ethnic populations. The present study aimed to detect the frequency of HLA B27 Antigen among Seronegative Spondyloarthropathy (SpA) patients by PCR. The study was conducted over a period of one year. A total of 40 blood samples were collected from suspected Spondyloarthropathy subjects, these blood samples were subjected to HLA B27 detection by PCR. The results were analyzed. 20% of subjects in study group were HLA B27 positive. So prevalence of HLA B27 in Spondyloarthropathy in this region is 20%. This study highlights the prevalence of HLA B27 antigen in Seronegative Spondyloarthropathy patients, strength of association is more between HLA B27 antigen and Spondyloarthropathy. HLA typing is helpful in early diagnosis of SpA before the development of Radiological features. Detection of HLA B27 is important for institution of early treatment and to investigate for other manifestations of Spondyloarthropathy. It is also useful in screening of other family members for HLA B27. HLA typing is also important to exclude other causes of inflammatory arthritis.

Introduction

Spondyloarthropathy (SpA)s are multifactorial diseases that constitute a cluster of interrelated overlapping chronic inflammatory diseases, such as reactive arthritis (ReA), psoriatic arthritis (PsA), Enteropathic arthritis, a subgroup of juvenile chronic arthritis, ankylosing spondylitis (AS), and undifferentiated Spondyloarthropathy (USpA). They seem to have an immune-mediated pathogenesis that share a number of clinical, radiographic, and genetic characteristics with a well-defined group of rheumatic disorders (Rudwaleit *et al.,* 1996).

Association of HLA-B27 with the entire group of Spondyloarthropathy is well known and its association varies markedly among different forms of SpA and among different ethnic populations (Akkoc and Khan, 2006). Prevalence of SpA worldwide varies from 0.1% to1.4% in general population depending on the geographical region studied (Ehrenfeld, 2010). Despite the fact that SpA tends to affect males more frequently and severely than

it does females, an increasing proportion of female patients have been observed during the last decade among patients with less severe clinical manifestations (Haroon *et al.,* 2014).

However there are other factors that have an impact on the onset of the disease, such as environmental factors, and other genetic factors, including those within the major histocompatibility complex (MHC) class I HLA B60/HLA-B40, HLA-B39, HLA-B14, and HLA-B15and class II DRB1-alleles (John Londono and Ana Maria Santos, 2015). It has been estimated that HLA B27 allele is responsible for up to 28% of the aetiology of SpA (Reveille, 2012). Association between Ankylosing spondylitis and HLA B27 is more (92%) (Khan, 2002). However, the prevalence of HLA-B27 varies among populations and ethnic groups worldwide. For example, about 8% of Caucasians, 4% of North Africans, 2-9%of Chinese and 0.1-0.5% of Japanese descent possess this gene (Khan, 1995). The diagnosis of SpA is made principally based on clinical, radiological and laboratory findings. Clinically Amor criteria is used for diagnosis.

For a definitive diagnosis of Spondyloarthritis, ≥6 points are required; 5 points indicates probable Spondyloarthritis. In the laboratory it can be diagnosed by nonspecific tests like ESR, CRP and a specific test like HLAB27 detection. There are 3 methods available for detection of HLAB27. These are 1.Microlymphocytotoxicity (MLCT) 2. Flowcytometry 3. Polymerase chain reaction (PCR). Of which PCR is the "gold standard method" with sensitivity of 100% and specificity of 100%. Hence the present study was undertaken to detect the frequency of HLA B27 Antigen among Seronegative Spondyloarthropathy (SpA) patients by PCR. (Khan, 2002).

Materials and Methods

In the present study 40 cases with History suggestive of Spondyloarthropathy attending Rheumatology clinic in Tirunelveli Medical College, Tirunelveli were selected as study group during the period of 2016- 2017.

Inclusion criteria

Patients in the age group of 14years to 65years of both sexes with history suggestive of Spondyloarthropathy and fulfilling the Amor criteria for Spondyloarthropathy.

Exclusion criteria

Patients with back pain diagnosed as having rheumatoid arthritis, SLE and other Connective tissue diseases. Patients having other infectious cause for back pain like tuberculosis, syphilis, pelvic inflammatory diseases and osteoporosis.

The Ethical committee clearance was obtained from our institution and informed consent was obtained from all patients included in the study.

Sample collection

After getting informed consent median cubital vein was selected for sample collection, area was cleaned with surgical spirit and allowed to air dry, 2ml of blood was collected for HLA B27 PCR in test tube with EDTA.

Sample storage

2ml of whole blood with EDTA was collected and stored at -20°C for HLAB27 detection.

Polymerase chain reaction

Real-time PCR kit is an *in vitro* nucleic acid amplification kit for the detection of HLA-B27 Genotype [HLA-B 27:01 to 27:73] in Human blood samples obtained from Helini Biomolecules, Chennai. HLA-B27 primer and Probe have been designed for the specific and

exclusive in vitro detection of HLA-B27 genotypes. The Primer Probe is able to detect all the subtypes ranging from B27:01 to B27:73.

The target sequence is highly conserved and has previously been shown to be a good genetic marker for the detection of HLA-B27 genotyping. The Primers and probe sequences in this kit have 100% homology with a broad range of clinically relevant reference sequences based on a comprehensive bioinformatics analysis. PCR for detection of HLA B27 antigen was done as per manufactures instruction.

Results and Discussion

Study group cases were analyzed for Amor clinical criteria and results of HLA B27 PCR results. Among the 40 cases of Spondyloarthropathy, 20 (50%) were males and 20 were females (50%).

Age groups less than 20 years, 20-40 years, 40-60 years and more than 60 years included 10 (20%) cases in each age group (Table 1).

Among the 40 suspected cases of Spondyloarthropathy 8 were positive for HLA B27 antigen by PCR.

Table 2 showed the age and sex wise distribution of HLA B27 positive cases.

Among the 8 positive cases two were in the age group of less than 20 years, three were in the age group of 20-40 years, two were between the age group of 40-60 years, one case was in the age group of more than 60 years. Sex wise HLA B27 positivity was more common in males.

HLAB27 is an antigen expressed on the surface of all nucleated cells which is encoded by B27 allele in the B locus of MHC I region present in the chromosome 6. The presence of this antigen in some individuals predisposes to development of Spondyloarthropathy (SpA) in any part of their life. SpA constitutes a spectrum of diseases which includes Ankylosing spondylitis (AS), Reactive arthritis (ReA), Psoriatic arthritis (PsA), Enteropathic arthritis, a subgroup of juvenile chronic arthritis, and Undifferentiated Spondyloarthropathy (USpA). Prototype among all these is Ankylosing spondylitis (AS) which is most commonly associated with HLAB27.

The common pathology of all these diseases is inflammation of sacroiliac joint thus the common symptom is back pain which has some specific characters.

Diagnosis of these diseases is delayed due to late appearance of features of SPA in conventional radiography however MRI can detect the early changes of inflammatory arthritis nowadays. Detection of HLA B27 antigen in peripheral blood in early stages of these diseases will help to exclude other causes of inflammatory arthritis and those individuals are classified as SpA and to start treatment early which includes physical exercise, NSAIDS, DMARDS, Anti-tumor necrosis factor therapy.

Early diagnosis of SPA is also important in screening of other diseases like anterior uveitis, cardiac problems, pulmonary consolidation, renal diseases, etc, in which HLA B27 will be a predisposing factor and it is particularly more helpful in diagnosis of SpA in young individuals before the appearance of radiological changes. HLA B27 detection is also useful for screening of relatives who may have HLA B27 antigen and at risk of developing SpA.

Table.1 Age and Sex wise distribution among cases of Spondyloarthropathy

Age group	Male	Female	Total
< 20 years	5	5	10
20-40 years	5	5	10
40-60 years	5	5	10
>60 years	5	5	10
Total	20	20	40

Table.2 Age and sex wise distribution of HLA B27 positive individuals

Age group	Male	Female	Total
< 20 years	1	1	2
20-40 years	2	1	3
40-60 years	2	0	2
>60 years	1	0	1

Amor criteria is used for diagnosis

Amor Criteria for Spondyloarthritis	Score
Lumbar or dorsal pain during the night, or morning stiffness of lumbar or dorsal spine	1
Asymmetric oligoarthritis	2
Buttock pain	1
Alternating buttock pain	2
Dactylitis of finger or toe	2
Heel pain or other well-defined Enthesopathy	2
Iritis	2
Nongonococcal urethritis or cervicitis within 1 month of arthritis onset	1
Acute diarrhea within 1 mo of arthritis onset	1
Psoriasis, balanitis, or inflammatory bowel disease	2
Radiology Sacroiliitis (grade ≥2 if bilateral; grade ≥3 if unilateral)	3
Genetic Background HLA-B27+ or family history of ankylosing spondylitis, reactive arthritis, uveitis, psoriasis, or inflammatory bowel disease	2
Response to Treatment Good response to NSAIDs within 48 hr, or relapse within 48 hr if NSAIDs withdrawn	2

In the present study among the 40 suspected cases of Spondyloarthropathy, 8 cases (20%) were positive for HLA B27 antigen by PCR. In a study conducted by Reveille JD, it has been estimated that this allele is responsible for up to 28% of the aetiology of SpA (Chopra *et al.,* 1990). In a study conducted by Seager K *et al.,* and other study done by Weber *et al.,* Clinically SpA is more common in males, with a reported male-to-female ratio of about 2: 1 to 3: 1 and disease manifested commonly in the age group of 20-40 years (Weber *et al.,* 2010; Seager *et al.,* 1979). In the present study, among the 8 positive cases two were in the age group of less than 20 years, three were in the age group of 20-40 years, two were between the age group of 40-60 years, one case was in the age group of more than 60 years. Sex wise HLA B27 positivity was more common in males.

In young patients with inflammatory chronic back pain, a positive HLA-B27test increases the likelihood of having AS later, particularly if imaging of the SI joints does not provide conclusive results (Seager *et al.,* 1979). HLA-B27 may be a useful prognostic indicator for development of complications like acute anterior uveitis, ascending aortitis, aortic valve incompetence, cardiac conduction abnormalities, etc. and it is a highly useful predictor of early sacroiliitis (Tyrrell *et al.,* 1994). Genes that are passed from parents to their children control the production of those antigens. If two members of the same family are HLA-B27 positive and one of them develops a disease associated with HLA-B27, then the other person is at an increased risk of developing a similar disease.

HLA B27 positive individuals should be screened for other manifestations like acute anterior uveitis, ascending aortitis, aortic valve incompetence, cardiac conduction abnormalities, etc. and their family members also should be tested for HLA B27 antigen. Testing for HLA B27 is of clinical importance for early diagnosis of Spondyloarthropathies. Understanding the effects of these known genes will open up promising new avenues of research into clarifying their role in pathogenesis of Spondyloarthropathy and in discovering a cure for these patients. Our observation confirmed the significance of HLA B27 allele as a novel and rapid molecular marker for diagnosis of Spondyloarthropathies.

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