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Is Seronegative Spondyloarthritides (SSA) Truly Seronegative? IgG -Rheumatoid Factor, Anti-Nuclear Ab, and Anticardiolipin Ab can be Positive in SSA also

Niladri[1*], Usha[1], Kavyanjali Sharma[1], Shailja Singh[1] , Pramod Kumar Verma[1], Nand Kumar Singh[2], Shyam Kumar Saraf[3], Ranjan Singh Rana[1], Saurabh Singh[3], Neha Chaurasiya[1]

[1]Department of Pathology, Institute of Medical Science, Banaras Hindu University, Varanasi, India
[2]Department of Medicine, Institute of Medical Science, Banaras Hindu University, Varanasi, India
[3]Department of Orthopaedics, Institute of Medical Science, Banaras Hindu University, Varanasi, India

***Corresponding author:** Niladri, Research Scholar, Department of Pathology, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221005, Uttar Pradesh, India, Tel: (91) 9628703222; E-mail: niladri0225@gmail.com

Abstract

Background : Seronegative Spondyloarthritides is a very common problem in our area. Etiopathogenesis of the disease is not clear. Rheumatoid arthritis factor (RA factor) and anti CCP antibody which are diagnostic marker of RA are absent in Seronegative Spondyloarthritides. Hence it is called Seronegative.

Aim of the present study is to see some autoantibodies of RA and SLE in SSA patients.

Method: HLA B27 was done by PCR SSP method. Only HLA B27 positive cases were taken. IgM RF, IgA RF, IgG RF, anti CCP antibodies, ANA, ds DNA were done by ELISA technique. Total 90 cases of SSA and 43 healthy control cases were studied within a period of 1 year.

Result: IgM RF, IgA RF and IgG RF was positive in 5.5%, 8.9% and 37.8% cases of SSA respectively while in control only 4.7% were IgA RF positive and 18.6% were IgG RF positive. Anticyclic citrullinated peptide(CCP) Abs were not detected in healthy control but it was positive in 11.11% cases of SSA. All these patients had polyarthritis. ANA and ds DNA was positive in 13.3% and 7.8% cases of SSA respectively. Taking together 5.6% were positive for both ANA and ds DNA.

Conclusion: Although clinically none of the patients had feature of SLE. Positivity of IgG RF and ANA was statistically significant. Anticardiolipin Ab (ACLA) was positive in 15.6% of SSA and 11.6% cases of control cases which was non significant. Thus our study concludes that SSA also have autoimmune basis.

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Introduction

The Seronegative Spondyloarthritides (SSA) are the group of disorders characterized by involvement of mainly spine, hip, sacroiliac joint with 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[1-4]. 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, Camphylobacter coli, Ureaplasma ureolyticum, 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[2-4]. 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[2].

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)[5]. 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 CCA CGT TCA CC-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 1hour 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 1: Rheumatoid Factor positivity in SSA and Healthy control

Groups (No.Of cases)	IgM RF Positive cases		IgA RF Positive cases		IgG RF Positive cases	
	Number of patients	Percent	Number of patients	Percent	Number of patients	Percent
Group A - SSA (90)	5	5.5	8	8.9	34	37.8
Group B- Control(43)	0	0	2	4.7	8	18.6
Chi Square Group A vs Group B	2.487		0.751		4.951	
P value Group A vs Group B	0.115 (NS)		0.386 (NS)		0.026 (S)	

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

Table 2: Anti-CCP Ab positivity in SSA and Healthy control

Groups (No.Of cases)	Anti CCP2 Ab Positive cases		Anti CCP2 Ab Negative cases	
	Number of patients	Percent	Number of patients	Percent
Group A - SSA (90)	10	11.1	80	88.9
Group B - Control (43)	0	0	43	100
Chi Square Group A vs Group B	5.166			
P value Group A vs Group B	0.023 (S)			

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

Table 3: ANA and ds DNA positivity in SSA and Healthy control

Groups (No.Of cases)	ANA Positive cases		ds DNA Positive cases		Both ANA and ds DNA Positive cases	
	Number of patients	Percent	Number of patients	Percent	Number of patients	Percent
Group A- SSA (90)	12	13.3	7	7.8	5	5.6
Group B- Control(43)	0	0	0	0	0	0
Chi Square Group A vs Group B	6.302		3.53		2.482	
P value Group A vs Group B	0.012 (S)		0.06 (NS)		0.115(NS)	

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

Groups (No.Of cases)	Anti cardiolipin Ab Positive cases		Anti cardiolipin Ab Negative cases	
	Number of patients	Percent	Number of patients	Percent
Group A- SSA (90)	14	15.6	76	84.4
Group B- Control (43)	5	11.6	38	88.4
Chi Square Group A vs Group B	0.367			
P value Group A vs Group B	0.545 (NS)			

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

Discussion

Etiopathogenesis of sacroiliitis is not known. It is supposed 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 cases. IgM RF is an old marker for diagnosis of RA where it is positive in 50 to 80 % cases. In the present study IgM RF was detected 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 control (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 undifferentiated 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 peripheral joints including bilateral sacroiliitis. There is variable report of anti-CCP2 Ab in SSA. Ates et al.[15], 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 diseases[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 coexistence of SLE with SSA. Olivieri et al. [16], 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 elevated 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.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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