Seronegative Spondyloarthritis and its association with HLA B27

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ABSTRACT

Introduction: Seronegative Spondyloarthritis comprises of various arthritis like rheumatoid arthritis, ankylosing spondylitis, inflammatory arthritis, reactive arthritis and etc. Seronegative spondyloarthritis is a frequently observed condition. Most of these cases are HLA B27 positive.

Aims and Objectives: The aim of our study was to assess the association of the HLA B27 antigen in the population of Seronegative Spondyloarthritis and also distribution of Seronegative Spondyloarthritis in different age group.

Materials and Methods: The study was carried out in the Pathology department in a Tertiary Care Hospital. The patients attending in the department of Pathology from August 2016 to August 2023 with Seronegative Spondyloarthritis for evaluation of HLA B27 were included in the study. EDTA blood was collected. Then DNA extraction was done manually by DNA extraction kit. The HLA B27 were assessed by Real time PCR (RTPCR) by the help of kit.

Results: Out of total 615 patients with seronegative arthritis 446 (72%) patients were positive for HLA B27. The male patients outnumbered the females. 461 (75%) were male and the most frequent age group was 16 to 50 year. Low back pain and cervical pain were most prevalent complaints. The most common extraarticular manifestation was uveitis (red eye), seen in 89 (20%) patients. The most frequent MRI finding was Sacroilitis. 423 (95%) out of 446 HLA B27 positive patients presented with Sacroilitis.

Conclusion: Seronegative Spondyloarthritis is often associated with HLA B27. It is found mainly in male. Most common age group was 16- 50 year.

Introduction

Low back pain (LBP) is the most common complaint of the people suffering from joint pain. These people live with disability if not treated in the right time. A large number of people all over the world, irrespective of age and sex are presenting with low back pain. LBP means the pain between 12th rib and gluteal fold lasting for more than one day. Now 619 million people are suffering from low back pain. This condition is going to increase gradually and in 2050 this number will be more than 800 million [1]. Young patients both male and female also complained of LBP, joint pain, small joint pain. Some of the patients were diagnosed as Rheumatoid arthritis, some as Systemic Lupus Erythematosus (SLE). All the investigations of arthritis like Rheumatoid Factor (RF) and Anti Cyclic Citrullinated Peptide (Anti CCP) are negative. But the test for HLA B27 is positive [2].

Seronegative Spondyloarthritis (SpA) comprises of various types of arthritis where Rheumatoid Factor (RF) and Anti Cyclic Citrullinated Peptie (anti CCP) are negative but HLA B27 is

positive. Seronegative SpA is a group of arthritis with similar clinical features, laboratory test results and genetic features like HLA B27 antigen association (2). There are various types of arthritis. Osteoarthritis, ankylosing spondylitis, Seropositive arthritis, Psoriatic arthritis, Reactive arthritis, Rheumatoid arthritis, Arthritis associated with Inflammatory Bowel Disease, arthritis due to Systemic Lupus Erythematosus and etc. This SpA can be classified as Axial Spondyloarthritis and priphral Spondyloarthritis. They are HLA B27 positive [3-5].

In general population HLA B27 is present in 7% population. But in this SpA HLA B27 positive in 50% to 80% cases. Ankylosing Spondylitis is a major disease in the group of seronegative spondyloarhritis presenting with low back pain and cervical pain and radiographic features of sacroilitis [6]. Another associated feature of this condition is presence of HLA B27 antigen. The incidence of SpA all over the world is 0.5 to 1.9% [5].

We conducted the study on the patients with low back pain and morning stiffness and who tested negative for RF and Anti CCP.

Materials and Methods

The study was carried out in the Pathology department in a Tertiary Care Hospital for a period of 7 years from August 2016 to August 2023 to find out the presenting features, its distribution among the different age group and the association of HLA B27 with seronegative Spondyloarthritis. The patients who were attending in the Rheumatology outpatient department were sent to the Pathology department for evaluation of HLA B27. Various tests like CBC, CRP, ESR, Rheumatoid Factor, Anti CCP were carried out and then HLA B27 test was assessed. The patients who tested negative for RF and anti CCP were assessed for HLA B27 antigen. We took the entire history, chief complaints along with radiological investigation. The HLA B27 antigen test was done by using MyLab HLA B27 RTPCR Detection kit.

We carried out the following steps for evaluation of HLA B27 by Real Time PCR (RT PCR). During this procedure EDTA blood was collected. DNA extraction was done by DNA extraction kit manually. DNA extraction consists of a few steps like washing of DNA, eluting and storing of DNA. In this method the blood sample was first added to lysis enhancer buffer, then lysis buffer and incubated at 56 degree celsius. Afterwards binding buffer and wash buffer were added step by step. After adding elution buffer, microcentrifugation was done and DNA was collected. Finally, PCR mix and HLA B27 detection mix were taken together. This final mix is added to the wells of the PCR plate. Positive control well and negative control well were also set. Afterwards real time PCR CFX Connect was run. After Real Time PCR run Amplification curve (S shaped curve) of the reaction is viewed by a software on the computer screen. It was detected in FAM and VIC channel. A clear, early S-curve and a specific melting peak, confirming the presence of HLA-B27 DNA (Figure.1). If the curve is more than the threshold level within 37 seconds then it is taken as positive.
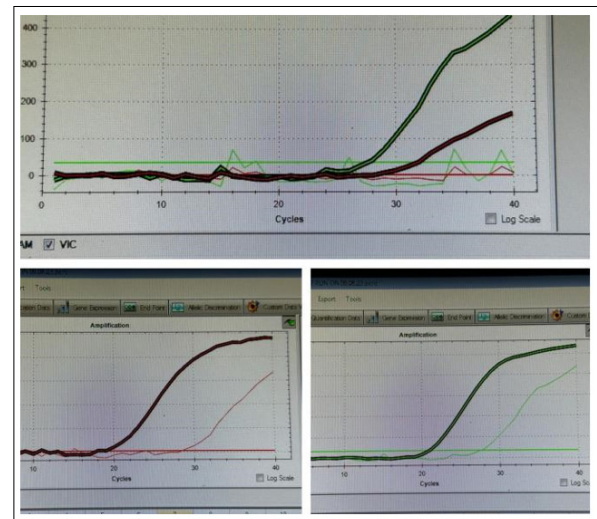
Result and Analysis

This study was conducted on 615 patients from August 2016 to August 2023. These patients had negative Rheumatoid factor and Anti CCP. All these patients were tested for HLA B27 by RTPCR.

Out of 615 patients 446 (72%) patients were positive for HLA B27. And 169 (28%) patents were negative for HLA B27. Among HLA B27 positive patients 334 (75%) were male and rest female. Seronegative Spondyloarthritis were seen commonly in the age group of 16- 50 year. The patients were predominantly male. A few patients 20 (4%) were under the age of 15 year. Even in this age group males were more than females. 391 (87%) patients were in the age group of 16- 50. 35 (8%) patients were in the age group of above 50 years. The age and sex distribution of the patients are shown in the Table no. 1.

Table 1: Age and Sex Distribution of the Patients

AGE IN YEARS	NO. OF PATIENTS	MALE	FEMALE
< 15	20	15	5
16 - 30	190	142	48
31 - 50	201	151	50
>50	35	26	9
total	446	334	112

Figure 1: Amplification Curve of Positive Assay Control- Above Amplification Curve Plot of Patient Positive for HLA B 27- BELOW

All the patients had low back pain. Cervical pain was very common followed by knee pain. Some also complained of shoulder pain and ankle pain. Small joint pain,hip joint pain,elbow and sometimes wrist joint pain. (Table no. 2).

Table 2: Different Joints Affected Along with LBP

Serial no	Joint pain	No. of patients
1.	Cervical	97
2.	Knee	63
3.	Shoulder	27
4.	Ankle	19
5.	Hip	8
6	Small joint	6
7.	Wrist	4
8.	Elbow	4

The most common extra articular manifestation was uveitis (Red eye). It was present in 89 (20%) patients. 8 (2%) patients had psoriasis and 2 patients presented with Inflammatory bowel disease. All the patients had positive C Reactive protein and raised ESR.

The CT/MRI findings in these patients were Sacroilitis. Unilateral Sacroilitis was more common than bilateral sacroilitis. 423 (95%) out of 446 HLA B27 positive patients presented with Sacroilitis. It was seen that 236 (56%) patients had Acute Sacroilitis and 187 (42%) had chronic sacroilitis.

Discussion

Seronegative Spondyloarthritis is commonly encountered below 40 years. They usually present with low back pain and morning stiffness. Previously the definitive diagnosis of SpA used to depend upon the radiographic findings of sacroilitis. But it developed late. By MRI the features of sacroilitis can be detected more promptly and accurately than conventional radiography [7-9]. In our study also the patients were within 16 to 50 years of age. Assessment of Spondyloarthropathy of International Society (ASAS) enumerated a few criteria for diagnosis of SpA. The criterias are radiographic findings of Sacroilitis , positive HLA B27 result, family history of SpA or Crohn colitis, psoriasis or positive C reactive Protein result [10]. In our study 423 (95%) out of 446 HLA B27 positive patients presented with Sacroilitis . HLA B27 was present in 72% of these patients. All the patients had positive C reactive protein. Uma Maheswari et al. found 26% positivity of HLA B27 in Spondyloarthritis patients [11]. The incidence and prevalence of SpA which includes Ankylosing Spondylitis and Rheumatoid arthritis vary from country to country. In some countries the incidence of Ankylosing Spondylitis was more common and in other countries Rheumatoid Arthritis was more common. But both of them were included under Seronegative SpA [12]. The seronegative Spondyloarthritis consists of a group of diseases which have similar clinical features, laboratory tests and genetic features. All these patients are positive for HLA B27 antigen [12,13]. Malviya et al. found that the incidence of SpA is increasing India. The incidence of SpA in India is 7-9/10,000 [14]. We also found the patients of seronegative SpA attending the Rheumatology OPD were increasing yearly. In India 6% people in the general population are positive for HLA B27. But more than 90% positive HLA B27 antigen is seen in Seronegative SpA patients. He reported that Male to Female ratio is 3:1 [14]. Jayaprakash et al. in his study found that HLA B27 was positive in 54% cases of SpA. He also used RTPCR for detection [4]. In our study there is 72% Seronegative SpA patients showed positive HLA B27 antigen and the male to female ratio was 2.9:1, which is similar to above studies. We also used RTPCR for its detection.These group of diseases have same MRI findings of Sacroilitis. These diseases (SpA) have been classified as Axial Spondyloarthritis and Peripheral Spondyloarthritis [14]. 95% of our patients presented with sacroilitis.The extra articular manifestation of seronegative SpA was uveitis, inflammatory bowel disease, psoriasis and etc. [15]. In our patients the extra articular manifestation, uveitis was seen in 89 (20%) patients, 8 (2%) patients had psoriasis and 2 patients presented with inflammatory bowel disease.

Early diagnosis and early treatment is necessary for this seronegative SpA. For this, Clinical, radiological preferably MRI findings and above all genetic testing is necessary. We can do the genetic testing by HLA B27 [16]. We also tested HLA B27 anigen by RTPCR of the patients who came to the Pathology department with the clinical and radiographical features of Seronegative SpA. If the HLA B27 test was positive and MRI features of sacroilitis was present the patients could be diagnosed as seronegative SpA. and managed accordingly. The first-degree relatives of Ankylosing Spondylitis patients also have symptoms of Spondyloarthritis The treatment of these diseases means to control inflammation, prevent joint damage, preserve the mobility of the joints and to keep the patients active. The Tumor necrosis factor alpha inhibitors are used for this purpose. [16-19]. In our cases 89 (20%) seronegatve SpA patients gave the history of similar clinical features in their first-degree relatives.

Conclusion

Seronegative SpA is a heterogenous group of diseases. They have similar clinical, radiological and genetic features. Most common symptom is low back pain. The tests for anti CCP and Rheumatoid Factor are negative. HLA B27 antigen test is positive. If the cases are diagnosed early and proper treatment can be given, the joint destruction can be prevented and patient's pain and difficulty can be controlled and management will be easier.

Conflict Of Interest Statement

None.

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