USING CLINICAL PROFILE AND INITIAL LABORATORY RESULTS TO DIFFERENTIATE LEPTOSPIROSIS, SCRUB TYPHUS AND DENGUE VIRAL INFECTIONS AMONG CHILDREN WITH AN ACUTE FEBRILE ILLNESS

Surapat Assawawiroonhakarn[1], Alan Geater[2] and Kamolwish Laoprasopwattana[1]

[1]Department of Pediatrics, [2]Epidemiology Unit, Faculty of Medicine,
Prince of Songkla University, Hat Yai, Songkhla, Thailand

Abstract. The clinical manifestations of dengue viral infection (DVI), leptospirosis and scrub typhus are similar, especially during the early stage of illness. We aimed to compare initial clinical profiles and laboratory results in order to determine if these infections can be reliably differentiated using only this information. We conducted a retrospective observational study of patients aged <18 years serologically diagnosed with DVI, leptospirosis or scrub typhus who were treated at Songklanagarind Hospital between 1992 and 2012. The initial clinical presentations and laboratory results of 142 cases were analyzed to identify factors significantly associated with leptospirosis, scrub typhus, and DVI, with 24, 23 and 95 cases, respectively. The median (IQR) age was 9.5 (6.4-12.7) years. Patients with a history of being exposed to a flood/contaminated water, who had a fever for ≥3 days prior to presentation to the hospital or who had ≥70% neutrophils on a peripheral blood smear were significantly more likely to have leptospirosis with relative probability ratios (RPRs) of 72.8, 10.1 and 28.4, respectively (p <0.01). Patients with a fever lasting ≥4 days before the first visit and a body temperature >40°C were more likely to have scrub typhus or leptospirosis with RPRs of 26.7 and 4.5, respectively (p <0.01). Patients with >50% lymphocytes or ≤50% lymphocytes with a WBC count <5,000 cells/mm^3 were significantly less likely to have leptospirosis or scrub typhus with RPRs of 0 and 0.5, respectively (p <0.01).

Keywords: dengue viral infection, leptospirosis, scrub typhus

INTRODUCTION

In Thailand, fever is a common presenting symptom. Important causes of fever in Thailand include dengue viral infection (DVI), leptospirosis and scrub typhus (Leelarasamee *et al*, 2004; Suttinont *et al*, 2006). Studies have reported incidences of DVI, leptospirosis and scrub typhus in Thailand to be 80-200 cases, 5-10 cases and 2-11 cases respectively per 100,000 people (Leelarasamee *et al*, 2004; Sabchareon *et al*, 2012; Bureau of Epidemiology, 2011). These diseases can be specifically diagnosed with laboratory investigations, but in resource-limited countries such as in Thailand, it is important to use

Correspondence: Dr Kamolwish Laoprasopwattana, Department of Pediatrics, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand.
Tel: +66 (0) 74 451250; Fax: +66 (0) 74 429618
E-mail: kamolwish@gmail.com, lkamolwi@medicine.psu.ac.th

a detailed medical history, an accurate physical examination and basic laboratory testing to guide management. However, using only a clinical diagnosis without specific laboratory testing often results in misdiagnosis (Levett *et al*, 2000; Sergio *et al*, 2012). Previous studies have reported clinical factors to help differentiate among these diseases, such as finding an eschar is more likely to indicate scrub typhus than the other two diseases (Chang *et al*, 2012) and finding thrombocytopenia is more likely to indicate DVI (Watt *et al*, 2003; Libraty *et al*, 2007; Chrispal *et al*, 2010; Zaki and Shanbag, 2010; Chang *et al*, 2012). However, studies of this type are limited and do not provide enough information to allow physicians to reliably distinguish between these serious diseases and start the correct treatment in a timely fashion to minimize the occurrence of serious associated complications, especially among pediatric patients (Libraty *et al*, 2007; Zaki and Shanbag, 2010).

In our study we aimed to identify any combinations of initial clinical and basic laboratory factors significantly associated with DVI, leptospirosis and/or scrub typhus among pediatric patients in Thailand which would allow the most effective treatment to be undertaken before confirming laboratory studies are completed.

MATERIALS AND METHODS

We conducted a retrospective observational study by reviewing the records of patients aged <18 years who attended the pediatric outpatient department and/or were admitted to a pediatric ward at Prince of Songkla University Hospital, the major tertiary care and referral center in southern Thailand. Included patients were those with a positive immunofluorescence assay (IFA) for leptospirosis or scrub typhus during 1992-2012 or a positive enzyme-linked immunosorbent assay (ELISA) for DVI during 2008-2012. Immunocompromised patients and patients with coinfections were excluded from the study. Data obtained from the medical records were: demographic information, patient signs and symptoms, initial laboratory test results, treatment outcomes, complications, date of fever onset, date of first visit and date of first complete blood count (CBC). The presence of acute kidney injury (AKI) was recorded if the patient met the following criteria: an increase in serum creatinine (Cr) level to >2 mg/dl or a serum creatinine level increase of at least 2 times higher than a previously recorded value prior to illness if it was higher than the upper limit of a normal value for the patient's age (Chan *et al*, 2002).

Statistical analysis

Means, standard deviations, medians, interquartile ranges, frequencies and percentages were calculated where appropriate. Patient characteristics, signs, symptoms and initial laboratory results were compared among groups to identify factors significantly associated with each disease. The ANOVA and Kruskal-Wallis tests were used to compare normally distributed and non-normally distributed continuous variables, respectively. The chi-square test was used to compare categorical variables. Multivariate analysis was performed using multinomial logistic regression with backward elimination of non-significant variables, in which relative probability ratios (RPRs) of significant variables were compared among the three groups. Only the initial CBC results were included in the multivariate analysis due to missing data. Statistical significance

Table 1
Comparison of clinical characteristics of leptospirosis, scrub typhus and DVI patients.

Clinical characteristic	Leptospirosis (N = 24)	Scrub typhus (N = 23)	DVI (N = 95)	p-value
Age in years, median (IQR)	13.3 (11.5-14.5)[a]	10.6 (7.2-13.2)[b]	8.6 (6.0-11.4)[b]	<0.01
Males, *n* (%)	19.0 (79.2)[a]	17.0 (73.9)[a,b]	52.0 (54.7)[b]	0.04
Fever duration before first visit in days, median (IQR)	2.5 (1.0-3.0)[a]	3.0 (1.0-5.0)[a]	1 (0-2.0)[b]	<0.01
History of exposure to flood or contaminated water, *n* (%)	16 (66.7)[a]	6 (26.1)[b]	6 (6.3)[c]	<0.01
Cough, *n* (%)	9 (37.5)	12 (52.2)	39 (41.1)	0.36
Skin rash, *n* (%)	0 (0)[a]	6 (26.1)[b]	7 (7.4)[a]	0.02
Vomiting, *n* (%)	15 (62.5)[a]	7 (30.4)[b]	65 (68.4)[a]	<0.01
Anorexia, *n* (%)	21 (87.5)[a]	16 (69.6)[a]	86 (90.5)[b]	<0.01
Bleeding, *n* (%)	4 (16.7)	1 (4.3)	24 (25.3)	0.13
Conjunctivitis, *n* (%)	10 (41.7)[a]	4 (17.4)[a]	2 (2.1)[b]	<0.01
Hepatomegaly, *n* (%)	8 (33.3)	11 (47.8)	33 (34.7)	0.47
Lymphadenopathy, *n* (%)	1 (4.2)[a]	7 (30.4)[b]	6 (6.3)[a]	<0.01
BT in °C, mean (SD)	39.3 (1.1)[a,b]	39.6 (0.9)[a]	39.1 (0.9)[b]	<0.01
BT >40°C, *n* (%)	9 (37.5)[a]	14 (60.9)[a]	20 (21.1)[b]	<0.01
Fever duration in days, median (IQR)	6.0 (4.0-8.0)[a]	11.0 (8.0-12.0)[b]	6.0 (5.0-6.0)[a]	<0.01

[a,b,c] Values within rows not having a superscript in common differ significantly (p <0.05). DVI, dengue viral infection; IQR, interquartile range; BT, body temperature; SD, standard deviation.

was set at p <0.05. Stata version 14 (StataCorp, College Station, TX) was used for statistical analysis.

RESULTS

During the twenty-one year study period there were 24 cases of leptospirosis and 23 cases of scrub typhus that were serologically confirmed. During the 5-year study period for DVI, there were 95 cases of DVI that were serologically confirmed.

The median age of all the patients was 9.5 years (IQR 6.4-12.7 years). The DVI patients had a younger median age (IQR) than the leptospirosis patients [8.6 (6.0-11.4) *vs* 13.3 (11.5-14.5), respectively, p <0.01]. The DVI patients had sought medical care sooner than patients from the other two disease groups, with medians of 1.0 *vs* 2.5-3 days, respectively, p <0.01. Patients with scrub typhus had a longer duration of fever than the other two diseases with median days of 11.0 *vs* 6.0 days, p <0.01 (Table 1).

Comparing clinical characteristics among the 3 groups, leptospirosis had the highest proportion of patients with flood/contaminated water exposure history (66.7% compared to 26.1% in the scrub typhus group and 6.3% in the DVI group, p <0.01) and a higher proportion of patients with conjunctivitis than the dengue group (41.7% *vs* 2.1%, respectively, p <0.01). Scrub typhus had the highest proportion of patients with skin rash (26.1% compared to 0% in the leptospirosis group and 7.4% in the DVI group, p =0.02), lymph-

adenopathy (30.4% compared to 4.2% in the leptospirosis group and 6.3% in the DVI group, p <0.01) and a higher proportion of patients with body temperature >40°C than DVI patients (60.9% compared to 21.1%, respectively, p <0.01) (Table 1).

Comparing initial CBCs among the 3 groups, leptospirosis had a higher proportion of patients with a white blood cell count (WBC) >12,000/mm^3 than DVI patients (41.7% *vs* 7.4%, respectively, p <0.01) and the highest proportion of patients with neutrophils >70% (87.5% compared to 36.4% in the scrub typhus group and 19.0% in the DVI group, p <0.01). The DVI group had the highest proportion of patients with hematocrit >40% (47.4% compared to 16.7% in the leptospirosis group and 21.7% in the scrub typhus group, p <0.01), WBC <5,000/mm^3 (62.1% compared to 0% in the leptospirosis group and 21.7% in the scrub typhus group, p <0.01), and lymphocytes >50% (14.7% compared to 0% in both the leptospirosis and scrub typhus groups, p =0.02). The median (IQR) platelet count in the DVI group was significantly lower than in the leptospirosis group [120 (67-173) compared to 177 (103-248) x 10^3/mm^3], respectively; the proportions of patients with a platelet count <100,000/mm^3 were not significantly different among the three groups (Table 2).

Comparing complications among the 3 groups, the leptospirosis group had a higher proportion of patients with acute kidney injury (56.5% compared to 7.1% in the scrub typhus group and 2.4% in the DVI group, p <0.01) (Table 2). The proportions of patients with a bleeding disorder among the 3 group were not significantly different. In the DVI group, 54% (13/24) patients had bleeding from the oral cavity. Half the leptospirosis patients with bleeding (2/4) had pulmonary hemorrhage. Only one case of scrub typhus had bleeding and this was from the oral cavity. The only group with deaths was the leptospirosis group (3 cases); all died from sepsis and one of these had pulmonary hemorrhage as well.

Multivariate analysis was performed on only 138 of the 142 study patients due to missing data. These 138 patients were 23 with leptospirosis, 21 with scrub typhus and 94 with DVI. Patients with a history of exposure to flood or contaminated water, with a fever lasting ≥3 days before their first visit or a neutrophil count >70% were more likely to have leptospirosis (p <0.01). Patients with a fever lasting ≥4 days before the first visit and a body temperature >40°C were more likely to have scrub typhus (p <0.01). Patients with a lymphocyte count >50% or a lymphocyte count ≤50% along with a WBC count <5,000 cells/mm^3 were more likely to have DVI (p <0.01) (Table 3).

DISCUSSION

In our study, patients exposed to flood or contaminated water were more likely to have leptospirosis than scrub typhus or DVI. A study by Zaki and Shanbag (2010) conducted among children in Mumbai, comparing leptospirosis patients with DVI patients, also found that patients exposed to flood or contaminated water were more likely to have leptospirosis. The same study also found that conjunctivitis was also more likely in patients with leptospirosis compared to patients with DVI. Our study also found similar result although on multivariate analysis conjunctivitis was neither significantly associated with leptospirosis nor differentiable from the other two diseases. Neutrophils >70% was significantly more likely to be

Table 2
Comparison of CBC, urine RBC, BUN, Cr, and LFT in leptospirosis, scrub typhus and DVI.

Variable	Leptospirosis (N = 24)	Scrub typhus (N = 23)	DVI (N = 95)	p-value
Duration from fever to 1st CBC in days, mean (SD)	3.5 (1.7)[a]	5.9 (3.0)[b]	3.6 (1.6)[a]	<0.01
Hct, %, mean (SD)	33.7 (6.7)[a]	36.6 (5.2)[a]	39.3 (5.1)[b]	<0.01
Hct >40%, *n* (%)	4 (16.7)[a]	5 (21.7)[a]	45 (47.4)[b]	<0.01
WBC/mm^3, median (IQR)	11,300[a] (8,052-14,650)	8,270[a] (5,350-14,500)	4,410[b] (2,760-6,190)	<0.01
WBC <5,000/mm^3, *n* (%)	0 (0)[a]	5 (21.7)[b]	59 (62.1)[c]	<0.01
WBC >12,000/mm^3, *n* (%)	10 (41.7)[a]	7 (30.4)[a]	7 (7.4)[b]	<0.01
Neutrophils >70%, *n* (%)	21 (87.5)[a]	8 (36.4)[b] (*n*=22)	18 (19.0)[c]	<0.01
Lymphocytes >50%, *n* (%)	0 (0)[a]	0 (0)[a]	14 (14.7)[b]	0.02
Atypical lymphocytes >5%, *n* (%)	0 (0)[a]	4 (17.3)[a]	25 (26.9)[b]	0.04
Platelets x10^3/mm^3, median (IQR)	177 (103-248)[a]	151 (101-203)[a, b]	120 (67-173)[b]	0.02
Platelets <100 x10^3/mm^3, *n* (%)	5 (20.8)	5 (21.7)	33 (34.7)	0.25
BUN in mg/dl, median (IQR)	19 (14-44.0)[a] (*n* =23)	9.2 (7-11)[b] (*n* =14)	14 (10-20)c (*n* =40)	<0.01
Cr in mg/dl, median (IQR)	1.1 (0.9-2.7)[a]	0.6 (0.5-0.7)[b]	0.5 (0.4-0.7)[b]	<0.01
Acute kidney injury, *n* (%)	13 (56.5)[a] (*n* =23)	1 (7.1)[b] (*n* =14)	1 (2.4)[b] (*n* =41)	<0.01
DB in mg/dl, median (IQR)	0.4 (0.2-1.1)[a] (*n* =20)	0.1 (0.1-1.2)[b] (*n* =14)	0.1 (0.0-0.3)[b] (*n* =26)	<0.01
TB in mg/dl, median (IQR)	1.0 (0.4-2.3)[a] (*n* =20)	0.3 (0.2-0.5)[b] (*n* =14)	0.4 (0.2-0.7)[a] (*n* =29)	<0.01
AST in U/l, median (IQR)	27 (24-66)[a] (*n* =20)	106 (66-137)[b] (*n* =14)	148 (102-424)c (*n* =35)	<0.01
ALT in U/l, median (IQR)	40 (16-62)[a] (*n* =20)	105 (31-133)[b] (*n* =14)	58 (39-159)[b] (*n* =20)	0.02
Albumin in g/dl, median (IQR)	3.1 (2.6-3.5) (*n* =18)	3.2 (2.6-3.8) (*n* =12)	3.3 (2.9-3.9) (*n* =30)	0.55
Urine RBC >3/mm^3	5 (20.8)	2 (9.5) (*n* =23)	6 (7.8) (*n* =77)	0.19

[a,b,c] Values within rows not having a superscript in common differ significantly (p <0.05). CBC, complete blood count; RBC, red blood cells; BUN, blood urea nitrogen; Cr, creatinine; DVI, dengue viral infection; SD, standard deviation; Hct, hematocrit; WBC, white blood cell count; IQR, interquartile range; Cr, serum creatinine; DB, direct bilirubin; TB, total bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

found in patients with leptospirosis in our study, a finding supported by a study conducted in Thai children by Libraty *et al* (2007) which found that every 1,000 cells/mm^3 of absolute neutrophil count (ANC) increased the OR of leptospirosis 2-fold when compared to DVI.

In our study, patients with body temperature >40°C were more likely to have scrub typhus than leptospirosis or DVI.

Patients with lymphocytes >50% or lymphocytes ≤50% with WBC <5,000/mm^3 were more likely to have DVI than leptospirosis or scrub typhus in our study.

Table 3
Multinomial logistic regression, comparing leptospirosis and scrub typhus with DVI.

Variable	Relative probability ratio (95% CI)		*p*-value
	Leptospirosis	Scrub typhus	
History of exposure to flood or contaminated water	72.8 (8.9-591.0)	4.9 (0.9-27.6)	<0.01
Fever duration before first visit			<0.01
≤2 days	1	1	
3 days	10.1 (1.6-63.6)	1	
≥4 days	10.1 (1.6-63.6)	26.7 (4.7-150.4)	
BT >40°C	1	4.5 (1.3-16.3)	<0.01
Neutrophils >70%	28.4 (4.1-198.2)	1	<0.01
Lymphocytes >50% or	0*	0*	<0.01
Lymphocytes ≤50% with WBC <5,000/mm^3	0.05 (0.01-0.3)	0.05 (0.01-0.3)	

* No lymphocyte counts >50% among patients with leptospirosis or scrub typhus. DVI, dengue viral infection; CI, confidence interval; BT, body temperature; WBC, white blood cell count.

Comparable with our study, Zaki and Shanbag (2010) also found that leucopenia was more likely in children with DVI compared to children with leptospirosis. Moreover, a study in adults by Watt *et al* (2003) which compared scrub typhus patients with DVI patients also found that WBC <5,000/mm^3 was more likely in DVI patients. However, studies by Zaki and Shanbag (2010) and Watt *et al* (2003) did not find significant differences in lymphocyte percentages between patients with DVI and leptospirosis and patients with DVI and scrub typhus, respectively.

Surprisingly, in our study, thrombocytopenia was not helpful in differentiating DVI from the other two diseases. Thrombocytopenia is common among DVI patients (Watt *et al*, 2003; Libraty *et al*, 2007; Chrispal *et al*, 2010; Zaki and Shanbag, 2010; Chang *et al*, 2012). Another study also reported thrombocytopenia was not helpful in differentiating DVI from leptospirosis (Libraty *et al*, 2007). These differences may be related to when during the course of the disease the CBCs were performed. In our study and the study by Libraty *et al* (2007), the CBCs used for our evaluation was performed during the first 2-3 days of the onset of fever when the platelet count may not have decreased much.

This study had some limitations. There were missing data of renal and liver function tests, especially in patients with mild symptoms or who were not suspected of having acute kidney injury or acute hepatitis. Also, our findings need to be considered with care if applied to other settings because the study institution is a tertiary care referral center and more likely to have more severe cases. A further prospective cohort study is needed to confirm our findings.

In conclusion, among the 3 diseases of leptospirosis, scrub typhus, and DVI, patients with leptospirosis were more likely to have a history of exposure to flood or contaminated water, along with fever lasting ≥3 days before their first visit

and/or a neutrophil count >70%. Patients with scrub typhus were more likely to have fever lasting ≥4 days before their first hospital visit and a body temperature >40°C. Patients with DVI were more likely to have a lymphocyte count >50% or a lymphocyte count ≤50% along with a WBC count <5,000/mm^3.

ACKNOWLEDGEMENTS

The authors wish to thank Miss Walailuck Jitpiboon for her assistance with data analysis and Mr David Patterson, an English teacher and writing consultant, for help with English; both work at the Faculty of Medicine, Prince of Songkla University.

REFERENCES

Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, Thailand. Scrub typhus. Annual epidemiological surveillance report. Nonthaburi: Bureau of Epidemiology, 2011. [Cited 2015 Dec 25]. Available from: http://boe.moph.go.th/

Chan JC, Williams DM, Roth KS. Kidney failure in infants and children. *Pediatr Rev* 2002; 23: 47-60.

Chang HC, Lin MC, Liu SF, *et al*. The clinical differences between dengue and scrub typhus with acute respiratory failure in southern Taiwan. *Infection* 2012; 40: 359-65.

Chrispal A, Boorugu H, Gopinath KG, *et al*. Acute undifferentiated febrile illness in adult hospitalized patients: the disease spectrum and diagnostic predictors - an experience from a tertiary care hospital in South India. *Trop Doct* 2010; 40: 230-4.

Leelarasamee A, Chupaprawan C, Chenchittikul M, Udompanthurat S. Etiologies of acute undifferentiated febrile illness in Thailand. *J Med Assoc Thai* 2004; 87: 464-72.

Levett PN, Branch SL, Edwards CN. Detection of dengue infection in patients investigated for leptospirosis in Barbados. *Am J Trop Med Hyg* 2000; 62: 112-4.

Libraty DH, Myint KS, Murray CK, *et al*. A comparative study of leptospirosis and dengue in Thai children. *PLOS Negl Trop Dis* 2007; 1: e111.

Sabchareon A, Sirivichayakul C, Limkittikul K, *et al*. Dengue infection in children in Ratchaburi, Thailand: a cohort study. I. Epidemiology of symptomatic acute dengue infection in children, 2006-2009. *PLOS Negl Trop Dis* 2012; 6: e1732.

Sergio ADM, Figueroa EA, Saadia VGM, *et al*. Leptospirosis prevalence in patients with initial diagnosis of dengue. *J Trop Med* 2012; 2012: 519701.

Suttinont C, Losuwanaluk K, Niwatayakul K, *et al*. Causes of acute, undifferentiated, febrile illness in rural Thailand: results of a prospective observational study. *Ann Trop Med Parasitol* 2006; 100: 363-70.

Watt G, Jongsakul K, Chouriyagune C, Paris R. Differentiating dengue virus infection from scrub typhus in Thai adults with fever. *Am J Trop Med Hyg* 2003; 68: 536-8.

Zaki SA, Shanbag P. Clinical manifestations of dengue and leptospirosis in children in Mumbai: an observational study. *Infection* 2010; 38: 285-91.