SPORADIC REACTIVE ARTHRITIS IN NORTH INDIA: LACK OF IgA RESPONSE
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Abstract:
Objectives: In this hospital based study, we describe the clinical profile and prevalence of IgA antibodies against a panel of bacterial antigens in sera of patients with reactive arthritis (Rea).
Methods: Thirty-one patients with Rea were included, 21 with preceding history of diarrhea (eRea) and 10 with symptoms suggestive of genitourinary infection (gRea). Fifty age and sex matched healthy controls from the same community were used as control. Whole bacterial lysate of Salmonella enteritidis, Shigella flexneri 2b, Yersinia enterocolitica 0.3 and Campylobacter jejuni and Escherachia coli were used as antigens. IgA antibodies to Chlamydia trachomatis were assayed using a commercial ELISA kit.
Results: The median age at presentation was 28 years (11-52 yrs) and there was male predominance. The median duration of disease was 4 months (15 day – 16 yrs). Majority of patients on follow-up had either chronic (44%) or relapsing (28%) course Sera from 19 patients (11-eRea, 8-gRea) were analysed for antibacterial antibodies. Two patients with history of urethritis had elevated level of IgA antibodies against Chlamydia trachomatis. None of the 11 patients with eRea had raised levels of IgA antibodies against any of the bacterial antigens tested.
Conclusion: The clinical profile of sporadic Rea is similar to other parts of the world but the organisms or strains responsible for triggering eRea may be different.
Key Words: Seronegative spondyloarthropathy, clinical, immunological tests

Introduction:
Reactive arthritis (Rea) is an aseptic arthritis triggered by infection at a distant mucosal site. Enteric infections by Salmonella typhimurium, Salmonella enteritidis, Shigella flexneri, Yersinia enterocolitica and Campylobacter jejuni and genitourinary infection by Chlamydia trachomatis and Ureaplasma urealytica are implicated in triggering Rea. Escherachia coli and various other organisms have been implicated in only few case reports.
Prevalence of these organisms varies depending on geographic region. Amongst the enteric organisms responsible for Rea C jejuni and Salmonella are more common in Australia, whereas, Y. enterocolitica is common in USA and Europe. A report from South India indicated Shigella and Salmonella to be responsible for triggering enteric Rea (eRea) in 14% and 1.2% respectively. Studies regarding other organisms are lacking.

The clinical profile, outcome and HLA-B27 association differ according to the triggering organisms. The detection of the triggering agent responsible for sporadic Rea is difficult. The stool and urinary cultures are sterile when the patients present with arthritis. IgA antibodies against these organisms persist in sera for prolonged period in patients developing Rea and serve as serological evidence of infection. The level of IgA antibody response has been correlated with severity of Rea.

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To study the clinical profile and bacteria triggering Rea in our part of the country we undertook analysis of the cases attending our department. In a referral center like our hospital, patients present late in their disease when stool and urethral swabs cultures are rarely positive. So we used persisting IgA antibodies as a marker of infection.

**Patients and Methods:**

Thirty-one consecutive patients fulfilling Wilken’s criteria for diagnosis of Rea attending the Department of Immunology from 1989 to January 1996 were included in this study. All patients had either history of diarrhea or urethritis within 1 month prior to the onset of arthritis. The patients with definite history of diarrhea were grouped as enteric Rea (eRea) and those with urethritis as genitourinary infection triggered Rea (gRea). Clinical details at presentation and during follow-up were retrieved from hospital records. Sera were collected at their first visit and stored at –70°C till analysed. Fifty age and sex matched healthy blood donors from the same demographic background were taken as control for antibacterial antibody analysis. Patients followed up till complete remission or for a period of more than 6 months were included in analyzing the outcome.

The outcomes were categorized as: complete remission absence of arthritis enthesitis or tenosynovitis and other extra-articular manifestation with normal ESR and C-reactive protein values. Relapsing when the patient had complete remission in between relapses. Chronic arthritis when the patient had persisting disease activity.

**Bacterial Antigen:**

Bacterial antigens were prepared from S. flexneri 2b, S. typhimurium Y, enterocolitica 0.3, (kind gift from P. Toivanen, Turk, Finland) C. Jejuni and E.coli as described earlier. Briefly bacteria were grown in peptone broth for 24 hours. They were washed thoroughly in phosphate buffer saline (PBS) 0.15 M, pH 7.4. Bacteria were lysed by ultrasonication, 10 cycles at 8000 cps (each cycle of 60 sec duration with 30 sec of rest) at 0-4°C. Lysate was centrifuged at 10,000 rpm for 10 minutes and supernatant was collected. The protein content of the antigen filtrate was estimated by Lawry’s method and aliquoted and stored at –40°C till further use.

**ELISA for IgA antibacterial antibodies:**

ELISA was done as described earlier. Ninety-six well plates were coated with 50 µl per well of 10 µg/ml of antigen concentration in carbonate-bicarbonate buffer pH 9.6 and left overnight at 4°C. The following morning plates were washed with PBS pH 7.4, 0.15 M and blocked with 150 µl of 2% bovine serum albumin (BSA) SRL India) in PBS. Plates were incubated for 1 hour at 37°C. Plates were washed with PBS containing 0.1% Tween 20 (PBS-T). After washing 50 µl of sera diluted to 1:100 in PBS with 1% tween-20 and 1% BSA (PBS-BSA-T) were added to each well in duplicate. Following incubation for 3 hr at 37°C the plates were washed with PBS-T. Fifty µl of anti-human IgA antibodies, conjugated with horse radish peroxidase (Dako-patts, Denmark), diluted to 1:4000 in PBS-BSA-T were added to each well. Plates were incubated for 1 hour at 37°C. Plates were washed with PBS-T. Color was developed by adding 50 µl of 0.4 mg/l of orthophenyl diamine in citrate-phosphate buffer pH 5 and 0.04% of hydrogen peroxide. Reaction was stopped by adding 25 µl of 1NH₂SO₄ and absorbance was read at 492 nm. An optical density (OD) value of more than mean plus two standard deviations of normal control was considered as positive.
ELISA for *Chlamydia trachomatis* was performed using commercial kit (Eurogenetics, UK) according to the manufacturer’s instructions. The samples having absorbance value 15% higher than the mean absorbance of the control serum (provided in the kit) were classified as positive.

The study included 31 patients of Rea with median age of 28 years (11-52 yrs). The median duration of illness prior to presentation was 4 months (30 days – 16 yrs). The male to female ratio was 7.2:1. There was no difference in the clinical manifestation between the eRea and the gRea (Table-1). Only one patient had classical Reiter’s syndrome. None had erythema nodosum, iridocyclitis, cardiac and neurological involvement. Two of patients underwent colonoscopic biopsy and they were normal except for mild lymphocytic infiltration.

**Table I : Comparison of clinical variables between two reactive arthritis**

<table>
<thead>
<tr>
<th>Type of arthritis</th>
<th>Enteric</th>
<th>Genitourinary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Range)</td>
<td>26 (11-45) yrs</td>
<td>32 (22-52) yrs</td>
</tr>
<tr>
<td>Sex M:F</td>
<td>18:3</td>
<td>8:2</td>
</tr>
<tr>
<td>Median Duration (yrs)</td>
<td>0.25 (0.08-10)</td>
<td>0.45 (0.03-16)</td>
</tr>
</tbody>
</table>

**Treatment and follow-up:**

All the patients received NSAID and intra-articular injections wherever necessary. Five of the patients in eRea group and one in gRea were put on sulphasalazine. One patient with gRea was treated with low dose methotrexate. One patient received demicyclin. The patients were followed for a median period of 24 months (1-72 months). Six patients (4 eRea and 2 gRea) were lost to follow-up. In eRea, 5 had self-limiting disease, 5 had relapsing course and 7 had chronic persistent disease. The corresponding numbers for gRea were 2, 2 and 4 respectively. Thus there was no difference in the clinical outcome between the two groups.

**IgA Antibacterial Antibodies:**

None of the 11 patients with preceding history of diarrhea had significantly elevated IgA antibodies against any of the organism tested in the panel (Figure-1). Eight patients had history of urethritis preceding arthritis. Two of the eight patients had high level of antibodies against *C. trachomatis*. One another patient with history of urethritis was positive for *C. trachomatis* as well for *S. typhimurium*.

**Discussion:**

This hospital based study of Asian Indian patients with Rea revealed that the clinical picture is similar to that described from other parts of the world. But in majority of our patients IgA antibodies to implicated enteric and genitourinary microbes were not elevated. This cohort of 21 patients represented about 16% of seronegative spondyloarthropathy seen during the same period. Ours is a referral center, hence, it is likely that patients having more recurrent and chronic form are referred. The incidence of Rea has been de-
terminated to be 4.6 to 5 per year per 1,00,000 population in a two year study. Even if the half of this figure is extended to our population of 30 million coming under our referral preview, about 8000 to 9000 cases per year are expected. Our study thus represents only 0.33% of the annual cases in our community.

Gastrointestinal infections are quite common in India and hence there were twice as many cases of eRea as compared to gRea. The clinical features of both the groups did not differ significantly. The clinical features of eRea group closely resembled as that has been described of Shigella triggered Rea. The gRea resembled the previous descriptions. In contrast no EN, iridocyclitis, cardiac and neurological complications were found.

While in eRea male to female ratio reaches unity (except for Shigella associated Rea), in gRea the ratio is 28:1. Multiple episodes were noted in sexually acquired Rea and in Campylobacter associated eRea. Chronicity was a feature of Shigella and sexually acquired Rea. Clinical features of our cohort closely In In Southern India, S. flexneri accounts for 14% of eRea, a study from our center more than 50% of the isolate in diarrheal episodes were protozoa. Shigella, Campylobacteria and Salmonella accounted for 7.5%, 5% and 3.7% diarrheal episodes respectively.

Sero logical study done for these organisms did not yield any positive results in our study. We took the mean plus two standard deviation as cut-off value for positivity. The prevalence of these organisms is high in our population. Hence the mean was more skewed to higher values in spite of fifty controls especially for Shigella. Shigella serology may be not useful for epidemiological study for the same reason. In addition the delay in patient referral might have accounted for few negative results.

The organisms tested in our panel were isolated in only 23% of acute bacterial diarrhea studied in our center. Whether organisms like, Entamoeba histolytica, Giardia and other parasitic infections, commonly found in the subcontinent, are responsible for Rea in India needs to be probed. Alternatively different strains of the same organisms may be responsible.

A careful epidemiological study and application of newer modalities as evidence for presence of infection like antigen specific lymphocyte stimulation and PCR should be of help in identifying the the organisms triggering Rea.

References:


**ASIA PACIFIC LEAGUE OF ASSOCIATIONS FOR RHEUMATOLOGY (APLAR)**

**APLAR FELLOWSHIP 2005**

The Asia Pacific League of Association for Rheumatology (APLAR) invites applications from science and medical graduates for its APLAR FELLOWSHIP. The grant of US$10,000 is to assist graduates to undertake intensive or advanced study in the research or clinical aspects of either adult or paediatric rheumatology in a rheumatic disease unit in any country within (preferable) or outside the Asia Pacific area for a minimum period of six months. The successful candidate is expected to have a long-term commitment to continue research or clinical work in his / her own country at the conclusion of the Fellowship. The grant is to cover air fares, accommodation and subsistence costs. Three APLAR Fellowship grants are available for the year 2005.

**The offer is valid only for:**

1. Medical or science graduate of less than 40 years of age. 2. Nationals of countries in the Asia Pacific area.

The following documents need to be enclosed with the application:

1. Photocopy of birth certificate.
2. Recommendation of the Head of Department where the applicant works at present.
3. Curriculum vitae including a recent 2 x 2 inch photograph.
4. A written statement that the institute where the applicant wishes to work is able to accommodate him / her and the willingness of an instructor to supervise the programme.
5. A general outline of the clinical or laboratory course or research work the applicant wishes to undertake.
6. A certificate of fluency in the host country’s language.
7. A certificate of ability to write the country’s language at tertiary level.
8. A reference of good standing from the Dean of the candidate’s medical school / university.

All successful fellows are required to submit a full report to the IRA Executive Committee and APLAR Executive Committee upon the completion of the Fellowship. Additionally, they are expected to submit data of the work carried out during the Fellowship for consideration for presentation at a subsequent IRACON/APLAR Congress of Rheumatology, and for publication as a full scientific article in JIRA/APLAR Journal of Rheumatology.

Seven copies of the application and other documents need to be received before **10 March 2005** by the IRA Secretariat (documents sent by facsimile or email will **NOT** be accepted). Applications received after the closing date shall NOT be entertained. The IRA Secretariat shall forward the applications received by the last date (10-03-05) along with the recommendations of the President IRA to the APLAR authorities for final selection.

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