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Association of HLA-B27 with Ankylosing Spondylitis in Jharkhand

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Abstract:
1. ***Introduction:*** *Ankylosing spondylitis (AS) affects one in 200 individuals and is usually diagnosed many years after onset of symptoms. Chronic back pain is common and recognition of early disease requires clinical experience and a high index of suspicion. Further, inflammatory markers are not invariably elevated and radiographic changes are often late findings.*
2. ***Material and Method****: Fifty patients were selected for study in which 25 with AS 25 controls. Using flow cell cytometry method by BD Fascantto Machine detection of antigen HLA B 27 done at Department of Laboratory Medicine, RIMS, Ranchi.*
3. ***Results:*** *The HLA-B27 antigen was identified in 21 out of 25 patients (84%) with classical ankylosing spondylitis (AS), compared to 3 out of 25 controls (12%) (P < 0.005).*
4. ***Conclusion:*** *There is a strong correlation between HLA B 27 positivity with ankylosing spondylitis (AS).*

Keywords:*Ankylosing Spondylitis, HLA-B27, Seronegative Spondyloarthropathies.*

I. Introduction

A link between the HLA-B27 histocompatibility antigen and several forms of seronegative spondyloarthropathies including ankylosing spondylitis. Reiter's disease and reactive arthritis is now finally estahlished.[1.2]Ankylosing spondylitis (AS) shows a strong correlation with human leukocyte antigen (HLA-B27), and number of evidence suggests that the B27 gene may have a pathogenic role in the development of AS and HLA-B27 has a tendency for familial association[3,4,5]. The association of HLA-B27 with Ankylosing spondylitis was first described in 1973[6], and is among the strongest described for a HLA locus. The frequency of HLA-B27 with AS among Indian population varies from 40 to 94% as compared to 1.4-8% of the general population[7]. HLA-B27 is a unique HLA class I molecule, not only because of its high association with AS but also has characteristically different amino acid composition from other class I molecules. There are two important characteristic structures which are different from others: the presence of B pocket and the free thiol group of Cys67[8, 9].

Ankylosing spondylitis (**AS**) is a type of arthritis in which there is long term inflammation of the joints of the spine. Typically the joints where the spine joins the pelvis are also affected. Occasionally other joints such as the shoulders or hips are involved. Eye and bowel problems may also occur. Back pain is a characteristic symptom of AS, and it often comes and goes. Stiffness of the affected joints generally worsens over time.
Although the cause of ankylosing spondylitis is unknown, it is believed to involve a combination of genetic and environmental factors. More than 90% of those affected have a specific human leukocyte antigen known as the HLA-B27 antigen. The underlying mechanism is believed to be autoimmune or autoinflammatory. Diagnosis is typically based on the symptoms with support from medical imaging and blood tests. AS is a type of seronegative spondyloarthropathy, meaning that tests show no presence of rheumatoid factor (RF) antibodies. It is also within a broader category known as axial spondyloarthritis.

II. Materials And Methodology

All of the patients visited to the Laboratory Medicine Department of RIMS, Ranchi for HLA B 27 typing and had been under observation in orthopedic department for at least one year. Some had advanced overt disease with restriction or chest and spinal movement while others had only lumbar and pelvic pain. The patient's radiographs were studied by orthopedican and those having at least graded 3 bilateral sacroiliitis were accepted as definite patients.
All patients had classical clinical and radiological findings of ankylosing spondylitis according to the New York criteria.[10]
The patients consisted of 25 subjects out of these adults, 20 men (80%) and 5 women (20%), with an age range of 16 to 38 years (average 25 years). Among the patients, 62% had a raised erythrocyte sedimentation rate. 25

other patients (15 men and 10 women) who referred to the lab with arthritis due to Reiter's disease, ulcerative colitis, psoriasis, rheumatoid arthritis and other non-AS disease were also typed for the HLA-B27 antigen.
Sample Collection: Collect the venous blood 3ml in fresh EDTA vial.
Sample Preparation: In a Test tube take 30ml HLAB27 Reagent and 50ml whole blood. Mix it in vortex and keep the sample tube in dark place for 15 minutes.
Preparation of HLAB27 lysing solution: In a test tube take 9ml of Distilled water and 1ml HLAB lysing solution. This make solution in 1:10 Ratio.

Alert 15minutes: In a sample tube take 2ml lysing solution mix properly keep the sample tube again in dark place for 10min, Centrifuge the sample tube for 5min in 1500 RPM. Discard the all supernant fluid Add 2ml ship fluid in sample tube mix properly by vortex. This solution centrifuge again mixes 500ml ship fluid mix properly. Load the sample tube in the loaded station of machine and take reading.

III. Results

HLA-B27 was found in 21 of 25 patients (84%). The 4 HLA-B27 negative patients had definite ankylosing spondylitis. 3 out of 25 controls were HLA-B27 positive (12%).
One of the HLA-B27 positive normal controls was found to have an increased sedimentation rate and antistreptolysin 0 titer on further investigation. He did not have a positive family history of definite AS, and did not give a history of rheumatic disease or recent streptococcal infection.
Among the 25 patients with other types of arthritis, 3 had the HLA-B27 antigen (12%). In our study, men were more often affected by disease than women, and we had a male to female sex ratio of 4.

IV. Discussion

There are marked differences in the prevalence of AS among various ethnic and racial groups. These races related differences are very obvious between white and black populations.[10] In general, most patients with AS possess HLA·B27 and the prevalence of the disease roughly corresponds to the prevalence of HLA-B27 in the population.[11]
The present study was performed to determine the prevalence of HLA-B27 in the normal population of Jharkhand and to compare its prevalence with that in AS patients. Aside from this the prevalence of all HLA group one antigens was also determined in controls and patients.
By studying the results, one can easily claim the there is a statistically significant difference between the two groups in the prevalence of the HLA-B27 antigen ($P<0.005$). Other HLA group one antigens were not significantly different between the two groups.
Our finding of HLA-B27 in 84% of individuals with AS in Jharkhand population is similar to the findings of Davatchi and Nikbin in Iran who found HLA-B27 positivity in 92% of AS patients, very close to UK (88%) and the US white population (88%). Our results are in contrast with the findings of Sonozaki et al from Japan who found 67% HLA-B27 positivity among patients and 0% HLA-B27 positivity among controls.
Our data show a male to female sex ratio of 4. Carter et al found similar results (4:1), but Polley et al. reported a 10:1 ratio in his earlier study. However most investigators reported this ratio to be 3 fold greater in males but the importance and cause of this finding has yet to be explained.

V. Conclusion

The discovery of the link between HLA-B27 and a large family of inflammatory rheumatic diseases was one of the seminal advances in rheumatology in the last century. Associations have subsequently been identified with other musculoskeletal and non-rheumatic diseases. The distinction between the disease-associated and non-associated subtypes may give an insight into precisely how HLA-B27 contributes to pathogenesis in the seronegative spondarthritides, whilst some of the pathogenesis hypotheses may help to elucidate the mechanisms of disease susceptibility, initiation and progression. New ways to employ HLA-B27 as a diagnostic and prognostic aid will continue to emerge.

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