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Antinuclear antibodies in scrub typhus: Transient occurrence during acute illness

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ABSTRACT

Background & objectives: The pathological hallmark of scrub typhus infection is focal or disseminated vasculitis. As with other infections, antinuclear antibodies (ANA) have been previously described in scrub typhus. However, the underlying mechanisms and implications of this immunological phenomenon is not well understood. In the present work it was assessed whether ANA is associated with illness severity and outcomes.

Methods: In this prospective study spanning one year, patients fulfilling the diagnostic criteria for scrub typhus were recruited. Patients with other acute infective febrile illnesses were taken as controls. ANA positivity was compared between the cases and controls. ANA in scrub typhus was assessed for correlation with disease severity, organ dysfunction and outcomes.

Results: The cohort comprised of 149 patients (scrub 89; controls 60) with mean age 46.5 (SD=16.9) yr; 48.3% were female. ANA was detected in 48 (53.9%) patients with scrub typhus and 9(15%) controls ($p < 0.001$). The ANA pattern was predominantly speckled (93.8%) in both scrub typhus patients and controls. In patients with scrub typhus, ANA positivity was associated with increasing APACHE-III score [Odds ratio (OR) 1.01; 95% CI 0.99–1.03; $p = 0.09$]. On bivariate analysis, ANA tended to be correlated with acute respiratory distress syndrome (OR 2.32; 95% CI 0.98–5.46; $p = 0.06$), hepatic dysfunction (OR 2.25; 95% CI 0.94–5.39, $p = 0.06$) and aseptic meningitis (OR 6.83; 95% CI 0.80–58.05, $p = 0.08$). The presence of these antibodies did not correlate with duration of hospitalization or mortality. Convalescent sera on 31 ANA positive scrub typhus patients demonstrated persistence of ANA in only 5 (16.1%) patients.

Interpretation & conclusion: The disappearance of ANA during the convalescent phase suggests that ANA is expressed during the acute phase of scrub typhus infection. Its association with organ dysfunction warrants further study of the mechanisms and impact of autoantibody formation in scrub typhus.

Key words Immunology; rickettsial diseases; scrub typhus; tropical medicine

INTRODUCTION

Scrub typhus, a zoonotic disease, endemic in Southeast Asia and Northern Australia, is caused by *Orientia tsutsugamushi*, a gram-negative, obligate intracellular parasite[1]. Transmission to human occurs through the bite of the larval form of trombiculid mites called Chiggers[2]. Infection results in an acute febrile illness, with clinical features ranging from mild self-limiting illness to multiorgan dysfunction[3-4]. The pathophysiological hallmark of scrub typhus is focal and disseminated vasculitis. Proliferation of the organism in the endothelium of small blood vessels results in endothelial disruption which causes fluid leak, platelet aggregation and neutrophil proliferation. This leads to micro-infarction, secondary to focal occlusive angiitis[5].

Antinuclear antibodies (ANAs) are autoantibodies that target intranuclear proteins, commonly seen in the setting of autoimmune disease. Their positivity can occur in 5% of normal individuals with infections and drug intake[6]. Antinuclear antibodies have also been previously observed in patients with scrub typhus. In a pilot study of 40 patients with severe scrub typhus infection admitted to the intensive care unit (ICU), 16 (40%) patients tested positive for ANA, with a nonsignificant protective effect on organ dysfunction[7]. As scrub typhus is an infectious vasculitis, we postulate that ANA positivity may be either causal (contributing to organ dysfunction) or an epiphenomenon (a non-specific immunological expression).

This prospective study was undertaken to evaluate the prevalence of ANA in patients admitted to hospital with

scrub typhus infection. We also assessed if the presence of ANA correlated with illness severity and outcomes.

MATERIAL & METHODS

Study design

This prospective observational study was conducted in the medical wards and medical ICU of Christian Medical College, Vellore, a tertiary care referral teaching hospital in India, for a period of one year from January to December 2013.

All patients aged 16 year and above, who presented with an acute febrile illness of up to 21 days duration (documented temperature >100.4 °F), who fulfilled diagnostic criteria for scrub typhus were considered for inclusion as cases. Scrub typhus was defined as an acute febrile illness with (a) presence of an eschar and positive scrub IgM ELISA, or (b) a positive scrub IgM ELISA and defervescence within 48 h of initiation of doxycycline or azithromycin[3]. Exclusion criteria were immuno-compromised state (HIV infection, pre-existing immuno-suppressive medication), autoimmune disease, malignancy and use of medication known to induce ANAs (*e.g.* sulfasalazine, praziquantel, isoniazid, hydralazine). As ANAs are also known to occur non-specifically with other acute infective febrile illnesses, controls were included for the study. Controls were patients with acute infective febrile illness, not fulfilling diagnostic criteria for scrub typhus. Every attempt was made to recruit age-sex matched controls admitted around the same time as a case; however, this was not always possible.

All patients were recruited within 24 h of admission and demographic details and laboratory data were documented. Acute physiology and chronic health evaluation III (APACHE-III) score was calculated on the day of admission and sequential organ failure assessment (SOFA) scores were calculated on Days 1, 3 and 5[8-9]. Scrub typhus detect IgM ELISA (InBios International Inc., Seattle, WA, USA) was used for the serological diagnosis of scrub typhus; as per the manufacturer's instructions an optical density of ≥0.5 was considered to be diagnostic of scrub typhus[10]. Antinuclear antibodies, and IgG, A and M were detected by an indirect immunofluorescent test (IIFT) using a commercial kit (Mosaic HEp-20-10/Monkey Liver, Euroimmun AG, Lubeck, Germany) as per the protocol followed in the laboratory. The patients' sera were assessed at a 1:100 dilution for the presence or absence of antibodies[11]. As this test is a qualitative test, a negative control, a 2+ control (50% staining intensity) and a 4+ control was used for quality control. In addition, an internal quality control (1+ or 2+) was used to validate the results. Reactions were rated negative when no fluorescent or a weak fluorescent signal was observed. Positives were graded 1+ (25% intensity) to 4+ for staining (maximum 100% intensity of staining). Antinuclear antibodies fluorescence patterns were reported based on the staining pattern of the nuclei of interphase and mitotic cells[10, 12].

The disease outcomes assessed were hospital mortality, ICU and hospital length of stay, need for mechanical ventilation and incidence of organ dysfunction (myocarditis, renal failure, acute respiratory distress syndrome, aseptic meningitis and circulatory shock).

Myocarditis was defined as the presence of either elevated cardiac biomarkers, or ECG features of myocardial involvement, or ECHO features of regional wall motion abnormalities or reduced ejection fraction. Acute kidney injury was defined as an increase in serum creatinine by ≥0.3 mg/dl within 48 h or >1.5 times baseline within the prior seven days (KDIGO definition)[13]. Acute respiratory distress syndrome was diagnosed based on the Berlin definition: Respiratory failure occurring within one week of a known clinical insult, not explained by cardiac failure or fluid overload, evidenced by bilateral opacities on chest imaging and drop in P/F ration to ≤300 mm Hg[14]. Aseptic meningitis was defined by the presence of altered sensorium, headache or neck rigidity with CSF analysis showing lymphocytic pleocytosis, elevated protein and low glucose. Convalescent sera were tested to evaluate for persistence of ANA following recovery from the infection.

Statistical methods

Sample size calculation was done as follows: The prevalence of ANA in scrub typhus was taken as 40% as reported previously[7]. The prevalence of ANA in other acute febrile illness was estimated at approximately 15%[6]. The sample size for cases was thus 60. It was decided to recruit 60 controls.

All study variables were described using descriptive statistical methods. Continuous variables which were normally distributed were summarized using mean and standard deviation (SD). Continuous variables, not normally distributed, were summarized using median with inter-quartile ranges (IQR). Cases and controls were assessed for the presence of antinuclear antibodies. Scrub typhus patients with and without ANAs were compared using chi-squared test. Quantitative variables not normally distributed were analysed using Mann-Whitney U-test. Antinuclear antibodies positivity was correlated with severity of illness (SOFA and APACHE-III), organ dysfunction and outcomes (length of ICU and hospital stay, mortality) using bivariate logistic regression analy-

sis. Associations were expressed as odds ratio (OR) with 95% confidence intervals (CI). SPSS v20 for windows (IBM Corp., NY) was used for statistical analysis.

Ethical statements

The study was approved by the Institutional Review Board and ethics committee (IRB Approval no. 8158). Written and verbal informed consent was obtained from the study patients.

RESULTS

The cohort comprised of 149 patients (scrub 89; controls 60) with mean age 46.5 (SD = 16.9) yr; and 48.3% were females. The majority of the patients (89.9%) were from within a 50 km radius from the hospital. The mean duration of fever prior to presentation was 7.7 (95% CI; 7.1–8.5) days. An eschar was noted in 75.3% (n = 67) of the cases. Demographic data, treatment and outcome of patients with scrub typhus (cases) are summarized in Table 1. The control group comprised of patients with urinary tract infection (n = 22), liver abscess (n = 9), pneumonia (n = 9), dengue (n = 8), malaria (n = 6), enteric fever (n = 3), pyogenic meningitis (n = 2) and cellulitis (n = 1). When cases were compared with controls, although the mean age was similar, there were significantly more females in cases than controls (Table 2). Cases were sicker than controls with a significantly larger proportion of cases requiring mechanical ventilation (Table 2).

Antinuclear antibodies data is summarized in Table 2. Of the 89 patients with scrub typhus, ANA was detected in 48 (53.9%) patients. In contrast, in controls with other acute febrile illnesses, only 9 (15%) patients were ANA positive ($p < 0.001$). The ANA pattern was universally (100%) speckled in controls, while in those with scrub typhus, a majority (n = 45; 93.8%) had a speckled pattern and 3 (6.3%) had a nucleolar pattern of ANAs.

In patients with scrub typhus, acute respiratory distress syndrome (57.3%) and renal dysfunction (36%) were frequent, while complications such as aseptic meningitis (9%) and myocarditis (7.9%) were less commonly observed. Need for ventilation and vasoactive agents was in 26 (29.2%) and 20 (22.4%) patients respectively. Mortality was 3.3% (n = 3). In the control group, two patients required ventilation (3.3%). The duration of hospital stay and mortality in controls was similar to patients with scrub typhus (Table 2).

Repeat ANA was done during the convalescent phase

Table 1. Demographics, treatment and outcomes of patients with scrub typhus

Parameters	All patients (n = 89)	ANA positive (n = 48)	ANA negative (n = 41)	*p*-value*
Age (yr)	46.5 (16.9)	47.5 (16.1)	45.2 (17.9)	0.51
Gender, Male : Female	38 : 51	23 : 25	15 : 26	0.28
Profession				
Professional/Skilled	7	5	2	0.66
Unskilled	32	16	16	
Unemployed	50	27	23	
Duration of symptoms (Days)†	7 (5–10)	7 (5–10)	7 (7–10)	0.70
Eschar (*n*)	67 (75.2%)	36 (75%)	31 (75.6%)	0.94
Admission				
SOFA score	5.6 (3.6)	5.9 (3.7)	5.3 (3.5)	0.43
APACHE III score	42.4 (24.3)	46.5 (28.4)	37.6 (17.6)	0.23
Complications (*n*)				
Shock	25	14	11	0.80
ARDS	51	32	19	0.05
Myocarditis	7	5	2	0.33
Renal failure	32	17	15	0.90
Aseptic meningitis	8	7	1	0.04
Number ventilated (%)	26 (29.2%)	14 (29.1%)	12 (29.2%)	0.99
Use of vasoactive agents (*n*)	20 (22.4%)	10 (20.8%)	10 (24.3%)	0.68
Admission to ICU (*n*)	17 (19.1%)	7 (14.5%)	10 (24.3%)	0.24
Hospital length of stay (Days)†	6 (5–8)	6 (5–8)	6 (4.5–9)	0.29
Hospital mortality (*n*)	3 (3.3%)	2 (4.1%)	1 (2.4%)	0.65

**p*-value comparisons are between anti-nuclear antibody (ANA) positive and ANA negative patients using chi-square test; †Median, inter-quartile range; *p*-value was calculated by comparing means using chi-square and *t*-test; SOFA–Sequential organ failure assessment; APACHE–Acute physiology and chronic health evaluation; ARDS–Acute respiratory distress syndrome; (*n*) indicates number of patients; Values in parentheses indicate standard deviation unless specified.

(around 6 wk) in 31 out of the 46 survivors (67.3%) with scrub typhus who were tested positive for ANA during the acute illness. Only 5 (16.1%) patients persisted to have ANAs and even in these patients there was a decrease in the intensity of ANA positivity on immunofluorescence.

Table 2. Comparison of characteristics of scrub typhus patients with controls with acute febrile illnesses

Parameters	Scrub typhus (n = 89)	Controls (n = 60)*	*p*-value**
Age (yr)	46.5 (16.9)	46.6 (18)	0.48
Gender Male : Female	38 : 51	39 : 21	0.01
ANA			
Positive (*n*)	48 (53.9%)	9 (15%)	<0.001
Negative (*n*)	41 (46%)	51 (85%)	
ANA titer			<0.001
(–)ve or weakly (+)ve	41	51	
1+	23	3	
2+	22	6	
3+	3	0	
ANA pattern			
Speckled	45 (93.8%)	15 (100%)	1
Speckled and nucleolar	3 (6.2%)	0	
Admission SOFA	5.6 (3.6)	3.24 (2.5)‡	<0.001
Ventilation (*n*)	26 (29.2%)	2 (3.3%)‡	<0.001
Duration of stay†	6 (5–8)	8 (5.7–13)	0.016
Mortality	3 (3.3%)	1 (1.7%)	0.45

*Controls were patients with acute infective febrile illness other than scrub typhus; ***p*-value comparions between patients and controls using chi-square test; †Median, interquartile range; ‡Data available for 58 patients; ANA–Anti-nuclear antibody; SOFA–Sequential organ failure assessment; (*n*) indicates number of patients; Values in parentheses indicate standard deviation unless specified.

Table 3. Bivariate analysis of antinuclear antibody (ANA) positivity and its association with severity of illness scores, organ dysfunction and outcomes

Parameters	Odds ratio	95% Confidence interval	*p*-value
Age (yr)	1	0.98–1.03	0.50
Scoring systems			
APACHE III	1.01	0.99–1.03	0.09
SOFA (Day 1)	1.04	0.93–1.17	0.42
MODS	1.25	0.92–1.69	0.14
Organ dysfunction			
Myocarditis	2.27	0.43–12.36	0.34
Renal failure	0.95	0.40–2.26	0.91
Circulatory shock	1.12	0.44–2.85	0.81
ARDS	2.32	0.98–5.46	0.06
Aseptic meningitis	6.83	0.80–58.05	0.08
Hepatic dysfunction	2.25	0.94–5.39	0.06
Outcomes			
Duration of ICU stay	0.87	0.71–1.06	0.17
Duration of hospital stay	0.94	0.85–1.04	0.29
Mortality	0.57	0.05–6.5	0.65

APACHE–Acute physiology and chronic health evaluation; SOFA–Sequential organ failure assessment; MODS–Multiple organ dysfunction score; ARDS–Acute respiratory distress syndrome.

Logistic regression analysis (Table 3) of ANA with organ dysfunction, illness severity and outcomes showed that SOFA scores did not correlate with ANA positivity on Day 1 (OR 1.04; 95% CI 0.93–1.17; $p = 0.42$), Day 3 (OR 1.06; 95% CI 0.94–1.20; $p = 0.32$) or Day 5 (OR 1; 95% CI 0.85–1.18; $p = 0.93$). However, a statistically insignificant positive association between ANA positivity and APACHE-III score was found (OR 1.01; 95% CI 0.99–1.03; $p = 0.09$).

Antinuclear antibodies positivity tended ($p = 0.06$) to be correlated, with the development of acute respiratory distress syndrome (OR 2.32; 95% CI 0.98–5.46) and hepatic dysfunction (OR 2.25; 95% CI 0.94–5.39); this association was however weak ($p = 0.08$) for aseptic meningitis (OR 6.83; 95% CI 0.80–58.05). It did not correlate with length of intensive care ($p = 0.17$), hospital stay ($p = 0.29$) or mortality ($p = 0.65$).

DISCUSSION

This study explored the prevalence of ANA in scrub typhus infection and assessed its relationship with disease outcomes. A significantly ($p < 0.001$) higher prevalence of ANA was found in scrub typhus patients (53.9%) in comparison to patients with other febrile illnesses (15%). These antibodies appear to develop rapidly, occurring in about a week following onset of fever. During the convalescent phase, only 16.1% of the scrub typhus patients assessed, continued to be ANA positive, suggesting that ANA positivity was a transient occurrence during acute illness. The prevalence of ANA positivity during the convalescent phase of illness was similar to that reported in the general population[6] of 5–15%. Although, ANA positivity was not strongly correlated with illness severity at admission, it was associated, albeit weakly, with acute respiratory distress syndrome, hepatic dysfunction and aseptic meningitis. ANA positivity did not impact mortality or length of stay. The findings of this study suggest a possible association between these nonspecific autoantibodies and the severity of illness, the significance of which is unclear and needs to be evaluated further.

The prevalence of ANA in infections such as malaria, tuberculosis and hepatitis B is well known and attributed to be an epiphenomenon[13–15]. The high prevalence of such antibodies in scrub typhus is not surprising since the pathogenesis is that of an infectious systemic vasculitis. The principle site of involvement in scrub typhus is the endothelial cell which results in a vasculopathy and increased microvascular permeability. This may account for the protean manifestations of this

disease, ranging from skin to central nervous system involvement[16–18]. Inflammation is mediated *via* cytokines with a Th1 response and formation of IFN-γ. Recent studies have demonstrated that TNF-α level can predict severity of illness[19]. Autoantibody expression in scrub typhus could be a result of molecular mimicry and a non-specific activation of the immune system. Inflammation and stimulation of polyclonal antibody formation probably induces autoantibody formation against self-antigens. The speckled pattern, which is usually seen in systemic auto-immune diseases such as lupus, mixed connective tissue disease, polymyositis and Sjogren's syndrome[20] was observed in the scrub typhus patients. However, the speckled pattern was also universally observed in patients with other infections.

The decrease in antibody titres during the convalescent phase supports the view that autoantibody formation is a transient phenomenon during the active phase of the infection. In a study of African immigrants, the presence of ANA was attributed to, living in an infectious environment[14]. In this study[14], none of the ANA positive subjects developed any symptoms of autoimmune disease during a mean follow up of 18 months.

Thus, ANAs in scrub typhus may be an epiphenomenon. This is supported by the fact that positivity resolves with resolution of the infection. This may be akin to what happens with an acute phase reactant, in that it is a non-specific marker of an infective process. On the other hand, the association of ANA with acute respiratory distress syndrome, hepatic dysfunction and neurological manifestations, was albeit weak, suggesting that specific ANAs may play a role in the pathogenesis of organ dysfunction.

This study is the first to have assessed the correlation of disease severity and outcomes with the presence of autoantibodies in scrub typhus. The prospective nature of the study allowed for analysis of convalescent sera thus, demonstrating the transient nature of autoantibody formation. None of the patients in this study developed autoimmune disease during follow up.

Limitations

The study had few limitations meriting mention. The sample size was relatively small and this could have limited the association between ANA and organ dysfunction. Strain virulence of *Orientia* and genetic variation was not explored in this study and these could have been potential confounding factors. The control group was heterogeneous and it was not possible to recruit perfectly age-sex matched controls at the time of recruitment of cases. The high prevalence of ANA in this study, in cases and controls, could have been an overestimation, due to the use of a commercial kit that assessed IgG, IgM and IgA antibodies[21]. Most laboratories perform ANA tests that detect the IgG isotype, as it is more relevant in the screening for autoimmune diseases[18], and IgM isotype antinuclear antibodies are seen in normal individuals and are non-specific[22-23]. Convalescent samples for ANA could not be collected in 15 survivors despite multiple efforts.

This study highlights the need for further studies to understand the underlying immune mechanisms driving autoantibody formation in scrub typhus. Studies which look at humoral immunity, with analysis of immune markers along with complement levels, may throw more light on the pathogenesis of this infectious vasculitis.

CONCLUSION

The prevalence of ANA positivity was 53.9% in patients admitted to the hospital with scrub typhus infection. Its disappearance during the convalescent phase suggests that it may be a transient phenomenon. The association with organ dysfunction warrants further study of the mechanisms and impact of autoantibody formation in scrub typhus.

Conflict of interest: None to declare.

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