Prevalence of *Chlamydia trachomatis* infection in Ankylosing Spondylitis

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ABSTRACT ***Objective:*** *To investigate the prevalence of Chlamydia trachomatis IgA and IgG antibodies in serum of Ankylosing Spondylitis patients and healthy control.* ***Method:*** *The prevalence of Chlamydia trachomatis IgA and IgG antibodies was assessed in 232 cases of Ankylosing spondylitis and 100 age and sex matched control. The presence of serum IgA and IgG antibodies to Chlamydia trachomatis was estimated using commercial ELISA kit.* ***Result:*** *No significant differences were seen between AS patients and control in the prevalence of Chlamydia trachomatis IgA and IgG antibodies. Moreover, there was no significant association between HLA-B27 gene and Chlamydia trachomatis infection.* ***Conclusion:*** *This study did not show a significant difference between AS patients and control in the prevalence of Chlamydia trachomatis. There was no significant association between HLA-B27 gene and Chlamydia trachomatis infection.*

Keywords*: Ankylosing Spondylitis, Chlamydia trachomatis, HLA-B27*

Introduction

AS is a multifactorial disease with genetic and environmental factors playing main role. In this disease, innate immunity could be disturbed making individuals prone to abnormal reactions after bacterial infections. Though the role of infections is established in reactive arthritis but so far serological studies have not confirmed a chlamydial involvement in AS. A hypothesis has already been proposed that HLA-B27 promotes the continued existence of bacterial components, may be because of identification between HLA-B27 and Gram-negative bacteria. [1]

Chlamydia trachomatis is the most common obligate intracellular bacterium that infects more than 90 million people each year globally by sexual transmission [2]. It is a gram -negative intra-cellular obligate bacteria with bi-phasic life cycle: intra-cellular metabolic form known as Reticulate bodies (RBs) while extra-cellular infections forms are called as Elementary Bodies (EBs). Chlamydial infections are generally asymptomatic resulting in frequent irreversible complications which further leads to great clinical and epidemiological problem. It is the most commonly prevalent sexually transmitted pathogen in the world including India [3, 4, 5]. Genital Chlamydial infection causes serial sequelae, one of which is Chronic reactive arthritis. Some groups reported that approximately 5% genital Chlamydial infection develops acute reactive arthritis, and half of them progress to chronicity [6].

Patients

In the present study, a total of 232 cases of Ankylosing Spondylitis and 100 controls were taken from the Out-patient Division of Rheumatology, Department of Medicine and Orthopedics' of Sir Sunder lal Hospital, Banaras Hindu University, Varanasi. The venous blood was collected from patients and healthy controls in sterile tubes and was used for various immunological investigations and 3 ml blood was taken in EDTA tube for HLA-B27 typing. The study protocol was approved by the ethical committee of the Institute of Medical Sciences, Banaras Hindu University. 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 Ankylosing spondylitis was done by criteria laid down ASAS for Axial Spondyloarthritis. [7]

Methods

Anti- *Chlamydia trachomatis* antibody detection by ELISA

The presence of serum IgG and IgA antibodies to *Chlamydia trachomatis* was estimated using commercial ELISA kit Nova Tec Immunodiagnostica GmbH, Germany following the manufacturers' guidelines. As per the

kit manual, Cut-off value for both IgA and IgG was 10 NTU (Nova Tec-Units). Samples are considered positive if the absorbance value is higher than 10% over the cut-off.

HLA-B27 typing

Genomic DNA was extracted by phenol-chloroform method. 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.

Statistical Analysis

All data were analyzed using Statistical Package for Social Sciences (SPSS, Chicago, Illinos, USA), version 16. Pearson's Chi-square and Fisher exact test were used to compare differences between the frequencies as per the requirement. A p-value <0.05 was considered significant for all analysis.

Results

The antibodies to *Chlamydia trachomatis* antigen, IgA and IgG were positive in only 3.9% and 3.4% of AS patients respectively while in control these were 2.0% and 1.0% respectively. (Table 1) *Chlamydia* IgA were insignificantly increased in HLA-B27 positive cases of Ankylosing Spondylitis. On the contrary, Chlamydia IgG antibodies were insignificantly higher in HLA-B27 negative cases of AS. *Chlamydia* IgA was not detected in HLA-B27 negative cases. (Table 2). No significant differences were found for *Chlamydia trachomatis* antibodies between males and female's patients of AS. (Table 3)

Table 1: Showing *Chlamydia trachomatis* (IgA and IgG) positivity in AS patients & Control.

Group (No of Cases)	*Chlamydia trachomatis* IgA				*Chlamydia trachomatis* IgG			
	Positive		Negative		Positive		Negative	
	No	%	No	%	No	%	No	%
AS (n=232)	9	3.9	223	96.1	8	3.4	224	96.6
Control (n=100)	2	2.0	98	98	1	1.0	99	99
Fisher's exact P value	0.515(NS)				0.287(NS)			

*statistically significant (p<0.05)

Note: NS: Not Significant; S: Significant

Table 2: Showing correlation of *Chlamydia* IgA and IgG with HLA-B27 positivity in AS patients.

Group (No of Cases)	*Chlamydia trachomatis* IgA				*Chlamydia trachomatis* IgG			
	Positive		Negative		Positive		Negative	
	No	%	No	%	No	%	No	%
HLA-B27 positive (162)	9	5.6	153	94.4	4	2.5	158	97.5
HLA-B27 Negative (70)	0	0	70	100	4	5.7	66	94.3
Fisher's exact P value	0.060(NS)				0.247(NS)			

*statistically significant (p<0.05)

Note: NS: Not Significant; S: Significant

Table 3: Correlation of *Chlamydia* (IgA, IgG) antibodies with the gender of AS patients

Groups (Gender)		*Chlamydia trachomatis* IgA		*Chlamydia trachomatis* IgG	
	Total	No	%	No	%
Male	190	8	4.2	6	3.2
Female	42	1	2.4	2	4.8
Chi-Square		0.309		0.27	
p-value		0.578(NS)		0.606(NS)	

*statistically significant (p<0.05)

Note: NS: Not Significant; S: Significant

Discussion

Most of the published data in literature establishes presence of *Chlamydia trachomatis* in reactive arthritis (ReA) and undifferentiated spondyloarthritis (uSpA). But still its underestimated in various parts of world. Kumar et al. [8] reported presence of *Chlamydia trachomatis* infection in joint fluid by Sn PCR and n PCR method in both ReA and uSpA patients in India [8]. According to our data, the antibodies to *Chlamydia trachomatis* antigen, IgA and IgG were positive in only 3.9% and 3.4% of AS patients respectively while in control these were 2.0% and 1.0% respectively. Thus, this study didn't show a significant difference between AS cases and controls in the prevalence of *Chlamydia trachomatis* infection. *Chlamydia* IgA antibodies were seen more in HLA-B27 positive cases of Ankylosing Spondylitis whereas Chlamydia IgG antibodies were seen more in HLA-B27 negative cases.

Very few reports in literature shows co-relations of Ankylosing Spondylitis with *Chlamydia trachomatis*. Lange et al. [9] in their study on 164 known patients of AS concluded: 1) evidence of *Chlamydia trachomatis* infection is frequent in male & female patients with Ankylosing Spondylitis, (2) patients with genitourinary infection tend to have HLA-B27 and 3) presence of genitourinary infection was not significantly associated with chronic illness [9]. Similar to our study, a report on Dutch patients did not reveal an increased prevalence of *Chlamydia trachomatis* infections in AS males compared with a group of healthy men. [10].

Conclusion

Our study did not show a significant difference between AS patients and control in the prevalence of *Chlamydia trachomatis*. Also, no significant association was found between HLA-B27 gene and *Chlamydia trachomatis* infection. Thus, our study concludes that genetic factors rather than environmental factors, are far more important in the etiology of Ankylosing Spondylitis.

Conflict of Interest

The authors declare that they have no conflict of interest.

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