Introduction

The Seronegative Spondyloarthritides (SSA) are the group of disorders characterized by involvement of mainly spine, hip, sacroiliac joint and enthesopathy. It may involve peripheral joints like shoulder, knees, ankles and extraarticular tissue. Peripheral joint involvement is usually below the waist, oligoarticular and asymmetrical\(^1,2\). This is a group of arthritis which includes ankylosing spondylitis, reactive arthritis, psoriatic arthritis, enteropathic arthritis, juvenile onset spondyloarthritides and undifferentiated arthritis. It is seen mostly in males below 40 years of age. A common feature of this group is involvement of axial spine, low backache and HLA B27 positivity\(^1\).\(^2\)\(^3\)\(^4\). Etiopathogenesis of the disease is not clear. Inflammation of sacroiliac joint with T helper cell, especially Th17 cells, cytotoxic T cells, macrophages, increased expression of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and good response to anti-TNF therapy suggest that cell-mediated immunity is the predominant mode of destruction of joints. The presence of certain infection like Salmonella, Yersinia enterocolitica, Yersinia pseudotuberculosis, Campylobacter jejuni, Chlamydia trachomatis, Campylobacter coli, Ureaplasma urealyticum, Chlamydia pneumonia, Shigella dysenteriae in many cases of reactive arthritis and enteropathic arthritis suggest that infection play a role in genetically susceptible person and causes inflammation and fibrosis\(^2\). The main laboratory marker of these diseases is HLA B27 which is positive in 30% to 90% cases of SSA. HLA B27 heavy chains have a tendency to misfold and are supposed to present arthritogenic antigen to T cells. HLA B27 may have molecular mimicry and prolong survival of Yersinia enterocolitica and Salmonella enteritides in Human and mouse cell line\(^2\)\(^3\)\(^4\). This is called Seronegative arthritis because Rheumatoid factor (RF) and anti-CCP
Ab, a diagnostic marker of rheumatoid arthritis and antinuclear antibody (ANA), marker of collagen disorder are absent unless caused by a coexistent disease. In the present study, we have done RF (IgG, IgA, IgM), anti-CCP2 Ab, ANA, ds-DNA and anticardiolipin Ab in SSA patient to see whether these markers are truly negative.

Materials and Methods

A total of 90 cases of SSA and 43 healthy controls were recruited from the Rheumatology Clinic of Department of Medicine and Orthopaedics, Sir Sunderlal Hospital of Banaras Hindu University, India, during a period of one year from July 2011 to June 2012. In all these cases, detailed clinical and radiological findings were noted. Informed consent was taken from all the patients and the work was approved by the Institute ethical committee of this University. Diagnosis of SSA was done by criteria laid down by European Spondyloarthropathy study group (ESSG). In all cases, clinical details were noted. HLA B27 was done by PCR-SSP method. The primers designed to amplify codons 91-136 of B-27 specific exon 3 of B gene were E91S (5’-GGG TCT CAC ACC CTC CAG AAT-3’) and 136AS (5’-CGG CGG TCC AGG AGC T-3’), which produces a 135-bp PCR product from genomic DNA. As an internal control for exon 3 amplification β-globin primers PCO4 (5’CAA CTT CAT CGA AAT CTA GCT CTA-3’) and GH20 (5’-GAA GAG CCA AGG ACA GGT AC-3’), which produces a 268-bp PCR product. The thermal cycler program for PCR was as follows: denaturation at 94°C for 2 min, followed by 30 cycles of denaturation at 94°C for 10s, annealing at 61°C for 50s, and extension at 72°C for 30s. It takes roughly 1 hour to complete the PCR program.

Amplified PCR products were directly loaded on 2% Agarose gel with 0.5 mg/ml of ethidium bromide and electrophoresed in 1X TBE buffer for 30 min at 100 V. Rheumatoid factor IgG, IgA, IgM was done by ELISA Kit of DSI, Italy, Genesis diagnostics supplied by Thermoscientific UK. The value of IgG RF above 85 IU/ml, IgM RF above 20 IU/ml, IgA RF above 30 IU/ml was taken as positive. Anti-nuclear antibody (ANA), anti-double stranded DNA antibody (anti-ds-DNA Ab), anti-cardiolipin antibody (ACLA Ab) was done by the kit of Varelisa supplied by EIA. ANA above 1.4 ratio, ds-DNA Ab above 55 IU/ml and ACLA above 10 IU/ml was taken as a positive. Anti CCP 2 Ab was done by ELISA kit supplied by IMMCO diagnostic supplied by M/S Transcourier Co. Anti CCP 2 above 25U/ml was taken as positive.

Results

IgM RF was positive in only 5.5% cases of SSA whereas it was not detected in healthy control between 15-40 years. IgA RF was detected in 8.9% cases of SSA and 4.7% cases of control while IgG RF was detected in 37.8% cases of SSA while in control it was elevated in only 18.6% cases. The rise of IgM RF and IgA RF in SSA was not significant while the rising of IgG RF in SSA was significant as compared to control (Table 1). Anti CCP2 Ab was positive in 10 cases which were statistically significant as compared to controls (p-value = 0.023). Out of these 10 cases 2 were males and 8 were females. Peripheral joints were also involved in these cases, and all patients clinically had polyarthritis involving both small joints of hands, feet, larger joint and sacroiliac joint. In 8 cases, more than four joints were involved in addition to bilateral sacroiliitis (Table 2). ANA was positive in 13.3% cases and ds-DNA was positive in 7.8% cases and in 5.6% cases both ANA and ds-DNA Ab were positive (Table 3). Although clinically none of the patient had features of SLE, Scleroderma or any other Connective tissue disease but peripheral joints were involved. Anti-Cardiolipin Ab IgG was positive in only 15.6% cases which were not significant (p-value = 0.545) (Table 4).

<table>
<thead>
<tr>
<th>Groups (No. of cases)</th>
<th>IgM RF Positive cases</th>
<th>IgA RF Positive cases</th>
<th>IgG RF Positive cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A - SSA (90)</td>
<td>5</td>
<td>8</td>
<td>34</td>
</tr>
<tr>
<td>Group B - Control(43)</td>
<td>2</td>
<td>2</td>
<td>8</td>
</tr>
</tbody>
</table>

| Chi Square Group A vs Group B | 0.751 | 4.951 |
| P value Group A vs Group B   | 0.026 |

Note: Group A-SSA (90 cases), Group B-Healthy individuals (43 cases); NS: Not Significant; S: Significant

<table>
<thead>
<tr>
<th>Groups (No. of cases)</th>
<th>Anti CCP2 Ab Positive cases</th>
<th>Anti CCP2 Ab Negative cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A - SSA (90)</td>
<td>10</td>
<td>80</td>
</tr>
<tr>
<td>Group B - Control (43)</td>
<td>0</td>
<td>43</td>
</tr>
<tr>
<td>Chi Square Group A vs Group B</td>
<td>5.166</td>
<td>100</td>
</tr>
<tr>
<td>P value Group A vs Group B</td>
<td>0.023</td>
<td></td>
</tr>
</tbody>
</table>

Note: Group A-SSA (90 cases), Group B-Healthy individuals (43 cases); NS: Not Significant; S: Significant
positive for both ANA and ds-DNA Ab. This may be due to the
and ds-DNA was positive in 7.8% cases. Out of these 5.6% were
more sensitive and specific marker for diagnosis of RA
as anti-cyclic citrullinated peptide antibody (anti-CCP 2 Ab) is
A study from India also reported a case that had
anti-CCP2 Ab can be positive in some cases of SSA positive for anti-CCP 2 Ab but studies conducted
in India found that anti-CCP 2 Ab can be positive in some cases of SSA, Streptococcal infection, Tuberculosis, SLE and after
trauma. Probably in these cases RA and SSA coexists. ANA and
ds-DNA Ab are markers for SLE and other connective tissue dis-
eseases[11]. In the present study ANA was positive in 13.3 % cases
and ds-DNA was positive in 7.8% cases. Out of these 5.6% were
positive for both ANA and ds-DNA Ab. This may be due to the
coeistence of SLE with SSA. Olivieri et al. [10], reported a case of
ankylosing spondylitis who developed the clinical feature of
SLE after four years. In this patient both ANA and HLA B27
were positive. A study from India also reported a case that had
full brown SLE with ankylosing spondylitis[10].

In our study, anti-CCP 2 Ab was positive in 12.9%
In the present study ANA was positive in 13.3 % cases
of SSA, Streptococcal infection, Tuberculosis, SLE and after
trauma. Probably in these cases RA and SSA coexists. ANA and
ds-DNA Ab are markers for SLE and other connective tissue dis-
es[11]. In the present study ANA was positive in 13.3 % cases
and ds-DNA was positive in 7.8% cases. Out of these 5.6% were
positive for both ANA and ds-DNA Ab. This may be due to the
coeistence of SLE with SSA. Olivieri et al. [10], reported a case of
ankylosing spondylitis who developed the clinical feature of
SLE after four years. In this patient both ANA and HLA B27
were positive. A study from India also reported a case that had
full brown SLE with ankylosing spondylitis[10].

In the present study, we found non-significantly el-
evated ACLA in SSA patients, who did not have evidence of abortions or thrombosis. More or less similar to our study, some
workers in Spain also found that 5% of healthy control and 29%
patients of ankylosing spondylitis had positive IgG ACLA but
without manifestation of Thrombosis[17]. Mateo et al.[18], reported
two cases of ankylosing spondylitis who were ACLA positive.
The first case had an infarct in PONS while second case had
deep vein thrombosis. Thus, our present study concludes that
many cases of SSA are autoimmune in nature. Our study also
shows that only IgM RF is specific for diagnosis of RA while
IgG RF is an autoimmune marker which can be positive in many
diseases. Probably infection of low grade initiated autoimmunity
in SSA.

Discussion

Etiopathogenesis of sacroiliitis is not known. It is sup-
posed to be more inflammatory in nature, although it is immune
mediated. Autoimmunity as a cause of SSA has not been given
importance because no distinct antibody is detected in these cas-
es. IgM RF is an old marker for diagnosis of RA where it is posi-
tive in 50 to 80 % cases. In the present study IgM RF was detect-
ed in only 5.5% cases of SSA which is a more or less described
in healthy population in several studies[6-10]. IgG RF was detected
in 37.8 % cases of SSA which was significantly higher than con-
trol (p value = 0.026). Diagnostic value of IgG RF for RA is
not clear. One study of 16 cases of nonspecific arthritis including
tubercular arthritis, sacroiliitis, streptococcal arthritis and un-
differentiated arthritis involving one of two joints showed that
IgG RF was positive in 62.5% cases[11]. A recent marker called
as anti-cyclic citrullinated peptide antibody (anti-CCP 2 Ab) is
more sensitive and specific marker for diagnosis of RA[12-14].

In our study, anti-CCP 2 Ab was positive in 12.9%
cases and all these cases had involvement of more than 4 pe-
ripheral joints including bilateral sacroiliitis. There is variable
report of anti-CCP2 Ab in SSA. Ates et al.[13], did not find any
cases of SSA positive for anti-CCP 2 Ab but studies conducted
in India found that anti-CCP 2 Ab can be positive in some cases
of SSA, Streptococcal infection, Tuberculosis, SLE and after
trauma. Probably in these cases RA and SSA coexists. ANA and
ds-DNA Ab are markers for SLE and other connective tissue dis-
eses[11]. In the present study ANA was positive in 13.3 % cases
and ds-DNA was positive in 7.8% cases. Out of these 5.6% were
positive for both ANA and ds-DNA Ab. This may be due to the
coeistence of SLE with SSA. Olivieri et al. [10], reported a case of
ankylosing spondylitis who developed the clinical feature of
SLE after four years. In this patient both ANA and HLA B27
were positive. A study from India also reported a case that had
full brown SLE with ankylosing spondylitis[10].

In the present study, we found non-significantly el-
evated ACLA in SSA patients, who did not have evidence of abortions or thrombosis. More or less similar to our study, some

Note: Group A-SSA (90 cases), Group B-Healthy individuals (43 cases); NS: Not Significant; S: Significant

Table 4: Anti-cardiolipin Ab positivity in SSA and Healthy control

<table>
<thead>
<tr>
<th>Groups (No.Of cases)</th>
<th>Anti cardiolipin Ab Positive cases</th>
<th>Anti cardiolipin Ab Negative cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of patients</td>
<td>Percent</td>
</tr>
<tr>
<td>Group A- SSA (90)</td>
<td>14</td>
<td>15.6</td>
</tr>
<tr>
<td>Group B- Control (43)</td>
<td>5</td>
<td>11.6</td>
</tr>
</tbody>
</table>

Chi Square Group A vs Group B 0.367
P value Group A vs Group B 0.06 (NS) 0.115(NS)

Note: Group A-SSA (90 cases), Group B-Healthy individuals (43 cases); NS: Not Significant; S: Significant

References

tis associated autoantibodies in patients with synovitis of recent onset.