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ORIGINAL ARTICLE

Associated factors and clinical implications of serum aminotransferase elevation in scrub typhus



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Background/Purpose: Timely diagnosis and prompt treatment can reduce the complications of scrub typhus. It is thus important to find easy laboratory tests to help in the diagnosis, especially in patients without eschar at initial presentation. Because serum aminotransferase elevation is common in scrub typhus, its associated factors and clinical implications need further investigations.

Methods: We conducted a retrospective study in Kinmen, Taiwan, to collect clinically suspected scrub typhus patients notified to Taiwan Centers for Disease Control for confirmation during 2005–2010. Scrub typhus was diagnosed and *Orientia tsutsugamushi* was genotyped by serological or molecular assays. The laboratory data and clinical information were recorded for analysis.

Results: Overall, 344 suspected scrub typhus patients were reported to Taiwan Centers for Disease Control and 288 of them were certified scrub typhus. Scrub typhus patients had significantly more thrombocytopenia, serum aminotransferase elevation (76% vs. 54%, $p = 0.001$), higher frequency of fever, eschar, and lymphadenopathy, compared with nontyphus patients. Hepatic dysfunction in scrub typhus was associated with older age, longer fever duration, and

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absence of lymphadenopathy, but seemed to be unrelated to the rickettsial genotypes. Multivariate analysis showed that serum aminotransferase elevation (odds ratio: 3.75; $p = 0.003$; 95% confidence interval: 1.56–9.01) independently predicted scrub typhus. Furthermore, in suspected scrub typhus patients without eschar, 92% of true typhus patients had serum aminotransferase elevation compared with the nontyphus ones (odds ratio: 6.47; $p = 0.028$, 95% confidence interval: 1.23–34.11).

Conclusion: Hepatic dysfunction in scrub typhus patients is associated with older age, longer fever duration, and absence of lymphadenopathy. Serum aminotransferase elevation can aid in the diagnosis of scrub typhus, especially in suspected patients without eschar.

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Introduction

Scrub typhus is a zoonotic disease caused by *Orientia tsutsugamushi*, which is transmitted vertically in the life cycle of mites (especially *Leptotrombidium* species); rodents are common animal hosts of scrub typhus. Humans contract scrub typhus after being bitten by mites in the trombiculid larval stage (chiggers) during recreational activities or agricultural works.¹ Scrub typhus prevails in eastern and southern Asia, the Western Pacific islands, and eastern Australia.² Taiwan is located within this region; the eastern parts of this country and its offshore islands have been reported to be endemic for scrub typhus.^{3,4} Scrub typhus has been listed as a notifiable infectious disease in Taiwan since 1955; therefore, all clinically suspected patients of scrub typhus should be reported to the Taiwan Centers for Disease Control (CDC) for registration and confirmation.

After an incubation period of 9–12 days, the clinical features including eschar, fever, headache, skin rash, lymphadenopathy, myalgia, arthralgia, malaise, and cough start to occur.⁵ The initial presentations of scrub typhus are nonspecific, and the more specific signs (e.g., eschar and skin rash) develop several days after fever. Despite being a pathognomonic sign of scrub typhus, however, eschar could not be found in a portion of patients, making the diagnosis challenging.⁶ The definite diagnosis relies largely on serological or molecular assays on paired sera collected 2 weeks apart.¹ Therefore, a correct diagnosis of scrub typhus may be delayed, occasionally leading to severe complications such as acute respiratory distress, acute renal failure, interstitial pneumonitis, encephalitis, and even death.⁷ It is important to find inexpensive and quick laboratory tests to aid in the diagnosis, especially in endemic areas with constrained medical resources.

Of particular note, hepatic dysfunction has been occasionally reported in 77–96% of patients with scrub typhus in some studies with small case numbers.^{7,8} Although serum aminotransferase elevation was shown to serve as a distinguishing parameter for scrub typhus associated with acute hepatitis A,⁹ the associated factors and clinical implications of hepatic dysfunction for scrub typhus remains largely unclear.

Kinmen island (24°44'N and 118°33'E) is an offshore island of Taiwan, located between Taiwan and southern

China. The climate is subtropical, and the rainy season is between April and September, with average temperatures of 19–28°C. Kinmen is reported to be highly endemic for scrub typhus with a bimodal summer–autumn distribution.⁴ Taking advantage of this fact, with a large cohort of scrub typhus patients in Kinmen, this study aimed to evaluate the associated factors and clinical implications of serum aminotransferase elevation in scrub typhus, especially in patients without eschar.

Methods

Patients

We retrospectively included all clinically suspected cases of scrub typhus notified to Taiwan CDC by Kinmen Hospital, Kinmen, Taiwan between January 1, 2005 and December 31, 2010. The clinical information, laboratory data, and relevant medical history of these patients were reviewed from the medical records. Serum aminotransferase elevation was defined as either elevated aspartate aminotransferase level (> 40 U/L) or elevated alanine aminotransferase level (> 65 U/L) at the initial clinical visit with the suspicion of scrub typhus. Leukopenia was defined as a white blood cell count of $< 4000/\mu\text{L}$ and thrombocytopenia as a platelet count of $< 120\text{k}/\mu\text{L}$. This study was performed in accordance with the principles of the Declaration of Helsinki and had been approved by the Institutional Review Board of the National Taiwan University Hospital (200904057R), Taipei, Taiwan.

Serological and molecular diagnosis of scrub typhus

As the standard procedure of Taiwan CDC, serum and whole blood were collected in the acute and convalescent phases (within 7 days and 14 days after symptoms onset, respectively) from each patient, and were sent to the laboratory of Taiwan CDC for serological or molecular assays. The indirect immunofluorescence antibody assay with antigens of major strains of *O. tsutsugamushi* (Karp, Kato, Kawasaki, and Gilliam strains) was used. Scrub typhus was diagnosed as an initial immunoglobulin M titer of $>1:80$ or a greater than four-fold rise of immunoglobulin G titer in paired serum.¹ In addition, a positive polymerase chain reaction of

specific primer for 56-kDa-type-specific antigen gene of *O. tsutsugamushi* was used to confirm the diagnosis^{10,11} and for genotyping as described elsewhere.¹¹ For the non-scrub typhus patients, other common rickettsial diseases in Taiwan (e.g., acute Q fever and murine typhus) would be investigated by polymerase chain reaction-based assays.¹

Statistical analysis

Descriptive values were expressed as mean (standard deviation) and number (percentage) accordingly. The Student *t* test was used for data comparison between continuous variables. The Chi-square test was used for categorical data. In order to find the clinical predictors of scrub typhus in patients, parameters in univariate analysis with statistical significance were entered into the multivariate logistic regression analysis, and odds ratios (OR) with 95% confidence intervals (CI) were reported. The *p* values were two tailed, and *p* < 0.05 were considered statistically significant. The statistical analysis was performed by the STATA software (STATA 11.0; STATA Corp., College Station, TX, USA).

Results

A total of 423 suspected cases of scrub typhus were notified to Taiwan CDC during 2005–2010. We excluded 17 patients with incomplete clinical profiles, 29 with indeterminate diagnosis of scrub typhus (without follow-up serum for confirmation), and 33 without available baseline serum transaminases data for analysis. Finally, 344 clinically suspected scrub typhus patients were enrolled: 288 were certified cases and the remaining 56 were non-scrub typhus cases. In the patients without scrub typhus, one actually had murine typhus.

Clinical manifestations of scrub typhus

The baseline characteristics of certified scrub typhus (*n* = 288) and non-scrub typhus patients (*n* = 56) are shown in Table 1. Compared with the nontyphus patients, the true scrub typhus cases had a significantly higher percentage of thrombocytopenia (45% vs. 22%, *p* = 0.001) and serum aminotransferase elevation (76% vs. 54%, *p* = 0.001). They also had a significantly higher frequency of fever (97% vs. 91%, *p* = 0.025), eschar/eschar-like lesions (91% vs. 34%, *p* < 0.001), and lymphadenopathy (20% vs. 5%, *p* = 0.009) compared with nontyphus ones.

Factors associated with serum aminotransferase elevation

We further investigated factors associated with serum aminotransferase elevation in certified scrub typhus patients (*n* = 288; Table 2). Patients with serum aminotransferase elevation were significantly older (45 years vs. 34 years, *p* < 0.001), had longer fever duration prior to diagnosis (4.1 days vs. 2.5 days, *p* < 0.001), and less lymphadenopathy (17% vs. 30%, *p* = 0.018). The genotype of *O. tsutsugamushi* had been evaluated in 71 patients,

Table 1 Baseline parameters of all 344 clinically suspected scrub typhus patients notified to Taiwan CDC

Parameters	Scrub typhus	Non-scrub typhus	<i>p</i>
	<i>N</i> = 288	<i>N</i> = 56	
Age, y	42 (21)	42 (21)	0.918
Male	210 (73)	35 (63)	0.115
Laboratory abnormality			
Leukopenia	65 (23)	8 (15)	0.190
Thrombocytopenia	129 (45)	12 (22)	0.001
Serum aminotransferase elevation	218 (76)	30 (54)	0.001
Clinical symptoms and signs			
Fever	280 (97)	50 (91)	0.025
Eschar/eschar-like lesions	263 (91)	19 (34)	< 0.001
Headache	157 (55)	26 (47)	0.324
Chills	111 (39)	28 (51)	0.091
Myalgia	93 (32)	18 (33)	0.950
Lymphadenopathy	58 (20)	3 (5)	0.009
Rash	35 (12)	8 (15)	0.623

Data are presented as *n* (%) or mean (standard deviation). CDC = Centers for Disease Control.

showing Karp in 33, Kato in 20, and Kawasaki in 18. The genotypic distribution was similar among patients with elevated and normal serum aminotransferase.

Serum aminotransferase elevation as a predictor for scrub typhus especially in patients without eschar

In order to find clinical predictors for the diagnosis of scrub typhus, multivariate logistic regression analysis was performed for all 344 clinically suspected scrub typhus patients. After adjusted for age, sex, and other variables, we found male sex (OR: 2.59; *p* = 0.046; 95% CI: 1.02–6.61), thrombocytopenia (OR: 3.23; *p* = 0.011; 95% CI: 1.31–7.94), serum aminotransferase elevation (OR: 3.75; *p* = 0.003; 95% CI: 1.56–9.01), fever (OR: 6.10; *p* = 0.025; 95% CI: 1.26–29.50), eschar/eschar-like lesions (OR: 38.43; *p* < 0.001; 95% CI: 15.64–94.41), and lymphadenopathy (OR: 4.15; *p* = 0.041; 95% CI: 1.06–16.26) were independent predictors for the diagnosis of scrub typhus (Table 3).

Among our cohort, 62 suspected scrub typhus patients did not have eschar or eschar-like lesions at the initial presentation, making prompt diagnosis even more challenging. We compared the parameters of all suspected cases of scrub typhus without eschar (Table 4). Patients of true scrub typhus were predominantly male, had significantly more thrombocytopenia (56% vs. 24%, *p* = 0.011), and exhibited more serum aminotransferase elevation (92% vs. 54%, *p* = 0.002) compared with the nontyphus patients. The clinical symptoms and signs were comparable between both subgroups. After adjustment for age, sex, thrombocytopenia, and lymphadenopathy, we found that serum aminotransferase elevation (OR: 6.47; *p* = 0.028; 95% CI: 1.23–34.11) was the only independent predictor for scrub typhus in patients without eschar.

Table 2 Factors associated with serum aminotransferase elevation in 288 certified scrub typhus patients

Factor	Serum aminotransferase elevation		p
	N = 218	Normal serum aminotransferase N = 70	
Age, y	45 (21)	34 (18)	< 0.001
Male	155 (71)	55 (79)	0.221
Fever	212 (97)	68 (97)	> 0.99
Fever to diagnosis, d	4.1 (2.2)	2.5 (2.2)	< 0.001
Eschar/eschar-like lesions	195 (89)	68 (97)	0.051
Lymphadenopathy	37 (17)	21 (30)	0.018
Genotype (Karp:Kato:Kawasaki) ^a	26:18:16	7:2:2	0.530

^a The genotype of *Orientia tsutsugamushi* had been determined in 71 patients. Data are presented as n (%) or mean (standard deviation).

Discussion

In this study, we found hepatic dysfunction is common in scrub typhus, especially in patients without eschar. The risk factors associated with serum aminotransferase elevation included older age, longer fever duration, and absence of lymphadenopathy, but not probably the genotypes of *O. tsutsugamushi*. Furthermore, serum aminotransferase elevation can aid in the diagnosis for scrub typhus, especially in those without eschar.

A few previous studies demonstrated that serum aminotransferase elevation was common in scrub typhus patients, ranging from 77% to 96%, both in adults and in children^{7,8,12}; however, the case numbers were relatively small and there were no nontyphus control groups. The associated factors and clinical implications of abnormal liver function were not well characterized. We found that older age, longer fever duration, and less lymphadenopathy were associated with serum aminotransferase elevation. Longer fever duration suggests a prolonged systemic inflammation, including hepatic inflammation. A recent

study demonstrated that infection of dendritic cells and activated inflammatory monocytes offers a potential route for dissemination of *O. tsutsugamushi* from the initial eschar.¹³ However, being an obligate intracellular pathogen, *O. tsutsugamushi* had been visualized in infected hepatocytes.¹⁴ Watanabe et al¹⁵ suggested a direct hepatocellular cytopathic effect by *Rickettsiae*, rather than indirect immunoreactions. These lines of evidence suggested that host immune reaction, rickettsial load, and virulence may all play some role in hepatic dysfunction. Various clinical features (presence of eschar or rash) were reported in different genotypes of *O. tsutsugamushi*¹⁶; however, we failed to find a significant association between elevated serum aminotransferase and the genotypes of *O. tsutsugamushi*, which probably is related to only 25% of the study cohort with recognized genotypes.

The clinical implication of serum aminotransferase elevation as a diagnostic predictor was further investigated. Multivariate logistic regression showed that serum aminotransferase elevation independently predicts scrub typhus (OR: 3.75; $p = 0.003$; 95% CI: 1.56–9.01). Although eschar is pathognomonic for scrub typhus, its existence varies from 7% to 97% in different geographic regions.^{3,17,18} Patients are often unaware of eschars because these are painless and nonpruritic. Furthermore, eschars are difficult to detect in patients with dark skin and are hard to distinguish from scabs caused by trauma.¹⁹ In our patients, 19 (34%) nontyphus patients were reported to have eschar-like lesions at the initial presentation. Scrub typhus remains a diagnostic challenge even in the presence of eschar, not to mention those without eschar. The possibility of eschar or lymphadenopathy being neglected in our cohort was low because a detailed physical examination would be performed for any suspected scrub typhus cases to be notified to Taiwan CDC. In our study, we found that, among suspected scrub typhus patients without eschar, up to 92% of true scrub typhus patients would have serum aminotransferase elevation. After adjusted for other variables, serum aminotransferase elevation remained the only predictor for scrub typhus in patients without eschar, further supporting the diagnostic role of hepatic dysfunction in clinical use.

Our study had a few limitations. First, this is a retrospective study, and the information bias cannot be excluded. The genotypic information of *O. tsutsugamushi* was evaluated only in 25% of the patients; therefore, its influences were not well characterized. Clinicians may

Table 3 Predictors for scrub typhus, detected by multivariate logistic regression analysis in 344 clinically suspected scrub typhus cases

Parameter	Odds ratio	p	95% CI
Age (1 y increment)	1.01	0.606	0.99–1.03
Sex	2.59	0.046	1.02–6.61
Male vs female			
Thrombocytopenia	3.23	0.011	1.31–7.94
Platelet ≥ 120 k/ μ L			
vs < 120 k/ μ L			
Serum aminotransferase	3.75	0.003	1.56–9.01
Elevated versus normal			
Fever	6.10	0.025	1.26–29.50
Presence vs absence			
Eschar/eschar-like lesions	38.43	<0.001	15.64–94.41
Presence vs absence			
Lymphadenopathy	4.15	0.041	1.06–16.26
Presence vs absence			

CI = confidence interval.

Table 4 Characteristics of 62 clinically suspected patients of scrub typhus without eschar

Parameter	Scrub typhus N = 25	Non-scrub typhus N = 37	Univariate	Multivariate		
			p	OR	p	95% CI
Age, y	38 (20)	44 (22)	0.291	0.99	0.682	0.96–1.02
Male	22 (88)	23 (62)	0.025	3.63	0.121	0.71–18.53
Laboratory abnormality						
Leukopenia	7 (28)	4 (11)	0.082			
Thrombocytopenia	14 (56)	9 (24)	0.011	3.39	0.058	0.96–12.03
Serum aminotransferase elevation	23 (92)	20 (54)	0.002	6.47	0.028	1.23–34.11
Clinical symptoms and signs						
Fever	25 (100)	36 (97)	> 0.99			
Headache	13 (52)	18 (49)	0.796			
Chills	12 (48)	20 (54)	0.640			
Myalgia	8 (32)	12 (32)	0.971			
Lymphadenopathy	4 (16)	2 (5)	0.210	2.65	0.348	0.35–20.21
Rash	2 (8)	6 (16)	0.456			

Data are presented as n (%) or mean (standard deviation).
CI = confidence interval; OR = odds ratio.

notify Taiwan CDC for scrub typhus in febrile patients without an obvious infection focus but hepatic dysfunction; therefore, we observed a higher (54%) than expected (30–40%) nontyphus patients with aminotransferase elevation. However, an overestimation of hepatic dysfunction in scrub typhus was unlikely because a missed diagnosis in scrub typhus patients with normal liver function would lead to unremitting fever.

In summary, serum aminotransferase elevation is common in patients with scrub typhus, and hepatic dysfunction is associated with older age, longer fever duration, and absence of lymphadenopathy. Serum aminotransferase elevation independently predicted scrub typhus, especially in suspected scrub typhus patients without eschar. Our findings suggest an easy, inexpensive liver function test to aid in the diagnosis of scrub typhus.

Conflicts of interest

The authors declare no competing financial interests.

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