Infectious Medicine 3 (2024) 100088

Contents lists available at ScienceDirect

Infectious Medicine

journal homepage: www.elsevier.com/locate/imj

Original Article

Viremia and clinical manifestations in acute febrile patients of Chikungunya infection during the 2016 CHIKV outbreak in Delhi, India

Rohit Sagar[a,b], Siva Raghavendhar[c], Vineet Jain[d], Naushad Khan[a], Anmol Chandele[e], Ashok Kumar Patel[c], Murali Kaja[e,f], Pratima Ray[a], Neera Kapoor[b,*]

[a] Department of Biotechnology, School of Chemical and Life Sciences, Jamia Hamdard, New Delhi 110062, India
[b] Department of Life Sciences, School of Sciences, IGNOU, New Delhi 110068, India
[c] Kusuma School of Biological Sciences, Indian Institute of Technology, New Delhi 110016, India
[d] HAH Centenary Hospital, Jamia Hamdard, New Delhi 110062, India
[e] ICGEB-Emory Vaccine Center, ICGEB, New Delhi 110067, India
[f] Department of Pediatrics, Emory University School of Medicine, 30322 Atlanta, GA, USA

ARTICLE INFO

Keywords:
CHIKV
Clinical features
Viral load
Febrile patients
Outbreak

ABSTRACT

Background: Chikungunya virus (CHIKV) is an infectious agent that caused several outbreaks among different countries and affected approximately 1.3 million Indian populations. It is transmitted by *Aedes* mosquito–either *A. albopictus* or *A. aegypti*. Generally, the clinical manifestations of CHIKV infection involve high-grade fever, joint pain, skin rashes, headache, and myalgia. The present study aims to investigate the relationship between the CHIKV virus load and clinical symptoms of the CHIKV infection so that better patient management can be done in the background of the CHIKV outbreak as there is no licensed anti-viral drug and approved vaccines available against CHIKV.

Methods: CHIKV RTPCR positive samples ($n = 18$) (Acute febrile patients having D.O.F ≤ 7 days) were taken for the quantification of CHIKV viremia by Real-Time PCR. Clinical features of the febrile patients were recorded during the collection of blood samples.

Results: The log mean virus load of 18 RT-PCR-positive samples was 1.3×10^6 copies/mL (1.21×10^3–2.33×10^8 copies/mL). Among the observed clinical features, the log mean virus load (CHIKV) of the patients without skin rash is higher than in the patients with skin rash (6.61 vs 5.5, $P = 0.0435$).

Conclusion: The conclusion of the study was that the patients with skin rashes had lower viral load and those without skin rashes had higher viral load.

1. Introduction

Chikungunya virus (CHIKV) is an infectious agent that caused several outbreaks among different countries in Southeast Asia, Africa, the Indian Ocean islands, and India and affected approximately 1.3 million Indian populations [1]. Amongst the several arboviral infections, CHIKV has the potential to re-emerge [2] and is considered a neglected tropical disease. CHIKV is a single-stranded RNA virus that belongs to the Alphavirus genus of the Togaviridae family [3]. It is transmitted by *Aedes* mosquito – either *Aedes albopictus* or *Aedes aegypti* [4]. Generally, the clinical manifestations of CHIKV infection involve high-grade fever, joint pain, skin rashes, headache, and myalgia [2,5]. There are approximately 50%–97% of individuals infected with the chikungunya virus develop clinical disease [6–8]. The most common clinical symptom observed in CHIKV infection is polyarthralgia [4]. Approximately in 50% of CHIKV-infected patients, skin rashes are reported in the acute phase [9].

The manifestation of clinical symptoms is the outcome of virus replication in host cells, the immune response generated in response to viremia, and interactions between virus and host factors depending on the dura-

* Corresponding author.
E-mail address: neerakapoor@ignou.ac.in (N. Kapoor).

https://doi.org/10.1016/j.imj.2024.100088
Received 28 September 2023; Received in revised form 16 November 2023; Accepted 25 January 2024

tion of illness [10,11]. Severe CHIKV infection is associated with increased plasma levels of proinflammatory cytokines like IL-6, IL-12, and Interferon α [12,13]. In the case of humans, the plasma level of Interferon α is correlated with viral load. The viremic phase persists for 1-4 days [14] and after that seroconversion phase starts. Host innate immune responses are triggered by viral replication but virus spreads in several sites of replication like lymphoid organs, skin (rash), muscles, peripheral joints [15–17]. Replication of CHIKV in peripheral tissues results in a high viral load in plasma ($>10^9$ virus particles/mL) [18]. There is no licensed antiviral drugs that can target the primary and secondary site of replication of CHIKV. During the CHIKV outbreak and in the area where viremia cannot be determined, in such conditions, clinical symptoms can be used as a marker for viremia to guide treatment. The present study aims to investigate the relationship between the CHIKV virus load and clinical symptoms of the CHIKV infection so that better patient management can be performed in the background of the CHIKV outbreak as there is no licensed antiviral drug and approved vaccines available against CHIKV.

2. Materials and methods

2.1. Sample collection

Blood samples of suspected Chikungunya febrile patients (D.O.F ≤7 days) were collected from HAH Centenary Hospital, Jamia Hamdard, Hamdard Nagar, New Delhi from August 2016 to November 2016 ($n = 325$) during CHIKV outbreak in Delhi. Out of 325, only 29 patients were CHIKV positive by RT-PCR. Moreover, All the samples included in this study are CHIKV IgM and CHIKV IgG negative. As, we were left with plasma of only 18 samples, so 18 samples were taken for estimation of chikungunya viral load.

2.2. RNA extraction and real time PCR

CHIKV was isolated from plasma samples by using a kit (Qiagen mini viral RNA, Cat. No 52906). A complementary DNA kit (RevertAid H Minus First Strand cDNA synthesis kit; Cat. No K1632)) was used to construct cDNA from isolated RNA. For the determination of CHIKV virus load in plasma, another commercially available kit (Maxima SYBR Green/ROX qPCR; Cat. No K0223) was used. A total of 20 μL reaction mixture was prepared, out of which 10 μL was RNA template and 10 μL compromised of other reaction components. The primers used for the PCR were designed with the help of software (Primer Express, Thermo Fisher Scientific, Waltham, MA, USA). Specific primers targeting 120 bp region of E1 envelope gene of CHIKV were used for amplification (Forward primer 5′-3′ TACAGCCCCATGGTACTGCA and reverse primer 5′-3′ CTGTACCGCAGCATTTCACG). Real-time PCR was performed at different thermal conditions of 95°C for 3 min, 95°C for 10 s, 55°C for 1 min, 65°C for 10 s, and 95°C for 10 min. The size of the amplification product was 120 bp. The CHIKV S27 strain (African prototype) was used as a positive control during the experiment. All the samples were run in duplicate. For construction of the standard curve, the control cDNA (865.3 ng/ul) was serially diluted 10-fold up to 6 dilutions and obtained cycle threshold (ct) values were plotted against log dilution of the DNA (Fig. 1). The copy numbers in test samples were determined from the intersection point on standard plot. For calculating genome copy number for standard, cDNA concentration was used and calculated by using online tool (https://eu.idtdna.com/pages/education/decoded/article/calculations-converting-from-nanograms-to-copy-number). The clinical symptoms of CHIKV-positive samples were recorded at the time of blood sample collection.

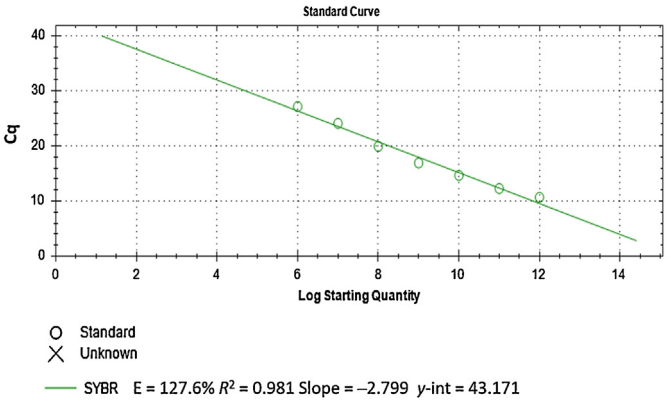

Fig. 1. The standard curve was generated from the Cq values obtained against the known concentration of six-fold serially diluted CHIKV cDNA. The standard curve shows a correlation coefficient of 0.981 and efficiency = 127.6%).

2.3. Statistical analysis

Categorical variables were analyzed by chi-square test. p- values less than 0.05 were considered to be statistically significant. GraphPad Prism 8.4.3 was used for statistical analysis.

3. Results

3.1. Clinical features

Clinical features of CHIKV patients ($n = 18$) were recorded during the first 7 days of illness. Out of 18, 10 were males and 8 were females. The age of the patients varied from 19 to 54 years. The symptoms that were frequently observed in CHIKV patients were joint pain (94.44%), joint swelling (61.11%), headache (94.44%), and vomiting (55.56%). Other clinical symptoms include skin rash (38.89%), diarrhea (22.22%), abdomi-

Table 1
Demographic and clinical characteristics of CHIKV RT-PCR positive febrile patients (n = 18).

Characteristics	Number (n = 18)	Percentage (%)
Age median range in years	38 (19–54)	
Male	10	55.56
Female	8	44.44
Rash	7	38.89
Joint Pain	17	94.44
Joint swelling	11	61.11
Headache	17	94.44
Vomiting	10	55.56
Diarrhea	4	22.22
Abdominal pain	3	16.67
Rhinitis	6	33.33
Cough	5	16.66

nal pain (16.67%), Rhinitis (33.33) and cough (16.66%) (Table 1).

3.2. Viral load

RT-PCR positive samples (n = 18) were taken for the quantification of viral load in samples by SYBR-Green-based real-time RT-PCR assay. RNA templates from CHIKV S-27 strain was used as a positive control. Based on the standard curve plotted, the highest amount of viral load was detected in serum #11 with a quantity of genomic RNA of 2.33×10^8 copies/mL (Table 2). The lowest number of viral genomic RNA copies was estimated to be 1.21×10^3 copies/mL for serum #1 (Table 2).

CHIKV viral load of acute febrile patients (D.O.F:1-7 days) was estimated by qRT-PCR and the log mean virus load of 18 RT-PCR positive samples was 1.3×10^6 copies/mL. Clinical symptoms were correlated with the CHIKV virus load. A significant difference in CHIKV virus load was found between patients with or without skin rash (P = 0.0435). The log mean CHIKV virus load of the patients without skin rashes is higher than in the patients with skin rashes (6.61 vs 5.15) (Fig. 2). On the other hand, a significant difference in CHIKV virus load was observed between subjects with or without headache (P = 0.033). The log mean CHIKV virus load of the subjects without headache is higher than the subjects with headache (8.06 vs 5.79) (Fig. 2).

4. Discussion

CHIKV infection is one of the mosquito-borne viral diseases and is considered to be a major public threat globally. It has re-emerged in different parts of the world and caused several outbreaks [19–22]. CHIKV symptomatic patients are characterized by symptoms like high-grade fever, polyarthralgia, headache, myalgia, and Skin rash [23–25]. Clinical manifestations like arthralgia may be responsible for the increase in the duration of illness [26].

In this study, the most frequently observed clinical manifestations in acute febrile patients were joint pain and headache [10,14,27]. The clinical manifestations of CHIKV infection is the outcome of the cumulative effect of viral load and the host immune response against the virus by generating neutralizing antibodies [10].

In this study, among all the clinical features recorded, skin rash (P = 0.04) and headache (P = 0.03) varied with the differences in viral load. Interestingly, the patients who had no rashes had more chikungunya virus load as compared to patients that have rashes. This finding is in concordance with a report where skin rashes were negatively correlated with high CHIKV viral load [28]. It may appear that skin rash is experienced relatively more by CHIKV patients, during the seroconversion phase instead of the viremic phase of acute infection. A report showed

Table 2
Determination of Sera viral load from CHIKV RT-PCR positive samples (n = 18).

Patients' serum specimen number	Patient sex	Patient age (yr)	Mean genomic RNA copy number (copies/mL)
#1	M	50	1.21×10^3
#2	F	52	5.28×10^4
#3	F	45	5.03×10^6
#4	F	21	5.42×10^5
#5	M	38	6.67×10^5
#6	M	28	5.01×10^7
#7	F	19	1.28×10^5
#8	F	30	1.08×10^7
#9	M	43	1.43×10^8
#10	M	31	1.36×10^6
#11	M	25	2.33×10^8
#12	F	50	9.55×10^7
#13	M	38	6.49×10^6
#14	M	40	5.32×10^3
#15	M	20	3.38×10^5
#16	F	54	3.48×10^6
#17	M	35	3.29×10^5
#18	M	24	1.45×10^4
Positive control	–	–	1.16×10^{12}
Negative control	–	–	Undetermined

Note: The equation of the standard curve constructed for the SYBR Green real-time RT-PCR assay was R^2 value = 0.981, Efficiency = 127.6%).

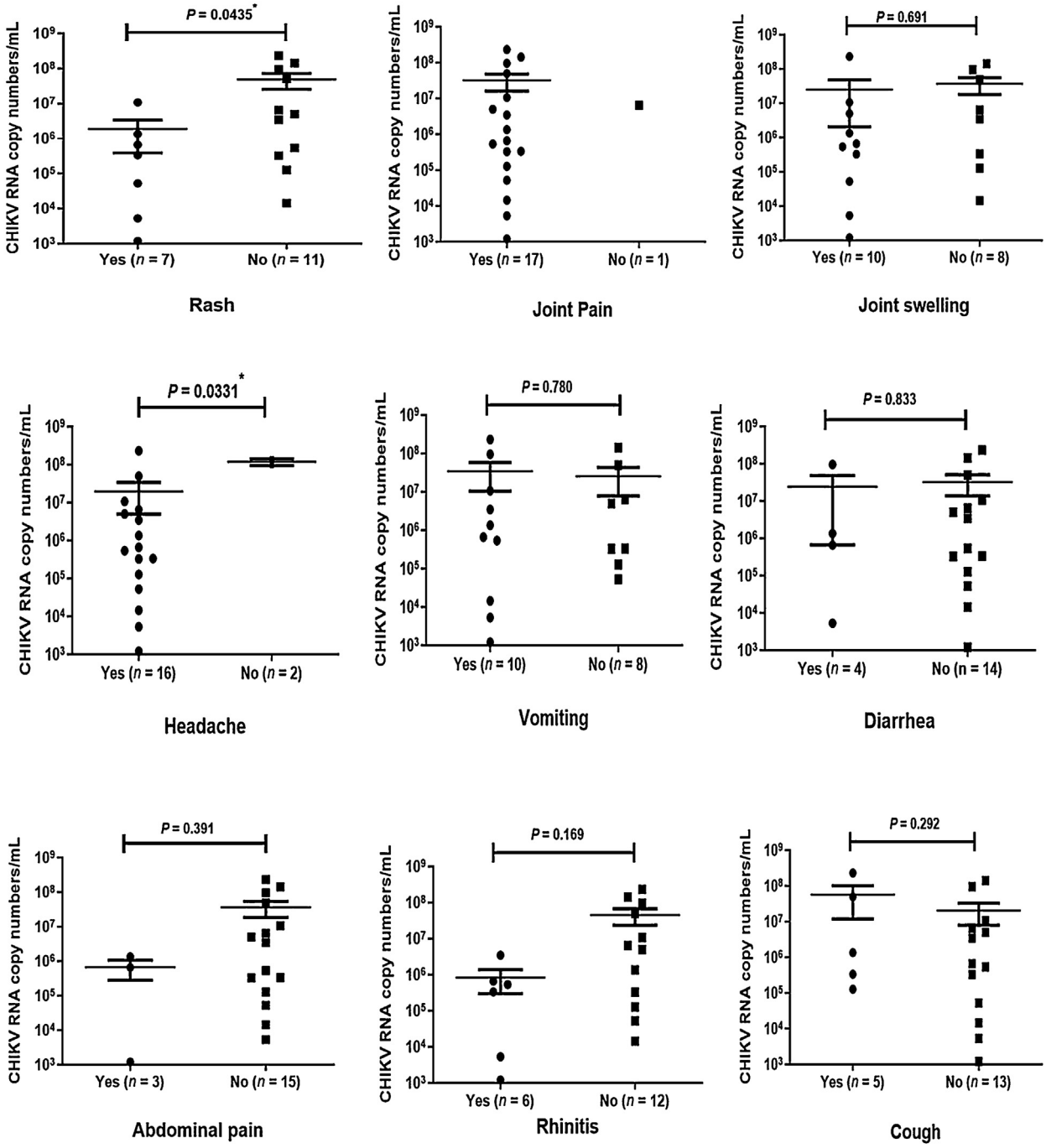

Fig. 2. Association of CHIKV viral load (n = 18) with different clinical features (rash, joint pain, joint swelling, headache, vomiting, diarrhea, abdominal pain, rhinitis and cough) where rash (P = 0.0435) and headache (P = 0.0331) were found statistically significant as compared to other clinical symptoms. P-value found statistically significant was marked by asterick.

a pictorial representation of the temporal expression of clinical symptoms of CHIKV-infected patients where skin rash is experienced after 4–5 days of onset of illness by the patients [11]. In one of the other studies on CHIKV patients, it was reported that clinical manifestations like skin rashes and itching appeared between 4 to 6 days of fever onset after the development of arthralgia and myalgia [27]. Also, joint pain was observed in almost all subjects and the mean viral load of patients having joint pain is 3.2×10^7 copies/mL (n = 17) and it is generally high ($>10^5$ Copies/mL) [28,29]. In a report it was observed that a high viral load was significantly associated with the development of arthritis [30].

In this study, it has been also observed that the patients who have no headache, had more chikungunya virus load as compared to subjects having a headache (8.06 vs 5.79). This finding is not aligned with a study where chikungunya-infected patients in Thai patients who have headaches had more virus load (P = 0.028) as compared to patients having no headache (6.83 vs 5.06) [30]. One

of the possible reasons for this is due to the difference in the sample sizes, therefore our result did not align with this study. Limited data is available to show the relationship between clinical symptoms of CHIKV patients and viral load. More studies are required to make a conclusive remark.

5. Conclusions

In our study, to know the relationship between virus load and clinical features, we estimated the chikungunya virus load in acute febrile patients infected during the CHIKV outbreak in Delhi during 2016. It has been found that virus load might be an association with skin rashes. The patients having higher viral load had no rashes as compared to patients having rashes. It appears that this finding suggests skin rashes may be the indicator of the fading of the viremic phase Currently, in the light of little understanding of the pathogenesis and progression of the disease and lack of approved vaccine and licensed antiviral drug, our study highlighted that clinical symptoms and viral load of CHIKV may be helpful for clinical decision-making and available possible CHIKV management during the acute phase of infection during outbreak condition so that progression of the disease can be prevented timely.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors during the study period.

Author contributions

R.S. performed the experiments and analyzed the data and wrote the initial manuscript. V.J. and N.K. helped in sample collection. A.C., M.K., and A.K.P. helped in the clinical study design. S.R. reviewed the initial manuscript. N.K. and P.R. supervised the whole study including study design and final editing of the manuscript. All authors read and approved the final manuscript.

Acknowledgments

R.S. acknowledges the JRF fellowship from the University Grant Commission, Govt. of India.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data available statement

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

Ethics statement

This study was approved by the Institutional Ethical Committee of Jamia Hamdard, New Delhi. The procedure used in this study adheres to the tenets of the declaration of Helsinki. Human patient's blood samples were collected from all patients by taking written informed consent.

Informed consent

Informed written consent was taken from all patients who participated in the study.

References

[1] M. Enserink, Infectious diseases. Massive outbreak draws fresh attention to little-known virus, Science 311 (5764) (2006) 1085, doi:10.1126/science.311.5764.1085a.
[2] L. Thaikruea, O. Charearnsook, S. Reanphumkarnkit, et al., Chikungunya in Thailand: a re-emerging disease? Southeast Asian J Trop Med Public Health 28 (2) (1997) 359–364.
[3] A.M. Powers, A.C. Brault, R.B. Tesh, et al., re-emergence of Chikungunya and O'nyong-nyong viruses: evidence for distinct geographical lineages and distant evolutionary relationships, J Gen Virol 81 (Pt 2) (2000) 471–479, doi:10.1099/0022-1317-81-2-471.
[4] T.E. Morrison, L. Oko, S.A. Montgomery, et al., A mouse model of chikungunya virus-induced musculoskeletal inflammatory disease: evidence of arthritis, tenosynovitis, myositis, and persistence, Am J Pathol 178 (1) (2011) 32–40, doi:10.1016/j.ajpath.2010.11.018.
[5] J.D. Druce, D.F. Johnson, T. Tran, et al., Chikungunya virus infection in traveler to Australia, Emerg Infect Dis 13 (3) (2007) 509–510, doi:10.3201/eid1303.060761.
[6] S.M. Ayu, L.R. Lai, Y.F. Chan, et al., Seroprevalence survey of Chikungunya virus in Bagan Panchor, Malaysia, Am J Trop Med Hyg 83 (6) (2010) 1245–1248, doi:10.4269/ajtmh.2010.10-0279.
[7] P. Nakkhara, V. Chongsuvivatwong, S. Thammapalo, Risk factors for symptomatic and asymptomatic chikungunya infection, Trans R Soc Trop Med Hyg 107 (12) (2013) 789–796, doi:10.1093/trstmh/trt083.
[8] J.E. Staples, R.F. Breiman, A.M. Powers, Chikungunya fever: an epidemiological review of a re-emerging infectious disease, Clin Infect Dis 49 (6) (2009) 942–948, doi:10.1086/605496.
[9] J.V.J. Silva, L.F. Ludwig-Begall, E.F. de Oliveira-Filho, et al., A scoping review of Chikungunya virus infection: epidemiology, clinical characteristics, viral co-circulation complications, and control, Acta Trop 188 (2018) 213–224, doi:10.1016/j.actatropica.2018.09.003.
[10] B. Siva Raghavendhar, A.K. Patel, S.K. Kabra, et al., Virus load and clinical features during the acute phase of Chikungunya infection in children, PLoS One 14 (2) (2019) e0211036, doi:10.1371/journal.pone.0211036.
[11] S.P. Kalantri, R. Joshi, L.W. Riley, Chikungunya epidemic: an Indian perspective, Natl Med J India 19 (6) (2006) 315–322.
[12] J.J. Hoarau, M.C. Jaffar Bandjee, P. Krejbich Trotot, et al., Persistent chronic inflammation and infection by Chikungunya arthritogenic alphavirus in spite of a robust host immune response, J Immunol 184 (10) (2010) 5914–5927, doi:10.4049/jimmunol.0900255.
[13] N. Wauquier, P. Becquart, D. Nkoghe, et al., The acute phase of Chikungunya virus infection in humans is associated with strong innate immunity and T CD8 cell activation, J Infect Dis 204 (1) (2011) 115–123, doi:10.1093/infdis/jiq006.
[14] P. Ray, V.H. Ratagiri, S.K. Kabra, et al., Chikungunya infection in India: results of a prospective hospital based multi-centric study, PLoS One 7 (2) (2012) e30025, doi:10.1371/journal.pone.0030025.
[15] T. Couderc, F. Chrétien, C. Schilte, et al., A mouse model for Chikungunya: young age and inefficient type-I interferon signaling are risk factors for severe disease, PLoS Pathog 4 (2) (2008) e29, doi:10.1371/journal.ppat.0040029.
[16] K. Labadie, T. Larcher, C. Joubert, et al., Chikungunya disease in nonhuman primates involves long-term viral persistence in macrophages, J Clin Invest 120 (3) (2010) 894–906, doi:10.1172/JCI40104.

[17] C.R. Morrison, K.S. Plante, M.T. Heise, Chikungunya virus: current perspectives on a reemerging virus, Microbiol Spectr 4 (3) (2016) 143–161, doi:10.1128/microbiolspec.EI10-0017-2016.
[18] T. Das, M.C. Jaffar-Bandjee, J.J. Hoarau, et al., Chikungunya fever: CNS infection and pathologies of a re-emerging arbovirus, Prog Neurobiol 91 (2) (2010) 121–129, doi:10.1016/j.pneurobio.2009.12.006.
[19] M.S.. Cunha, P.A.G. Costa, I.A. Correa, et al., Chikungunya virus: an emergent arbovirus to the South American continent and a continuous threat to the world, Front Microbiol 26 (11) (2020) 1297.
[20] G. Russo, L. Subissi, G. Rezza, Chikungunya fever in Africa: a systematic review, Pathog Glob Health 114 (3) (2020) 136–144, doi:10.1080/20477724.2020.1748965.
[21] S. Sengupta, S. Mukherjee, S.K. Haldar, et al., Re-emergence of Chikungunya virus infection in Eastern India, Braz J Microbiol 51 (1) (2020) 177–182, doi:10.1007/s42770-019-00212-0.
[22] B. Wahid, A. Ali, S. Rafique, et al., Global expansion of chikungunya virus: mapping the 64-year history, Int J Infect Dis 58 (2017) 69–76, doi:10.1016/j.ijid.2017.03.006.
[23] F.J. Burt, W. Chen, J.J. Miner, et al., Chikungunya virus: an update on the biology and pathogenesis of this emerging pathogen, Lancet Infect Dis 17 (4) (2017) e107–e117, doi:10.1016/S1473-3099(16)30385-1.
[24] A. Singal, Chikungunya and skin: current perspective, Indian Dermatol Online J 8 (5) (2017) 307–309, doi:10.4103/idoj.IDOJ_93_17.
[25] S.D. Thiberville, N. Moyen, L. Dupuis-Maguiraga, et al., Chikungunya fever: epidemiology, clinical syndrome, pathogenesis and therapy, Antiviral Res 99 (3) (2013) 345–370, doi:10.1016/j.antiviral.2013.06.009.
[26] A. Mohan, D.H.N. Kiran, I.C. Manohar, et al., Epidemiology, clinical manifestations, and diagnosis of Chikungunya fever: lessons learned from the re-emerging epidemic, Indian J Dermatol 55 (1) (2010) 54–63, doi:10.4103/0019-5154.60355.
[27] S. Anwar, J. Taslem Mourosi, M.F. Khan, et al., Chikungunya outbreak in Bangladesh (2017): clinical and hematological findings, PLoS Negl Trop Dis 14 (2) (2020) e0007466, doi:10.1371/journal.pntd.0007466.
[28] S.K. Dutta, T. Pal, B. Saha, et al., Copy number variation of Chikungunya ECSA virus with disease symptoms among Indian patients, J Med Virol 86 (8) (2014) 1386–1392, doi:10.1002/jmv.23794.
[29] R. Huits, J. De Kort, R. Van Den Berg, et al., Chikungunya virus infection in Aruba: diagnosis, clinical features and predictors of post-chikungunya chronic polyarthralgia, PLoS One 13 (4) (2018) e0196630, doi:10.1371/journal.pone.0196630.
[30] Y.M. Tun, P. Charunwatthana, C. Duangdee, et al., Virological, serological and clinical analysis of chikungunya virus infection in Thai patients, Viruses 14 (8) (2022) 1805, doi:10.3390/v14081805.