DNA diagnostics: Using the polymerase chain reaction for detecting HLA B27 in patients of acute anterior uveitis

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ABSTRACT
Objective: The human leukocyte antigens (HLA) are a group of cell surface molecules encoded for by the major histocompatibility locus on the short arm of human chromosome six. Individuals carrying the HLA-B27 gene, have been shown to have a higher incidence of isolated acute anterior uveitis (AAU). This study aims to examine the incidence of the HLAB27 gene in individuals presenting with AAU at GMCHRC

Material and Methods: This pilot study was carried out for a period from May 2013 to July 2013 at GMCHRC Ajman UAE. Four patients of clinically diagnosed AAU were examined for the presence of the HLA B27 gene. Genomic DNA was amplified using primers and cycling parameters were as described by Bunce et al. using exon-2, B-locus- specific primers. The PCR products were run in a 1% agarose gel stained with ethidium bromide (0.5 µg/ml) and visualized under U.V. light.

Results: Four cases of AAU were subjects in this study. Of the four cases examined two (50%) were positive for the HLAB27 gene. The M:F ratio of the cases 3:1. All the HLA B27 positive cases were males. The average age of the positive cases was 37.5 years as compared to 53.5 years in the negative cases. There was no racial predisposition seen. Using the above set of primers, DNA from HLA B27 positive individuals gave a 150 bp band.

Conclusion: AAU is the most common form of uveitis. Of the cases reported in literature half of all cases of AAU are HLA-B27 positive and this is similar to the results displayed in our series of four cases. Cases positive for HLAB27 in AAU require active treatment using immunomodulators for early resolution and topical cycloplegic agents and steroids are the cornerstones of treatment. Methotrexate, Salazopyrine, anti-TNF and anti-CD20 therapy may be used to prevent recurrent attacks. This study exemplifies the use of PCR and DNA diagnostics in the identification of HLAB27 gene in cases of AAU. Further studies using the Dideoxy Sequencing are proposed to be carried out.

Keywords: polymerase chain reaction, human leukocyte antigens(HLA), acute anterior uveitis

INTRODUCTION
The human leukocyte antigens (HLA) are a group of cell surface molecules encoded for by the major histocompatibility locus on the short arm of human chromosome six. These genes show various polymorphic forms, one of which is the B27. People who carry the HLA-B27 gene have been estimated that up to 20% have one of several associated conditions like ankylosing arthritis (AS), undifferentiated spondyloarthopathies (SP-U), reactive arthritis (ReA) and psoriatic arthritis (PsA). Individuals carrying the HLA-B27 gene, have been also been shown to have a higher incidence of isolated acute anterior uveitis (AAU)1. The role of HLA-B27 in triggering disease has not yet been precisely defined. The postulations for disease include arthritogenic peptide, molecular mimicry, affinity to peptides from infectious agents, and an innate etiology wherein HLA-B27 represents a locus, closely linked to the gene causing the inflammatory response. Many cases of uveitis or reactive arthritis follow gram-
negative bacillary dysentery or chlamydial infection. These gram-negative organisms include *Shigella*, *Salmonella*, *Klebsiella*, and *Yersinia* species.

Opinions vary as to the importance of the diagnostic testing for the HLA-B27 gene in arthropathies and anterior uveitis. It is recommended that all patients with above conditions should be tested because of its prognostic implications and use in therapeutic planning. Patients who are HLA B27 positive should be actively managed with immunomodulatory drugs for efficient control of their disease symptoms and to ensure better long term prognosis. This study aims to examine the incidence of the HLAB27 gene in individuals presenting with AAU at GMCHRC.

**MATERIALS AND METHODS**

This pilot study was carried out for a period from May 2013 to July 2013 at the Ophthalmology Department and the center for Advanced Biomedical research and Innovation (CABRI) at GMCHRC and GMU Ajman respectively. Four consecutive patients of clinically diagnosed AAU tested for the presence of HLA B27 gene are reported in the study. Genomic DNA was extracted by using a DNA extraction kit from whole blood. Primers used and cycling parameters were as described by Bunce et al (4) and are exon-2, B-locus- specific primers. The sense primer used was 5’-GCT ACG TGG ACG ACA CGC T -3’ and antisense primer used was 5’-CTC GGT CAG TCT GTG CCT T-3’. DNA samples were amplified in an ABI 9700 PCR machine with a heated lid. For a 14 µL mix used the cycling parameters were 1 min at 96°C followed by 5 cycles of 25 sec at 96°C, 45 sec at 70°C and 45 sec at 72°C followed by 21 cycles of 25 sec at 96°C, 50 sec at 65°C and 45 sec at 72°C. The 14µL polymerase chain reaction (PCR) reaction mixtures consisted of 3.4µM of each primer, 1µL of genomic DNA; 1µL of a mixture of dATP, dCTP, dGTP, and dTTP; 0.5 µL of (3U/µL) of Taq DNA polymerase; 1.4 µL of 10X PCR buffer; and double distilled water to a final volume of 14µL. The PCR products were run in a 1% agarose gel stained with ethidium bromide (0.5 µg/ml) and visualized under U.V. light. Positive and negative controls were used for each PCR run. In negative controls the template DNA was replaced by an equal volume of DDW. 5µL of a 100 bp DNA ladder was loaded on each gel as a molecular weight marker.

**RESULTS**

Four cases of AAU were subjects in this study. Of the four cases examined two (50%) were positive for the HLAB27 gene. The M:F ratio of the cases 3:1. All the HLA B27 positive cases were males. The average age of the positive cases was 37.5 years as compared to 53.5 years in the negative cases. There was no racial predisposition seen. Using the above set of primers, DNA from HLA B27 positive individuals gave a 150 bp band. (Table 1 & Figure 1)

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<td>4</td>
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DISCUSSION
Traditionally, the microcytolymphotoxicity assay was used to determine HLA status (5). With the development of monoclonal antibodies to HLA B27, flow cytometry became the alternative procedure (6). The technique using microlymphocytotoxicity is costly and can give false negative results. False negatives are also observed with flow cytometry. We have used Polymerase Chain Reaction using sequence specific primers (PCR-SSP) which is the standard of care, to detect the presence of HLA B27 gene. PCR is superior to serological techniques to determine HLA-B27 positivity unequivocally, since it is based on the detection of HLA-B27 gene sequences (8). PCR is also fast, reliable, cost effective and well adapted for routine laboratory testing and can resolve cases unsatisfactorily defined by microlymphocytotoxicity assay or, flowcytometry.

The HLA-B27 antigen is an important genetic marker in AAU. In this study we have used PCR SSP typing as described by Bunce et al (4). HLA B27 has varied racial/ethnic prevalence worldwide. Our study shows HLA B27 gene in 50% of cases of AAU. This is similar to results reported elsewhere (7). Further studies using sequencing methodology and molecular modeling are required to understand the HLA B27 subtypes and underlying mechanisms responsible for the pathogenesis of AAU and its linkage with the HLA B27 gene. More than 70 subtypes of HLA B27 have been described. It is proposed to carry out further studies on patients of AAU from different ethnic populations so as to determine the incidence of genetic subtypes and understand molecular mechanisms underlying the disease.

CONCLUSION
AAU is the most common form of uveitis. Of the cases reported in literature half of all cases of AAU are HLA-B27 positive and this is similar to the results displayed in our series of four cases. Cases positive for HLAB27 in AAU require active treatment using immunomodulators for early resolution and topical cycloplegic agents and steroids are the cornerstones of treatment. Methotrexate, Salazopyrine, anti-TNF and anti-CD20 therapy may be used to prevent recurrent attacks. This study exemplifies the use of PCR and DNA diagnostics in the identification of HLAB27 gene in cases of AAU. Further studies using sequencing and molecular modeling are proposed to be carried out to exactly understand the genetic subtypes of HLA B27 and molecular pathogenesis responsible for AAU.

REFERENCES

Figure 1: PCR for HLA B27 in AAU