ORIGINAL ARTICLES

Diagnosis of circulatory antibodies to *Chlamydia trachomatis* among asymptomatic undifferentiated spondyloarthropathy patients in India

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Abstract

Background

C. trachomatis infection has been reported in patients with undifferentiated spondyloarthropathy (uSpA). However, it is underdiagnosed globally, including India.

Aim

The aim of the study was to determine circulatory anti-*Chlamydia trachomatis* antibodies in uSpA patients.

Materials and methods

Twenty two uSpA patients were included in the study following the European Spondylarthropathy Study Group (ESSG) criteria and 12 age- and sex- matched osteoarthritis patients were also included for comparison. From each patient, 5 ml of non-heparinized intravenous blood was collected for analysis. Anti-*C. trachomatis* IgG and IgA antibodies were detected in serum using commercially available ELISA kits and the data was statistically evaluated.

Results

During the study, 18.1% (4/ 22) uSpA patients were found positive for anti-*C. trachomatis* IgA antibodies, while 4.5% (1/ 22) patients were positive for anti-*C.trachomatis* IgG antibodies in serum. None of the control patients was positive for these antibodies.

Conclusion

C. trachomatis may be an unrecognized cause for uSpA even among Indian patients. Further studies involving larger study population are significant to substantiate the findings.

Introduction

Reactive arthritis (ReA), a non-canonical septic, inflammatory arthritis commonly associated with *Chlamydia trachomatis* infection, represents a significant health burden, yet it is poorly understood and underestimated.[1] Another important entity, *viz.* undifferentiated spondyloarthropathy (uSpA) encompasses a group of arthritic patients who do not get categorized into any defined spondyloarthropathy, *viz.*: ankylosing spondylitis, psoriatic arthritis, ReA or arthritis associated with chronic inflammatory bowel disease and are considered to be '*forme fruste*' of ReA.[2, 3] Patients with uSpA may or may not present with typical clinical symptoms like synovitis in joints. Broadly, the only difference with ReA is the absence of urogenital symptoms. *C. trachomatis* causes chronic infection and it becomes persistent and untreatable in joint and this asymptomatic form makes this pathogen an unrecognized cause of ReA.[4] Numerous published

studies on ReA/uSpA from our country have focused on enteric infections.[4, 5] As the prevalence of genital *C. trachomatis i*s found to be high in India, there is a definite need to investigate the presence of C. trachomatis in ReA/uSpA patients.[6, 7] Recently, our group reported identifying intra-articular *C. trachomatis* infection by both PCR and immunofluorescence in genitourinary ReA as well as in uSpA patients.[8, 9] Since screening is necessary, the aim of the study was to screen asymptomatic uSpA patients for the presence of serum anti-*C. trachomatis* IgG/IgA antibodies.

Materials and methods

With the permission of hospital ethics committee, 34 age-matched uSpA (mean age - 30.7 years) and OA patients (mean age - 34.6 years) attending Department of Orthopedics at Safdarjung Hospital, New Delhi were enrolled. Among these, 22 were uSpA patients while the control group of OA comprised of 12 patients. A detailed clinical history including any previous infection, treatment or any other extra-articular symptoms was recorded. uSpA patients were recruited following ESSG criteria.[10] Clinical and radiological diagnosis was done for OA patients. Informed written consent was obtained from each patient. Patients with enteric/ tubercular/ viral infection or any other defined arthropathies were excluded.

Intravenous blood of 5.0 ml was drawn under aseptic condition and the serum was separated and stored at -20°C for conducting various assays. The detection of circulatory IgG and IgA antibodies to *C. trachomatis* was done by ELISA in the sera of arthritic patients using commercially available kits (IBL International, Germany and Savyon Diagnostics, Israel, respectively), following the manufacturer's guidelines. Briefly, 10 µl of diluted sera of patients and controls were pipetted into wells, incubated (for binding of *C. trachomatis*-specific IgG/IgA antibodies) and washed. This was followed by the addition of horseradish peroxidase-conjugated anti-human IgG/IgA antibodies, incubation, and washing. 3,3',5,5'-tetramethylbenzidine (TMB) substrate was added for color development and the reaction was stopped by sulphuric acid and the plate was read at an absorbance of 450 nm. Anti-*C. trachomatis* IgG antibodies were calculated in Units (U) according to the formula: patient (mean) absorbance value x 10/ cut-off, where cut-off = 10 U. Patients with >11 U were considered positive, <9 U were negative and between 9-11 U were in the grey zone. The cut-off value (COV) was calculated for *C. trachomatis*-specific IgA antibodies according to the formula: COV= NC x 2, where NC= average absorbance at 450 nm of the negative control run in duplicate, while the cut-off index (COI) was calculated according to the formula: COI= absorbance of serum sample at 450 nm divided by COV. Patients with COI >1.1 were considered as positive, COI <1.0 as negative, while those with COI between 1 - 1.1 were borderline cases.

Non-parametric Mann-Whitney test was performed for comparing different groups. Fisher exact test was performed for different variables. Statistical analysis was performed with GraphPad Prism software version 5.0 (GraphPad Software, Inc., San Diego, California, USA).

Results

Age-matched 34 arthritic patients (22 uSpA, 12 OA) were subjected to *C. trachomatis* screening by commercially available antibody detection kit in blood. The mean age of uSpA patients belonging to the study group was 30.7 years. M:F ratio was 15:7 and the mean disease duration was 18.6 months. Oligoarthritis was seen in 68% (15) patients and synovitis of major joints (knee/ ankle) in 45.5% (10) patients. Majority (95.4%) of the patients (n=21) in the study group had low backache and enthesitis was reported in 9% (n=2) of the subjects. Serum C-reactive protein was found to be 29.6 + 16.1 µg/ml (mean + S.D.) in uSpA patients, while it was 4.6 + 1.2 µg/ml (mean + S.D.) in patients with OA. Overall, 18.1% (4/ 22) uSpA patients found positive for anti-*C. trachomatis* antibodies in the serum. All positive patients had an oligoarthritic pattern in joints and had high level of C-reactive protein (> 57 µg/ml) (Table 1). 18.1% (4/ 22) patients were positive for anti-*C. trachomatis* IgA antibodies, while 4.5% (1/ 22) patients were found positive for anti-*C. trachomatis* IgG antibodies in serum. One patient was found to be positive for both antibodies. Comparison of IgA cut-off index value is shown in Fig. 1.

Discussion

uSpA is the most common presentation among various spondylarthritides.[11] However, the exact prevalence of uSpA is difficult to assess quantitatively as the disease is often unrecognized.[3] In this regard, *C. trachomatis* was found to be the most important pathogen in patients diagnosed with an asymmetrical oligoarthritis and having no preceding history of infection. Although, the serodiagnosis of *C. trachomatis* is not well appreciated in various diseases, it can serve as an initial non-invasive diagnostic method and can be utilized in uSpA patients without having any symptoms suggestive of

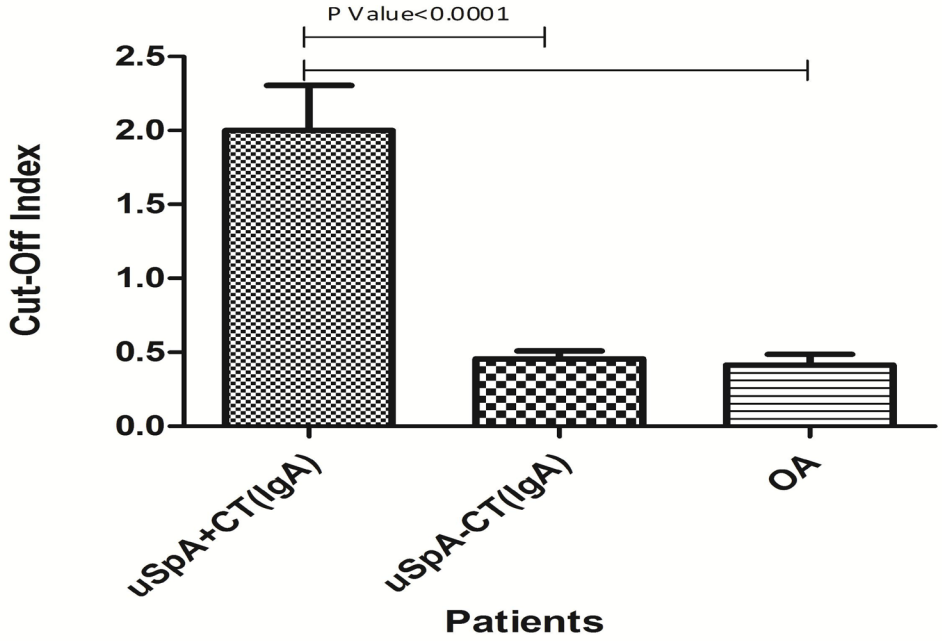
chlamydia infections, which may cause persistent infection in later stages. It can also be used in cases where nucleic acids are either undetectable or absent. The specificity and sensitivity for determination of IgG antibodies in joints is 80%. The specificity reaches 90% with the determination of synovial fluid IgA antibodies.[12] Diagnosis of *C. trachomatis* infection in uSpA is difficult even in active or silent phase of infection. During antibiotic treatment or in self-limiting condition/relapsing phase, antibody screening may assist in C. trachomatis detection in the serum as the DNA of this pathogen becomes undetectable by PCR method. Screening for anti-*C. trachomatis* antibodies may work as a prognostic marker and can be performed at intermittent periods

Table 1:Clinical characteristics of chlamydia trachomatis-positive undifferentiated spondyloarthropathy patients

Age(yrs) / Sex	Disease Duration (months)	Type of arthritis	IgG Abs to Ct	IgA Abs to Ct	CRP (µg/ml)	IgG Units	IgA COI
18/ F	6	O	+	+	60	12	1.7
21/ M	6	O	-	+	63	-	1.69
21/ M	6	O	-	+	83	-	2.6
21/ M	6	P	-	-	57	-	1.8

F:female; M: male yrs: years; abs: antibodies; Ct: *chlamydia trachomatis*; CRP: C-reactive protein; COI: cut-off index; O: oligoarthritis; P: polyarthritis

Fig 1: Bar diagram showing difference in cut-off index value in study and control groups



uSpA+CT: undifferentiated spondyloarthropathy patients with *C. trachomatis* infection

uSpA-CT: undifferentiated spondyloarthropathy patients without *C. trachomatis* infection

OA: Osteoarthritis

'p' value <0.05 considered to be significant

for diagnosis of antibodies to circulatory *C. trachomatis* antigens like lipopolysaccharide (major outer membrane protein) in serum. If any patient is found positive for *C. trachomatis* antibodies in serum, further molecular diagnostic tests can be performed for confirmation. This is particularly important for the male patients who are at higher risk of developing ReA/uSpA in comparison to female due to its asymptomatic nature in male. Our observations on detection of *C. trachomatis* IgG/IgA antibodies in asymptomatic uSpA patients were further strengthened by the fact that all positive subjects had higher serum C-reactive protein levels, thereby indicating bacterial infection. These patients were also assessed for extra-articular infection. However, none had any such manifestations

Current study showed that uSpA patients who were asymptomatic for urogenital infection were also at risk of developing *C. trachomatis*-induced ReA. It is significant to understand the magnitude of disease burden in larger number of asymptomatic uSpA patients in our country to prevent chlamydial infection at an earlier stage by employing improved management strategies.

However, in the present study, the results obtained only in OA patients were reported. Since OA patients serve as non-inflammatory controls, it would have been more appropriate to enroll inflammatory disorder patients such as those with rheumatoid arthritis also as controls for assessing non-specific antibody production. Another limitation of the study is that serology alone cannot convincingly diagnose chlamydial infection and also cannot replace the sensitivity and specificity associated with molecular diagnostic tests. However, it can serve as an initial non-invasive screening method during an early diagnosis of *C. trachomatis* infection, but the results obtained will require further confirmation before therapeutic intervention.

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Competing interests

The authors declare that they have no competing interests.

Citation

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