Hyperferritinemia in pediatric scrub typhus: A prospective observational study

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Abstract

Background: Hyperferritinemia is increasingly associated with mortality in sepsis. Studies estimating the prevalence of hyperferritinemia in pediatric scrub typhus is limited.

Methods: We conducted a prospective observational study from a tertiary care teaching hospital in North India where 72 children with confirmed scrub typhus; 17(24%)-PCR positive, 68(94%)-IgM ELISA positive and 13(18%)-both PCR and ELISA positive. Serum ferritin was measured in 62 children to identify the prevalence of hyperferritinemia and determine its association with mortality.

Results: Hyperferritinemia ($>500\mu\text{g/L}$) was seen in 72.6% [$n=45$] children; 26(42%) were mild ($500\text{--}2000\mu\text{g/L}$), 13(21%) were moderate ($2000\text{--}10000\mu\text{g/L}$) and 6(9.7%) were severe ($>10000\mu\text{g/L}$). A biphasic pattern of hyperferritinemia was seen with two peaks when plotted against duration since symptoms onset; one between 2 and 3 days (early onset hyperferritinemia) and second peak between 12 and 14 days (late onset hyperferritinemia). Early onset hyperferritinemia had more survivors than late onset hyperferritinemia. Non survivors had significantly higher PRISM III, PELOD-2, hyperlactatemia, hypoalbuminemia, organ dysfunction, need for mechanical ventilation and need of RRT. Ferritin had poor sensitivity and specificity in predicting survival with AUC of 0.56. Organ dysfunction and risk scores as PRISM III, PELOD 2 and VIS at admission were better predictors with AUC(95%CI) of 0.72(0.56,0.89), 0.77(0.63,0.92) and 0.90(0.78,1.0) respectively.

Conclusions: Hyperferritinemia is common in scrub typhus. A distinct biphasic response was observed with worse outcome in late onset hyperferritinemia. Organ dysfunction and risk scores were better predictors of mortality than ferritin.

Background

Scrub typhus is a common cause of acute undifferentiated fever in Asian countries [1]. The disease manifests with protean clinical features varying from undifferentiated fever to several organ dysfunctions which include acute respiratory distress syndrome (ARDS), myocarditis, acute kidney injury (AKI), encephalitis, acute hepatic failure, and culminating in multiorgan dysfunction syndrome (MODS) [2, 3]. Early suspicion and treatment is essential as delay increases morbidity and mortality multifold. A significant overlap of symptoms with other viral and certain bacterial sepsis makes the diagnosis difficult and hence, a syndromic approach is recommended [4].

The pathogenesis differs from other bacterial causes of sepsis in early and severe involvement of vascular endothelium [5]. Hematological dysfunction is prominent; most have thrombocytopenia with varying degrees of anemia and disseminated intravascular coagulation (DIC) [6]. Other organ dysfunctions are not uncommon and have an additive association with mortality [7–10].

Several clinical and laboratory parameters have been studied to predict organ dysfunction and survival in children with scrub typhus with inconsistent results [2, 10–12]. Ferritin can be an attractive marker as

hematological dysfunction is prominent in scrub typhus especially in those with MODS [13]. From a mere marker of iron stores, ferritin's role in disease pathogenesis and immunomodulation is increasingly being explored in sepsis [13–16]. Hyperferritinemic syndrome seen in critically ill children is attributed secondary to hypercytokinemia and macrophage activation, both well known in scrub typhus [17]. However, robust studies on ferritin measurements in this cohort is lacking.

We hypothesized that in pediatric scrub typhus, early elevated ferritin may help identify the severe cases that progress to MODS and eventual death. Hence, we planned this observational study to measure serum ferritin in children with confirmed scrub typhus and determine its association with disease severity and outcome.

Methods

This was a single center, prospective study at a tertiary care teaching hospital in North India. Children aged between 1 month and 12 years admitted to Pediatric Emergency Department and Pediatric Intensive Care Unit (PICU) of the Advanced Pediatrics Center, Postgraduate Institute of Medical Education and Research were consecutively enrolled from July 2019 to December 2019 (6 months), the season with peak incidence of tropical infections. We chose this age cut off as the upper age limit for pediatric admission is 12 years as per hospital policy.

Children with acute febrile illness (fever $>38.3^{\circ}\text{C}$ for more than 48 hours' duration and onset <14 days) with either (a) cytopenia (platelet count $<1,00,000/\text{mm}^3$, and/or hemoglobin $<10\text{gm/dl}$), (b) organomegaly (hepatomegaly/splenomegaly), (c) lymphadenopathy or (d) systemic signs (rash, edema) were screened for eligibility and included if they were confirmed scrub typhus either by PCR or IgM ELISA. Those who received a transfusion were excluded. Children who met the eligibility criteria were enrolled consecutively after obtaining a written informed consent. Demographic, clinical and laboratory data were collected. Complete blood counts, renal and liver function tests were performed at presentation for all cases and were repeated if necessary. Acute kidney injury was staged as per KDIGO guidelines [18]. Elevation in transaminases was defined as >5 times elevation of Aspartate transaminase (AST) and/or Alanine transaminase (ALT) from baseline. Treatment related variables, severity score (PRISM III, PELOD2), details of organ supportive therapies, length of PICU stay and hospital outcome were recorded.

Diagnosis of scrub typhus was confirmed by PCR and/or IgM ELISA. Nested PCR was performed using the DNA extracted from the whole blood and was amplified to detect *Orientia tsutsugamushi* DNA. We used the oligonucleotide primers that were based on the nucleotide sequences of a gene encoding for the 56-kDa antigen of a Gilliam strain of *Orientia tsutsugamushi*. ELISA for scrub typhus IgM antibody was performed using Scrub Typhus Detect IgM ELISA (InBios International Inc., Seattle, WA). An optical density of ≥ 0.5 was considered as diagnostic for scrub typhus.

Serum ferritin was estimated from the 2-3ml EDTA blood sample collected at admission. Plasma was separated after centrifugation at 3000rpm for 15 minutes 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 manufacturer's instructions. If clinical suspicion of hemophagocytic lymphohistiocytosis (HLH) (persistence of fever, worsening cytopenia and organ dysfunction) and/or elevated serum ferritin ≥ 2000 $\mu\text{g/L}$, then other relevant tests such as triglycerides, fibrinogen and serum soluble CD25 estimation and/or bone marrow examination was performed as per clinician's decision. Decision to treat HLH with immunomodulatory therapy (steroids, Ivlg or both) was at the treating clinician's discretion. The study protocol was approved by Institute Ethics Committee of the Postgraduate Institute of Medical Education and Research ((NK/5433/Study/594).

Definitions

Hyperferritinemia was defined as ferritin levels more than 500 $\mu\text{g/L}$ [13,17,19]. We stratified hyperferritinemia into mild (500-2000), moderate (>2000-10,000) and severe (>10,000) categories. We classified the subjects into early presenters (<7 days of febrile illness) and late presenters (>7 days of febrile illness) to see if day of presentation since fever onset affected degree of hyperferritinemia. Organ dysfunction was defined as per Pediatric Logistic Organ Dysfunction score (PELOD-2) [20]. PELOD-2 was done daily till 5 days or till discharge which ever was earlier. The vasoactive drugs used during therapy were monitored and daily Vasoactive inotrope score (VIS) was calculated [21].

Outcome

The **primary outcome** was to identify the prevalence of hyperferritinemia in children with scrub typhus. The **secondary outcomes** were to study the predictors of mortality in scrub typhus and determine the association of serum ferritin levels with duration of illness and mortality.

Statistical analysis

Descriptive statistics including frequency, mean, median, interquartile range (IQR), and standard deviation (SD) were calculated for the demographic data and laboratory parameters. The chi-square test was used to compare categorical data and the Mann Whitney tests were used to compare nonparametric, continuous data. Predictors of mortality were identified using bivariate and multivariate logistic regression. Ferritin values of survivors were compared with non survivors. The sensitivity and specificity for different cut off values of ferritin were analyzed using a receiver-operating characteristic (ROC) curve and were compared with organ dysfunction and risk scores. For all tests, a two-sided *P* value of < 0.05 was considered statistically significant. All statistical analyses were performed using SPSS software version 23.0 (SPSS Inc., Chicago, IL).

Results

Baseline characteristics

During the 6 months of study period, 10158 children visited Pediatric Emergency Department and 4130 of them required admission. A total of 242 children were eligible and only 72 children met the inclusion criteria. Of them, 17 (23.6%) were PCR positive, 68 (94.4%) were ELISA positive and 13 (18%) were both PCR and ELISA positive. The median (IQR) age of presentation was 60 (36,96) months with a male preponderance (n = 45, 62.5%) (Table 1). The median (IQR) PRISM III and PELOD 2 score at admission were 8 (5, 12) and 3 (2, 7) respectively. Fever was present in all; generalized edema, fast breathing and abdominal pain were other common symptoms. Hepatomegaly was seen in 66 (91.7%) and splenomegaly in 43 (59.7%) children. Eschar, a pathognomonic clue to trombiculid bite was seen only in 14 (19.4%) children. A rash was noted in 11% patients which varied from macular, blanching erythematous rash to petechial purpuric lesions.

Table 1
Clinical profile, investigations and outcomes

Parameter	N = 72
Age in months	60 (36,96)
Boys, n(%)	45 (62.5)
Weight in kg	16.0 (11.4, 20.75)
Weight in Z score	-1.37 (-2.18, -0.24)
PRISM III	8 (5, 12)
Duration of illness in days	7 (5, 10)
Clinical features	
Fever, n(%)	72 (100)
Edema, n(%)	35 (48.6)
Fast breathing, n(%)	27 (37.5)
Abdominal pain, n(%)	25 (34.7)
Bleeding manifestations, n(%)	17 (23.6)
Altered sensorium, n(%)	16 (22.2)
Seizures, n(%)	16 (22.2)
Headache, n(%)	14 (19.4)
Purpuric rash, n(%)	8 (11.1)
Oliguria, n(%)	6 (8.3)
Hepatomegaly, n(%)	66 (91.7)
Splenomegaly, n(%)	43 (59.7)
Eschar, n(%)	14 (19.4)
Investigations	
Hemoglobin, g/dl	8.7 (7.4, 10.2)
Platelet count cells/mm ³	44000 (18000, 80000)
Sodium, mmol/L	133 (130, 137)
Albumin, g/dl	2.2 (2.1, 2.6)

All values expressed as Median (IQR)], PRISM - Pediatric Risk of Mortality

Parameter	N = 72
Total bilirubin, mg/dl	0.8 (0.6, 1.9)
Aspartate transaminase, U/L	185 (94, 285)
Alanine transaminase, U/L	79 (49, 125)
Creatinine, mg/dl	0.4 (0.25,0.5)
International Normalized Ratio	1.2 (1.0, 1.5)
Treatment	
Doxycycline, n(%)	65 (90.3)
Azithromycin, n(%)	7 (9.7)
Ivlg only, n(%)	2 (2.8)
Ivlg with steroid, n(%)	2 (2.8)
Outcome	
Survivors, n(%)	60 (83.3)
Length of PICU stay, days	5 (4, 9)
Length of hospital stay, days	5 (3, 8)
All values expressed as Median (IQR)], PRISM - Pediatric Risk of Mortality	

The median (IQR) Hemoglobin was 8.7 (7.4, 10.2) g/dL (Table 2). The median (IQR) platelet count was 44000 (18000, 80000) cells/mm³. Severe hypoalbuminemia was common; more than half had serum albumin < 2.5 g/dL at admission. Elevated liver enzymes (> 5 times elevation of AST/ ALT) was seen in 27 of 65 children (41.5%).

Table 2
Univariate analysis comparing survivors with non survivors

Parameter	N	Total (n = 72)	Survivors (n = 60)	Non survivors (n = 12)	P value
Age, years	72	60 (36,96)	60 (30,98)	36 (31, 105)	0.60
Boys, n(%)	72	45 (62.5)	41 (68.3)	4 (33.3)	0.04
PRISM III	72	8 (5, 12)	8 (5, 11)	12 (8.7, 17.5)	0.005
Duration of illness, days	72	7 (5, 10)	7 (5, 9.5)	9 (5, 12)	0.38
Investigations					
Ferritin, µg/L	62	668 (420, 2714)	666 (382, 2653)	1806 (443,4727)	0.53
Normal (< 500),n(%)		17 (27.4)	15 (28.8)	2 (20)	0.42
Mild (> 500–2000),n(%)		26 (41.9)	23 (44.2)	3 (30)	
Moderate (> 2000–10000),n(%)		13 (21)	10 (19.2)	3 (30)	
Severe (> 10000), n(%)		6 (9.7)	4 (7.8)	2 (20)	
Early onset hyperferritinemia, n(%)	45	25 (55.5)	22 (59.5)	3 (37.5)	0.22
Late onset hyperferritinemia, n(%)		20 (44.5)	15 (40.5)	5 (62.5)	
Hemoglobin, g/dl	72	8.7 (7.4, 10.2)	8.6 (7.5, 10.5)	8.1 (6.8, 10.1)	0.48
Total leukocyte count cells/mm ³	72	14000 (9820, 18000)	14090 (10200, 17500)	12465 (7020, 21080)	0.39
Platelet count cells/mm ³	72	18000 (44000, 80000)	19000 (48000, 80000)	31000 (11500, 100000)	0.39
Sodium, mmol/L	72	133 (130, 137)	133 (130, 136)	138 (133, 141)	0.03
International Normalized Ratio	56	1.2 (1.0, 1.5)	1.19 (1, 1.5)	1.3 (1.1, 1.6)	0.36
Aspartate transaminase, U/L	65	185 (94, 285)	179 (94, 257)	239 (88, 378)	0.26

All values expressed as Median (IQR)], PRISM - Pediatric Risk of Mortality, PELOD- Pediatric Logistic Organ Dysfunction, ARDS- Acute Respiratory distress syndrome, VIS- Vasoactive inotrope score, AKI- Acute Kidney Injury, RRT- Renal Replacement therapy

Parameter	N	Total (n = 72)	Survivors (n = 60)	Non survivors (n = 12)	P value
Alanine transaminase, U/L	65	79 (49, 125)	69 (45, 122)	88 (52, 139)	0.58
Albumin, g/dl	58	2.2 (2.1, 2.6)	2.3 (2.1, 2.6)	2.1 (1.9, 2.2)	0.05
Severe hypoalbuminemia, n(%)	58	39 (54.2)	30 (50)	9 (75)	0.14
Lactate, mmol/L	72	1.6 (1.1, 2.6)	1.6 (1, 1.8)	3.0 (1.85, 9.8)	0.04
Organ dysfunction					
Hematological dysfunction	72	68 (94.4)	56 (93.3)	12 (100)	0.60
Respiratory failure	72	40 (55.5)	28 (46.6)	12 (100)	0.001
ARDS, n(%)	72	23 (31.9)	13 (21.7)	10 (83.3)	< 0.0001
Mechanical ventilation, n(%)	72	34 (47.2)	22 (36.7)	12 (100)	0.0001
Shock, n(%)	72	20 (28.2)	12 (20.3)	8 (66.7)	0.003
VIS on day 1	72	0 (0,10)	0 (0,0)	10 (0,30)	0.0001
VIS on day 3	67	0 (0,10)	0 (0,0)	20 (10,86)	0.0001
VIS on day 5	64	0 (0,0)	0 (0,0)	20 (12,27)	0.0001
AKI, n(%)	72	15 (20.8)	10 (16.7)	5 (41.7)	0.02
RRT, n(%)	72	5 (6.9)	2 (3.3)	3 (25)	0.03
CNS dysfunction, n(%)	72	10 (13.9)	6 (10)	4 (33.3)	0.05
Coagulopathy (INR > 1.5),n(%)	56	12 (21.4)	10 (21.3)	2 (22.2)	1.0
Elevated transaminases, n(%)	65	27 (41.5)	20 (37.7)	7 (58.3)	0.21
PELOD-2 score					
Day 1	72	3 (2,7)	2 (2, 5)	7 (7,9)	< 0.0001
Day 2	67	2 (1,5)	2 (1, 5)	6 (4,7)	0.001
Day 3	64	2 (1,5)	2 (1,4)	4 (1,6)	0.32
All values expressed as Median (IQR)], PRISM - Pediatric Risk of Mortality, PELOD- Pediatric Logistic Organ Dysfunction, ARDS- Acute Respiratory distress syndrome, VIS- Vasoactive inotrope score, AKI- Acute Kidney Injury, RRT- Renal Replacement therapy					

Clinical course

A total of 36 (50%) children required PICU admission. Hematological dysfunction was the commonest organ dysfunction [n = 68, 94.4%]. Respiratory failure [n = 40, 56%] was the next common organ dysfunction; ARDS was seen in 31.9% children. Nearly half (n = 34, 47%) received mechanical ventilation. Twenty children (28.2%) had shock requiring vasoactive drug support. AKI was seen in 15 (20.8%) children and 5 (7%) required RRT. Encephalopathy with GCS < 10, was seen in 10 (13.9%) children. Sixty-five children were treated with doxycycline and 7 received azithromycin. The median (IQR) length of hospital stay was 5 (3,8) days. Sixty children survived to hospital discharge and all [n = 60, 83.3%] were alive at 28 day follow up. Four children (5.5%) had clinical features and investigations that fulfilled HLH diagnostic criteria. All 4 received intravenous immunoglobulin (Ivlg) and 2 of them were also treated with methylprednisolone. Of them, 2 survived and 2 died.

Hyperferritinemia

Ferritin estimation was possible in 62 children. The median (IQR) day of ferritin measurement since symptom onset was 7 (5,10) days. The median (IQR) ferritin level was 668 (42, 2714) µg/L. Seventeen children (27.4%) had ferritin in normal range. Hyperferritinemia (> 500 µg/L) was seen in 45 (72.6%) children; 26 (41.9%) were mild, 13 (21%) were moderate and 6 (9.7%) were severe. Ferritin levels were not significantly different between survivors and non survivors (p = 0.53).

We found a biphasic trend in ferritin elevation when plotted against the day of measurement since symptoms onset (Fig. 2). Children had two peak levels one between 2 and 3 days (early onset hyperferritinemia) and second peak between 12 and 14 days (late onset hyperferritinemia). Of the 25 children who had early onset hyperferritinemia, 22 (88%) whereas of 20 children who had late onset hyperferritinemia 15 (75%) survived. When the group was analyzed with respect to outcome, we found that early onset hyperferritinemia had more survivors than late onset hyperferritinemia, though this did not reach statistical significance [OR (95%): 2.4 (0.5, 11.4)]. (Additional file 1).

Predictors of outcome

On univariate analysis comparing survivors with non survivors, higher PRISM III, PELOD 2 scores, hyperlactatemia, hypoalbuminemia, presence of any organ dysfunction, need for mechanical ventilation and need of RRT were associated with increased mortality (Table 2). Ferritin though higher in non survivors [1806 (443,4727) µg/L] than survivors [666 (382,2653) µg/L], the difference was statistically not significant. We found that ferritin has poor sensitivity and specificity in predicting mortality with AUC (95%CI) of 0.56 (0.35,0.78) (Fig. 3). It was observed that higher ferritin values increased specificity to predict mortality. Ferritin value above 496 µg/L had 80% sensitivity and 29% specificity and value above 2000 µg/L had 50% sensitivity 73% specificity while value above 7880 µg/L had 20% sensitivity and 93% specificity. We found that organ dysfunction and risk score as PRISM III, PELOD 2 and VIS at admission were better predictors with AUC (95%CI) of 0.72 (0.56,0.89), 0.77 (0.63,0.92) and 0.90 (0.78,1.0) respectively. VIS score > 16.5 was 80% sensitive and 88% specific to predict mortality. A PELOD-2 > 7 had 80% sensitivity and 91% specificity while a PRISM III > 10 had 70% sensitivity and 71% specificity in predicting mortality.

Discussion

In our study we found that hyperferritinemia is common in scrub typhus and about a third (30.7%) had ferritin values more than 2000 µg/L. Severe hyperferritinemia (> 10,000 µg/L) was seen in 10%. Ferritin did not have good predictive ability for outcome though the non survivors had higher proportion of moderate and severe hyperferritinemia than survivors. We also observed a distinct biphasic trend with greater early onset hyperferritinemia in survivors and late onset hyperferritinemia in non survivors. PELOD-2 and VIS score fared better than ferritin in predicting mortality. Presence of ARDS was independently associated with poor outcome. The reported case fatality rate in scrub typhus varies between 1.5–30% with a median of about 8% [7, 22]. Our reported case fatality rate in this study is higher (16.7%). This may be due to the referral bias and the inclusion criteria selecting cases with high severity of illness as evident by a higher PRISM III and PELOD-2 scores.

Organ dysfunction is common in scrub typhus. The mortality is proportionate to the severity and number of organ systems involved [9]. An elevated SOFA score in adult studies have been independently associated with mortality [9]. We found organ dysfunction especially ARDS as an independent predictors of mortality. The median (IQR) PELOD2 scores at admission was 2 (2, 5) in survivors vs. 7 (7,9) in non survivors.

Ferritin has been measured as a marker to determine the body iron stores since 1970s [23] and ever since our understanding of ferritin has diversified with its role in inflammation, infection and malignancy. Surprisingly, the understanding about this molecule is still enigmatic despite 5 decades of its utility in clinical medicine. Despite the mention in 2004 HLH criteria, elevation of ferritin greater than 500 µg/L could still be nonspecific in infections. In our study too, hyperferritinemia was seen in 72.6%, reiterating its function as an acute phase reactant to infection. More than half had mild elevation, but severe hyperferritinemia was seen only in 10% of children. Be it changing epidemiology or increased recognition, hyperferritinemia as a syndrome is increasingly diagnosed in critically-ill children [24]. The pathogenesis of hyperferritinemia in sepsis/systemic inflammation continue to evolve in recent literature [25]. With growing evidence, ferritin is now believed to play a key role in normal host response to infection and immunomodulation rather than a nonspecific marker with sole function of sequestering iron from the circulation [15, 26].

Illness-appropriate response in ferritin is believed to correlate with favorable outcome. Both high and very low response were shown to be associated with increased mortality in patients with sepsis. Garcia et al observed a mortality of 23% and 58% when ferritin values were < 200 µg/L and > 500 µg/L respectively. However, patients with ferritin between 200 and 500 had only 9% mortality [14]. Bennett et al observed a stepwise increase in mortality risk in hospitalized children with ferritin $\geq$ 1,000 µg/L and $\geq$ 3,000 µg/L [27]. Our findings in children with scrub typhus are concurrent with these observations as non-survivors had greater frequency of moderate to severe hyperferritinemia when compared to survivors.

The biphasic response of ferritin elevation is an interesting observation in our cohort. There is limited scientific data available to reason out the possible explanation for an early and late peak for

hyperferritinemia in scrub typhus. However, review of available literature prompts us to suggest the following possible mechanism. It has been highlighted that iron may not serve as an essential nutrient for intracellular obligate bacteria like Chlamydia and Rickettsial species since they tend to grow in an iron deficient media to avoid excessive free iron stress and also do not have functional homology sequences to siderophore synthesis enzymes in their genomes [28, 29]. Another experimental study supporting this is by Ellison et al who proved that there is minimal transcriptional response after iron depletion in rickettsial culture exposed to iron chelator 24 hours after in vitro treatment [30]. Moreover, it is observed that *Orientia tsutsugamushi* infect human monocytic cells and increases expression of Interferon type-1 genes resulting in a M1 type of Monocyte-macrophage phenotype which sequesters iron within macrophages [31, 32].

With this knowledge, it is likely that in individuals who have relatively normal/ adequate iron stores, the M1 macrophage phenotype response following rickettsial infection leads to transient early hyperferritinemia due to macrophage sequestration of iron and increased transcription of ferritin gene as acute phase reactant. This response also leads to better Interferon and pro-inflammatory cytokine secretion by macrophage- resulting in better control of infection and hence a favourable outcome. However, in those with low or absent stores to start with, the same peak for hyperferritinemia is possibly delayed and is compounded by multiple other host and bacterial genome factors which need to be elucidated yet. The initial elevation may be due to the robust cytokine release during the early phase to clear the infection. Those with elevated ferritin at later phase (> 7 days) may be due to secondary macrophage activation and organ dysfunction that could explain the high mortality in this subgroup. Also, it is logical to appreciate that the higher mortality is strongly linked to the delay in receiving specific antimicrobial therapy in the late presenters.

Four children (5.5%) were treated for secondary HLH, of whom 2 survived. Diagnosis of HLH is often challenging in scrub typhus as children would present with fever, cytopenia and organomegaly at the outset. Ferritin elevation > 500 µg/L can be very nonspecific as seen in our cohort. All 4 children in our study had fulfilled HLH-2004 criteria. Secondary HLH may not require aggressive management when compared to primary HLH. Demirkol et al. showed 100% survival in children with hyperferritinemic sepsis MODS/MAS/HLH who were treated with methylprednisolone, IVIG, and plasma exchange, whereas those treated with the HLH-94 protocol using chemotherapy and dexamethasone had only 50% survival with all deaths occurring from overwhelming sepsis [17]. Our cohort received Ivlg as first line; steroids were added in 2 children.

Our study has some important strengths. This is one of largest prospective pediatric studies on scrub typhus evaluating the prevalence of hyperferritinemia. We included children with laboratory confirmed scrub typhus and followed them up to 28 days with daily evaluation of organ dysfunction. However, a few limitations need mention. Our cohort had greater proportion of severe scrub typhus cases which could have led to increase in the prevalence of hyperferritinemia. Serial ferritin measurements and its trend with respect to clinical course and treatment may give more answers regarding the pathogenesis.

Further studies are needed to elucidate the molecular basis behind elevation of ferritin at different time points of illness and understand its possible role for targeted therapy.

Conclusions

Hyperferritinemia is common in scrub typhus. Ferritin elevation showed a distinct biphasic response with worse outcome in late onset hyperferritinemia. Organ dysfunction, especially ARDS, was independently associated with mortality. Organ dysfunction scores were found to be better predictors of mortality than ferritin.

Declarations

Ethical approval and consent to participate

Trial was initiated after approval from the Institute Ethics Committee, Postgraduate Institute of Medical Education & Research, Chandigarh, India (NK/5433/Study/594). A written informed consent was obtained from a parent or guardian for all participants.

Consent for publication:

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request

Competing interest:

The authors declare that they have no competing interests

Funding:

None

Author's contributions

KN had full access to all the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis. All authors participated in the study concept and design. VW and NM had collected, analysed and interpreted study data. VW, NM and KN drafted the manuscript. PB helped in

ferritin estimation. MB helped in laboratory confirmation of scrub typhus through PCR and ELISA. SS did bone marrow examination in those suspected with HLH. JM and SS gave critical inputs during revision of the manuscript. JM and KN provided administrative, technical, or material support. All authors read and approved the final manuscript.

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Abbreviations

- AKI- Acute Kidney Injury
- ARDS- Acute Respiratory Distress Syndrome
- DIC- Disseminated Intravascular Coagulation
- ELISA- Enzyme linked immunosorbent assay
- Ivlg- Intravenous Immunoglobulin
- KDIGO- Kidney Diseases Improving Global Outcomes
- MODS- Multiorgan dysfunction syndrome
- PCR- Polymerase chain reaction
- PELOD- Pediatric Logistic Organ Dysfunction
- PICU- Pediatric Intensive Care Unit
- PRISM- Pediatric risk of mortality
- VIS- Vasoactive inotrope score

Tables

Table 1. Clinical profile, investigations and outcomes

Parameter	N=72
Age in months	60 (36,96)
Boys, n(%)	45 (62.5)
Weight in kg	16.0 (11.4, 20.75)
Weight in Z score	-1.37 (-2.18, -0.24)
PRISM III	8 (5, 12)
Duration of illness in days	7 (5, 10)
Clinical features	
Fever, n(%)	72 (100)
Edema, n(%)	35 (48.6)
Fast breathing, n(%)	27 (37.5)
Abdominal pain, n(%)	25 (34.7)
Bleeding manifestations, n(%)	17 (23.6)
Altered sensorium, n(%)	16 (22.2)
Seizures, n(%)	16 (22.2)
Headache, n(%)	14 (19.4)
Purpuric rash, n(%)	8 (11.1)
Oliguria, n(%)	6 (8.3)
Hepatomegaly, n(%)	66 (91.7)
Splenomegaly, n(%)	43 (59.7)
Eschar, n(%)	14 (19.4)
Investigations	
Hemoglobin, g/dl	8.7 (7.4, 10.2)
Platelet count cells/mm ³	44000 (18000, 80000)
Sodium, mmol/L	133 (130, 137)
Albumin, g/dl	2.2 (2.1, 2.6)
Total bilirubin, mg/dl	0.8 (0.6, 1.9)
Aspartate transaminase, U/L	185 (94, 285)
Alanine transaminase, U/L	79 (49, 125)
Creatinine, mg/dl	0.4 (0.25,0.5)
International Normalized Ratio	1.2 (1.0, 1.5)
Treatment	
Doxycycline, n(%)	65 (90.3)
Azithromycin, n(%)	7 (9.7)
IvIg only, n(%)	2 (2.8)
IvIg with steroid, n(%)	2 (2.8)
Outcome	
Survivors, n(%)	60 (83.3)
Length of PICU stay, days	5 (4, 9)
Length of hospital stay, days	5 (3, 8)

All values expressed as Median (IQR)], PRISM - Pediatric Risk of Mortality

Table 2. Univariate analysis comparing survivors with non survivors

Parameter	N	Total (n=72)	Survivors (n=60)	Non survivors (n=12)	P value
Age, years	72	60 (36,96)	60 (30,98)	36 (31, 105)	0.60
Boys, n(%)	72	45 (62.5)	41 (68.3)	4 (33.3)	0.04
PRISM III	72	8 (5, 12)	8 (5, 11)	12 (8.7, 17.5)	0.005
Duration of illness, days	72	7 (5, 10)	7 (5, 9.5)	9 (5, 12)	0.38
Investigations					
Ferritin, µg/L	62	668 (420, 2714)	666 (382, 2653)	1806 (443,4727)	0.53
Normal (<500),n(%)		17 (27.4)	15 (28.8)	2 (20)	0.42
Mild (>500-2000),n(%)		26 (41.9)	23 (44.2)	3 (30)	
Moderate (>2000-10000),n(%)		13 (21)	10 (19.2)	3 (30)	
Severe (>10000), n(%)		6 (9.7)	4 (7.8)	2 (20)	
Early onset hyperferritinemia, n(%)	45	25 (55.5)	22 (59.5)	3 (37.5)	0.22
Late onset hyperferritinemia, n(%)		20 (44.5)	15 (40.5)	5 (62.5)	
Hemoglobin, g/dl	72	8.7 (7.4, 10.2)	8.6 (7.5, 10.5)	8.1 (6.8, 10.1)	0.48
Total leukocyte count cells/mm ³	72	14000 (9820, 18000)	14090 (10200, 17500)	12465 (7020, 21080)	0.39
Platelet count cells/mm ³	72	18000 (44000, 80000)	19000 (48000, 80000)	31000 (11500, 100000)	0.39
Sodium, mmol/L	72	133 (130, 137)	133 (130, 136)	138 (133, 141)	0.03
International Normalized Ratio	56	1.2 (1.0, 1.5)	1.19 (1, 1.5)	1.3 (1.1, 1.6)	0.36
Aspartate transaminase, U/L	65	185 (94, 285)	179 (94, 257)	239 (88, 378)	0.26
Alanine transaminase, U/L	65	79 (49, 125)	69 (45, 122)	88 (52, 139)	0.58
Albumin, g/dl	58	2.2 (2.1, 2.6)	2.3 (2.1, 2.6)	2.1 (1.9, 2.2)	0.05
Severe hypoalbuminemia, n(%)	58	39 (54.2)	30 (50)	9 (75)	0.14
Lactate, mmol/L	72	1.6 (1.1, 2.6)	1.6 (1, 1.8)	3.0 (1.85, 9.8)	0.04
Organ dysfunction					
Hematological dysfunction	72	68 (94.4)	56 (93.3)	12 (100)	0.60
Respiratory failure	72	40 (55.5)	28 (46.6)	12 (100)	0.001
ARDS, n(%)	72	23 (31.9)	13 (21.7)	10 (83.3)	<0.0001
Mechanical ventilation, n(%)	72	34 (47.2)	22 (36.7)	12 (100)	0.0001
Shock, n(%)	72	20 (28.2)	12 (20.3)	8 (66.7)	0.003
VIS on day 1	72	0 (0,10)	0 (0,0)	10 (0,30)	0.0001
VIS on day 3	67	0 (0,10)	0 (0,0)	20 (10,86)	0.0001
VIS on day 5	64	0 (0,0)	0 (0,0)	20 (12,27)	0.0001
AKI, n(%)	72	15 (20.8)	10 (16.7)	5 (41.7)	0.02
RRT, n(%)	72	5 (6.9)	2 (3.3)	3 (25)	0.03
CNS dysfunction, n(%)	72	10 (13.9)	6 (10)	4 (33.3)	0.05
Coagulopathy (INR>1.5),n(%)	56	12 (21.4)	10 (21.3)	2 (22.2)	1.0
Elevated transaminases, n(%)	65	27 (41.5)	20 (37.7)	7 (58.3)	0.21
PELOD-2 score					
Day 1	72	3 (2,7)	2 (2, 5)	7 (7,9)	<0.0001
Day 2	67	2 (1,5)	2 (1, 5)	6 (4,7)	0.001
Day 3	64	2 (1,5)	2 (1,4)	4 (1,6)	0.32

All values expressed as Median (IQR)], PRISM - Pediatric Risk of Mortality, PELOD- Pediatric Logistic Organ Dysfunction, ARDS- Acute Respiratory distress syndrome, VIS- Vasoactive inotrope score, AKI- Acute Kidney Injury, RRT- Renal Replacement therapy

Figures

Figure 1

Figure 2

Panel A: Median ferritin levels plotted with day of illness for entire cohort; Panel B: Median ferritin levels plotted with day of illness for survivors and non survivors

Figure 3

Receiver operator characteristic curve comparing ferritin, PELOD 2 score and VIS for predicting mortality

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