

Evaluation of renal markers and liver enzymes in patients infected with the Chikungunya virus

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April 24, 2023

Abstract

Chikungunya virus (CHIKV) is an arbovirus (*Togaviridae* family, *Alphavirus* genus), first identified in 1953 in Tanzania. In 2005, CHIKV emerged in India, and later caused outbreaks in Southeast Asia, Oceania and the Americas. Some Clinical signs are associated with CHIKV infection include fever and/or concomitant arthralgia, neurological manifestations and death. However, in infections caused by other arboviruses, such as the Dengue virus and West Nile virus, its often observed changes in liver enzymes. This study aims to evaluate the profile of the biochemical markers for kidney and liver injury patients infected with CHIKV in acute phase of infection. We found a significant elevation on the levels of creatinine in CHIKV-infected people, possibly associated with myalgia and indicative of muscle damage. The novelty was the elevated levels of creatinine found during a long period.

Introduction

Chikungunya fever is featured as an acute febrile illness and associated to a severe and debilitating polyarthralgias.^[1] Caused by Chikungunya virus (CHIKV), that belong of the *Togaviridae* family and genus Alphavirus, discovered in 1950s in Tanzania on the African continent, the name Chikungunya derivate from the idiom Makonde, that means "that which bends up", due to the severe polyarthralgia that make the infected people walk with a stooped posture, with the spine curved. This virus is transmitted by the bite of an infected mosquito of the genus *Aedes* to humans. CHIKV virus is small, with icosahedral symmetry and a capsid size of 65-70 nanometers in diameter. The viral genome consists of a single positive-strand RNA molecule, 12 kb-long with a methylguanosine cap at 5' UTR (untranslated region) and a polyadenylated at 3' UTR. It has two ORF (open reading frame). One that encode for non-structural proteins involved in virus replication and pathogenesis, and other encodes for structural proteins that compose the virion structure.^[2] Phylogenetic inferences on the envelope protein (E1) gene sequences suggest that the virus Chikungunya can be subdivided in three genotypes: (i) Asian, (ii) East/Central/South/African (ECSA), and (iii) West African.^[3-5]

In 2004, CHIKV re-emerged and spread to new regions, such as Europe, and caused millions of infections throughout countries in the Indian subcontinent. It has been proposed that the introduction of CHIKV into new area and its current worldwide distribution has been facilitated by: (i) the high attack rates associated high levels of viremia in infected humans,^[9] and, (ii) the high competence of the *Aedes spp* vectors, responsible for transmitting CHIKV in epidemiologic settings.^[1,5,6]

Serum levels of the liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are elevated in several diseases, such as chronic viral hepatitis, non-alcoholic fatty liver disease, autoimmune

hepatitis, hemochromatosis, and alcoholic liver disease. Therefore, both are taken as markers of liver damage. On the other hand, creatinine and urea are renal markers that indicate renal injury levels.^[7]

Moreover, high levels of uric acid are a well-established risk factor for gout and renal problems.^[8,9] Clinical and laboratory evidence indicated muscular dysfunctions in patients infected by Dengue virus (DENV), with high blood levels of creatine kinase (CK), enzyme that indicate muscular lesion.^[9] Likewise, CHIKV infects progenitor muscular cells, which associates with muscle tissue damage.^[9]

In infections caused by other arboviruses such as DENV and Yellow Fever virus (YFV), changes in liver enzymes are observed, due to viral tropism for hepatocytes, which causes cell death and an increase in ALT and AST levels in the serum. Although clinical studies evidenced the severe complications during CHIKV infection^[10], in our work we tried describe the course of infection in eighty-six CHIKV-viremic patients. Therefore, we evaluated clinical findings comparing to biochemical test results for: (i) hepatic enzymes, such as alanine aminotransferase and aspartate aminotransferase (ALT, AST) and, (ii) renal markers, such as: urea, creatinine and uric acid.

Objective

Evaluate the biochemical markers profile in serum samples of acute patients with CHIKV, to study the course of infection and suggest tissue damage.

Material and methods

Serum samples. Serum samples were collected using vacuum tube systems after a garroting period of 1 minute or less. Five ml of total blood was harvested with a dry tube without additives and without gel seed. After clot retraction was observed, the samples were centrifuged at 3000 rpm for 10 minutes at room temperature. Samples were stored at -80 °C.^[11] All samples were classified and catalogued, including clinical and demographic information from each patient.

Molecular diagnosis. The CHIVK infection status previously confirmed by qRT-PCR by our group.^[12]

Biochemical analysis. The biochemical analysis was performed on a fully automatic analyzer, Cobas® 6000 module (Cobas C501, Roche Diagnostics International Ltd., Rotkreuz, SWZ), using kits provided by Roche Diagnostics International Ltd. The dosage of the biochemical profile was made by kinect enzymatic and colorimetric methods as dictated by Roche Diagnostics International Ltd.^[13,14,15]

Statistical analysis. To evaluate clinical association among renal markers and hepatic alterations, we performed the Student's t-test, on data from patients that presented symptoms and markers alterations under a statistic significance of $p < 0.05$. Some comparisons were not done because of a lack of proper patient records or a lack of sample material. Because gene expression and viral genome replication show logarithmic variation, we used a Kolmogorov-Smirnov test (K-S test) to verify correlations of clinical test results with Cts obtained from the same patients. The K-S, is a non-parametric test on the equality of continuous and one-dimensional probability distributions that can be used to compare a sample with a reference probability distribution (uniamostral K-S test) or two samples with each other (bi-lateral K-S test).^[16]

A critical $p > 0.05$ level was assumed as indication of difference in distributions between changes in clinical markers and Cts of patients.

Results

We evaluated the biochemical profile of serum samples, from 86 CHIKV viremic patients, confirmed by qRT-PCR, which also presented headache, fever, severe joint pains, myalgia, and rash. As shown in Table 1, the biochemical profiles obtained indicated: (i) an elevation of creatinine in 54/86 (63%) patients, (ii) AST was altered in 37/87 (29%) patients, (iii) ALT was elevated in 6/86 (7%) patients, (iv) high urea was observed in 11/86 (13%) patients and, (v) 31/87 (36%) patients presented high levels of uric acid. Table 1 shows a correlation between symptoms and hepatic lesion indicators. The correlations were obtained with 85 samples for the dosage of ALT, since one sample had insufficient volume to dose AST. For fever, we had a

77, because nine patients did not provide reliable answers. Thirty one percent of 86 patients had alterations on AST levels and 7 % patients (n= 86) presented alterations in ALT. The correlation of the AST/ALT with symptoms did not show statistical significance. Table 2 shows that 13% (n=86) of the patients had alterations in urea, 36% (n=86) had alterations in uric acid and 63% (n=86) with alterations in creatinine. We also observed statistical significance on the correlation of renal markers with creatinine, myalgia and uric acid with asthenia. Moreover, we also found significant correlation between vomiting and hemorrhagic signs. From a clinical standpoint, the correlation of creatinine with myalgia can be relevant, but the uric acid and symptoms correlations did not appear to have clinical relevance.

Figure shows the distribution between of the biochemical test results, and viral Ct. We chose to use a Kolmogorov-Smirnov test to evaluate the significance of correlations based on the differences between distributions, because box plots were suggestive of differences in the distributions of Cts when comparing infected patients, with and without alterations in enzymatic levels and, because Cts indicate viral load variations in logarithmic space.

Discussion

CHIKV infection causes several clinical manifestations such as, fever, myalgia, headache and severe joint pains.^[17] In general; the manifestations are like those in ZIKV and DENV infections. Consequently, accurate clinical diagnostics are difficult and laborious.^[17] The biochemical parameters showed alterations in all parameters analyzed. Based on our data, it could be argued that, overall the alterations in the biochemical markers were associated with clinical manifestations. The study population, and the results showed no statistical significance in AST, ALT and uric acid elevations, but a significant association was found between high levels of creatinine and myalgia. Importantly, myalgia was one of the symptoms most referred to by our patients during infection.^[18]

Creatinine alterations are indicative of severe renal impairment.^[19] However, creatinine is also a product of the degradation of the muscular creatine. This could help explain why we obtained a high level of this metabolite in the serum of the infected patients. It is known that CHIKV can replicate in muscular cells^[19], causing intense cell lysis, elevating creatine levels in the blood stream, which will be later converted in creatinine and excreted by the kidneys.^[19] Another hypothesis could be that CHIKV is able to cause renal lesions, causing renal function imbalance during infection, as observed on murine models. Fittingly, CHIKV is known to replicate in baby hamster kidney (BHK-21) cells, and was found in their urine, suggesting a possible viral replication on mice kidneys.^[20] Moreover, the virus was found in urine and semen of patients during infection, also indicative of renal tropism.^[20]

Ethics

Ethics. This study is part of a project for arbovirus research in Sergipe, Brazil approved by the Ethics Committee of the Department of Microbiology of the Institute of Biomedical Sciences of the University of São Paulo (Protocol 1284/CEPSH – CAAE: 54937216.5.0000.5467).

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Table 1: clinical manifestations and hepatic lesions markers.

AST	AST	AST	AST	ALT	ALT	ALT
Clinical manifestations	n	%	p value	n	%	p value
Fever	77	23,37	1.000	77	2,59	0,168

AST	AST	AST	AST	ALT	ALT	ALT
Headache	86	25,58	0.150	85	7,05	1.000
Myalgia	86	19,76	0.315	85	3,52	0,372
Joint pain	86	29,06	0.668	85	5,88	0,408
Asthenia	86	11,62	0.196	85	1,17	0,662
Hemorrhagic Signs	86	1,16	1.000	85	1,17	0,255
Meningo-encephalitis	86	1,16	0.294	85	0	1.000
Vomiting	86	2,32	0.056	85	1,17	1.000
Retro-orbital pain	86	10,46	0.286	85	1,17	1.000
Abdominal pain	86	2,32	1.000	85	0	1.000
Rash	86	6,97	0.781	85	1,17	1.000
Itching	86	8,13	0.167	85	0	0,588

Table 2: Clinical manifestations and renal markers

UREA	UREA	UREA	UREA	CREATININE	CREATININE	CREATININE	UR
Clinical manifestations	n	%	p value	N	%	p value	n
Fever	77	11..68	0.676	76	41.86	1.000	77
Headache	86	10.46	0.609	86	55.81	1.000	86
Myalgia	86	8.13	0.734	86	37.2	0.017*	86
Joint pain	86	10.46	0.218	86	58.13	1.000	86
Asthenia	86	3.48	1.000	86	19.76	0.811	86
Hemorrhagic Signs	86	0	1.000	86	3.48	1.000	86
Meningo-encephalitis	86	0	1.000	86	1.16	1.000	86
Vomiting	86	1.16	0.683	86	10.46	1.000	86
Retro-orbital pain	86	4.65	0.491	86	17.44	1.000	86
Abdominal pain	86	0	0.596	86	8.13	0.741	86
Rash	86	4.65	0.272	86	11.62	0.196	86
Itching	86	0	0.349	86	8.13	0.755	86

*Statistically significant.

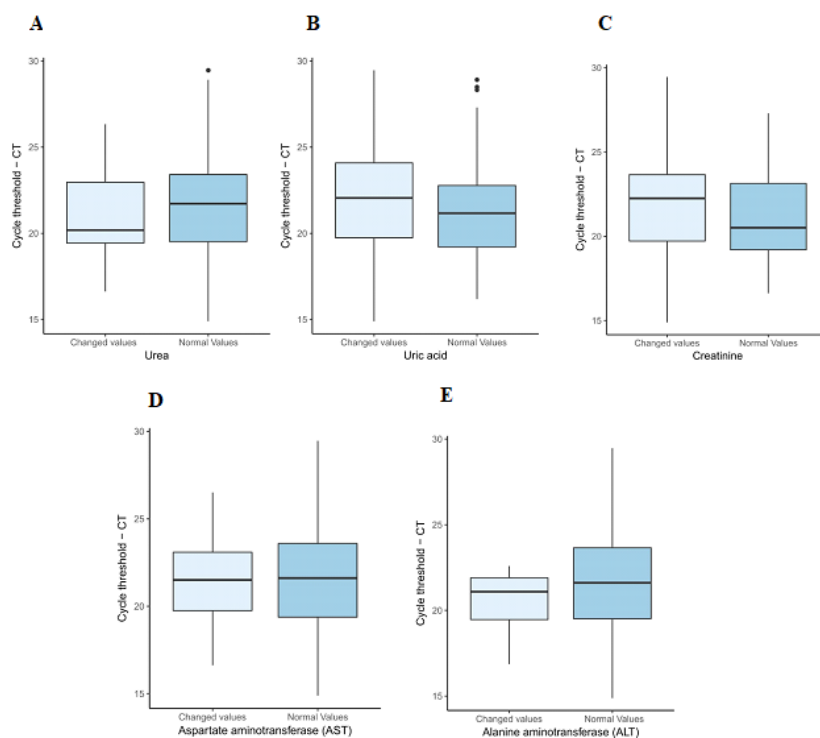


Figure:Boxplot graphs showing the distribution of the biochemical tests and CT values. In A, B and C is represented the renal markers, urea, uric acid and creatinine normal and altered values, correlated with different CT values. In D and E is represented the hepatic markers, AST, ALT and the correlation with CT values.