Received: 21 April 2023 | Accepted: 17 November 2023

DOI: 10.1002/jmv.29276

SHORT COMMUNICATION

JOURNAL OF MEDICAL VIROLOGY WILEY

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

Anderson Pereira Soares[1] | Daniel Ferreira de Lima Neto[1] | Shahab Zaki Pour[1] | Saulo Duarte Passos[2] | Marielton dos Passos Cunha[1] | Paolo Marinho de Andrade Zanotto[1]

[1]Laboratory of Molecular Evolution and Bioinformatics, Department of Microbiology, Biomedical Sciences Institute, University of São Paulo, São Paulo, São Paulo, Brazil

[2]Department of Pediatrics, Jundiaí School of Medicine, Jundiaí, São Paulo, Brazil

Correspondence
Paolo Marinho de Andrade Zanotto, Laboratory of Molecular Evolution and Bioinformatics, Department of Microbiology, Biomedical Sciences Institute, University of São Paulo, São Paulo, Brazil.
Email: pzanotto@usp.br

Funding information
Fundação de Amparo à Pesquisa do Estado de São Paulo

Abstract

Chikungunya virus (CHIKV) is an arbovirus (*Togaviridae* family, *Alphavirus* genus) that was first identified in 1953 in Tanzania. In 2014, the Asian and East/Central/South/African (ECSA) genotypes were identified in Brazil, although the genotype that spread the most in the following years across the Brazilian territory was the ECSA. The clinical symptoms associated with the infection caused by CHIKV include mainly fever, myalgia, headache, and arthralgia. In infections caused by other arboviruses (such as the ones caused by Dengue and West Nile viruses), changes in biochemical markers are often observed. This study aims to evaluate the biochemical markers profile of kidney and liver injury in acute patients infected with CHIKV. Two groups of correlations were found between the variables analyzed, namely, one between liver enzymes ($r = 0.91$), and another for kidney markers ($r = 0.54–0.66$). A significant elevation in the percentage of altered creatinine in CHIKV-infected patients was observed, followed by uric acid and AST. Altogether, in 8 different comparisons, it was possible to observe statistically significant differences between the levels of the markers when compared to the manifestation of symptoms (presence and absence). These noticeable changes in marker measurements could potentially be connected to the range of clinical symptoms seen in the disease.

KEYWORDS
Chikungunya virus infection, liver enzymes, renal markers

1 | INTRODUCTION

Chikungunya fever is an acute febrile illness associated with severe and debilitating polyarthralgia[1,2] caused by the Chikungunya virus (CHIKV), which belongs to the *Togaviridae* family and genus *Alphavirus*, discovered in the 1950s in Tanzania.[3] The virus is transmitted to humans during feeding of infected *Aedes* mosquitoes.[4,5] The viral genome consists of a single positive-strand RNA molecule, 12 kb, with a 7-methylguanosine cap at the 5′ UTR (untranslated region) and a polyadenylated region at the 3′ UTR, and the coding region includes two ORFs (open reading frames) coding for nonstructural and structural proteins, respectively, all of which are

Marielton dos Passos Cunha and Paolo Marinho de Andrade Zanotto contributed equally to this article.

arranged as a 5′-cap-nsP1-nsP2-nsP3-nsP4-(junction-region)-C-E3-E2-6K-E1-poly(A)-3′.[6]

Evolutionary analysis based on genomic sequences suggested that the CHIKV can be subdivided into three genotypes: (*i*) Asian, (*ii*) East/Central/South/African (ECSA), and (*iii*) West African.[7–10] In 2004, CHIKV re-emerged and spread to new regions, such as Europe, and caused millions of infections throughout countries in the Indian subcontinent.[7,11] It has been proposed that the introduction of CHIKV into new areas and its current worldwide distribution have been facilitated by: (*i*) the high attack rates associated with high levels of viremia in infected human patients[12]; and (*ii*) the high competence of the *Aedes spp*. vectors, responsible for transmitting CHIKV in epidemiologic settings.[4,13–17] In Brazil, the first cases identified are from 2014, when the ECSA and Asian genotypes were identified simultaneously,[18] however, the vast majority of cases subsequently identified were characterized as belonging to the ECSA genotype.[18–21]

Serum levels of the liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are elevated in several diseases, such as chronic viral hepatitis, nonalcoholic fatty liver disease, autoimmune hepatitis, hemochromatosis, and alcoholic liver disease.[22–24] On the other hand, creatinine and urea are renal markers that indicate renal injury levels.[25] Moreover, high levels of uric acid are a well-established risk factor for gout and renal perturbations.[26,27] In some arbovirus infections (such as those caused by the Dengue virus), muscular dysfunctions occur in infected patients and correlate with high blood levels of creatine kinase (CK), an enzyme that indicates muscular lesions.[28] Likewise, CHIKV infects progenitor muscular cells, which are associated with muscle tissue damage.[29] In other infections caused by arboviruses, such as Dengue virus (DENV) and Yellow Fever virus (YFV), changes in the liver enzymes are observed due to viral tropism towards hepatocyte cells, which causes cell death. Moreover, an increase in ALT and AST levels is observed in the serum samples. Although clinical studies have evidenced severe complications during CHIKV infection,[30] little is known about the quantitative distribution of these markers in infected patients. Here, we described the course of infection in CHIKV-viraemic patients ($n = 85$), comparing the clinical and laboratory findings to evaluate: (*i*) hepatic enzymes, such as alanine aminotransferase and aspartate aminotransferase (ALT and AST); and (*ii*) renal markers, such as urea, creatinine, and uric acid.

2 | MATERIAL AND METHODS

2.1 | Ethical statement

This study is part of a project for arbovirus research initiated in Sergipe State, 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). Informed consent was provided and obtained from all research subjects enrolled in the study.

2.2 | Patients and samples

The samples were collected from symptomatic human patients in Sergipe State, Brazil, in February 2016. Serum samples were collected using vacuum tube systems after a garrotting period of 1 min or less. Five ml of total blood was collected in a dry tube without additives. After clot retraction, the samples were centrifuged at 3000 rpm for 10 min at room temperature. Samples were stored at −80°C.[31–33] All samples were classified and cataloged, including clinical and demographic information from each patient.

2.3 | Molecular diagnosis

The CHIKV RNA identification was previously confirmed by RT-qPCR[19] using the protocol previously validated. The viral RNA was extracted using the QIAamp Viral RNA Mini Kit (Qiagen, Valencia, CA, USA), and the molecular detection of CHIKV RNA[34] was performed with the use of the Quantitative RT-PCR ReadyMix™ reagents (Sigma-Aldrich, St. Louis, MO, USA). The RT-qPCR reactions consisted of a step of reverse transcription at 44°C for 30 min followed by enzyme activation at 94°C for 2 min, and 40 cycles at 94°C for 15 s and 60°C for 1 min for hybridization and extension with the use of ABI7500 equipment (Thermo Fisher Scientific, Waltham, MA, USA).

2.4 | 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.[35–39]

2.5 | Statistical analysis

We performed the correlation analysis between the CHIKV Ct values and the measurements of the renal markers and liver enzymes to determine the degree of relationship among these variables. We also performed descriptive statistical analysis to estimate the percentage of changes observed for each marker or their combination in renal markers and liver enzymes. The Shapiro-Wilk normality test was used to guide the analytical strategy. The two-sample *t*-test was conducted if both samples had passed the preliminary Shapiro-Wilk test for normality; otherwise, we applied Mann-Whitney's *U* test, assuming a significance level of 0.05, to evaluate the clinical profile of the patients in comparison with the measurements obtained from renal markers and liver enzymes. The analyses were performed using R programming version 4.2.2 and RStudio version 2023.06.0 + 421,[40] and the R scripts are available upon request.

2.6 | Experimental design

The inclusion of controls would undoubtedly provide an ideal choice for a baseline for the study. However, due to the observational nature of this fieldwork, we collected samples from symptomatic patients, as determined by the MD *in loco*, and only did clinical exams on CHIKV PCR-positive patients. But to establish a much-needed parameter for comparison we used the canonical and well-established clinical diagnostic reference values that, therefore, fulfill an equivalent function as that of the *bonafide* control for establishing a baseline (the range considered altered or normal). Furthermore, we used the gathered information on symptoms during sample collection, and one could assume that baseline information is also provided within this positive group for Chikungunya, for each symptom studied, which allows us to obtain comparisons. All data involved in the development of the study will be made available by the authors upon request.

3 | RESULTS

We evaluated the biochemical profile in serum samples from 85 acute CHIKV viraemic patients, confirmed by RT-qPCR, which also presented headache, fever, joint pain, and myalgia.[19] The quantitative data obtained for the markers considered in the study (urea, creatinine, uric acid, AST, ALT, and the Ct value obtained for viral RNA detection) revealed the existence of two correlation groups: one for the group of enzyme liver markers (AST and ALT) and the other for the group of renal markers (urea, creatinine, and uric acid) (Figure 1A). Based on these findings, we considered two types of classes: (*i*) changes in liver enzymes (AST Reference Range [RR]: Male: <40 U/L, Female and child: <32 U/L; ALT RR: Male: <50 U/L, Female and child: <35 U/L), and (*ii*) changes in renal markers (Creatinine RR: Male: 0.70–1.20 mg/dl, Female and child: 0.40–0.60 mg/dl; Urea RR: 17–49 mg/dl, and Uric Acid RR: Male: 3.4–7.0 mg/dl, Female and child: 2.4–5.7 mg/dl). This class considered in combination with the union of patients who have one or more of these alterations (renal or hepatic alterations), indicated that alterations in renal markers are more frequent (83.5%) than alterations in liver enzymes (32.9%). The biochemical profiles obtained indicated: (*i*) alteration in the creatinine levels in 65/85 (76.5%) patients; (*ii*) AST altered in 27/85 (31.8%) patients; (*iii*) ALT elevated in 6/85 (7.1%) patients; (*iv*) altered urea observed in 11/85 (12.9%) patients; and (*v*) 30/85 (35.3%) patients that presented alteration in levels of uric acid (Figure 1B).

No significant differences were found when addressing the distribution of Ct values and the days after the onset of symptoms when compared to changes in renal markers and liver enzymes, although there is a tendency of difference to the renal markers (altered and non-altered) considering the duration of symptoms ($p = 0.05789$) (Figure 1C–F). When considering the highest values of the Pearson's correlation coefficient, among correlations for renal markers, patients with or without alteration overlap, while for liver enzymes, two well-defined and distinct data points clusters in the graphs (Figure 1) occur in the CHIKV-positive patients, considering alteration and absence of alteration in these (Figure 1G–J).

Exploring the clinical presentation of the symptoms and the markers measurements, statistically significant differences (i.e., with *p*-values less than 0.05) were observed in 8 comparisons (Table 1). It was found that the presence of symptoms was directly associated with the marker levels, including headache and creatinine ($p = 0.0335$), fever and uric acid ($p = 0.0145$), and hemorrhagic signs and uric acid ($p = 0.0195$). On the other hand, the presence of symptoms was negatively associated with the levels of the markers analyzed in five comparisons, including rash and urea ($p = 0.0144$), rash and creatinine ($p = 0.0461$), vomiting and AST ($p = 0.0208$), abdominal pain and AST ($p = 0.0444$), and vomiting and ALT ($p = 0.0490$) (Figure 2).

4 | DISCUSSION

The CHIKV infection causes several clinical manifestations, such as headache, fever, joint pain, and myalgia.[19,41,42] In general, the manifestations overlap with those observed in the infections caused by other arboviruses, including ZIKV and DENV.[43] Consequently, accurate predictive clinical diagnostics based on symptoms are difficult.[44] In this context, biochemical clinical exams, usually performed in clinical laboratories, can be of great importance to characterize the patient's physiological profile and also to collaborate at the beginning of the patient's symptomatic treatment.[45,46] Here, our results indicated that the biochemical parameters showed alterations in all parameters analyzed, with alterations in renal markers being more frequent than in liver enzymes. Nevertheless, the correlation and organization between the alteration profile and the absence of alteration are more noticeable in the distinctions of patterns of data points clustering in the graph(s) of these patients. However, this can be justified by: (*i*) the reference values are more distinct in renal markers according to the patients; (*ii*) most of the CHIKV-positive patients have renal alterations.

Creatinine alterations are indicative of severe renal impairment.[47] However, creatinine is also a product of the degradation of muscular creatinine. 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,[29] causing intense cell lysis and elevating creatinine levels in the bloodstream, which will be later converted into creatinine and excreted by the kidneys.[29,48,49] Another hypothesis could be that CHIKV can cause renal lesions, causing a renal function imbalance during infection, as observed in murine models. Fittingly, CHIKV is known to replicate in baby hamster kidney cells (BHK-21 cells) and was found in urine samples, suggesting a possible viral replication in the kidney.[48,49] Moreover, the virus was found in the urine and semen of patients during infection, which is also suggestive of renal tropism.[50,51] Although the frequency of alterations was much lower for liver markers, they had a higher correlation than all the others, and better spatially organized. In severe cases of infection caused by CHIKV, the liver appears to be an organ involved in this outcome.[52,53]

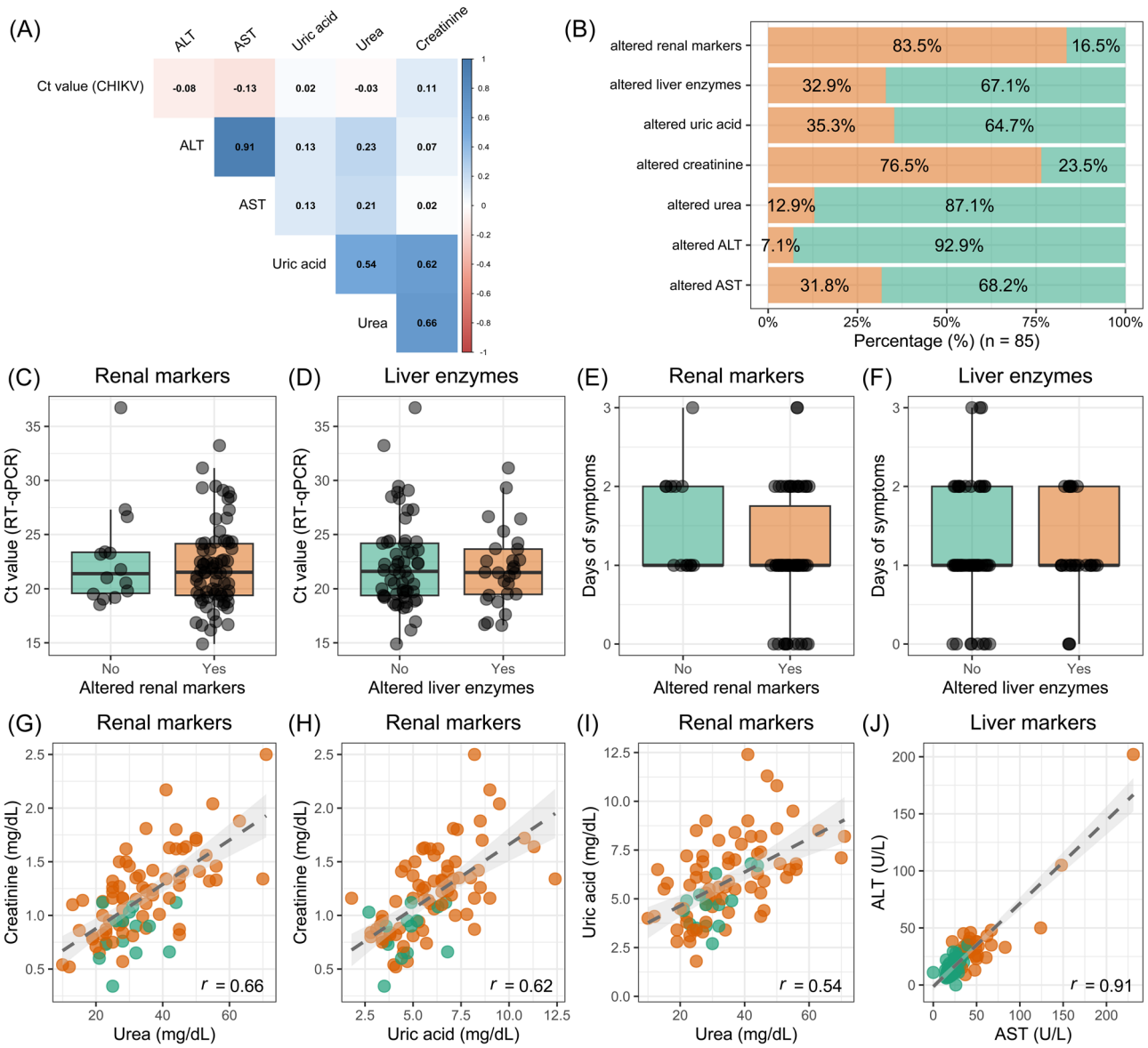

FIGURE 1 Evaluation of renal markers and liver enzymes in the studied patients ($n = 85$). (A) Pearson's correlations comparing the levels of renal markers, liver enzymes, and Ct values obtained for the RNA detection of Chikungunya virus (CHIKV); (B) Relative frequency for changes observed in the studied population, considering each condition considered (changes in renal markers, changes in liver enzymes, changes in uric acid levels, changes in creatinine levels, changes in urea levels, changes in ALT levels, changes in AST levels). The green color represents an absence of alteration, and the orange color represents the presence of alteration. Boxplot graphs showing the profile of the biochemical tests compared with the Ct values obtained to the CHIKV RNA (C and D). The green color represents an absence of alteration, and the orange color represents the presence of alteration. Boxplot graphs showing the profile of the biochemical tests compared with the duration of the symptoms in days (E and F). The green color represents an absence of alteration, and the orange color represents the presence of alteration. (G–J) Pearson correlations comparing the correlation levels of renal markers and liver enzymes, showing the pattern of the samples considering the presence and absence of alteration – Urea versus Creatinine (G), Uric acid versus Creatinine (H), Urea versus Uric acid (I), and AST versus ALT (J). Boxplots represent the 75th percentile, median, and 25th percentile, and the whiskers extend to the highest and lowest value in the 1.5x interquartile range. The class presence and absence of alteration, were established based on reference range values for identifying these markers, which are as follows: to the liver enzymes (AST Reference Range [RR]: Male: <40 U/L, Female and child: <32 U/L; ALT RR: Male: <50 U/L, Female and child: <35 U/L), and to the renal markers (Creatinine RR: Male: 0.70–1.20 mg/dl, Female and child: 0.40–0.60 mg/dl; Urea RR: 17–49 mg/dl, and Uric Acid RR: Male: 3.4–7.0 mg/dl, Female and child: 2.4–5.7 mg/dl).

The assessment of renal markers further revealed noteworthy irregularities within the studied patient group, presenting captivating findings. In our study, patients who had a headache had considerably higher values of creatinine than those who did not have a headache. In the infection caused by SARS-CoV-2, the presence of headache at the beginning is associated with milder disease conditions and was directly associated with D-dimer and creatinine levels.[54] Notably, statistically significant deviations in uric acid levels came to light specifically among patients presenting with fever. This observation aligns with established clinical knowledge,[55] as individuals

TABLE 1 Statistical analysis of clinical manifestations and the measures obtained to the hepatic and renal markers.

		Urea	Creatinine	Uric Acid	AST	ALT
Clinical symptoms	**n**	***p*-value**	***p*-value**	***p*-value**	***p*-value**	***p*-value**
Fever	77	0.1147	0.2576	0.0145*	0.1871	0.4631
Headache	85	0.4568	0.03353*	0.1182	0.146	0.4012
Myalgia	85	0.2089	0.07909	0.5505	0.5145	0.6233
Joint pain	85	0.1841	0.3374	0.7919	0.701	0.2838
Asthenia	85	0.657	0.2956	0.282	0.3903	0.5336
Hemorrhagic Signs	85	0.9917	0.11	0.01952*	0.5821	0.3342
Meningoencephalitis	85	0.1717	0.3698	0.4882	0.3588	0.1918
Vomiting	85	0.9009	0.2221	0.0612	0.02077*	0.04904*
Retro-orbital pain	85	0.1735	0.08427	0.1456	0.4548	0.7703
Abdominal pain	85	0.5418	0.6929	0.1526	0.04435*	0.3662
Rash	85	0.01439*	0.04608*	0.07381	0.4648	0.3481
Itching	85	0.7188	0.9299	0.6862	0.3371	0.7714

Note: Significant values are highlighted by an asterisk (*p-value < 0.05).

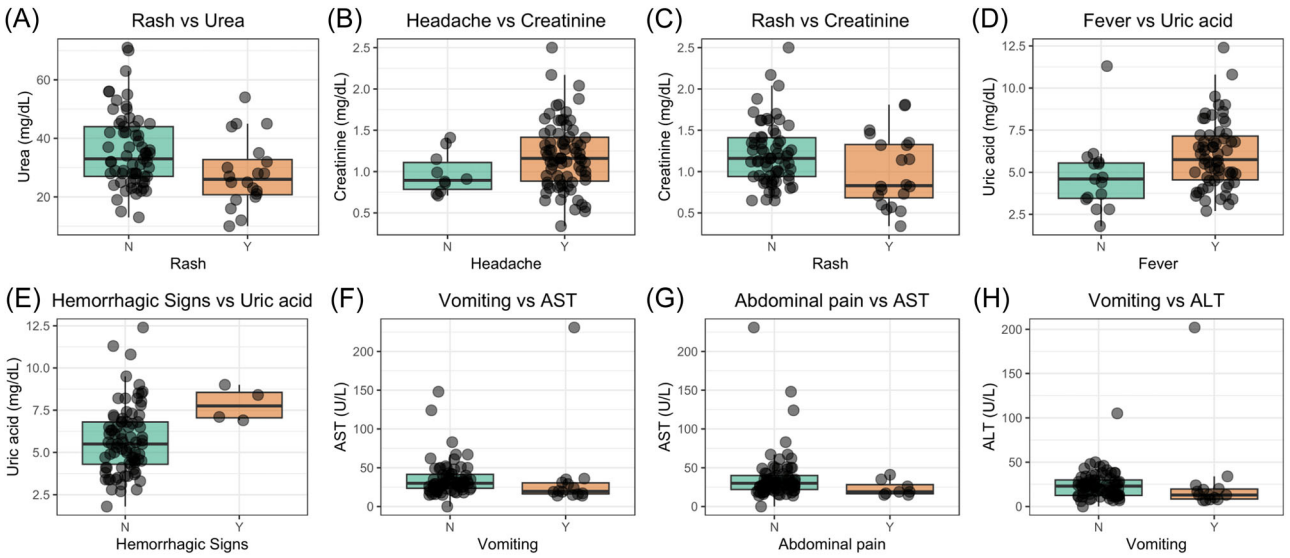

FIGURE 2 Differentially identification markers in comparison with the presentation of the symptoms. Boxplot graphs showing the profile of the biochemical tests compared with the presence and absence of symptoms (A–G). The green color represents the absence of symptoms, and the orange color represents the presence of the symptoms. Boxplots represent the 75th percentile, median, and 25th percentile, and the whiskers extend to the highest and lowest value in the 1.5x interquartile range.

undergoing febrile states due to chronic or reactive inflammatory conditions often display fluctuations in this specific marker. Uric acid, which results from purine metabolism, tends to surge during instances of inflammation.[56] This surge prompts an increased synthesis of cytokines and other inflammatory mediators, consequently setting off a cascade of adjustments in metabolic pathways, including alterations in nucleotide catabolism.[56,57] The association between the presence of hemorrhagic signs and increased uric acid levels needs to be treated with care, as only four of the 85 patients reported having any type of hemorrhage. In the literature, this indeed seems to be a possible association, such as, for example, reports of uric acid crystals being found in cases of hemorrhage.[26,58,59]

The presence of gastrointestinal symptoms (GIS) (including vomiting or abdominal pain) was significantly associated with lower AST and ALT levels. However, this needs to be treated with caution, as it was a small number of patients who showed a large numerical change in these values. Interestingly, there is an inverse relationship between low levels of urea and creatinine when compared to the

presentation of rash in the population. A rash is a clinical feature associated with exanthematous viruses and is even associated with the clinical condition caused by the Zika virus (ZIKV).[60,61]

Despite our hypotheses, drawing from our findings, a compelling suggestion can be made that the modifications in liver and renal biochemical markers manifest during Chikungunya infection. Particularly, these changes are more prominent in the renal markers when contrasted with the liver enzymes. The study is unprecedented because the cohort studied shows variability in age and gender, in addition to the fact that CHIKV infection was discovered during diagnostic research for ZIKV, two viruses that have shown previous interwoven transmission in the Pacific region. In addition, we focused on the study of biochemical parameters that were not yet described in the literature in the context of the CHIKV infection, and we showed several changes during the disease. These discernible shifts in measurements hold the potential to be linked to the clinical spectrum of the disease manifestation. In conclusion, we humbly believe that the information we provide is of relevance. It does not try to establish irrefutable proof but rather to shed light on relevant potential issues of CHIKV pathogenesis in humans by suggesting further research topics and focal points for potential treatment improvement.

AUTHOR CONTRIBUTIONS

All authors contributed to the study designing, and manuscript writing. Anderson Pereira Soares, Shahab Zaki Pour, and Marielton dos Passos Cunha performed the research and wrote the manuscript. All authors have revised and approved the final draft of the paper.

ACKNOWLEDGMENTS

PMAZ was supported by the Brazilian National Council of Scientific and Technological Development (CNPq) (grant #441105/2016-5), by the São Paulo Research Foundation (FAPESP) (grant #2017/23281-6), and by the Fiocruz/Pasteur/USP (grant #314502). MPC received a FAPESP fellowship (grant #2016/08204-2). The funders had no role in study design, data collection, and analysis, the decision to publish, or the preparation of the manuscript. We thank all the professionals from the Fundação de Saúde Parreiras Horta/LACEN from Sergipe, Brazil, for helping to organize the human sample collection used in this study. Also, we thank the professionals from the University Hospital (HU-USP) for collaborating with the execution of the assays for the quantification of the analyzed markers.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, PMAZ, upon reasonable request.

ETHICS STATEMENT

This study is part of a project for arbovirus research in Sergipe State, 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).

ORCID

Anderson Pereira Soares http://orcid.org/0000-0001-8066-818X
Daniel Ferreira de Lima Neto http://orcid.org/0000-0001-8501-2753
Shahab Zaki Pour http://orcid.org/0000-0002-7708-1406
Saulo Duarte Passos http://orcid.org/0000-0002-0908-2677
Marielton dos Passos Cunha http://orcid.org/0000-0002-2102-631X
Paolo Marinho de Andrade Zanotto http://orcid.org/0000-0002-8167-0625

REFERENCES

1. Borgherini G, Poubeau P, Jossaume A, et al. Persistent arthralgia associated with chikungunya virus: a study of 88 adult patients on Reunion Island. *Clin Infect Dis*. 2008;47:469-475. doi:10.1086/590003
2. Dupuis-Maguiraga L, Noret M, Brun S, Le Grand R, Gras G, Roques P. Chikungunya disease: infection-associated markers from the acute to the chronic phase of Arbovirus-Induced arthralgia. *PLoS Neglected Trop Dis*. 2012;6(3):e1446. doi:10.1371/journal.pntd.0001446
3. Ross RW. The newala epidemic: III. The virus: isolation, pathogenic properties and relationship to the epidemic. *J Hyg*. 1956;54(2):177-191. doi:10.1017/S0022172400044442
4. Vega-Rúa A, Zouache K, Girod R, Failloux A-B, Lourenço-De-Oliveira R. High level of vector competence of Aedes aegypti and Aedes albopictus from ten American countries as a crucial factor in the spread of chikungunya virus. *J Virol*. 2014;88:6294-6306. doi:10.1128/JVI.00370-14
5. Costa-da-silva AL, Ioshino RS, Petersen V, et al. First report of naturally infected Aedes aegypti with chikungunya virus genotype ECSA in the Americas. *PLoS Neglected Trop Dis*. 2017;11(6):e0005630.
6. Strauss JH, Strauss EG. The alphaviruses: gene expression, replication, and evolution. *Microbiol Rev*. 1994;58(3):491-562.
7. Cherian S, Walimbe A, Jadhav S, et al. Evolutionary rates and timescale comparison of chikungunya viruses inferred from the whole genome/E1 gene with special reference to the 2005-07 outbreak in the Indian subcontinent. *Infect Genet Evol*. 2009;9(1):16-23. doi:10.1016/j.meegid.2008.09.004
8. Pialoux G, Gaüzère B-A, Jauréguiberry S, Strobel M. Chikungunya, an epidemic arbovirosis. *Lancet Infect Dis*. 2007;7(5):319-327. doi:10.1099/0022-1317-83-12-3075
9. De Lamballerie X, Leroy E, Charrel RN, Ttsetsarkin K, Higgs S, Gould EA. Chikungunya virus adapts to tiger mosquito via evolutionary convergence: a sign of things to come? *Virol J*. 2008;5:33. doi:10.1186/1743-422X-5-33
10. Thiboutot MM, Kannan S, Kawalekar OU, et al. Chikungunya: a potentially emerging epidemic? *PLoS Neglected Trop Dis*. 2010;4(4):e623. doi:10.1371/journal.pntd.0000623
11. Hapuarachchi HC, Bandara KBAT, Sumanadasa SDM, et al. Re-emergence of chikungunya virus in South-east Asia: virological evidence from Sri Lanka and Singapore. *J Gen Virol*. 2010;91(4):1067-1076. doi:10.1099/vir.0.015743-0
12. Gratz NG. Critical review of the vector status of Aedes albopictus. *Med Vet Entomol*. 2004;18(3):215-227. doi:10.1111/j.0269-283X.2004.00513.x

13. Agha SB, Chepkorir E, Mulwa F, et al. Vector competence of populations of Aedes aegypti from three distinct cities in Kenya for chikungunya virus. *PLoS Neglected Trop Dis*. 2017;11(8):e0005860. doi:10.1371/journal.pntd.0005860
14. Nkoghe D, Kassa RF, Caron M, et al. Clinical forms of chikungunya in Gabon, 2010. *PLoS Neglected Trop Dis*. 2012;6(2):e1517. doi:10.1371/journal.pntd.0001517
15. Powers AM, Logue CH. Changing patterns of chikunya virus: re-emergence of a zoonotic arbovirus. *J Gen Virol*. 2007;88(9):2363-2377. doi:10.1099/vir.0.82858-0
16. Rezza G, Nicoletti L, Angelini R, et al. Infection with chikungunya virus in Italy: an outbreak in a temperate region. *The Lancet*. 2007;370:1840-1846.
17. Sergon K, Njuguna C, Kalani R, et al. Seroprevalence of chikungunya virus (CHIKV) infection on Lamu Island, Kenya, October 2004. *Am J Trop Med Hyg*. 2008;78(2):333-337. doi:10.4269/ajtmh.2008.78.333
18. Nunes MRT, Faria NR, de Vasconcelos JM, et al. Emergence and potential for spread of chikungunya virus in Brazil. *BMC Med*. 2015;13(1):102. doi:10.1186/s12916-015-0348-x
19. Cunha MP, Santos CA, Neto DFL, et al. Outbreak of chikungunya virus in a vulnerable population of Sergipe, Brazil—a molecular and serological survey. *J Clin Virol*. 2017;97:44-49. doi:10.1016/j.jcv.2017.10.015
20. Souza TML, Vieira YR, Delatorre E, et al. Emergence of the East-Central-South-African genotype of chikungunya virus in Brazil and the city of Rio de Janeiro may have occurred years before surveillance detection. *Sci Rep*. 2019;9:2760. doi:10.1038/s41598-019-39406-9
21. Teixeira MG, Andrade AMS, Da Costa MCN, et al. East/central/South African genotype chikungunya virus, Brazil, 2014. *Emerging Infect Dis*. 2015;21(5):906-907. doi:10.3201/eid2105.141727
22. Abdulrazzaq DNA, Ahmed MM, Hasan FT, Hasson DFC. The impacts of viral hepatitis on liver enzymes and bilrubin. *Int J Chem Math Phys*. 2022;6(6):01-06. doi:10.22161/ijcmp.6.6.1
23. Williams ALB, Hoofnagle JH. Ratio of serum aspartate to alanine aminotransferase in chronic hepatitis relationship to cirrhosis. *Gastroenterology*. 1988;95(3):734-739. doi:10.1016/S0016-5085(88)80022-2
24. Gurung R, Purbe B, Gyawali P, Risal P. The ratio of aspartate aminotransferase to alanine aminotransferase (AST/ALT): the correlation of value with underlying severity of alcoholic liver disease. *Kathmandu Univ Med J*. 2015;11(3):233-236. doi:10.3126/kumj.v11i3.12511
25. Bellomo R, Kellum J, Ronco C. Acute renal failure: time for consensus. *Intensive Care Med*. 2001;27(11):1685-1688. doi:10.1007/s00134-001-1120-6
26. Choi HK, Mount DB, Reginato AM. Pathogenesis of gout. *Ann Intern Med*. 2005;143(7):499. doi:10.7326/0003-4819-143-7-200510040-00009
27. Juraschek SP, Miller III ER, Gelber AC. Effect of oral vitamin C supplementation and serum uric acid: a meta-analysis of randomized controlled trials. *Arthritis Care Res*. 2011;63(9):1295-1306. doi:10.1002/acr.20519
28. Misra UK, Kalita J, Maurya PK, Kumar P, Shankar SK, Mahadevan A. Dengue-associated transient muscle dysfunction: clinical, electromyography and histopathological changes. *Infection*. 2012;40(2):125-130. doi:10.1007/s15010-011-0203-8
29. Ozden S, Huerre M, Riviere JP, et al. Human muscle satellite cells as targets of chikungunya virus infection. *PLoS One*. 2007;2(6):e527. doi:10.1371/journal.pone.0000527
30. Crosby L, Perreau C, Madeux B, et al. Severe manifestations of chikungunya virus in critically ill patients during the 2013-2014 Caribbean outbreak. *Int J Infect Dis*. 2016;48:78-80. doi:10.1016/j.ijid.2016.05.010
31. Melo MR, Martins AR, Barbosa IV, Romano P, Shcolnik W. Coleta, transporte e armazenamento de amostras para diagnóstico molecular. *J Bras Patol e Med Lab*. 2010;46(5):375-381. doi:10.1590/s1676-24442010000500006
32. Nakashima Y, Hata S, Yasuda T. Blood plasma separation and extraction from a minute amount of blood using dielectrophoretic and capillary forces. *Sens Actuators, B*. 2010;145(1):561-569. doi:10.1016/j.snb.2009.11.070
33. Anvisa. Manual de vigilância sanitária sobre o transporte de material biológico humano para fins de diagnóstico clínico. 2015.
34. Patel P, Abd El Wahed A, Faye O, et al. A field-deployable reverse transcription recombinase polymerase amplification assay for rapid detection of the chikungunya virus. *PLoS Neglected Trop Dis*. 2016;10(9):e0004953. doi:10.1371/journal.pntd.0004953
35. Jaffe M. Ueber den Niederschlag, welchen Pikrinsäure in normalem Harn erzeugt und über eine neue reaction des Kreatinins. *bchm*. 1886;10(5):391-400. doi:10.1515/bchm1.1886.10.5.391
36. Fabiny DL, Ertingshausen G. Automated reaction-rate method for determination of serum creatinine with the CentrifiChem. *Clin Chem*. 1971;17(8):696-700. doi:10.1093/clinchem/17.8.696
37. Tiffany TO, Jansen JM, Burtis CA, Overton JB, Scott CD. Enzymatic kinetic rate and end-point analyses of substrate, by use of a GeMSAEC fast analyzer. *Clin Chem*. 1972;18(8):829-840. doi:10.1093/clinchem/18.8.829
38. Leino A, Impivaara O, Irjala K, Mäki J, Peltola O, Järvisalo J. Health-based reference intervals for ALAT, ASAT and GT in serum, measured according to the recommendations of the european committee for clinical laboratory standards (ECCLS). *Scand J Clin Lab Invest*. 1995;55(3):243-250. doi:10.3109/00365519509089619
39. Supak Smolcic V, Bilic-Zulle L, Fisic E. Validation of methods performance for routine biochemistry analytes at cobas 6000 analyzer series module c501. *Biochem Med*. 2011;21(2):182-190. doi:10.11613/bm.2011.028
40. Gandrud C. *Reproducible research with R and RStudio*. Vol. 53; 2016. doi:10.1017/CBO9781107415324.004
41. Mercado M, Acosta-Reyes J, Parra E, et al. Clinical and histopathological features of fatal cases with dengue and chikungunya virus co-infection in Colombia, 2014 to 2015. *Euro Surveill*. 2016;21(22):30244. doi:10.2807/1560-7917.ES.2016.21.22.30244
42. Soares-Schanoski A, Baptista-Cruz N, De castro-Jorge LA, et al. Systems analysis of subjects acutely infected with the chikungunya virus. *PLoS Pathog*. 2019;15(6):e1007880. doi:10.1101/531921
43. Beltrán-Silva SL, Chacón-Hernández SS, Moreno-Palacios E, Pereyra-Molina JÁ. Clinical and differential diagnosis: dengue, chikungunya and zika. *Revista Médica del Hospital General de México*. 2018;81(3):146-153. doi:10.1016/j.hgmx.2016.09.011
44. Waggoner JJ, Gresh L, Vargas MJ, et al. Viremia and clinical presentation in Nicaraguan patients infected with zika virus, chikungunya virus, and dengue virus. *Clin Infect Dis*. 2016;63(12):1584-1590. doi:10.1093/cid/ciw589
45. Sheth SG, Flamm SL, Gordon FD, Chopra S. AST/ALT ratio predicts cirrhosis in patients with chronic hepatitis C virus infection. *Am J Gastroenterol*. 1998;93(1):44-48. doi:10.1111/j.1572-0241.1998.044_c.x
46. Liu Y, Yang Y, Zhang C, et al. Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. *Science China Life Sciences*. 2020;63(3):364-374. doi:10.1007/s11427-020-1643-8
47. Brosnan JT, Brosnan ME. Creatine metabolism and the urea cycle. *Mol Genet Metab*. 2010;100:S49-S52. doi:10.1016/j.ymgme.2010.02.020
48. Davis JL, Hodge HM, Campbell WE. Growth of chikungunya virus in baby hamster kidney cell (BHK-21-clone 13) suspension cultures. *Appl Microbiol*. 1971;21(2):338-341. doi:10.1128/am.21.2.338-341.1971
49. Paingankar MS, Arankalle VA. Identification of chikungunya virus interacting proteins in mammalian cells. *J Biosci*. 2014;39(3):389-399. doi:10.1007/s12038-014-9436-x

50. Bandeira AC, Campos GS, Rocha VFD, et al. Prolonged shedding of chikungunya virus in semen and urine: a new perspective for diagnosis and implications for transmission. *IDCases*. 2016;6:100-103. doi:10.1016/j.idcr.2016.10.007
51. Martins EB, de Bruycker-Nogueira F, Rodrigues CDS, et al. Chikungunya virus shedding in semen: a case series. *Viruses*. 2022;14(9):1879. doi:10.3390/v14091879
52. Srikirin P, Siripoon T, Charoenpong L, et al. Prevalence, risk factors, and prognosis of liver involvement in adult patients with chikungunya in Thailand. *Am J Trop Med Hyg*. 2022;107(5):1107-1113. doi:10.4269/ajtmh.22-0339
53. Chua HH, Abdul Rashid K, Law WC, et al. A fatal case of chikungunya virus infection with liver involvement. *Med J Malaysia*. 2010;65(1):83-84.
54. Gonzalez-Martinez A, Fanjul V, Ramos C, et al. Headache during SARS-CoV-2 infection as an early symptom associated with a more benign course of disease: a case–control study. *Eur J Neurol*. 2021;28(10):3426-3436. doi:10.1111/ene.14718
55. Ates B, Sazak S, Turkmenoglu Y, Irdem A, Dursun H. Relationship of serum vitamin D, D-dimer and uric acid levels with attacks in children with familial Mediterranean fever. *Egypt Rheumatol*. 2022;44(4):301-305. doi:10.1016/j.ejr.2022.03.004
56. Ngo TC, Assimos DG. Uric acid nephrolithiasis: recent progress and future directions. *Rev Urol*. 2007;9(1):17-27.
57. Baxmann ACA, Ahmed MS, Marques CAC, et al. Influence of muscle mass and physical activity on serum and urinary creatinine and serum cystatin C. *Clin J Am Soc Nephrol*. 2008;3(2):348-354. doi:10.2215/CJN.02870707
58. Ao J, Goldblatt F, Casson RJ. Review of the ophthalmic manifestations of gout and uric acid crystal deposition. *Clin Experiment Ophthalmol*. 2017;45(1):73-80. doi:10.1111/ceo.12749
59. Lin J, Zhao GQ, Che CY, Yang SS, Wang Q, Li CG. Characteristics of ocular abnormalities in gout patients. *Int J Ophthalmol*. 2013;6(3):307-311. doi:10.3980/j.issn.2222-3959.2013.03.09
60. Duffy MR, Chen T-H, Hancock WT, et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. *N Engl J Med*. 2009;360(24):2536-2543. doi:10.1056/nejmoa0805715
61. Cardoso CW, Paploski IAD, Kikuti M, et al. Outbreak of exanthematous illness associated with Zika, chikungunya, and dengue viruses, Salvador, Brazil. *Emerging Infect Dis*. 2015;21(12):2274-2276. doi:10.3201/eid2112.151167

How to cite this article: Soares AP, de Lima Neto DF, Pour SZ, Passos SD, Cunha MdP, Zanotto PMA. Evaluation of renal markers and liver enzymes in patients infected with the chikungunya virus. *J Med Virol*. 2023;95:e29276. doi:10.1002/jmv.29276