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RESEARCH ARTICLE
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EVALUATION OF HLA-B27 ANTIGEN IN SERONEGATIVE GROUP OF ARTHRITIS IN A TERTIARY CARE HOSPITAL IN THE STATE OF WEST BENGAL

Dr. Aniruddha Mondal[1], Dr. Shreyans Agrawal[2], Dr Satyaki Dandi[3], Dr. Arumoy Bhuimali[4*], Dr. Sanjay Saha[5]

[1]Assistant professor, Dept of Orthopaedics, NRSMCH, MBBS, DMRT Radiotherapy, DNB Ortho, +91 81006 35427, dranimondal@gmail.com
[2]Post Graduate Trainee, Dept of Orthopaedics NRSMCH, MBBS (Hons.), MRCS (Edinburgh), MS (Orthopaedics), +91 8340258933, shreyansagrawal85@gmail.com
[3]Senior Resident , Dept of Orthopaedics NRSMCH, MBBS, MS Ortho, DNB Ortho, +91 7439830615, satyakivck22@gmail.com
[4*]Assistant Professor, Dept of Orthopaedics, NRSMCH, MBBS (Hons.), MS (Orthopaedics) +91 89814 61189, arumoybhuimali@gmail.com
[5]Senior Resident, Dept of Orthopedics, NRSMCH, MBBS (Hons.), MS (Orthopaedics)

***Corresponding author-** Dr. Arumoy Bhuimali,
*Assistant Professor, Dept of Orthopaedics, NRSMCH, MBBS (Hons.), MS (Orthopaedics), arumoybhuimali@gmail.com, +91 89814 61189

ABSTRACT:

Introduction-Seronegative group of arthritis is a common arthropathy in Indian population ,second only to rheumatoid arthritis. HLA B27 has been seen to be strongly associated with this group of arthropathy. The major aim of this project is to asses the distribution of HLA B27 gene in our study population and its distribution along various other parameters.

Materials and methods-The various methods includes blood collection of the patient followed by DNA extraction and amplification by RTPCR and finally analysis of HLA-B27 antigen. Thereby, the necessary findings are reported and recorded accordingly .

Results and conclusion -HLA B27 gene was found to be present in 77.6% individuals presenting with seronegative arthritis. Out of all the patients who were found positive for HLA B27, majority were males (71%) and mainly distributed in the age group of 16-50years. An Acute red eye was found as the most common articular manifestation in these patients were as cervical pain was found to be the most common articular manifestation in addition to lower back pain. Sacroilitis was the most found MRI impression in these patients. Considering the variable distribution of the disease and various presentations, it becomes even more important to define its associations , so as to allow a better room for choosing treatment plans and change the prognosis of the disease.

INTRODUCTION

Lower back pain has been a problem since time immemorial. Recent reports suggested that in 2020, 619 million people were affected by chronic back pain globally which is expected to increase to 843 million till 2050 (1). Majority of this may be attributed to ageing, gradual changes in life style and increase in sedentary way of life style. Gradually, the problem of lower back pain has gained special attention in the last few decades. With gradual improvement in our

knowledge about joints and pain mechanism, we have classified the joint pains into various types, the most common ones being osteoarthritis which is related to wear and tear of join spaces and bones , seropositive arthritis- which have a background of rheumatological diseases like SLE and RA and yet another group namely seronegative arthritis. The term seronegative arthritis refer to a specific group of arthropathy where typically all serological tests are negative , a new specific indicator of other rheumatological diseases . These seronegative group of arthropathies are a group of immunologically mediated joint diseases that affect mainly the spine and are seen to have a crucial relation with MHC class1 molecule HLA B27(2). In contrast to other class of arthropathies, the seronegative arthritis involvement starts with sacroiliac joint and the involvement of cervical joints are less common. The seronegative group of arthropathy includes various types which are: Ankylosing spondylosis, psoriatic arthritis , reactive arthritis including Reiters disease and arthropathy associated with inflammatory bowel diseases.
The crucial importance of HLA B27 with seronegative arthritis needs to be studied in depth to understand the pathogenesis and progression of various subtypes of seronegative arthritis. The prevalence of seronegative spondyloarthropathy varies from 0.01% in japan to 2.5% in arctics with an average global prevalence of around 1% (3). A large hospital based study of seronegative arthritis patients revealed that 0.6% of people with lower back pain had AS and 87% of them had positivity of HLA B27 antigen (4). The early detection of seronegative arthritis may contribute to a decrease in burden of the disease and may contribute to slowing of the disease process. The goal of this project shall remain in the favour of evaluation of HLA B27 in seronegative spondyloarthropathy as observed in a tertiary health care hospital as observed in a time period within 2019(august)-2023(august).

Materials and methods:

Materials:

1. EDTA vials for blood collection
2. MyLab HLA-B27 Detection Kit.
3. BIO-RAD CFXConnect Real-Time System.

Methods:

The detection of HLAB27 antigen in done through

1) Blood collection
2) DNA-extraction
3) RT-PCR for detection
4) Analysis.

The DNA extraction process consists of 3 cardinal steps: washing DNA, eluting and storing the DNA.
The HLAB27 antigen status was determined via protocol of MyLab HLAB27 Antigen testing kit. The various steps involved are mentioned in chronological order-
The patient blood sample was taken and then mixed thoroughly if stored at 40C. 20uL of lysis enhancer buffer was dispensed into a 1.5ml microcentrifuge tube.
200uL of blood sample was then added to the microcentrifuge tube containing the lysis buffer. The solution was then briefly mixed.
200ul of lysis buffer was added to this mixture tube and was mixed by vortex for 10 seconds. The solution was incubated at 56 degree C for 10 mins on dry bath. In the meantime , a spin column was put into an empty collection tube.
The tube was removed from the dry bath followed by adding 250ul of binding buffer. This was mixed by vortexing for 10 seconds.
The contents were taken out from the mixer and was found to be dark green in colour. These contents were then added to the spin colum without touching the rim followed by centrifugaion. (13000rpm for 1 min), a continuous checking of the spin column is required at this point and if the

lysate is visible on top of the membrane, further centrifugation of 1 min is required.
Post centrifugation, the collection tube is removed and discarded. Meanwhile, the spin column was placed in a fresh collecting tube and 500ul of wash b uffer was added. This was further centrifuged at 13000rpm for 3 minutes. This step was repeated for total 3 washes.
The spin column with collection tube was centrifuged for yet another minute at 12000rpm at room temp to dry out the column with attached DNA.
The column was finally placed in a clean 1.5ml microcentrifuge tube and 100ul of elution buffer was added to ths column followed by centrifugation at 13000g for 1 minute. The spin column was discarded and the dilute was stored at 40 degree C (-20degreeC for long term storage).

AMPLIFICATION OF DNA USING RT-PCR-
The reaction mixtures are made and then dispensed into a 96- well reaction plate. The plate consists os samples as well as a positive and negative control for reference.
The PCR reaction mixtures are prepared using the components mentioned below:

REAGENT	VOLUME PER REACTION
PCR MIX	10uL
HLA-B27 DETECTION MIX	1uL

REAGENT	VOLUME PER REACTION
HLA-B27 DETECTION MIX	1uL
IPC DETECTION MIX	1uL
NUCLEASE FREE WATER	4uL
TOTAL VOLUME-	15uL

This reaction mixture is mixed by pipetting up and down along with centrifugation for a brief amount of time to remove air bubbles.
15uL reaction mixture is added to each well. As for the positive and negative control, the positive control well has no sample added whereas the negative control well contains only nuclease free water. After the PCR run, the amplification plots of the entire reaction plate is viewed in a software.
The sample of a patient was considered positive for HLAB27 genotype if the target reaction growth curve crossed the threshold line within 37 seconds.
The findings were noted, reported and added to data for analysis.

Results:
In the study as conducted on patients presenting to a tertiary health care centre in the period between august 2019- august 2023 a total of 300 patients of seronegative arthropathy was segregated following finding were noted:
A) Out of all the patients (300) presenting with lower back pain associated with early morning stiffness and non reactive to other serological assays, 77.6% of individuals were found to have HLA B27 presents when evaluated by RTPCR.

HLA B27 antigen status	Number of patinets
Positive	225
Negative	75

B) Majority of the individuals who were part of the study population were aged <50yrs (207), only a minority of patients were found to be aged above 50yrs (18) ***(HLA-B27 positive cases)***

Age group	Number of patients
>=15	10
16-30	96
31-50	101
>=51	18

C) -Majority of the individuals were found to be male (160 male and 65 females) giving a ratio of 2.46:1 ***(HLA B-27 positive cases)***

Sex	Number of patients
Male	160
Female	65
Others	0

D) A vast array of systemic issues were noted in the study population as follow: ***(Extra articular manifestation)***

1) acute red eye was found in 45 individuals of the study population
2) psoriasis was found as a history of 4 individuals of the study population
3) urethritis of unknown aetiology was noted in 1 individuals of the study population
4) 1 individual was found to have a combination of urethritis, acute red eye along with axial pain in the study population

Systemic features present along with arthritis	Number of patients
Acute red eyes	45
Psoriasis	4
Urethritis	1
Acute red eye + urethritis + axial pain	1

E) In addition to lower back pain the following ***articular manifestations*** were found:

Additional associated joint	Number of patients
Shoulder pain	10
Cervical pain	49
Elbow pain	2
Wrist pain	2
Knee pain	32
Hip pain	4
Ankle pain	10
Small joint pain	3
Jaw pain	0

F) The most common MRI finding inn the study population was found to be sacroilitis in various forms and distributions which are as follows:-

MRI finding	Number of patients
Acute sacroilitis	96
Chronic sacroilitis	86
Unilateral sacroilitis	120
Bilateral sacroilitis	60

Discussion:

The association between seronegative group of spondyloarthropathy is long known, HLA B27 has now started to been seen as a locus of spectrum of several disorders. Spondyloarthropathy represents the second most common kind of inflammatory type of arthritis following Rheumatoid arthritis. Our study found that 77.6% of all patients presenting with spondyloarthritis came out to be positive for HLA B27 antigen, with predominance in male sex. In a study conducted by umamaheshwari et al **(5)**, A study conducted among 386 patients of ankylosing spondylitis revealed an association of 90.7% of patients with HLA b27 antigen.**(10)** which involved a study population of 50patients only 26% were found to be HLA B27 positive where as in other studies namely Mishra et al**(6)** & Nessa et al**(7)** , the frequency of HLA B27 being positive among spondyloarthropathies were 56% and 49.2% respectively.

Our study concludes on a result that males have higher predominance of HLA B27 positive spondyloarthropathy, in contrast to Vaidya et al which observed no such difference in distribution among males and females**(8)**

A study from India concludes that the HLA B27 positive arthritis is more common in younger age , mainly in the second decade of life , however our study found out that the maximum number of patients fall in the 3rd decade of life followed by second decade of life**(9).**

Recent advancement in the field of imaging has given us scope to co-relate radiological findings **(11)** with the clinical picture of patients presenting with chronic lower back pain. A majority of patients who were seronegative and HLA B27 positive were found to have sacroilitis as a remarkable impression. A similar impression was found in Uma maheswari et al**(5)** as well.

With advancement in modern medicine and better screening techniques, we now stand a better opportunity to diagnose seronegative arthropathies at an early point and may be even able to change the course of disease with proper treatment.

Conclusion

The undertaken study gives us various conclusions both quantitative and qualitative. The study as undertaken in a tertiary healthcare centre for a period between 2019-2023 also gives us a few statistical conclusions. The study population consists of a wide array of patients of various demographs, age groups, symptomatic presentations distributed across the state of West Bengal. The primary conclusion found was that most of the patients who presented with chronic Lower back pain along with MRI suggesting of sacroilitis were found to be positive for HLAB27 antigen. The results give us a clue that HLAB27 positive seronegative group of arthropathies are more common in males than in females and are mainly restricted to an age group of <50 yrs. We also found out that apart from ,lower back pain which was a common symptom in our study population, neck pain was the most common associated articular manifestation, whereas an acute red eye was the most common extra-articular manifestation. Most of the patients underwent an imaging study via MRI scan which concluded sacroiliitis to be the most common radiological finding. The study gives us an idea about distribution of HLAB27 positive cases along various variables. We need further study and research in this background for a more holistic approach towards HLA-B27 seronegative spondyloarthropathy.

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Chronic lower back pain is a very prevalent condition in West Bengal with a significant portion attributed to seronegative group of arthropathy. there has been a wide array of symptomatology of the presenting patients. It is been observed that the HLA-B27 group of MHC CLASS 1 protein have a very crucial association with these kind of arthropathies. A majority of the patients were found to be positive for HLAB27. Unlike other arthropathies, the presenting features are very much profound varying from acute red eyes in a large group of study population, history of psoriasis, urethritis, IBD are relatively rare findings. Our study also finds that in a given study population, seronegative arthritis is relatively more prevalent in males than in females and the most affected age grp lies below 40 yrs of age. The continuous pain attributes to a poor quality of life and decrese in overall productivity. Further, research is hence required to improve the overall prognosis in terms of both life and quality of life in these patients. With the advent of modern diagnostic tools and monoclonal Abs, this can be achieved if worked upon in depth.