THE ETIOLOGY OF ACUTE PYREXIA OF UNKNOWN ORIGIN IN CHILDREN AFTER A FLOOD

Jintana Pradutkanchana[1], Sukone Pradutkanchana[1], Marisa Kemapanmanus[1], Nathakul Wuthipum[1], Kamkarn Silpapojakul[2]

[1]Department of Pathology, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla; [2]Department of Pediatrics, Hat Yai Hospital, Hat Yai, Songkhla, Thailand

Abstract. Acute pyrexia of unknown origin (PUO) is a major public health problem in Thailand. We studied the etiology of 180 cases of acute PUO in children after a sudden severe flood in Hat Yai city in 2000. Dengue infection and leptospirosis accounted for more than half of the total cases. Dengue hemorrhagic fever was the most common (29.4%) followed by leptospirosis (27.2%) and scrub typhus infection (1.1%). Five serovars of leptospires were involved in this study. *Leptospira interrogans bataviae* was the most common (86.5%). Acute serum antibody testing could detect only 52.8% and 40.8% of dengue and leptospirosis cases, respectively. This study showed both should be included in the presumptive diagnosis of acute PUO in patients after flooding.

INTRODUCTION

Acute pyrexia of unknown origin (acute PUO) is a public health problem in Thailand with a prevalence of 200,000-350,000 cases reported to the Ministry of Public Health each year. This prevalence was second only to acute diarrhea. Although dengue infection was the most common cause of acute PUO, the increased prevalence of scrub typhus and leptospirosis was documented recently (Division of Epidemiology, 1999).

During 21-25 November 2000, there was a sudden severe flood in Hat Yai, a city of ~200,000 inhabitants situated in southern Thailand. After the flood, the number of acute PUO cases increased dramatically. This study is an attempt to determine the etiology of these acute PUO cases using serologic methods to detect leptospirosis, dengue and rickettsial infections.

Correspondence: Jintana Pradutkanchana, Department of Pathology, Faculty of Medicine, Hat Yai, Songkhla 90110, Thailand.
Tel: 66 (0) 7445 1585; Fax: 66 (0) 7421 2908
E-mail: pjintana@ratree.psu.ac.th

MATERIALS AND METHODS

Study population

The study was conducted at Hat Yai Hospital, Songkhla Province, Thailand, a provincial referral hospital during December 2000. Serum samples were prospectively collected from pediatric patients who presenting with acute fever >38ºC for more than 1 day but not exceeding 3 weeks. Exclusion criteria were the presence of profused rhinorrhea, exudative pharyngitis, pneumonia, urethritis and diarrhea. All sera were stored at -80ºC until tested.

Hemagglutination inhibition assay (HI) for detection of dengue infection

HI modified to microtiter plate was carried out as previously described (Clarke and Casals, 1958). All sera tested were kaolin-treated with dilution starting at 1:20.

Microscopic agglutination test (MAT) for detection of leptospirosis

The assay was performed by a modified Galton technique (Galton *et al,* 1965), with the 23 serovars of *Leptospira interrogans* used as antigen: *bangkok, ballico, bratislava, akayami,*

rachamati, bataviae, canicola, cellidoni, djasiman, grippotyphosa, hebdomadis, hyos, tarassovi, icterohemorrhagiae, copenhageni, javanica, saigon, pomona, pyrogenes, sejroe, hardjo, wolffi and CH-11. Briefly, 25 µl of sera were diluted to 1:50 with phosphate buffer saline (PBS) and added into 23 wells of microtiter plate. Twenty-five microliters of each live leptospires serovars was added into each well. The specimens were mixed gently. After leaving for 2-3 hours at room temperature, 3 µl of the suspension were dropped on a slide. The agglutination was observed under dark field microscope (OLYMPUS model BH-2) at a final magnification of 100x. Sera showing positive reaction were then retested against the respective serovar to determine the endpoint titer which was the highest dilution giving more than 50% agglutination of leptospires. The serovar giving the highest titer was considered to be the infecting serovar.

Indirect immunofluorescent assay (IFA) for detection of leptospirosis and rickettsial infection

The assay was a modified Robinson microimmunofluorescence technique (Robinson *et al*, 1976). The leptospira antigen used was serovar *bataviae*. All three rickettsial antigens used (scrub, murine and tick typhus) were from rickettsiae propagated in yolk sac. Scrub typhus antigens used were pooled Karp, Kato and Gillium strain of *Orientia tsutsugamushi*. TT118 strain was used as tick typhus antigen. All four antigens were spotted at different areas on the same well of clean taflon-coated slide. After air drying at room temperature, the slide was fixed in acetone for 10 minutes then left to air-dry and stored at -70ºC until used.

Ten µl of diluted serum (start at 1:100) were added to the slide and incubated at 37ºC in a moist chamber for 30 minutes. The slide was washed three times in PBS pH 7.2, 5 minutes each time, then rinsed once with distilled water and air-dried. Ten microliters of optimal dilution of fluorescein isothiocyanate (FITC) conjugated rabbit anti-human immunoglobulins (IgG, IgM, IgA) (Dakopath, Code F200,

Denmark) was placed on the slide and incubated at 37ºC in moist chamber for 30 minutes. The slide was washed as previously. After mounting with glycerol buffer, the slide was examined under a fluorescence microscope (OLYMPUS model BH-2 with FITC filter and exciter filters at a 400x magnification). The serum was read as positive, where the organisms in the specific dotted antigen showed bright green colored. All positive sera were further diluted and final titers were determined. Positive and negative reference sera were included in every batch tested.

Diagnostic criteria

Based on WHO (1986) criteria for dengue infection, a four-fold or greater antibody rise or persistently high antibody titer (≥1:2,560) was considered as dengue positive. Antibody titers in convalescent sera ≤1:1,280 were classified as primary dengue infection while any sera titer ≥1:2,560 were considered as secondary dengue infection. The diagnostic criteria for leptospirosis were as follows. A four-fold rising in titer in paired serum or a titer ≥1:400 in single serum by either MAT or IFA were considered as indicative of current leptospiral infection (Galton *et al,* 1965; Appasakij, 1995). A four-fold rise in the IFA rickettsial antibody titer in paired sera or titer ≥1:400 in single serum were considered as diagnosis of corresponding rickettsial infection (scrub, murine or tick typhus) (Brown *et al*, 1988).

RESULTS

Between 1 and 31 December 2000, 281 serum samples (95 pairs and 85 singles) were collected from pediatric patients and tested. The etiology of acute PUOs is summarized in Table 1. Among the 180 pediatric patients, 53 (29.4%) patients had dengue infection. The male:female ratio was 1.52:1, with the mean age of 8.8 years (range 2-15 years). Forty-seven (88.7%) cases manifested as secondary dengue infection, while only 4 (7.5%) cases were interpreted as having primary dengue infection; 2 (3.8%) patients were unclassified.

Table 1

The etiologies of acute pyrexia of unknown origin in children after a sudden severe flood in Hat Yai.

Disease	Overall incidence (No. of positive patients/ Total no. of patients)	Etiologies detected with acute sera (No. of positive patients/ Total no. of patients)
Dengue infection	29.4% (53/180)	52.8% (28/53)
Leptospirosis	27.2% (49/180)	40.8% (20/49)
Scrub typhus	1.1% (2/180)	50% (1/2)
Murine typhus	0.0% (0/180)	0.0% (0/0)
Tick typhus	0.0% (0/180)	0.0% (0/0)
Coinfection of dengue and leptospirosis[a]	2.8% (5/180)	-

[a]cases who had positive antibodies against both dengue and leptospirosis.

Table 2

The serovar of *Leptospira* spp involved after a sudden severe flood of Hat Yai in 2000.

Serovar	Quantity (%)
L. bataviae	32 (86.5%)
L. bratislava	1 (2.7%)
L. canicola	1 (2.7%)
L. hyos	1 (2.7%)
L. wolffi	1 (2.7%)
Undefined[a]	1 (2.7%)
Total	37 (100.0%)

[a]More than 1 equal titers serovars shown by the MAT.

Forty-nine (27.2%) patients had leptospirosis. The sex ratio, male:female, was 2.67:1, with a mean age of 10.33 years (range 5-15 years). Of these 49 cases, 32 (65.3%) patients were positive on both MAT and IFA tests. Twelve (24.5%) patients were positive by IFA only, while only 5 (10.2%) cases were positive by MAT only. Five serovars were identified in this study (Table 2). *Leptospira interrogans* serovar *bataviae* was the most common (86.5%).

Only 2 (1.1%) patients had scrub typhus infection; no cases of murine typhus or tick typhus were detected.

DISCUSSION

Most studies of the causative agents of acute PUO in Thailand have demonstrated that dengue infection was the most common. In this study, dengue infection was the most common even though the overall prevalence of this disease in the year 2000 was lower than in previous years. However, after the flood, dengue cases continued to increase and eventually resulted in the biggest outbreak of dengue in 2001 (unpublished data).

Leptospirosis is known to be a common cause of acute PUO after flooding (Park *et al*, 1989; WHO, 2000). In this report, the prevalence of leptospirosis in children after a sudden flood was high as 27.2%, compared to Heisey's report that 36% of children who were admitted with fever after great flooding in Bangkok had leptospirosis (Heisey *et al*, 1988). Five serovars were involved in this study; the most common serovar was *bataviae*. This data confirmed previous reports that *Leptospira interrogans* serovar *bataviae* was the most common in southern Thailand (Sundharagiati, 1968; Kanjanapin, 2001).

In this study, we found that dengue fever was the major cause of acute PUO after flooding, followed by leptospirosis. Together, dengue fever and leptospirosis accounted for more than half of the total PUO cases (53.9%).

Similar to a previous report after hurricane in Puerto Rico that dengue fever and leptospirosis account for 68% of total acute PUO cases (Sanders *et al*, 1999). This comfirmed that both dengue infection and leptospirosis may have an outbreak in the same areas and at the same period of time. However, isolated outbreak of leptospirosis without dengue infection was reported after flooding (Trevejo *et al*, 1998). Misdiagnosis between two particular diseases may occurred. Ko *et al* (1999) reported that 42% of leptospirosis cases were misdiagnosed as dengue infection in the outpatient clinic. The differentiation between both diseases is essential because, in contrast to dengue fever, effective treatment of leptospirosis with antibiotics is available. Misdiagnosis may lead to complications or death of the patients.

The final identification of the etiologies of acute PUO in our study was based on serological diagnostic tests which in some cases, may required convalescent sera for comfirmation. We found that the sensitivity of the tests detected in first sera in our study was low. When acute sera were considered, only 52.8% of dengue cases and only 40.8% of leptospirosis cases were detected. In this situation, diagnosis using clinical score or new rapid diagnostic tests including PCR may be useful to reduce window period for interpretation of the serologic tests.

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