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Evidence of acute rickettsioses among patients presumed to have chikungunya fever during the chikungunya outbreak in Sri Lanka

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SUMMARY

Background: Chikungunya fever (CGF) and rickettsioses are known to cause acute onset febrile illnesses associated with severe arthritis. Rickettsial arthritis is curable with the use of appropriate anti-rickettsial antibiotics, however the arthritis of CGF tends to have a prolonged course leading to protracted disability. The aim of this study was to investigate the contribution of CGF and rickettsioses to cases of fever and arthritis during a presumed CGF outbreak in Sri Lanka.

Methods: Fifty-eight consecutive patients with presumed CGF were further investigated to determine the occurrence of rickettsioses among them, and to identify differences in clinical, hematological, and biochemical parameters between the two diseases.

Results: Nearly a third of the patients had serological evidence of rickettsioses accounting for their illness. The presence of a late onset major joint arthropathy sparing the small joints of the hands and feet, and the occurrence of a late onset discrete maculopapular rash over the trunk and extremities, suggested rickettsioses over CGF. White blood cell count, erythrocyte sedimentation rate, C-reactive protein, and liver function tests were not helpful in differentiating rickettsioses from CGF. Patients with rickettsioses and arthritis who received an empirical course of doxycycline recovered faster than those who did not receive specific treatment.

Conclusions: The establishment of rapid diagnostic methods able to differentiate the etiological agents of fever and arthritis, such as CGF and rickettsioses, would be beneficial in endemic settings.

1. Introduction

Chikungunya fever (CGF) is caused by an arbovirus transmitted by the *Aedes aegypti* mosquito.[1] The illness is characterized by an acute onset febrile illness associated with severe arthritis involving both major and small joints of the body. Although fever seems to subside over 5–7 days, similar to most other viral fevers, the incapacitating arthritis tends to persist for a variable period of time – commonly for about 3 months, but sometimes up to 1 year or more.[2–4] In addition to fever and arthritis, an erythematous blanching rash is seen during the early illness, and some patients also develop an erythematous maculopapular rash over the body, mainly involving the limbs and the trunk.[5] Since the illness is viral there is no effective specific treatment.

Rickettsioses are also known to cause a febrile illness associated with varying degrees of arthralgia and arthritis.[6] Some, especially the spotted fever group (SFG) rickettsioses, are associated with an erythematous maculopapular rash.[6,7] Although rickettsioses commonly cause arthralgia,[6] incapacitating major joint arthritis has also been recognized.[8] Unlike in CGF, patients with rickettsial arthritis recover rapidly with anti-rickettsial antibiotics.[8]

Sri Lanka experienced a major outbreak of CGF from mid 2006 to early 2008.[9] Patients presented with characteristic fever, severe arthritis, and sometimes a maculopapular rash. The outbreak was confirmed to be due to the chikungunya virus by isolation of the virus by PCR.[9] However due to the rapid spread of the illness throughout the island and the non-availability or non-affordability of confirmatory tests, most patients with fever and arthritis during this period were presumed to have CGF and were treated only symptomatically.

As rickettsioses are re-emerging in most parts of Sri Lanka, we carried out a retrospective study to investigate the contribution of rickettsioses in a group of patients with fever and arthritis, presumed to be due to CGF.

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2. Methods

Fifty-eight consecutive patients with presumed CGF who attended an outpatient clinic in Ragama, Western Sri Lanka, were further investigated. Of these patients, only 45 attended the second clinic visit at 2 weeks, and only 31 attended the third visit at 4 weeks. Clinical details were recorded at each clinic visit using a pre-tested questionnaire. A 3-ml serum sample was obtained during the first or second clinic visit (in order to ensure an interval of at least 7 days between the onset of the clinical illness and obtaining the serum) and follow-up serum sample were obtained at 8 weeks and 6 months after the initial presentation. All serum samples were stored at −70 °C before analysis for serological evidence of rickettsioses and CGF. The 31 patients were followed up by telephone interview until significant improvement of symptoms was reported.

Immuno fluorescent antibody assays (IFA) were carried out using rickettsial antigens prepared from cell culture-grown rickettsiae: *Rickettsia conorii* (Malish), *Rickettsia typhi* (Wilmington), and *Orientia tsutsugamushi* (Karp). IgM and IgG antibodies were detected using fluorescein-conjugated goat anti-human $IgG_{(\gamma)}$ and IgM (KPL, Inc., Gaithersburg, MD, USA). Serum was screened at 1/32, and positive samples were titered to an endpoint of 1:2048 for IgG. Acute rickettsial infection was defined by the presence of an IFA IgM titer of ≥1/64 combined with a positive IgG titer of ≥1:128 and either a four-fold increase or decrease in IgG titers in the follow-up samples.

Serum was also tested for chikungunya virus infection. Serological evidence of acute CGF was defined as a positive OnSite Chikungunya IgM Rapid Test (CTK Biotech Inc., USA), carried out in accordance with the manufacturer's instructions. Serological tests were carried out at the Rickettsial Disease Research and Diagnostic Laboratory, Faculty of Medicine, University of Kelaniya and Faculty of Veterinary Medicine, University of Peradeniya, Sri Lanka.

A full blood count, erythrocyte sedimentation rate, C-reactive protein levels, and liver function tests were also performed at the first clinic visit, if they had not already been done.

Statistical analysis of clinical, biochemical, and hematological parameters in the two populations of patients were done using Stata 8.2 (Stata Corp., College Station, TX, USA). Statistical significance was assessed using the Wilcoxon rank sum test for numeric variables. Ethical clearance for the study was obtained from the Ethics Review Committee, Faculty of Medicine, University of Kelaniya.

3. Results

Of the 31 patients who were investigated, 14 were male and 17 female; their mean age was 36.2 years (standard deviation 4.7 years). Ten of these patients had serological evidence of acute rickettsioses (Table 1), 16 had evidence of acute CGF, one had evidence of both CGF and acute *O. tsutsugamushi* infection (case 8), and four were negative for both. Out of the 10 who were positive for acute rickettsioses, eight were positive for acute SFG rickettsioses and two had serological evidence of acute *O. tsutsugamushi*; none were positive for *R. typhi*.

The comparison of clinical and biochemical parameters between rickettsioses and CGF is shown in Table 2.

Although all patients had a variable clinical recovery over 2 to 16 weeks, only five of the 10 cases of rickettsioses (four SFG and one *O. tsutsugamushi*) received doxycycline on an empirical basis due to the lack of diagnostic facilities (rickettsial disease diagnostics had not yet been established in Sri Lanka in June 2008). None of the patients with CGF received doxycycline. Among

Table 1
Serological titers of patients who were positive for rickettsioses

	Acute serology						Convalescent serology after 8 weeks/6 months	
	CKG	OT IgG	OT IgM	RC IgG	RC IgM	R. typhi IgG	OT-IgG	RC-IgG
1	Neg			1:256	1:64	Neg		1:128/1:64[a]
2	Neg			1:256	1:64	Neg		1:128/1:64[a]
3	Neg			1:256	1:64	Neg		ND/1:64[a]
4	Neg			1:256	1:128	Neg		1:64/1:64[a]
5		1:256	1:64	1:256	1:64	Neg	1:128[a]	ND/1:64[a]
6				1:512	1:128	Neg		1:64
7				1:1024	1:128	Neg		1:64
8	Pos	1:256	1:64			Neg	1:128/1:64[a]	
9		1:256	1:64			Neg	1:64	
10		1:2048	1:128			Neg	1:128	
11				1:256	1:64	Neg		1:128/1:64[a]

CKG, chikungunya virus; OT, *Orientia tsutsugamushi*; RC, *Rickettsia conorii*; R. typhi, *Rickettsia typhi*; ND, not determined.
[a] At 6 months.

Table 2
Comparison of clinical, hematological, and biochemical parameters between chikungunya fever and rickettsioses[a]

Clinical parameter	Rickettsioses	Chikungunya fever	*p*-Value
Duration of illness at the time of first presentation for medical care (days)	8.3 (3.2)	2.8 (0.9)	<0.001
Days to occurrence of arthritis from onset of fever	6.5 (1.2)	2.4 (0.9)	<0.001
Days to occurrence of rash from onset of fever	7.2 (2.4) (*n* = 5/10)	3.2 (1.1) (*n* = 9/16)	<0.001
Major joint involvement, *n*	10/10	14/16	0.6
Small joint involvement, *n*	4/10	13/16	0.01
WBC (after 4th day; cells × 10^9/l)	5.4 (1.3) (*n* = 10)	4.9 (1.9)	0.28
Platelet count (cells × 10^9/l)	128 (10)	92 (14)	<0.001
ESR (mm/h)	51.5 (5)	46.1 (8)	0.48
CRP (mg/dl)	52 (12)	59.4 (5.3)	0.45
AST (IU/l)	58.3 (12.4)	62.3 (10.5)	0.52
ALT (IU/l)	62.2(10.3)	59.7 (11.2)	0.58

WBC, white blood cell count; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; AST, aspartate aminotransferase; ALT, alanine aminotransferase.
[a] Results are mean (standard deviation) unless stated otherwise.

the patients with rickettsioses, the five who received doxycycline made a quicker recovery (within 4 days) compared to the patients who were not treated with doxycycline (mean 16 days).

4. Discussion

In this study, we found that among patients with clinically presumed CGF, 10 (32%) had confirmed rickettsioses. Patients with rickettsioses mainly had late onset, major joint arthritis, with lesser involvement of the small joints of the hands and feet, compared to patients with CGF. The early onset severe arthritis in CGF is the likely reason for these patients seeking treatment earlier in the illness than those with rickettsioses. In addition to arthritis, patients with CGF had a confluent erythematous maculopapular rash over the trunk and limbs compared to a more discrete rash in rickettsioses. We could not find any hematological or biochemical test that would be useful to differentiate rickettsioses from CGF, except that CGF patients had significantly lower platelet counts. In the patients with rickettsioses, those who were treated with doxycycline made a quicker recovery from the arthritis than those who did not receive specific treatment.

With regard to the hematological investigations, the platelet count in rickettsioses was significantly higher compared to that in CGF. However we could not establish a cut-off level for platelets for the prediction of rickettsioses over CGF due to the small number of cases in this study. Furthermore, we have seen very low platelet counts in patients with rickettsioses. Although rickettsioses are caused by bacteria and CGF is caused by a virus, we could not find any significant difference in white blood cell counts between the two groups. The reason behind this could be that patients with rickettsioses are known to present with a hematological picture similar to a viral infection, with low white blood cell counts and reactive lymphocytosis.[10] Similarly, none of the other biochemical tests used in this study were helpful in differentiating CGF and rickettsioses.

Therefore, we feel that the presence of early onset small joint arthritis in addition to major joint involvement and the occurrence of a more confluent early onset erythematous maculopapular rash over the trunk and limbs would help to differentiate CGF from rickettsioses among patients with acute onset fever and arthritis.

The early clinical response of arthritis in patients who were positive for rickettsioses and received doxycycline compared to those who did not receive doxycycline may have been due to early recovery from infection or the immunomodulatory or anti-inflammatory properties of doxycycline.[11–13] However none of the patients who were positive for CGF received doxycycline. Therefore, it is difficult to comment on the immunomodulatory properties of doxycycline in patients with CGF arthritis.

The role of serology in the diagnosis of rickettsioses during the acute phase of illness is doubtful until current deficiencies of the serological tests in relation to an endemic setting are sorted out. Furthermore, although microimmunofluorescence-based (IFA) IgM and IgG detection is considered the gold standard in the diagnosis of acute rickettsioses,[14] IFA diagnostics against *R. typhi* are known to cause non-specific cross-reactivity with other bacterial antigens such as *Proteus vulgaris* OX19 and *Legionella bozemanii*.[15] However such cross-reactivity has not been demonstrated between rickettsioses and arboviruses such as chikungunya virus. Therefore, it is likely that these patients were suffering from either rickettsioses or CGF. It is unlikely that immunomodulatory effects of doxycycline interfered with the initial serological results in patients with acute rickettsioses, because at the time the first serum sample was obtained, none of the patients had received doxycycline. It is difficult to comment on how such treatment could have interfered with follow-up serology. None of the patients who were positive for CGF received doxycycline. Considering the limitations of IFA-based diagnostics in the diagnosis of acute rickettsioses, PCR-based diagnostic facilities would be the best to differentiate the two illnesses early. However their availability and cost may interfere with their value in most endemic settings. Therefore, in areas where PCR-based diagnostic facilities are not available, an empirical treatment with doxycycline would be helpful in managing these patients.

Conflict of interest: No conflict of interest to declare.

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