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# Genotyping of HLA-B27 Sequence Specific Alleles in Ankylosing Spondylitis and Uveitis Patients

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## Abstract

**Background:** Proper prognosis in Ankylosing Spondylitis and Uveitis Patients is clinically significant and thus the role of *HLA-B27* genotyping is an important parameter to evaluate and follow up the patients.
**Aims**: We characterize the *HLA-B27* Sequence Specific Alleles (SSA) in Ankylosing Spondylitis and Uveitis Patients.
**Settings and Design:** Total of 153 cases were considered having symptoms which include stiffness in the lower back and buttocks, tenderness at the heel and 13 cases were with Inflammation of eye (Uveitis or Iritis).
**Methods and Material:** Molecular tools were employed, which included DNA extraction and sequence specific alleles amplification for all the cases to reveal the *HLA-B27 SSA* genotypic distribution.
**Results:** Out of 153 patients studied, 140 symptomatic for Ankylosing Spondylitis (AS) and 13 for Uveitis, 61(40%) were positive for *HLA-B27* PCR out of which 58(41.4%) were positive from AS category and 03(23%) cases for Uveitis. Molecular characterization of the alleles further revealed that in AS positive cases, Group A and Group B alleles got amplified in 55 (39.2%) patients, whereas only 02 (1.4%) and 01(0.7%) cases were with Group A and Group B respectively. In Uveitis, all the 03 positive cases were with both Group A and Group B alleles.
**Conclusions:** The presence of Group A (1.4%) and Group B (0.7%) alleles in the AS patients confirming it to be a necessary tool for AS screening. Present study suggest to use *HLA-B27* PCR for the diagnosis of AS, Reiter's Syndrome, Uveitis, Iritis, and inflammatory bowel disease at molecular level but must be done and considered with both the sequence specific alleles to avoid false results.
**Keywords:** Ankylosing Spondylitis, Polymerase Chain Reaction, Spondyloarthropathies, Rheumatic diseases, Amplicons

## 1. Introduction

The tendency to develop AS is believed to be genetically inherited; nearly 90% of people with AS are born with a gene known as the *HLA-B27* gene[1]. The *HLA-B27* gene appears only to increase the tendency of developing AS, while some additional factor(s), perhaps environmental, are necessary for the disease to appear or become expressed. Even among *HLA-B27* positive individuals, the risk of developing AS appears to be further related to heredity. In *HLA-B27* positive individuals who have relatives with the disease, the risk of developing AS is 12%, six times greater than for those whose relatives do not have ankylosing spondylitis[2,3]. The analysis of ankylosing

spondylitis is based on evaluating the patient's symptoms, X-ray findings (radiographs), physical assessment, and supported tests. Stiffness, pain, and decreased range of activity of the spine are characteristic of the inflammatory back pain of ankylosing spondylitis. Symptoms include pain and morning stiffness of the spine and sacral areas with or without accompanying inflammation in other joints, tendons, and organs [4]. Testing for *HLA-B27* is of clinical importance for the examination of AS. Excluding *HLA-B27* nearly excludes ankylosing spondylitis[5,6]. Serological techniques, like microcytotoxicity and flow cytometry for testing for *HLA-B27* require viable cells that adequately express *HLA-B27* and may give false negative results if *HLA-B27* is down regulated or "masked". Flow cytometry although is rapid and relatively inexpensive, but has been reported to lack specificity, especially in the presence of antigens that cross react with HLAB27, such as HLA-B7.4 [7,9].

The last two decades have seen a massive growth in the application of DNA technology in Histocompatibility and Immunogenetics. DNA based HLA typing is fast replacing conventional Microlymphocytotoxicity based method, which has been regarded as the gold standard. Many laboratories in India have already switched over to molecular methods, as the results are far superior PCR technique which is based on the detection of allelic differences at the nucleotide level and circumvents the common problems associated with MLCT (microlympho-cytotoxicity test) and Flow cytometry. Amplification of HLA loci with PCR-SSP has proved to be rapid and accurate method for genotyping HLA-B alleles and indicates that HLA typing by serology may not be sufficiently reliable [10]. Current study was done with Allele-specific PCR for HLA-B27, a direct genotyping method based on specific primer recognition of a unique HLA B-27 gene sequence.

## 2. Materials & Methods

Total of 153 whole blood specimens were considered for the proposed study, collected from different Departments of Shri Mahant Indiresh Hospital, Dehradun. The cases include the patients having symptoms which include stiffness in the lower back and buttocks, tenderness at the heel and 13 cases were with Inflammation of eye (Uveitis or Iritis). The study was approved by the institutional Ethical clearance committee and written consent from all the patients was taken. Olerup SSP kit was used for HLA genotyping [11,13]. PCR was performed and amplimers analyzed by agarose gel electrophoresis. For primer 1, appearance of 149-150 bp specific band indicates the presence of *HLA-B27* gene amplifying HLA-B*27:01-27:05:08, to HLA-B27:78-27:84 along with 439 bp band of internal control targeting Human Growth Hormone gene, which was used for the validation of the result as it is present in normal population as well as in diseased individuals. With the primer 2,95 bp specific band indicates the presence of *HLA-B27* gene amplifying *27:01-27:05:15 to 27:82-27:86 with 511 bp as an internal control (as shown in fig.1) [14,15].

## 3. Results

Total of 153 cases, 140 were suspected and symptomatic for AS and 13 for Uveitis. 61(40%) cases came positive for *HLA-B27* PCR; out of which 58(41.4%) were positive from AS category and 03(23%) cases come positive for Uvetis. When characterized further on the basis of alleles amplified it was seen that in AS positive cases Group A and Group B alleles amplifies in 55(39.2%) cases, whereas only 02 (1.4%) and 01(0.7%) cases were with only Group A and Group B respectively. In Uvetis, all the 03 positive cases were with both Group A and Group B alleles (Table 4). In the present study, the positivity rate of *HLA-B27* specific allele in different age groups and gender wise distribution of the *HLA-B27* specific alleles in these patients were also determined. When samples were analyzed in terms of age groups, it was found that out of 61 positive samples, the positivity rate was minimum i.e. 0% in the patients above 60 years of age. However, in the age group of 0-20 years, 21-40 years and 41-60 years, the positivity rate was 13%, 65% and 21.3%, respectively. Furthermore, it was observed that out of 111, 54 (48.6%) male, were positive for HLA-B27, whereas out of 42 female, 07 came positive for the same (Table 3). The study also includes the 13 cases of Uveitis or Iritis and seen that 03 cases with Uveitis came positive for *HLA-B27* specific alleles.

## 4. Discussion

It has been well established that association of the *HLA-B27* antigen in 90-95% of patients with AS are *HLA-B27* specific allele positive. Identification of *HLA-B27* by PCR supports the diagnosis of AS in symptomatic individuals and negative results exclude the diagnosis. Various studies showed that 90-94% of AS sufferers have *HLA-B27* allele positive, while 5-9% of the general population with AS may have other contributory factors for positivity of HLA-B27 [16]. The disease is most likely triggered in genetically predisposed

individuals by an environmental factor, since only 1% of the people with the *HLA-B27* allele develop AS. The exact mechanism of triggering the disease is unidentified, but many theories have been proposed to explain the contribution of *HLA-B27* in the disease[17]. SSP-PCR is a novel, rapid, cost effective, and standard method for the detection of *HLA-B27* alleles. Sequence based typing (SBT) or SSP technique is capable of detecting a single base difference in DNA sequence between two alleles but they are not likely to detect a new undefined allele, unless the variation happens to be at the specific site detected by the probe or the primer. To the best of our knowledge, alleles amplified on the basis of the groups have never been characterized in the Northern region of India. Thus, the utilization of the both the primers which will screen out alleles groups are of utmost significance. Although in the lower percentage, a single group can be expressed by an individual, which makes it very important for screening out the cases of AS and signifies about the role of both the alleles in diagnosis of AS and Uveitis and cannot be excluded during molecular diagnosis for the same. Serological techniques such as microcytotoxicity and flow cytometry for testing *HLA-B27* require viable cells that adequately express *HLA-B27* and may give false negative results if *HLA-B27* is down regulated or ‘‘masked’’. Flow cytometry is rapid and relatively inexpensive, but has been reported to lack specificity, especially in the presence of antigens that cross-react with HLA-B27, such as HLA-B27. Moreover, results of ongoing research will lead to a better understanding and treatment of the entire group of diseases collectively known as spondyloarthropathies. Present observations confirmed the significance of *HLA-B27* allele as a novel and rapid molecular marker for diagnosis of AS.

## 5. Conclusion

Amplification and detection of *HLA-B27* PCR is a simple, rapid and accurate method for the diagnosis of AS, Reiter’s Syndrome, certain eye disorders such as acute anterior Uveitis, Iritis, Behcets Syndrome, Psoriatic arthritis and inflammatory bowel disease at molecular level. Genetic counseling may be useful in assisting patients with questions regarding the risk of family members developing AS or other seronegative spondyloarthropathies which needs further confirmation on larger group of patients with AS and Uveitis.

**Figure1: Agarose gel Picture for HLA-B27 Alleles**

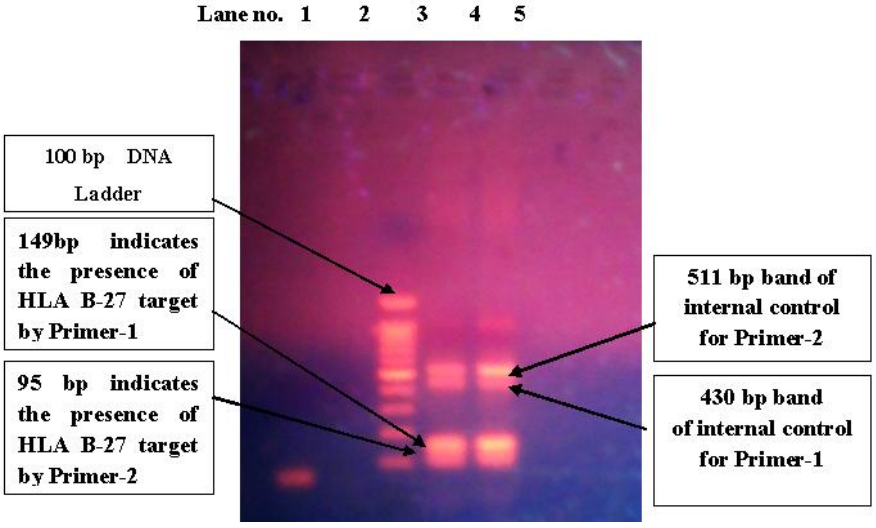


**Table 1: Results interpretation and amplicon size of the two primer mixes used for HLA-B*27 SSP typing by Sequence specific alleles.**

| Sr. No. | Primer Mix | Amplicon Size | Size of control band | Amplified HLA-B*27 alleles | Groups |
|---|---|---|---|---|---|
| 1 | 1 (For Group A) | 145 base pairs | 430 base pairs | *27:01-27:05:08, 27:05:10-27:11, 27:13-27:15, 27:17, 27:19-27:21, 27:24-27:25, 27:27-27:28, 27:30, 27:32-27:74, 27:76, 27:78-27:84 | **A** |
| 2 | 2 (For Group B) | 95 base pairs | 515 base pairs | *27:01-27:05:15, 27:05:17, 27:08, 27:10, 27:12-27:13, 27:15-27:18, 27:23, 27:25-27:26, 27:28-27:29, 27:31, 27:36-27:40, 27:42, 27:44-27:45, 27:47-27:69, 27:71-27:75, 27:77, 27:79-27:80, 27:82-27:86 | **B** |

**Table 2: Age wise distribution of HLA-B 27 alleles (2705*-2713*)**

| Sr. No. | Age Groups (in years) | No. of patients in the particular age group | No. of HLA-B27 positive | Positivity rate (%) |
|---|---|---|---|---|
| 1 | 0-20 | 22 | 8 | 13.0% |
| 2 | 21-40 | 108 | 40 | 65.5% |
| 3 | 41-60 | 21 | 13 | 21.3% |
| 4 | Above 60 | 02 | 0 | 0% |
| 5 | **Total (n) = 153** | | **61/153** | **40.0%** |

**Table 3: Gender wise accumulation of HLA- B27 SSP Alleles**

| Sr. No. | Gender | No. of positive patients | No. of Negative patients | Positivity rate (%) |
|---|---|---|---|---|
| 1 | Female (42) | 07 | 35 | 16.6 % |
| 2 | Male (111) | 54 | 57 | 48.6% |
| | **Total (n)= 153** | **61/153** | **92/153** | **40.0%** |

**Table 4: Results profile for the amplified alleles (group wise) in HLA-B27 Positive cases.**

| Total cases for HLA-B27 SSA PCR | Cases with AS profile | Cases with Uveitis/ Iritis | Total no of Cases Positive for HLA-B27 SSA PCR | Cases Positive for AS | Cases Positive for Uveitis | Amplified alleles for AS positive cases | Amplified alleles for Uveitis positive cases |
|---|---|---|---|---|---|---|---|
| 153 | 140 | 13 | 61 (40%) | 58 (41.4%) | 03 (23.0%) | Both Group A and Group B = 55 (39.2%)<br>Only Group A =02 (1.4%)<br>Only Group B= 01 (0.7%) | Both Group A and Group B = 03 (100%) |

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