Low levels of anti-histone antibodies in north Indian children with juvenile rheumatoid arthritis

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Background & objectives: Early onset pauciarticular disease with uveitis is distinctly uncommon in Indian children with juvenile rheumatoid arthritis (JRA). The occurrence of anti-histone antibodies (AHA) in serum is strongly associated with presence of uveitis. There is a paucity of information from India on the levels of AHA in patients of JRA. In this study, an attempt was made to evaluate the levels of IgG and IgM antibodies to histones in children with JRA in north India.

Methods: Serum samples of 148 children with JRA (84 boys, 64 girls) were collected. Clinical details including onset, symptoms and course of the disease in each patient were recorded. Detailed eye examination including slit lamp examination was done in all patients at presentation and yearly thereafter to rule out uveitis. The presence of antihistone IgG and IgM antibodies was studied by ELISA. Antinuclear antibodies (ANA) were measured by indirect immunofluorescence using HEP-2 cells as substrate at a screening dilution of 1:40.

Results: Of the 148 children, 54 had pauciarticular (12 early onset and 42 late onset), 64 polyarticular and 30 systemic onset disease respectively. ANA were present in two children. AHA were raised in 15 (10%) children, of whom 10 had IgM antibodies, 3 had IgG and 2 had both isotypes. None of the children with early onset pauciarticular disease had uveitis, ANA or AHA.

Interpretation & conclusion: The low occurrence of AHA and uveitis in our subset of patients with JRA is in contrast to that reported from Western countries. The low occurrence is unlikely due to technical reasons as the antigen that has been used consistently showed significant binding to serum from patients with systemic lupus erythematosus (SLE). This is in accordance with the rarity of early onset pauciarticular disease and chronic uveitis in these patients. More studies from other parts of the country are required to validate this observation.

Key words Antihistone antibodies - antinuclear antibodies - autoantibodies - juvenile idiopathic arthritis - uveitis

Juvenile rheumatoid arthritis (JRA) from the Indian subcontinent is different from that seen elsewhere, particularly in the West. The subset of children with early onset pauciarticular disease with uveitis is distinctly uncommon\(^1\)\(^-\)\(^3\). The presence of antinuclear antibodies (ANA) in children with JRA is strongly associated with the development of chronic uveitis\(^4\). More specifically, uveitis is associated with autoantibodies to histones\(^5\)\(^,\)\(^6\). Interestingly, there appears to be no correlation between the detection of antihistone antibodies (AHA) as detected by ELISA or immunoblotting and antinuclear antibodies detected by immunofluorescence in JRA\(^5\), unlike that seen in serum of SLE patients.

There are no systematic studies from India which have tried to analyze why early onset pauciarticular disease with uveitis is rare in India and whether this could be due to serological differences as compared to patients from the western countries. Also, there is paucity of data from India regarding the occurrence of antihistone
antibodies in patients with JRA and its relationship to the occurrence of uveitis. Therefore, in this hospital based prospective study, the presence of AHA in north Indian children with JRA was studied to see if there is any underlying serological difference that could explain paucity of uveitis in our population with JRA.

**Material & Methods**

**Patients:** A total of 148 children satisfying American Rheumatology Association (ARA) criteria for the diagnosis of JRA attending the Immunology Clinic from 1991 to 2001 at the Sanjay Gandhi Postgraduate Institute of Medical Sciences (SGPGIMS), Lucknow, Uttar Pradesh were included. Clinical details including onset, symptoms and course of the disease in each patient were recorded. Detailed eye examination including slit lamp examination was done in all patients at presentation and yearly thereafter to rule out uveitis. Serum samples were collected at presentation. Some of the patients were in the category of adults at the time of presentation but the onset of disease was in their childhood satisfying the criteria for JRA. The serum samples were stored at -80°C. The controls (n= 84) included 25 children and 59 healthy blood donors. The study was approved by the institutional ethics committee.

The minimum sample size was calculated based on a pre test probability of AHA positivity in patients of 0.5 and in controls of 0.05. The power of the study was kept at 99 per cent with alpha error at 0.01 using Epi Info software. With this the minimum number required was 39 in each group. We included more than double the number of controls and all samples available from patients with JRA.

**Antinuclear antibody (ANA):** ANA was detected by the indirect immunofluorescence using Hep-2 cells as substrate at a screening dilution of 1:40.

**Enzyme linked immunosorbent assay (ELISA) for anti-histone antibodies:** AHA were detected using Monestier's method with certain modifications. The modification was the use of calf thymus histone (Sigma, USA) as antigen at 10 µg/ml concentration.

A standard curve was plotted using doubling dilution of a serum sample selected from a panel of serum samples from patients with SLE, because of reproducible binding by the above assay. Doubling dilutions of the serum samples were used from 1:500 to 1:64,000 in each plate. The lower plateau was arbitrarily assigned 1 arbitrary unit (AU). The results are expressed in AU. A sample was considered positive when the value was more than 2 standard deviation above the mean in the control group. The average intra- and interplate variations were less than 10 per cent based on 12 assays.

The proportions of patients and controls with antibodies were compared by the Fisher’s exact test.

**Results & Discussion**

The study population included 148 north Indian patients (84 boys and 64 girls) with JRA, 54 (36.5%) having pauciarticular, 64 (43.2%) polyarticular and 30 (20.3%) with systemic onset disease. The median age of patients at presentation was 14 yr (range, 2-26 yr) and the median age at onset of disease was 9.5 yr (range, 1.5-14 yr). The median age of control children was 9 yr with a range of 3-12 yr, while the median age of control adults was 25 yr with a range of 18-35 yr. Five patients (all male) had uveitis and had late onset pauciarticular disease. There were 12 children (10 boys, 2 girls) with pauciarticular disease below 6 yr but none of them had uveitis at presentation or on follow up.

Only two patients (1.4%) had antinuclear antibodies and both had late onset seropositive polyarticular disease. IgA anti histone antibodies were elevated in 5 (3.4%) patients with JRA whereas among the controls, only two had mildly elevated values. The cut-off value was 3.95 AU/ml (mean ± 2SD, 2.38±1.57). IgG AHA were elevated in 12 patients as compared to one in the control subject (P<0.05). The cut-off value for IgM AHA was 9.56 AU/ml (mean±2SD, 4.87±4.69). None of these patients had uveitis at presentation or on follow up. Two children had both IgG and IgM AHA. The occurrence of AHA was not different in patients with uveitis (0/5) as compared to patients without uveitis (15/143).

The major observation of this study is low occurrence of uveitis, antinuclear and anti-histone antibodies in north Indian patients with JRA. Recurrent anterior uveitis was seen in only 5 patients with JRA, all of
onset pauciarticular disease and chronic uveitis in these with JRA, which is in accordance with the rarity of early onset pauciarticular disease and chronic uveitis in these patients. More studies from other parts of the country are required to validate this observation.

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