Is HLA-B27 a useful test in the diagnosis of juvenile spondyloarthropathies?

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ABSTRACT

Introduction: Seronegative spondyloarthritis (SSA) is a type of arthritis that involves joints in the spine, as well as the hips, shoulders, knees and ankles. The diagnosis of juvenile spondyloarthritis is rarely entertained in young children who present with back and leg pain. The aim of the present study was to assess the role of HLA-B27 as a diagnostic marker in children with spondyloarthropathy, and correlation of HLA-B27 with radiological features and tuberculosis.

Methods: Routine haematological and immunological tests were done by standard method and HLA-B27 typing was done by the lymphocytotoxicity method. A total of 70 cases of juvenile spondyloarthropathy were studied from May 2006 to September 2007. It included both males and females.

Results: Positivity of HLA-B27 in childhood SSA was only 71.4 percent (50/70). Gender-wise analysis showed that 76.7 percent (46/60) males and 20.0 percent (2/10) of the female patients were HLA-B27 positive. In HLA- B27-positive cases, the sacroiliac, hip, ankle, lower spine and knee joints were more involved. Urinary tract infection, diarrhoea and constipation were more common in HLA-B27-positive cases. None of the HLA-B27 cases were positive for rheumatoid factor; however, C-reactive protein was raised in 60.5 percent. In bilateral/unilateral sacroiliitis diagnosed by radiographs, only 81.5 percent patients were HLA-B27 positive. Tuberculosis was diagnosed in 14.3 percent (10/70) of total cases in which HLA-B27 positivity was seen in 60 percent of cases (6/10).

Conclusion: Our study concludes that both HLA-B27 and radiological tests should be done in male children suspected to have SSA, because it can indicate early cases of juvenile spondyloarthropathy when radiological changes are not present, and it produces a more severe disease. HLA-B27 positivity probably also predisposes to tuberculosis.

Keywords: ankylosing spondylitis, HLA-B27, juvenile spondyloarthropathies, seronegative spondyloarthritis

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INTRODUCTION

Seronegative spondyloarthropathies (SSA) are a group of arthritides that involve the sacroiliac joints, spine, hip, shoulders, knees and ankle joints. They comprise four distinct entities: ankylosing spondylitis (AS), psoriatic arthritis, SSA with inflammatory bowel disease, and reactive arthritis, including Reiter's disease and enteritis-associated SSA.[1] When this entity occurs in children, it is called as juvenile spondyloarthritides (JSpA). The concept of JSpA in children is emerging. Now, it is more recognised since more specific diagnostic criteria are present for young patients. It involves 20% of all juvenile chronic arthritis.[2]

JSpA has certain distinguishing features; viz: (1) It involves the spine and sacroiliac joints; (2) It affects particular joints on one side of the body rather than both sides; (3) It affects mostly larger joints; (4) It is associated frequently with eye inflammation (uveitis); (5) It causes enthesitis; and (6) Like adults, it is also common in HLA-B27-positive subjects. There is no single test to diagnose JSpA. If symptoms persists for more than six weeks, then a variety of tests, e.g. HLA-B27, radiological and clinical tests, are needed to confirm the diagnosis. Sometimes, the diagnosis of JSpA is difficult if the spine and lower back are not involved for several years after the other symptoms have begun. Hence, the aim of the present study was to assess the role of diagnostic markers, e.g. HLA-B27 antigen, radiological findings and tuberculosis, in the diagnosis of JSpA and correlation of HLA-B27 with disease activity markers, such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) and with extra-articular manifestations.

METHODS

A total of 70 cases of JSpA and 30 healthy children were recruited from the Rheumatology Clinic of Department of Medicine and Orthopaedics, Sir Sunderlal Hospital of Banaras Hindu University, India, during a period of 17 months from May 2006 to September 2007. In all these cases, detailed clinical and radiological findings were noted. Diagnosis of JSpA was made as defined by the

Table I. Involvement of joints in the juvenile SSA group.

Joints involvement	No. (%) B27-positive (n = 50) (A)	No. (%) B27-negative (n = 20) (B)	No. (%) total (n = 70)	χ^2
Cervical spine	15 (30.0)	6 (30.0)	21 (30.0)	13.53 (NS)
Lower spine	24 (48.0)	10 (50.0)	34 (48.57)	
Hip joint	35 (70.0)	16 (80.0)	51 (72.86)	
Sacroiliac	40 (80.0)	10 (50.0)	50 (71.43)	
Shoulder	16 (32.0)	9 (45.0)	25 (35.71)	
Elbow	4 (8.0)	3 (15.0)	7 (10.0)	
Wrist	15 (30.0)	5 (25.0)	20 (28.57)	
Knee	25 (50.0)	12 (60.0)	37 (52.86)	
Ankle	26 (52.0)	11 (55.0)	37 (52.86)	
Subtalar/metatarsophalangeal	11 (22.0)	5 (25.0)	16 (22.86)	
Interphalangeal	8 (16.0)	6 (30.0)	14 (20.0)	
Temperomandibular	4 (8.0)	4 (20.0)	8 (11.43)	

NS: not significant

Table II. Extra-articular manifestation in juvenile SSA.

Clinical features	No. (%) B27-positive (n = 50) (A)	No. (%) B27-negative (n = 20) (B)	No. (%) total (n = 70)
Uveitis	0	0	0
UTI	6 (12.0)	4 (20.0)	10 (14.29)
Diarrhoea	10 (20.0)	4 (20.0)	14 (20.0)
Constipation	8 (16.0)	3 (15.0)	11 (15.71)

European Spondyloarthropathy Study Group (ESSG).[3] In 57 out of a total of 70 JSpA cases, the total leucocyte count (TLC), haemoglobin (Hb) and ESR were done by the standard method, and in 52 cases of JSpA, rheumatoid factor (RF) and CRP were done by latex fixation method via a kit, Spinreact SA, Spain (Avadh Scientific, Lucknow, India). Radiological findings that were reported by radiologists were noted. HLA-B27 typing was done by the modified microcytotoxicity method of Terasaki as well-described in detail by Mehra in 1989.[4] HLA antisera B27, B7, Bw4 and Bw6 of BAG Company, Germany, were obtained from Shiva Scientific, New Delhi, India.

1 µL of HLA antisera was poured in duplicate into the wells of Terasaki plates containing 6 µL of light density liquid paraffin. About 5 ml blood was taken in a vial containing 500 U heparin. This was diluted using an equal amount of RPMI-1640 media. This diluted blood was carefully layered on 4 ml lymphoprep. It was centrifuged at 1,500 rpm for 30 minutes. A white buffy layer of lymphocytes was aspirated without disturbing the layers. It was then treated with 250 µL of adenosine diphosphate solution (0.1%) and centrifuged for one minute to remove the platelets. Cells were then washed three times in RPMI media at 1,500 rpm. 1 µL of the washed cells (2×10^6 cells/ml) were then poured into the respective wells and incubated for half an hour. 6 µL of the complement was added and incubated for one hour. 6 µL of eosin was then added to all the wells for staining, and after five minutes, 6 µL of neutral formalin was added to stop the reaction. The plate was kept at 4ºC overnight and reading was taken under an inverted phase contrast microscope. In positive wells, the cells were dead and appeared large and ghost-like. For statistical analysis, the values were given as mean ± standard deviation (SD). Where applicable, analysis was performed using Student's *t*-test and chi-square test using the Statistical Package for Social Sciences version 10.0 (SPSS Inc, Chicago, IL, USA). A p-value of less than 0.05 was considered to be significant.

RESULTS

In 17 months, a total of 170 cases of juvenile chronic arthritis were referred. Among these, only 70 cases (41.18%) had spondyloarthropathies. Out of 70 cases, only 50 patients (71.43%) were positive for HLA-B27. Gender-wise analysis showed that 60 patients were males (85.71%) and only ten patients were females (14.29%). Two of the female patients were positive for HLA-B27, whereas 46 out of 60 male patients (76.67%) were positive for HLA-B27. Multiple joints were involved. The hip joint was involved in 72.86% of cases, followed by the sacroiliac joint (71.43%), knee and ankle (52.86% each), cervical spine (30.0%), shoulder (35.71%), wrist (28.57%), interphalangeal (20.0%), subtalar and metatarsal (22.86%) and temperomandibular joints (11.43%).

Correlation of joint involvement with HLA-B27 antigen positivity revealed that in HLA-B27-positive

Table III. TLC, Hb, and ESR in HLA-B27-positive and B27-negative juvenile SSA.

Haematological parameters	Mean ± SD B27-positive (n = 40) (A)	Mean ± SD B27-negative (n = 17) (B)	p-value [(A) vs. (B)]
TLC (5,000–13,000 cells/ml)	10,899.63 ± 3,260.59	9,935.29 ± 3,536.76	> 0.05 [NS]
Hb (g/dL)	11.58 ± 1.93	11.45 ± 1.87	> 0.05 [NS]
ESR (mm in the 1st hr)	33.57 ± 20.39	26.82 ± 15.44	> 0.05 [NS]

TLC: total leucocyte count; Hb: haemoglobin; ESR: erythrocyte sedimentation rate; NS: not significant.

Table IV. RF and CRP in juvenile SSA.

Test	No. (%) B27-positive (n = 38)	No. (%) B27-negative (n = 14)	No. (%) total (n = 52)	χ^2
RF				2.40 (NS)
Positive	0	1 (7.14)	1 (1.92)	
Negative	38 (100)	13 (92.86)	51 (98.08)	
CRP				
Positive	23 (60.53)	3 (21.43)	26 (50.0)	7.13 (S)
Negative	15 (39.47)	11 (78.57)	26 (50.0)	

RF: rheumatoid factor; CRP: C-reactive proteins; NS: not significant; S: significant.

Table V. Radiological changes in juvenile SSA.

Radiological findings	No. (%) of cases	No. (%) B27-positive	No. (%) B27-negative	χ^2
Bilateral sacroiliitis	27 (50.0)	22 (81.48)	5 (18.52)	2.89 (NS)
Cervical and lumbar spondylitis	4 (7.41)	2 (50.0)	2 (50.0)	
No abnormality detected	23 (42.59)	13 (56.52)	10 (43.48)	
Total	54			

NS: not significant

cases, the sacroiliac joints (80% vs. 50%) and wrist joints (30% vs. 25%), were more involved. In HLA-B27-negative patients, the shoulder joint (45% vs. 32%), subtalar/metatarsal (25% vs. 22%), cervical spine (30% vs. 30%), lower spine (50% vs. 48%), hip joint (80% vs. 70%), elbow (15% vs. 8%), ankle (52% vs. 55%), interphalangeal (30% vs. 16%) and temperomandibular joints (20% vs. 8%) were more involved (Table I).

In our study, extra-articular manifestations were found only in 50.0% of cases. It was slightly more in HLA-B27-positive cases, but statistically it was non-significant (Table II). Haematological investigations revealed that the TLC was within normal limits in the majority of the patients. The mean level of TLC did not differ in HLA-B27-positive and negative cases, and it was statistically non-significant. Similarly, the mean haemoglobin level did not differ in both the groups. HLA-B27-positive patients had higher ESR, but it was statistically non-significant (Table III). RF was positive in only one HLA-B27-negative patient who was female, while CRP was positive in 50% of JSpA cases. Correlation of HLA-B27 positivity with CRP showed that HLA-B27-positive patients had more CRP positivity (60.53%), as compared to HLA-B27-negative patients (21.43%) (Table IV).

Radiological findings were available in only 54 cases of JSpA, in which only 57.41% patients had evidence of sacroiliitis or cervical or lumbar spondylitis. In 27 cases of bilateral/unilateral sacroiliitis, 81.48% of patients were HLA-B27-positive, while in lumbar and cervical spondylitis, only 50% of patients were HLA-B27-positive. In 23 cases, no radiological changes were reported, but 56.52% of these patients were HLA-B27-positive (Table V). Tuberculosis was diagnosed in 14.29% (10/70) of total cases in which HLA-B27-positivity was seen in 60% of cases (6/10). In a study done in 30 normal age- and gender-matched healthy control children, HLA-B27 was positive in 13.33% (4/30) and none of them was positive for tuberculosis.

DISCUSSION

Juvenile-onset spondyloarthritides is characterised by enthesitis, arthritis affecting mostly the lower extremities and in a variable portion of cases, the sacroiliac and spinal joints with various types of extra-articular manifestations.[5] We studied 70 cases of juvenile chronic arthritis with spondyloarthropathies over a 17-month period. Frequency of HLA-B27 positivity in juvenile SSA was in total 71.4%, but when gender-wise analysis was done, 76.7% of male children with SSA were positive for HLA-B27, but only 20% of the female patients were

HLA-B27-positive. No clear-cut role of HLA-B27 positivity in male childhood SSA has been found in the literature, but since AS is more common in male children, an increased frequency of HLA-B27 in these patients is understood. A study from Auckland noted a predominance of spondyloarthropathies in the male gender and found AS in a total of 41% HLA-B27-positive persons.[6]

Silva-Ramirez et al did HLA typing in 66 patients with JSpA, including 45 with AS and 21 undifferentiated JSpA, and 99 non-related healthy controls.[7] As a whole, frequency of HLA-B27 was positive in 53% (aetiological fraction 51%) and in well-defined AS, HLA-B27 was positive in 67.42% (OR) (aetiological fraction 57%), while the frequency of HLA-B44, B14, DR5 was significantly decreased in JSpA. These workers also found an increased frequency of B15, DR1. Like us, other workers have also reported involvement of the lower joints, especially the sacroiliac and spinal joints.[5,8] Some of the studies have also noted more involvement in the hip joints in JSpA.[9] In our study, we found a low incidence of extra-articular manifestations. Only 14.3% patients had evidence of urinary tract infection, 20.0% had diarrhoea with arthritis and 15.7% had a history of constipation. In western countries, Burgos-Vargas noted a higher incidence of extra-articular manifestations.[5]

None of our patients had uveitis. Similarly, other workers from India have also noted a low frequency of uveitis and extra-articular manifestation.[10,11] Contrary to this, in the Western literature, a higher incidence of uveitis have been reported. A study done in Helsinki, Finland found that 71% cases of anterior uveitis and 79% cases of acute/recurrent unilateral uveitis were positive for HLA-B27, as compared to posterior pan uveitis (7%), chronic or bilateral uveitis (7%).[12] Correlation of B27 positivity with Hb, ESR, RF, and CRP was noted. HLA-B27 positivity did not affect Hb, while ESR was more raised in HLA-B27-positive cases (70.1% vs. 29.8%). Similarly, CRP positivity was also high in HLA-B27-positive cases (65.5% vs. 21.4%). Both ESR and CRP are acute phase reactant probably in HLA-B27-positive cases, and either because there is more destruction of the joint or due to unknown microbial functions, there is increased synthesis of CRP which is responsible for the raised ESR. Studies from India and overseas also reported raised ESR and anaemia in SpA.[13,14]

Correlation of HLA-B27 positivity with radiological features suggested some interesting findings. Evidence of bilateral/unilateral sacroiliitis was found in only 50% cases, while 42.6% cases did not show any radiological change at all. 7.4% of cases showed evidence of cervical and lumbar spondylitis. Analysis of HLA-B27 positivity showed that 81.5% of cases with radiological evidence of sacroiliitis were HLA-B27 positive, and interestingly, 13 cases (56.5%) without any radiological evidence were also HLA-B27-positive. The association between HLA-B27 with spondyloarthropathies is very strong. In Galicia, Spain, its frequency in AS is reported to be 94.3%.[15] However, one study from Nuoro, Italy have not found an association between HLA-B27 expression with disease activity index or Bath Ankylosing Spondylitis Functional Index or with T-cell activation markers.[16]

Certain HLA-B27 subtype alleles bind arthritogenic peptides and cartilage antigen, and present it to CD8 T cells which produce tissue damage and ankylosis,[17] which may explain the early sacroiliitis visible on radiographs. A study of HLA-B27 done in juvenile chronic arthritis as a whole in Finland (Savolainen et al, 1998)[18] noted HLA-B27 positivity in only 27% of cases and also found that HLA-B27-positive children had more active disease, less remission, increased incidence of secondary amyloidosis and required orthroplasty 2.9 years earlier than HLA-B27-negative children. Similarly, a study conducted in Taipei, China in patients with juvenile rheumatoid arthritis (JRA) did not classify the JSpA, but also found that the patients with JRA having HLA-B27 positivity, raised CRP and thrombocytosis showed failure of first remission, and were hence drug-dependent.[19] van Rossum et al from the Netherlands studied 67 patients with juvenile chronic arthritis (JCA) and found the mean age of patients was 9.1 (range 2.5–17.6) years with a median disease duration of 24 months. Only 19.4% patients (13/67) were IgM RF positive and 16% were HLA-B27 positive. Contrary to our study, they reported radiological abnormalities in about 87% of cases.[20]

Huang et al had reported from their study that out of 169 AS cases, only 23 were positive for the purified protein derivative (PPD) reaction.[21] Flato et al from Oslo, Norway, studied prognostic factors in JRA, and found that female gender, early age of onset, large number of affected joints, long duration of raised ESR, DRB108 in symmetric arthritis, HLA-B27 and DRB101 in pauciarticular JCA IgM RF were risk factors for an unfavourable outcome.[22] A molecular study of HLA-B27 in patients with AS from Zulia, Venezuela showed that in AS, B705 was seen in 68.8% and B702 was seen in 31.2% of cases.[23] HLA-B27 and B705 types were seen in only male patients, while in healthy controls, the most common subtype was B702. Increased positivity of HLA-B27 in male children in our country also suggested that some HLA-B27 subtypes prevalent in males were responsible for JSpA.

In conclusion, our study concludes that HLA-B27 is a very important diagnostic test for male JSpA patients, as

it is found to be positive in a majority of our cases, even in those who did not develop any radiological change. HLA-B27-positive patients produce a more severe disease than HLA-B27-negative JSpA patients. In our study, we also found that HLA-B27 positivity to be associated with tuberculosis.

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