Research Article

Serological evidence for chikungunya and Zika virus infections in patients clinically diagnosed as dengue in Northern Sri Lanka

S Briyangari[1], J Kowsalavani[2], K Murugananthan[2], F Noordeen[3]

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Abstract

Background: Chikungunya (CHIKV), Zika (ZV) and dengue (DENV) viruses are transmitted by *Aedes* mosquitoes. CHIKV, ZV and DENV infections produce similar clinical features during the early phase of the illness. Disappearance of chikungunya for the last four decades and no reported evidence of Zika fever in Sri Lanka results in the diagnosis of any illnesses resembling dengue as DENV infection in the country.

Objectives: This study aimed to test for serological evidence for CHIKV and ZV infections in samples collected from patients suspected of having dengue fever.

Methods: Ninety-one sera were tested to determine past exposure to CHIKV and ZV through anti-CHIKV IgG and anti-ZV IgG positivity whilst recent infections were determined through anti-CHIKV IgM and ZV IgM positivity. Recent DENV & CHIKV and DENV & ZV co-infections were detected using anti-DENV IgM & anti-CHIKV IgM positivity and anti-DENV IgM & anti-ZV IgM positivity respectively.

Results: Of the 91 patients, 12% and 21.9% had recent and past CHIKV infection respectively, and 1.1% and 19.9% had recent and past exposure to ZV respectively. The overall exposure rate of CHIKV was 34.1% and that of ZV was 20.9%. Of the 91 patients with the clinical diagnosis of dengue, 69.2% had evidence for DENV infection. Nearly 8.8% had DENV and CHIKV co-infection and none of the patients had DENV and ZV co-infection. Of the patients with DENV infection, 14.3% had past

[1] *Postgraduate Institute of Sciences, University of Peradeniya, Peradeniya 20400, Sri Lanka*[2]
[2] *Department of Microbiology, Faculty of Medicine, University of Jaffna, Jaffna 40000, Sri Lanka*
[3] *Diagnostic and Research Virology Laboratory, Department of Microbiology, Faculty of Medicine, University of Peradeniya, Peradeniya 20400, Sri Lanka*

Address for correspondence: Prof Faseeha Noordeen, Diagnostic and Research Virology Laboratory, Department of Microbiology, Faculty of Medicine, University of Peradeniya, Peradeniya 20400, Sri Lanka
Telephone: +94772293301: email: faseeha.noordeen@med.pdn.ac.lk; faseeha.noordeen12@gmail.com
https://orcid.org/0000-0002-2018-0606

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exposure to CHIKV and 13.2% had past exposure to ZV. Approximately 63% of CHIKV and DENV co-infected patients had past exposure to DENV.

Conclusions: Findings of this study show evidence for exposure to CHIKV and ZV in the dengue suspected patients. Most of the clinical features were similar in recent DENV, CHIKV and ZV infections which will contribute to miss CHIKV and ZV infections in dengue endemic resource limited countries where testing for CHIKV and ZV infections are not routinely done.

Key words: *Anti-CHIKV IgM/IgG, anti-ZV IgM/IgG, Chikungunya virus, Dengue virus, Zika virus*

Introduction

Chikungunya (CHIKV) and Zika viral (ZV) diseases are arthropod borne infections transmitted to humans by infected mosquitoes – *Aedes aegypti* and *Aedes albopictus*.[1] CHIKV is a small enveloped, single-stranded RNA virus belonging to the genus *Alphavirus* in the *Togaviridae* family.[2] The virus was first isolated from human serum and then from *Aedes aegypti* mosquitoes in Tanzania in 1953.[1] A chikungunya epidemic was reported in the Western Province of Sri Lanka in 1965.[3] The Asian strain of CHIKV is believed to be the cause for the outbreaks that occurred prior to 2000, whereas the 2006 outbreak affecting many countries including Sri Lanka was caused by the African strain of the CHIKV.[2]

CHIKV fever was not considered a public health problem in Sri Lanka until 2006. During the 2006/2007 outbreak, 37,000 cases of suspected CHIKV fever were reported in highly populated areas in the north, east and western coastal areas of Sri Lanka.[4] The present study area, Jaffna, was one of the badly affected districts during the 2006/2007 outbreak.[3] CHIKV infection is characterized by high fever, chills and rigours, and severe arthralgia, myalgia, rash, headache, nausea and vomiting. Detection of anti-CHIKV IgM using an immuno-assay like ELISA is the commonly used method for laboratory-based diagnosis of CHIKV infection in symptomatic patients. CHIKV fever may be life threatening in its neurovirulent form and contributes to morbidity due to its clinical presentation resembling dengue during the early phase of the symptomatic infection. It is therefore possible for CHIKV infections to be mistakenly diagnosed as dengue clinically in dengue endemic countries.[5] Hence, laboratory confirmation is required to differentiate these infections from dengue infection.

ZV is an enveloped single-stranded, positive sense RNA virus belonging to the genus *Flavivirus* in the family *Flaviviridae* like DENV.[6] It was first identified from the serum of a rhesus monkey in the Zika forest in 1947.[7] Human cases were first identified in Uganda and in the United Republic of Tanzania in 1952.[7] The first Zika fever outbreak occurred in the Island of Yap in 2007.[6] Non-vector-mediated methods including sexual and congenital transmissions have also been reported for ZV infection.[6] ZV infection during pregnancy can cause congenital Zika syndrome, which gives birth defects such as microcephaly and several other severe foetal defects.[6]

Until now, no cases of ZV infection have been reported in Sri Lanka. However, the Epidemiology Unit of Sri Lanka is concerned that Sri Lanka is at risk for ZV infection as neighbouring countries (India and Maldives) have reported ZV transmission.[8] Since the principal vectors of ZV transmission are found across Sri Lanka, the danger of ZV disease through mosquito bites remains a noteworthy concern.[8] *Aedes* mosquitoes are the common vectors for DENV, CHIKV and ZV infections. In areas

with co-circulation of these viruses, these infections can be transmitted by *Aedes* mosquitoes resulting in viral co-infections.[9]

Despite the presence of *Aedes* vectors and the availability of breeding sites in the study area, the number of reported dengue cases was not high before 2009, the year of the cessation of war.[10] A high population mobility to Jaffna Peninsula from other parts of the island as well as from many other countries after the end of the conflict resulted in the increased number of both dengue and possible CHIKV infections in the study area.[10,11] The emergence and re-emergence of these viral infections has been documented from 2009-2012 during which the current study samples were also collected from patients with suspected dengue fever.[12] Epidemiological concern and non-availability of routine diagnostics for CHIKV and ZV infections in most Sri Lankan laboratories led us to test for serological evidence for DENV/CHIKV/ZV infections in samples collected during the 2009 and 2012 dengue outbreaks in the Jaffna district from patients clinically suspected of having dengue fever. Here, we report the serological evidence for CHIKV and ZV infections in patients with clinically diagnosed dengue fever from the Jaffna district of Sri Lanka.

Methods

Samples and data collected during two dengue outbreaks which occurred between 2009 and 2012 were used for the study. Ninety-one (n=91) blood samples from patients who presented to the Medical Wards of Teaching Hospital, Jaffna (THJ) with acute febrile illness of >7 days from the onset of fever, arthralgia, myalgia, rash, headache and retro-orbital pain were collected on the day of admission for analysis. Patients with other concurrent illnesses, immuno-suppression, and pregnant women were excluded. The serum samples stored at -40 ℃ were tested for anti-CHIKV IgM and IgG using an ELISA (Standard Diagnostics, Korea) and anti-ZV IgM and IgG using an ELISA, ABCAM (Standard Diagnostics, USA).

Samples were processed according to the manufacturer's instructions with the use of positive and negative controls. Clinical and demographic data were extracted from the questionnaires used during the collection of samples. Data were analysed using Chi square test by statistical package for social sciences (SPSS, Version 17).

The following definitions were used for different serological status of infections in this study:
Clinically suspected dengue – Patients exhibiting clinical features similar to dengue.
Recent infection – having dengue/CHIKV fever/Zika fever with clinical features suggestive of these infections with the presence of detectable IgM antibodies.
Co-infection – evidence for two or more viral infections (dengue/CHIKV fever/Zika fever) with simultaneous presence of detectable IgM antibodies.
Past exposure – Previous infection with DENV / CHILV / ZV identified by presence of detectable IgG antibodies.

Results

Of the serum samples tested (91 patients), CHIKV specific IgM antibodies were positive in 11 (12.1%) and CHIKV specific IgG antibodies were positive in 31 (34%) patients. However, the 11 patients positive for anti-CHIKV IgM also had anti-CHIKV IgG indicating a recent CHIKV infection in these

11 patients. Those with anti-CHIKV IgG only (n=20) would have had past exposure to CHIKV (Table 1).

Of the 91 patients with fever for more than 7 days, 11 had recent CHIKV infection (12.1%) and 20 had past exposure to the virus (21.9%), making an overall exposure rate of 34.1% to CHIKV in the study sample.

Anti-DENV IgM was present in 63 of the 91 patients (69.2%). Co-infection with CHIKV and DENV was detected in 8 patients (8.8%). Of the 8 co-infected patients, 5 (62.5%) had past infection with DENV. Thirteen patients (14.29%) with acute DENV infection had past exposure to CHIKV (Table 1).

Table 1. Serological status of CHIKV and DENV infections in the study sample (n=91).

Serological evidence for	No. of patients	Percentage (%)
Recent CHIKV infection	11	12.1
Past exposure to CHIKV infection	20	21.9
Recent DENV infection	63	69.2
Recent co-infection with CHIKV and DENV	8	8.8
Co-infected patients with past exposure to DENV	5	62.5
Recent DENV infection and past exposure to CHIKV	13	14.3
Negative for CHIKV and DENV infections	01	1.1

ZV specific IgM antibodies were detected in one patient (1.1%) and ZV specific IgG antibodies were detected in 18 patients (19.8%) giving an overall exposure rate of 20.9% to ZV infection in the present study sample (Table 2).

Of the 63 patients who were DENV specific IgM positive, 15 (16.5%) had past ZV infection. None of the patients had both anti-DENV IgM and anti-ZV IgM antibodies. However, 12 patients (13.2%) had both anti-DENV IgG and anti-ZV IgG antibodies (Table 2).

Table 2. Serological status of ZV and DENV infections in the study sample (n=91).

Serological evidence for	No. of patients	Percentage (%)
Recent ZV infection	1	1.1
Past exposure to ZV infection	18	19.8
Recent DENV infection	63	69.2
Recent co-infection with ZV and DENV	0	0
Co-infection based on past exposure to DENV and ZV	12	13.2
Recent DENV infection and past exposure to ZV	15	16.5

Of the 91 study participants, headache was experienced by all who had CHIKV fever (n=11) and 84% of the patients with dengue (p=0.0001). Dyspnoea was observed in more patients with CHIK fever compared to dengue (p=0.001). None of the patients with CHIKV fever had pleural effusion, while a substantial number of dengue patients had pleural effusion (p=0.095). Retro-orbital pain (p=0.005) and flushed skin (p=0.0001) were more prominent signs observed in dengue compared to CHIK fever. Arthralgia, myalgia, rash, haemorrhage, splenomegaly, and pallor were common in both DENV and CHICK infections (Table 3).

Of the 11 patients with CHIKV fever, 55% (p=0.01) had hepatomegaly although it is not specific to CHIKV infection. Hepatomegaly was noted in a significantly larger number of dengue patients (p=0.00). Almost 63% of patients co-infected with CHIKV and DENV had hepatomegaly. There is a significant association between hepatomegaly and CHIKV or DENV mono or CHIKV and DENV co-infections (p=0.01). About half of the co-infected patients suffered from arthralgia, myalgia and hepatomegaly and a substantial number had retro-orbital pain, flushed extremities, rash, haemorrhage, dyspnoea and splenomegaly. The severity of clinical illness appears to be moderate in CHIKV and DENV co-infected patients (n=8, 8.8%) compared to that in mono-infected patients with severe disease.

Table 3. Clinical features of sero-positive patients with recent CHIKV and DENV infections.

Clinical features in patients ≥7 days post-onset of fever	% patients with CHIK fever (n=11)	% patients with dengue (n= 63)	*p* value
Headache	100	84.1	**0.0001***
Retro-orbital pain	27.3	46.1	**0.005***
Myalgia	63.6	69.8	0.367
Arthralgia	45.5	49.2	0.571
Dyspnoea	18.2	3.2	**0.001***
Flushed skin	18.2	55.6	**0.0001***
Pallor	9.1	7.9	0.800
Rash	18.2	26.9	0.126
Splenomegaly	9.1	6.4	0.419
Hepatomegaly	54.6	38.1	**0.016***
Haemorrhage	27.3	20.6	0.320
Effusion	0	1.6	0.095

*$p < 0.05$ is taken as statistically significant.

Of the 91 patients in the study sample, headache was experienced by all patients with exposure to ZV and DENV while 84.1% of the patients with acute DENV infection reported headache. Fever was observed as the most common finding in patients with past exposure to ZV and acute DENV infection. Myalgia, arthralgia, flushed skin and retro-orbital pain were seen in more than 50% of the patients in both groups. Dyspnoea, pallor and splenomegaly were the less common signs observed in both groups. Effusion was more prominent in patients with dengue (Table 4).

Females were predominantly exposed to CHIKV (54.54%), ZV (52.63%) and DENV (55.56%) infection in the current study sample. The most exposed age group for CHIKV infection was 0-20 years and that for DENV was 21-30 years. For ZV infection, the most exposed females were 21-30 years and males were 11-20 years of age. Age groups of 0-10, 11-20 and 20-30 years were exposed to CHIKV and DENV co-infection more frequently compared to other age groups.

Table 4. Clinical features of sero-positive patients for ZV and DENV infections.

Clinical features	% patients with past exposure to ZV and acute DENV infection (n= 12)	% patients with acute DENV infection (n= 63)
Headache	100	84.1
Fever	100	100
Retro-orbital pain	58.3	46.1
Myalgia	83.3	69.8
Arthralgia	58.3	49.2
Dyspnoea	8.3	3.2
Flushed skin	58.3	55.6
Pallor	8.3	7.9
Rash	25	26.9
Splenomegaly	8.3	6.4
Hepatomegaly	41.6	38.1
Haemorrhage	25	20.6
Effusion	0	1.6

Discussion

In the present study sample, 12% had laboratory evidence for CHIKV infection (Table 1). However, all these patients were clinically diagnosed and managed as having DENV infection. Nearly one third of the present study sample had exposure to CHIKV infection. The burden of CHIKV infection cannot be commented upon using the current data as our study sample is small and it could have been high when considering the total case load during the dengue outbreaks in the study area. However, the current study data emphasise the importance of testing for CHIKV infection in the study area which was affected by the 2006/2007 chikungunya outbreaks.[3]

The results of this study show that less than three fourths of patients suspected of having dengue had DENV infection based on the anti-DENV IgM positivity. Of the anti-DENV IgM positive patients, a substantial number had past exposure to CHIKV infection. The presence of possible co-infection with both DENV and CHIKV by IgM positivity in the study population suggests the co-circulation of these viruses in *Aedes* mosquitoes as reported in other tropical regions.[9] Moreover, most of the co-infected patients had past exposure to DENV, supporting the hyper-endemicity of DENV in the study area.[13]

Co-infected patients had relatively less severe clinical disease compared to infections with CHIKV or DENV alone. Most of the clinical features of patients with CHIKV and DENV infections were similar during the non-specific clinical phase of the disease as also reported in previous studies.[5,9,14] Arthralgia, myalgia, rash, splenomegaly, haemorrhage and pallor were noted in both mono and co-infections and these features had no significant association to a particular infection.[14,15] Dyspnoea, hepatomegaly and headache were found to be the prominent signs in CHIKV infection whereas retro-orbital pain and flushed skin were noted as marked signs in dengue as has been observed in previous studies.[16,17,18,19] In both mono- and co-infections, hepatomegaly was associated with CHIKV infection which is in agreement with a previous study.[5]

Females were more affected by both CHIKV and DENV infections in the study sample compared to males. The female predominance in dengue has been reported in different areas of Sri Lanka.[10,16] More of those less than 30 years were affected with CHIKV mono-, DENV mono- and CHIKV and DENV co-infections compared to those older than 30 years. This can vary with different areas and populations as reported previously.[18,19,20.]

Detection of anti-ZV IgM antibodies in 1.1% of the study sample suggests an acute or a recent ZV infection and the finding of anti-ZV IgG antibodies in 19.8% patients indicates past infection with ZV, resulting in an overall exposure rate of 20.9%. Almost 13% of the study sample had past DENV and ZV infections. These finding suggest the possible co-circulation of ZV and DENV in the study area. However, it is difficult to comment whether these infections occurred simultaneously or resulted from initial DENV infection followed by ZV superinfection or vice versa as detection of IgG antibodies against DENV and ZV does not always mean the occurrence of simultaneous infections. It is also possible that DENV and ZV infections occur at different times, but the resulting IgG antibodies persist and become detectable at the time of testing. An overall exposure rate of 20.9% for ZV infection is significant and suggests the need to consider ZV infection in the differential diagnosis, at least in women in the reproductive age, including pregnant women.

Serological data from the current study reflects the status of the tested markers of CHIKV, ZV and DENV infections as the commercial assays used in this study (Standard Diagnostics, Korea & Standard Diagnostics, USA) have high sensitivity and specificity. Sensitivity and specificity of CHIKV IgM and CHIKV IgG detection are 93.6% and 95.9%, respectively with an overall accuracy of >90%. ZV IgM and ZV IgG detection has >90% sensitivity and specificity. CHIKV antibodies had no cross reactivity with DENV antibodies as these viruses belong to two different families (as documented by the manufacturer - Standard Diagnostics, Korea). However, cross reactivity between DENV and ZV antibodies cannot be excluded unless virus neutralization is performed to confirm the status as these viruses belong to the same family. Lack of virus neutralization studies to rule out cross reactivity between DENV and ZV antibodies is a limitation of the current study. The effects of freeze-thaw cycles of samples on the results can be ignored as original serum samples were aliquoted into three different micro-centrifuge vials and a new set of vials stored for serological testing were used for the analysis.

Most of the clinical features are similar during the non-specific clinical phase CHIKV, ZV and DENV infections and tend to overlap in co-infections. Clinical diagnosis of a particular infection is therefore difficult without laboratory evidence.[5] Thus in future dengue outbreaks, CHIKV and ZV infections should be considered in the differential diagnosis, at least in the risk groups. Rapid tests can be used to screen for CHIKV and ZV infections, similar to the point of care diagnosis used in DENV infection. Overall, this study also emphasizes the importance of improving viral diagnostics in the country[21] for the confirmation of CHIKV and ZV infection in dengue affected areas.

The current study findings also provide a warning for possible CHIKV and ZV outbreaks in the future and the need to initiate surveillance activities for CHIKV and ZV infections, at least in the provincial hospitals.[21] Moreover, vector surveillance for these viruses during pre-monsoon and monsoon periods can alert the authorities to prepare well to combat future outbreaks. Education and awareness programmes for the public are essential as CHIKV infection re-emerged in the country in 2006/2007

after 40 years since the 1965 outbreak.[4] Awareness among clinicians and public can facilitate early detection of CHIKV and ZV infections.

Conclusions

The current study reports exposure to CHIKV and ZV infection among patients clinically diagnosed as having dengue using serological testing in northern Sri Lanka. Exposure rate to CHIKV and ZV infection accounts for nearly 34% and 21%, respectively. The study also emphasises the need to test for CHIKV and ZV infection in dengue affected areas of Sri Lanka as these infections are clinically indistinguishable during the early phase of the illness.

Declarations

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