

Original Research Article

Prominent early adulthood hyperferritinemia in scrub typhus

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ABSTRACT

Background: Scrub typhus is an acute infectious disease, caused by *Orientia tsutsugamushi*. It is endemic to a geographically distinct region of Japan, Taiwan, China, and South Korea, known as the tsutsugamushi triangle, as well as in Nepal, Pakistan, and Papua New Guinea. In India, the incidence of this disease varies from 4-46% with high morbidity and mortality.

Methods: In this cross-sectional study at a tertiary care hospital in Kolkata, India we analysed ferritin blood levels along with different demographic and laboratory test parameters of 60 suspected scrub typhus patients who were also scrub typhus IgM antibody positive during a one-year study period.

Results: It was found that serum ferritin levels were unusually high in 21-40 years age group patients along with mildly increased urea and creatinine levels. Other important findings were below 40 years of age males were mostly affected, but above 40 years of age, females were generally affected. Haemoglobin and platelet levels were normal in all age groups. Although WBC count and neutrophils were normal in the 21-40 years age group, in other age groups increased levels were observed. CRP, SGPT, and SGOT levels were uniformly high above 20 years of age. Electrolytes were mildly decreased.

Conclusions: It appears that oxidative stress-induced acute kidney injury may be an important reason for the rising ferritin levels in the 21-40 years age group. These important findings may be helpful to clinicians in management of these patients.

Keywords: Acute kidney injury, Ferritin, *Orientia tsutsugamushi*, Scrub typhus

INTRODUCTION

Scrub typhus (also known as tsutsugamushi disease) is an acute dangerous infectious disease, caused by *Orientia tsutsugamushi*. It is transmitted by the bite of a larval *Leptotrombidium* mite (chigger).¹ *O. tsutsugamushi* is maintained by transovarial transmission in trombiculid mites. After hatching, infected larval mites (chiggers, the

only stage that feeds on a host) inoculate organisms into the skin. Infected chiggers are likely to be found in areas of heavy scrub vegetation during the wet season when mites lay eggs.²

It is endemic to a geographically distinct region, the so-called tsutsugamushi triangle, which includes Japan, Taiwan, China, and South Korea. It also occurs in Nepal,

Pakistan, and Papua New Guinea stationed in the rural or jungle areas in Pacific Theatre.³ During World War II scrub typhus killed thousands of troops who were stationed in the rural or jungle areas in the Pacific Theatre.⁴ In 1916 Weil and Felix described heterophilic antibody aggregation of OX-2 and OX-19 strains of *Proteus mirabilis* by typhus sera. Fletcher and Lesslar extended these to scrub typhus in 1926, they named another agglutinated variety strain OX-K. Ogata in 1931 isolated the organism and named it *Rickettsia tsutsugamushi*, it has been reclassified as *Orientia tsutsugamushi*.⁵

The target cells are endothelial cells, monocytes and other cell types and the disease is associated with vasculitis and endothelial dysfunction. Symptoms like headache, fever, rash, myalgia, cough and lymphadenopathy occur after 6-12 days of incubation.³

Clinical diagnosis of scrub typhus is often made by the presence of an eschar and the treatment is often started mainly on clinical grounds. Tetracycline 500 mg, QID or doxycycline 200 mg, OD for seven days is the treatment of choice. Chloramphenicol 500mg, QID is a choice. Rifampicin, 900 mg per day for a week, has been found effective in patients who respond poorly to conventional therapy.⁵ Identification of eschars in the Indian population is difficult due to dark skin with incidence ranging from 4%-46%.⁶

Few studies have reported scrub typhus-associated haemophagocytic lymphohistiocytosis (HLH) and multiple organ dysfunction syndrome (MODS). HLH is a rare and deadly hyperinflammatory syndrome, characterised by the over-activation of T lymphocytes and macrophages, along with the weakening of natural killer (NK) cells and cytotoxic T lymphocytes. This results in uncontrolled histiocytic phagocytosis of blood cells and cytokine-mediated multiple organ dysfunction.^{7,8}

The objectives of this study were to analyse blood levels of ferritin in patients suffering from scrub typhus and to find out any demographic variable associated with increased ferritin levels in blood in this disease.

METHODS

This is a cross-sectional study, conducted at Peerless Hospitex Hospital and Research Center Limited, Kolkata, West Bengal, India. The study was done according to Institutional Ethical Committee guidelines after getting permission for the study without revealing patients' identities. The period of study was for 12 months (August 2022 to July 2023). During this study period, several patients were admitted with fever and severe headache but were tested negative for commonly tested infective profiles according to local guidelines for infectious disease (dengue NS1 antigen, malaria parasite, typhidot IgM, SARS-CoV-2, hepatitis viruses, HIV etc.). Scrub typhus IgM Microelisa test was done using the patient's serum or plasma to detect the presence of IgM Scrub typhus

antibody. Out of all the suspected patients, a total of 60 patients were found to be positive for IgM Scrub typhus antibody during this study period.

Inclusion criteria

Inclusion criteria were patients with common symptoms of scrub typhus, all common tests for pyrexia were negative, and the IgM Scrub typhus antibody was positive.

Exclusion criteria

Exclusion criteria were positive common tests for pyrexia and IgM scrub typhus antibody negative results.

Serum ferritin was estimated from the EDTA blood sample of the patients. Plasma was separated after centrifugation at 3,000 rpm for 15 min and stored at -80°C. Samples were processed in batches of 10-15 samples every week using the chemiluminescence principle on the ADVIA Centaur Ferritin System (Siemens Healthcare Diagnostics, Los Angeles, CA) as per the manufacturer's instructions. Scrub Typhus IgM Microelisa test is an enzyme immunoassay based on "Indirect ELISA". Recombinant proteins representing immunodominant epitopes of Scrub Typhus were coated onto microtiter wells. Specimens and controls were added to the microtiter wells and incubated. IgM antibodies to scrub typhus if present in the specimen, bind to the specific Scrub Typhus antigen adsorbed onto the surface of the wells. The plate was then washed to remove unbound material. Horseradish peroxidase (HRP) conjugated Anti-Human IgM was added to each well. This conjugate binds the Scrub Typhus antigen-antibody complex present. Finally, a substrate solution containing chromogen and hydrogen peroxide was added to the wells and incubated. A blue colour developed in proportion to the amount of Scrub Typhus IgM antibodies present in the specimen. The colour reaction was stopped by a stop solution: The enzyme-substrate reaction was read by an EIA reader for absorbance at a wavelength of 450 nm along with 630 nm (reference filter). If the sample does not contain scrub typhus IgM antibodies then enzyme conjugate will not bind and the solution in the wells was either colourless or only a faint background colour developed.

RESULTS

It was found that below 40 years of age males were mostly affected, however above 40 years of age females were mostly affected. There was no significant difference in haemoglobin levels in different age groups and haemoglobin levels were largely normal. WBC count and neutrophils were normal in the 21-40 years age group, but in other age groups, WBC count and neutrophils were increased. Platelet count was within the normal range in all the age groups. SGPT and SGOT were normal in patients below 20 years of age but they were high in other groups. Alkaline phosphatase, total protein and albumin were more or less normal in all the age groups. CRP was increased in

all the age groups but the increase is less in the below 20 years age group. Sodium and potassium were mildly decreased. Urea and creatinine mildly increased in the 21-

40 years age group. Ferritin levels were markedly increased in this age group. Details of all results are given in Table 1, Table 2, Figure 1.

Table 1: Results of diverse laboratory tests in different age groups.

Age group (years) n=60	<20 n=11 M-10 (90.9%) F-1 (9.1%) Mean±SD±SEM	21-40 n=11 M-9 (81.8%) F-2 (18.2%) Mean±SD±SEM	41-60 n=21 M-7 (33.3%) F-14 (66.7%) Mean±SD±SEM	>61 n=17 M-9 (52.9%) F-8 (47.1%) Mean±SD±SEM
Hb (gm/dL)	11.03±1.18±0.35	11.86±2.32±0.8	15.4±20.79±4.65	15.31±20.22±4.90
WBC (/cu mm)	11221.81±4750.95±14 32.46	7793.75±3313.27±117 1.41	10585.78±4922.11±112 9.21	11745.29±4916.53±1192 .43
Neutrophil (%)	62.72±11.36±3.42	66.71±5.84±2.21	72.2±9.62±2.15	75.11±12.14±2.94
Platelet (/Cu mm)	228363.63±73791.19± 22248.88	149750±66156.53±23 389.86	167526.31±137718±31 594.67	133352.94±49879.78±12 097.62
SGPT (U/L)	36.12±7.67±2.71	112±72.06±25.47	93.64±78.97±19.15	101.05±103.86±25.19
SGOT (U/L)	52.62±11.25±3.98	104.5±54.63±19.31	168.5±201.75±47.55	123.88±119.80±29.05
Alkaline phosphatase (IU/L)	154.22±51.28±18.13	139±48.2±18.21	167.76±85.41±20.71	129.87±78.16±19.54
Total protein (gm/dL)	6.78±0.87±0.33	6.82±0.63±0.24	6.6±0.74±0.17	6.27±0.77±0.19
Albumin (gm/dL)	3.67±0.68±0.25	3.33±0.54±0.20	3.24±0.89±0.23	2.97±0.55±0.14
CRP (mg/dL)	70.45±50.89±15.34	136.83±126.55±51.66	120.47±75.80±18.36	132.86±74.58±19.25
Sodium (mEq/L)	135±1.78±0.8	130.37±4.09±1.44	131.38±4.04±0.95	129.82±13.13±3.18
Potassium (mEq/L)	4.28±0.81±.36	4.23±0.27±.09	4±0.68±0.16	3.74±0.70±0.17
Urea (mg/dL)	21.75±6.86±3.43	49.2±62.09±27.76	33.08±36.46±10.52	39.69±19.65±5.45
Creatinine (mg/dL)	0.51±0.19±0.067	1.35±1.18±0.48	0.89±0.72±0.16	1.12±0.46±0.11
Ferritin (ng/ml)	251.66±102.45±59.15	2061.66±2450.45±14.77	460.8±209.66±93.76	632.75±125.10±62.55

Table 2: Statistical analysis of the data between different groups.

	A-B	A-C	A-D	B-C	B-D	C-D
	P value					
Hb(gm/dL)	0.290	0.475	0.472	0.598	0.598	0.989
WBC (/cu mm)	0.069	0.720	0.776	0.116	0.033	0.474
Neutrophil (%)	0.338	0.0159	0.009	0.1084	0.051	0.414
Platelet	0.0169	0.167	0.0003	0.703	0.471	0.338
SGPT (iu/ml)	0.001	0.017	0.040	0.539	0.771	0.804
SGOT(iu/ml)	0.004	0.057	0.051	0.336	0.635	0.427
Alkaline phosphatase (u/dL)	0.487	0.619	0.356	0.331	0.742	0.166
Total protein (gm/dL)	0.904	0.532	0.107	0.425	0.067	0.187
Albumin (gm/dL)	0.215	0.158	0.004	0.771	0.110	0.282
CRP (mg/L)	0.110	0.050	0.018	0.655	0.918	0.616
Sodium (mEq/L)	0.002	0.006	0.188	0.522	0.899	0.608

Continued.

	A-B	A-C	A-D	P value	B-C	B-D	C-D
Potassium (mEq/L)	0.854	0.296	0.065		0.314	0.045	0.255
Urea (mg/dL)	0.142	0.297	0.005		0.368	0.560	0.506
Creatinine (mg/dL)	0.0241	0.084	0.0002		0.188	0.476	0.261
Ferritin	0.018	0.003	<0.0001		0.005	0.022	0.005

Group A: < 20 years; Group B: 21-40 years; Group C: 41-60 years; Group D: >60 years

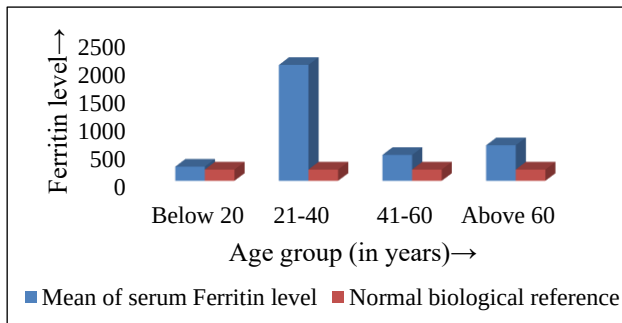


Figure 1: Mean serum ferritin levels of patients compared with normal biological reference in different age groups.

DISCUSSION

After its re-emergence, scrub typhus has become an important cause of morbidity and mortality in patients presenting with febrile illness during monsoon and post-monsoon season.⁹ According to WHO, scrub typhus is probably one of the most underdiagnosed and underreported febrile illnesses requiring hospitalization.¹⁰

We conducted a study in our institute, which is a tertiary care hospital in Kolkata on 300 febrile patients and performed a scrub typhus IgM test. 60 patients were found to be positive for scrub typhus. Common signs and symptoms in such patients were fever for more than a week, malaise, headache, nausea with or without vomiting, cough, and gastrointestinal symptoms. Few had maculopapular rash. Eschar was even rare to be found only in 4 patients.

Serological tests of scrub typhus are usually possible 5-10 days after disease onset. The Weil-Felix test is the most commonly used serological test, but it has poor specificity and sensitivity.¹¹ One study reported that the sensitivity and specificity of the Weil-Felix test and IgM enzyme-linked immunosorbent assay (ELISA) were 47%, 96%, 91%, and 100%, respectively.¹² Studies have reported that IgM ELISA and IgM capture ELISA techniques, and PCR-based tests have better predictive value in the diagnosis of scrub typhus.^{13,14}

It has been highlighted that iron may not serve as an essential nutrient for intracellular obligate bacteria like *Chlamydia* and *Rickettsial* species. These organisms tend to grow in an iron-deficient media to avoid excessive free

iron stress and do not have functional homology sequences to siderophore synthesis enzymes in their genomes.^{15,16} Ellison et al in their experimental study supported this hypothesis that there is a minimal transcriptional response after iron depletion in rickettsial culture exposed to iron chelator 24 hr after in vitro treatment.¹⁷ Moreover, it is observed that *Orientia tsutsugamushi* infects human monocytic cells and increases expression of interferon type-1 genes resulting in an M1 type of monocyte-macrophage phenotype, which sequesters iron within macrophages.¹⁸

Although it is challenging to elucidate why in 21-40 years age group ferritin levels were distinctly augmented studies indicated that there was both liver and kidney damage in this age group. It is well known that ferritin can guard from oxidative stress by requisitioning iron and thus limiting the formation of reactive oxygen species.¹⁹ Oxidative stress is known to happen in scrub typhus.²⁰ In oxidative stress-induced acute kidney injury, it is the protective mechanism of the body to escalate ferritin for recovery from acute kidney injury.¹⁹ Perhaps due to this, the ferritin levels were more in this age group (Figure 2).

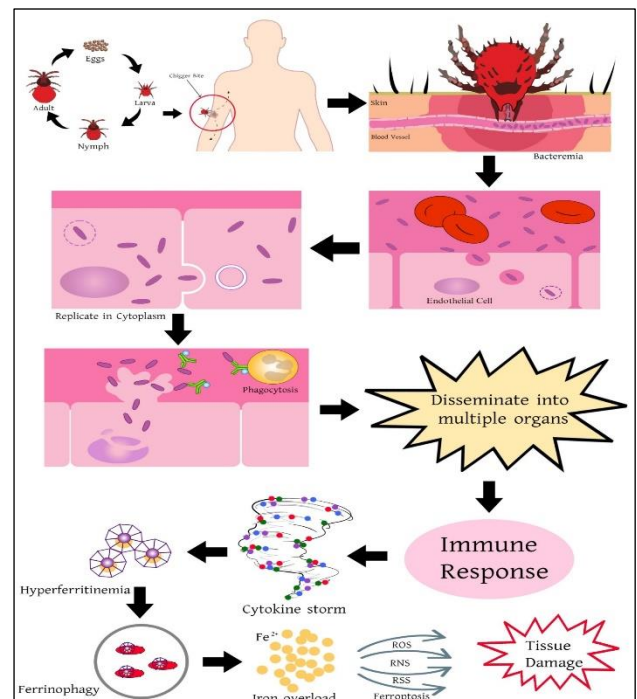


Figure 2: Mechanism of high ferritin levels in scrub typhus.

There were some limitations in this study. The study was done in a single tertiary care hospital; if the study were done in several hospitals, the results would be more appropriate. Similarly, analysis with more positive cases may give better results.

CONCLUSION

The study revealed significantly increased ferritin levels in the 21-40 years age group. Further analysis in this age group shows scrub typhus-associated oxidative stress-induced acute kidney injury may be an important reason behind the ferritin rise. These findings will be useful for clinicians in the management of such patients.

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