



## Co-circulation of Chikungunya virus and Dengue virus in Kassala, Sudan

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Article history: Recieved: March 2018

Accepted: May 2018

### Abstract:

Chikungunya fever was poorly documented and rarely considered in diagnosis of viral fevers according to the WHO, this study aimed to focus on co-circulation of chikungunya virus and dengue virus in Kassala state, Sudan, and highlights the clinical findings that are mutual in both viral infections, and laboratory investigations. This prospective study was conducted in Kassala state, Sudan. Upon 50 clinically suspected cases. The age group of the study population ranged from 14- 75 years old, all Chikungunya cases suffered from fever, (28%) of them were reported to have polyarthralgia. Both genders were affected. 50 serum samples were examined for serology and molecular techniques for the detection of both chikungunya and dengue virus. Only (10%) of suspected chikungunya cases were IgM positive, and (42%) of study population were DENV IgM positive, only one patient (2%) was both CHIKV/DENV IgM positive. This study proved that chikungunya viral infection existed in Kassala state, Sudan while there is ongoing endemic infections of dengue virus thus co-circulation, and one case was proved to have both viruses' IgM antibody, thus coinfection.

**Keywords:** Co-circulation, Co-infection, Chikungunya virus, Dengue virus.

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### المستخلص

ان حمى الشيكونغونيا موثقة بشكل ضعيف ، ونادراً ما تم أخذها في عين الاعتبار عند تشخيص الحميات الفيروسية وفقاً لمنظمة الصحة العالمية ، تهدف هذه الدراسة إلى التركيز على الانتقال المشترك لفيروس الشيكونغونيا وفيروس الضنك في ولاية كسلا بالسودان ، ويسلط الضوء على النتائج السريرية المشتركة في كل من العدوى الفيروسية ، بالإضافة الى

التحقيقات المخبرية. أجريت هذه الدراسة البحثية في ولاية كسلا ، السودان. على 50 عينة مصلية من الحالات المشتبه بها سريريا. كانت الفئات العمرية للدراسة من 14 إلى 75 عامًا ، جميع حالات الشيكونغونيا كانت تعاني من الحمى ، و (8٪) منها تم الإبلاغ بأنها تعاني من التهاب المفاصل المتعدد. تأثر كلا الجنسين ، تم فحص 50 عينة مصل لتقنيات الأمصال والتقنيات الجزيئية للكشف عن كل من فيروس الشيكونغونيا وفيروس الضنك. فقط (0٪) من حالات الشيكونغونيا لمشتبه بها كانت إيجابية للغلوبولين المناعي (م)، و (2٪) من مجتمع الدراسة للغلوبولين المناعي (م) موجبة لفيروس الضنك ، كان مريض واحد فقط (1٪) إيجابي للغلوبولين المناعي (م) لفيروس الشيكونغونيا وفيروس الضنك. أثبتت هذه الدراسة أن حمى الشيكونغونيا موجودة في ولاية كسلا ، السودان ، في حين أن هناك عدوى مسوطنة لفيروس حمى الضنك وبالتالي وجود الانتقال المشترك، وأثبتت وجود حالة واحدة إيجابية لكل من الغلوبولين المناعي (م) للفيروسان ، وبالتالي وجود الاصطيف.

**الكلمات المفتاحية:** الانتقال المشترك، العدوى المشتركة و فيروس حمى الضنك .

## INTRODACTION

Chikungunya virus (CHIKV) is an enzootic virus found in tropical and subtropical regions of Africa, in the Indian Ocean Islands, and in south and Southeast Asia. The virus was first isolated from a febrile patient during an outbreak On the Makonde Plateau in the southern province of Tanzania (formerly Tanganyika) in 1952–53. The name Chikungunya, which is used to describe both the virus and the disease, is derived from a Swahili or Makonde word Kun qunwala, meaning “that which bends up”. The disease is characterized by fever, headache, myalgia, rash, and joint pain. Although most symptoms resolve, some patients have joint pain that can continue for years and can be so severe that they adopt a bent or stooping posture<sup>1</sup>.

The virus is a member of the alphavirus in the family *Togaviridae*, which is endemic to Africa and Asia.<sup>2</sup> Many historical epidemics that were reported to be caused by dengue virus could have been caused by CHIKV. Virological or serological confirmations of CHIKV have been reported in countries in west, east, central, and southern Africa. Cases of naturally acquired human infection and virus isolation have been reported in Tanzania, Senegal, Guinea, Nigeria, Cameroon, Central African Republic, Gabon, Democratic Republic of the Congo, Uganda,

Kenya, Angola, southern Africa, and Madagascar. Serological evidence alone has been reported from Sierra Leone, Liberia, Benin, Malawi, Burundi, and Sudan.<sup>1</sup>

Dengue fever - caused by a flavivirus from the family *Flaviviridae* dengue virus (DENV) - is the most prevalent arbovirus in tropical and subtropical regions of Asia, the Pacific and Caribbean islands, and Central and South America.<sup>2</sup> As DENV and CHIKV have similar symptoms, CHIKV can be misdiagnosed as DENV. Both viruses are transmitted to humans by *Aedes aegypti* and *Aedes albopictus* mosquitoes - mosquitoes that bite mostly during the daytime<sup>2,6</sup> - and disease caused by these viruses have similar clinical symptoms, including fever, rash, and joint pains as well as headache, fatigue, nausea, vomiting, and muscle pain; Thus, many risk factors for Chikungunya virus (CHIKV) and dengue virus (DENV) infections are the same or similar.<sup>2</sup>

Chikungunya fever can be misdiagnosed in areas where dengue is common. There is no cure for the Chikungunya fever. Treatment is focused on relieving the symptoms. Also for Dengue fever there is no vaccine or any specific medicine to treat the disease.<sup>3</sup>

In Africa, the epidemiology and public health impact of both viruses is far from clear, but

the wide geographical distribution of their primary vectors (*Aedes aegypti* and *Aedes albopictus*), rapid human population growth, unplanned urbanization, and increased international travel make their transmission likely moreover.<sup>4</sup> CHIKV should be distinguished from DENV for they show similar clinical manifestations. Furthermore, where malaria is also endemic and the majority of febrile illnesses are diagnosed as such, often without laboratory confirmation, both viral infections may go undetected.<sup>5,6</sup>

In 2005 IgM antibodies to Chikungunya virus were detected in five ill individuals (27%) and three asymptomatic individuals (19%) during an outbreak of yellow fever in South Kordofan, Sudan.<sup>7</sup>

Seroprevalence studies and outbreak reports suggest endemic transmission of urban cycle of CHIKV, and at least indicates seroprevalence in the area. Despite the low quantity of CHIKV epidemiologic research in the region, this suggests that CHIKV transmission is currently under recognized.<sup>8</sup>

The global distribution of CHIKV shows that this virus is expanding at an alarming rate and has the potential to spread to new areas because it causes a travel-associated febrile illness.<sup>9</sup>

Preventing or reducing dengue virus transmission depends entirely in controlling the mosquito vectors or interruption of human–vector contact, world health organization (WHO) promotes the strategic approach known as Integrated Vector Management (IVM) to control mosquito vectors, including the vectors of dengue virus.<sup>10</sup>

Since *Aedes* species mosquitoes exist in Kassala state - the vectors of CHIKV as well as DENV, this study was constructed to detect the presence of CHIKV and DENV antibodies and viral particles in patients' sera.

And to observe presence of Chikungunya infection in patients misdiagnosed clinically as dengue fever, thus highlights co-circulation of both viruses along with possibility of coinfection in Kassala state, Sudan.

## **MATERIALS and METHODS**

### **Study design:**

This prospective study was conducted upon 50 blood samples received from Kassala State Public Health Laboratory. For detection of the presence of antibodies and viral particles to Dengue and Chikungunya virus. Patients' medical records were screened for further information regarding the clinical presentation and other investigation reports.

### **Study Area:**

This study and laboratory examinations were conducted in January to April 2014 at National Public Health laboratory, Khartoum, Sudan.

### **Study population:**

A total of 50 patients (aged more than 12 years old) attending Hospitals in Kassala state with clinical features of dengue fever were examined.

### **Inclusion criteria:**

Individuals suffering from fever, headache, loss of appetite, bleeding, jaundice and polyarthralgia were included in this study.

### **Exclusion criteria:**

Patients that did not show any clinical symptoms that are related to the above symptoms were excluded from this study.

### **Sampling technique:**

A total of 50 venous blood samples were collected during study period. 5 ml blood sample was collected from each in a plain tube and transported in ice immediately to the National Public Health Laboratory,

Department of Epidemiology and Virology,  
Khartoum.

### **Method:**

**Serology:** All the blood samples were centrifuged at 3500 rpm for 5 minutes to obtain serum. The serum from each patient was divided into two tubes labelled 1 and 2 with specifically assigned laboratory number. The sample in the first tube was used to perform dengue serology. Samples with negative and positive result for dengue serology: IgM and IgG were taken for further testing and performing Chikungunya serology. The samples from tube number 2 were used to perform Dengue and Chikungunya reverse transcriptase - Polymerase Chain Reaction, RT-PCR.

Test for dengue fever was done using Dengue IgM capture ELISA kit of Panbio diagnostics, Australia, Catalog number: E-DEN01M. Which is a qualitative enzyme immunoassay for detection of antibodies to Dengue virus. Serum antibodies of the IgM class, when present, combine with anti-human IgM antibodies attached to polystyrene surface of the microwell test strips. A concentrated pool of dengue 1-4 antigens was diluted to the correct working volume with antigen diluent. The antigens were produced using an insect cell expression system and purified utilizing a specific monoclonal antibody. An equal volume of the Horseradish Peroxidase (HRP) conjugated monoclonal antibody (MAb) was added to the diluted antigen, which allowed the formation of antigen- MAb complexes. Residual serum was removed from the assay plate by washing, and complexed antigen-MAb was added to the assay plate. After incubation, the microwells were washed and a colourless substrate system, tetramethyl benzidine/ hydrogen peroxide (TMB chromogen) was added. The substrate was hydrolyzed by the enzyme and the chromogen changes to blue colour. After

stopping the reaction with acid, the TMB became yellow. Colour development was indicative of the presence of anti-dengue IgM antibodies in the test sample.

Test for Chikungunya was done using Chikungunya IgM ELISA of SD, Germany, Catalog number: 46EK10-02-1, which was qualitative detection of IgM anti-chikungunya virus in human serum and plasma.

A microplate, which was pre-coated with recombinant chikungunya antigen on well. During first incubation, anti-chikungunya IgM in patient serum was bound to the recombinant chikungunya antigens. Following this incubation all unbound materials were removed by aspiration and washing. The anti-human IgM peroxidase-enzyme conjugate was bound to anti-chikungunya IgM. Following this incubation, all unbound materials were removed by aspiration and washing. The residual enzyme activity found in the wells would thus be directly proportional to the anti-chikungunya IgM concentration in patient serum and evidenced by incubating the solid-phase with TMB substrate A and B. Colorimetric reading was performed by using a spectrophotometer at 450nm.

**RNA Extraction:** RNA extraction from serum samples was performed using High Pure Viral Nucleic Acid Kit (QIAGEN), Catalog number: 52904, according to manufacturer's instructions.

### **Real time polymerase chain reaction (PCR):**

#### **Chikungunya Real Time Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)**

Chikungunya virus RT-PCR kit used in this study contained a specific ready-to-use system for the detection of the Chikungunya

virus by using Reverse Transcription Polymerase Chain Reaction in the real-time PCR system. The master contained a Super Mix for the specific amplification of Chikungunya structural polyprotein gene which covered gene sequence for both the African and Asian lineage strains.

The reaction was done in one step RT-PCR. The first step was a reverse transcription (RT), during which the Chikungunya virus RNA was transcribed into cDNA. Afterwards, a thermostable DNA polymerase was used to amplify the specific gene fragments by means of polymerase chain reaction. Fluorescence was emitted and measured by the real time systems' optical unit during PCR. The detection of amplified Chikungunya virus DNA fragment was performed in fluorimeter channel Cycling A.FAM of the PCR machine.

In addition, the kit contained a system to identify possible PCR inhibition by measuring the VIC/JOE fluorescence of the internal control (IC). The PCR system used was Rotor Gene™ 3000 (Corbett Research Australia).

#### **Dengue Reverse Transcription-PCR**

Initial step required viral RNA extraction, followed by amplification process similarly described in Chikungunya RT- PCR.

Initial step required viral RNA extraction, followed by amplification process similarly described in Chikungunya RT- PCR.

**Ethical Considerations:** The present study was approved by the regional health directors, and given the urgency of diagnosis, only individual verbal consents were obtained for blood sampling.

#### **Data analysis:**

Data were analyzed using Statistical Packages for Social Sciences (SPSS) version 12.0, and Microsoft office excel 2007.

### **RESULTS**

#### **Clinical diagnosis:**

Blood samples were obtained from 50 patients reported to hospitals in Kassala state those with clinically suspected dengue fever and serologically negative or positive were included in the study. (31) Patients were males, and (19) patients were females. The median age of the patients was 26 years (range 14 to 75 years old).

Clinical features were enlisted in Table 1; (100%) of the patients presented fever during 1<sup>st</sup> ten days of illness, (28%) of patients suffered from headache, (06%) of patients suffering from loss of appetite, (24%) from bleeding, (10%) from jaundice and (28%) from polyarthralgia.

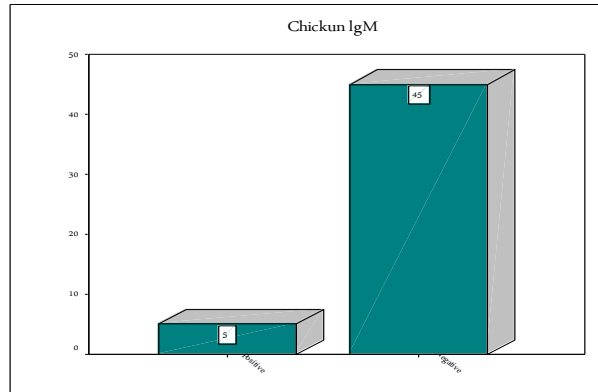
**Table 1:** General clinical features of patients

Symptom	No.pt	Percentage
Fever	50	100%
Polyarthralgia	14	28%
Headache	14	28%
Jaundice	5	10%
Bleeding	12	24%
loss of appetite	3	6%

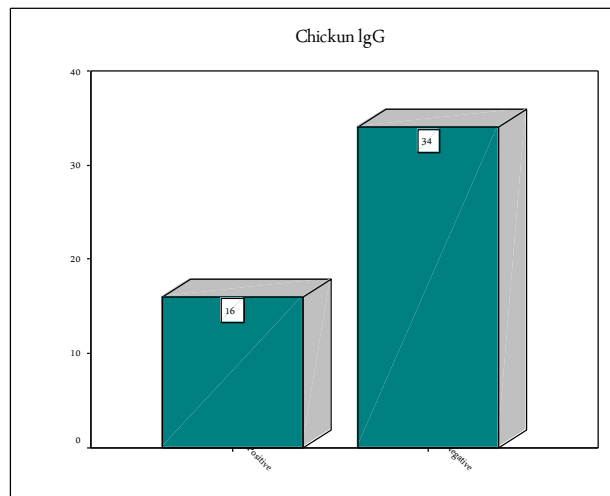
**Serological diagnosis:**

Out of 50 patients 5 (10%) were positive for Chikungunya IgM and 16 (32%) were

positive for Chikungunya IgG. Others were proved to be negative for Chikungunya by serology. (Figure 1) and (Figure 2)



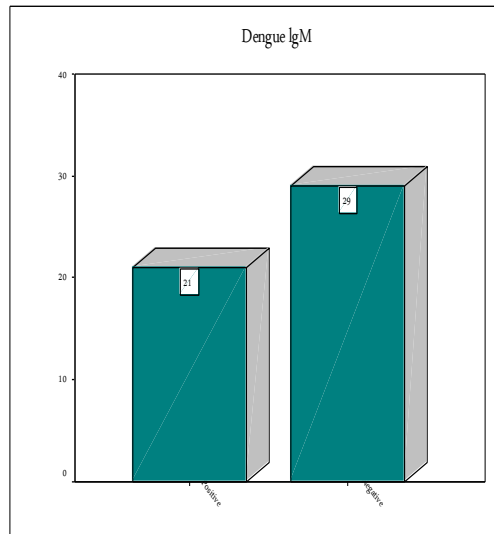
**Figure 1:** Chikungunya IgM (10%) positive.



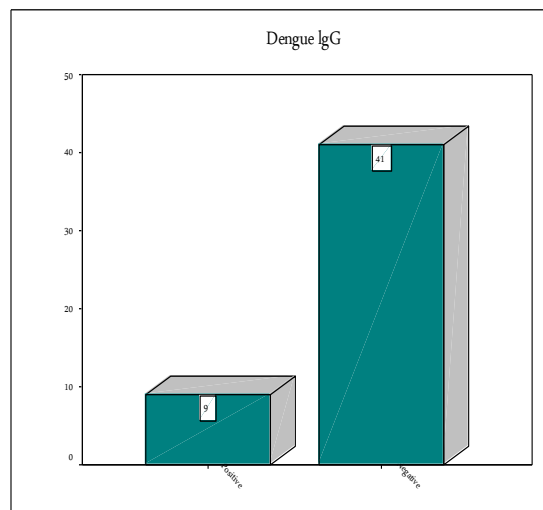
**Figure 2:** Chikungunya IgG (32%) positive.

Out of 50 patients 21 (42%) were positive for Dengue IgM, and 09 (18%) were positive for Dengue IgG. Others were proved to be

negative for Dengue by serology (Figure 3) and (Figure 4).



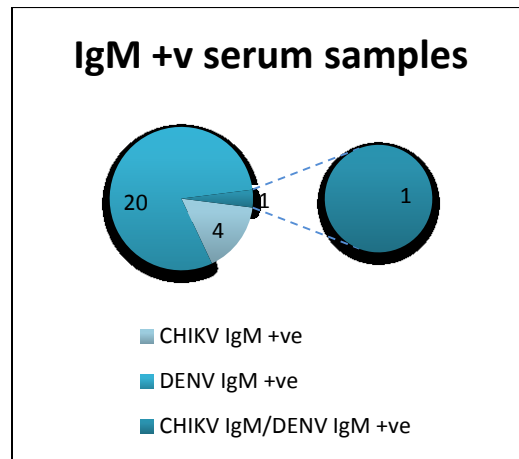
**Figure 3:** Dengue IgM (42% ) positive.



**Figure 4:** Dengue IgG (18%) positive.

One patient was positive for both CHIK IgM and DENV IgM, which probably could be co-infection

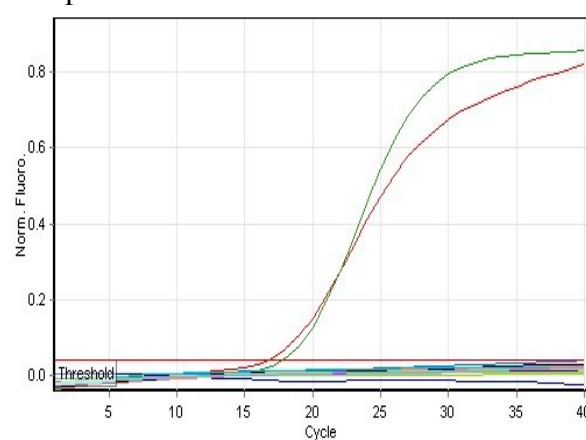




**Figure 5:** 4 CHIKV IgM +ve, 20 DENV IgM +ve, and 1 CHIKV IgM/ DENV IgM +ve

**Real Time-Polymerase Chain Reaction:** All the patients' sera were tested for Chikungunya and dengue by PCR. It was observed that all the patients those

serologically diagnosed as Chikungunya and Dengue were found to be negative by PCR as shown in (Figure 5).



**Figure 6:** Detection of dengue and Chikungunya by RT- PCR. Showed negative result for viral particles.

### DISCUSSION and CONCLUSION

CHIKV affects humans of all age groups worldwide. In the present study there was no mortality but the morbidity was high with loss of work as the population most affected belonged to the age group of 21- 45 years. The aim of this study was to focus on the importance of correct diagnosis with clinical features and confirmation of infections by laboratory methods. Although dengue fever had been notoriously involved in Kassala

public health problems, presence of Chikungunya fever with similar clinical features as a possible diagnosis should be considered.

Results have been described with figures and tables. Several other studies were published regarding early clinical and biological features of acute Chikungunya infection. Clinical diagnosis of Chikungunya based on clinical signs was examined by Staikowsky *et al.* (2009)<sup>12</sup>. However, as the clinical



manifestations of Chikungunya fever resembles to those of dengue and other fevers caused by arthropod-borne viruses of the genus alphavirus, laboratory confirmation is critical to establish the diagnosis<sup>3</sup>, in this study it has been proved that laboratory study is prerequisite to declare finally patients infected with either Chikungunya or dengue. The classical clinical features of acute Chikungunya infection are the triad of fever, arthralgia and inconstant skin rash<sup>11</sup>. Arthralgia is the key clinical features in clinical diagnosis of Chikungunya infection. In this study, it is the main symptom experienced by patients with Chikungunya IgM-positive group. Similar findings were reported by Staikowsky *et al.* (2009)<sup>12</sup>. Arthralgia in Chikungunya is usually symmetrical and involved more than one joint. The pain can be excruciating and involved fingers, wrist, elbows, toes, ankles and knees. This type of arthralgia was described in most alphaviruses infection. Robin *et al.* (2008) described the acute signs and symptoms were resolved after 2 weeks but arthralgia may persist for months or years. It has been reported persistent and disabling arthralgia in more than 60 % of patients after 18 months. In a study of Chikungunya virus infection, it was observed that most working adults were disabled with loss of mobility, hand handicap, and depressive reaction which lasted for weeks to months. Clinical impact was much more severe in older adults, progressively leading to complete loss of autonomy, health status deterioration, and sometimes death in debilitated or elderly people<sup>14</sup>. Compared to dengue fever, Chikungunya fever is responsible for long-lasting consequences in health, social organization and economy in epidemic areas.

Atypical presentation of Chikungunya was documented in several studies in large outbreak<sup>11</sup>. Atypical manifestations based on

the systems affected such as neurological (encephalitis, seizures, neuropathy, ocular) cardiovascular (myocarditis, heart failure, arrhythmia), dermatological, renal (nephritis) and other miscellaneous manifestations were not documented in this study. The lack of atypical presentation in this group of patients is likely because high representation of younger age group<sup>15</sup>. Most patients also did not require admission likely due to mild degree of severity in their clinical presentation.

Fatality was not documented in this study. Earlier outbreaks India had few crude deaths reported<sup>16</sup>. In Malaysia the first case of mortality due to CHIK virus infection in Malaysia was reported in Sarawak in January 2010<sup>17</sup>. This patient did not have pre-existing co-morbidity, developed hepatitis and succumbed likely due to cardiovascular collapse. Sam *et al.* 2010 later reported second death confirmed with viral culture in Kuala Lumpur in March 2010<sup>17</sup>.

Interpretation of results of serological tests; IgM positive results indicated acute infection were (10%) for CHIKV IgM when compared to (42%) for DENV IgM, refer to figure (1, 3), Although IgM was known to last for months, using WHO criteria for diagnosis, the result of positive CHIKV IgM was considered as evidence of acute infection because all patients suffered from fever<sup>3</sup>. According to figure (2, 4) results for CHIKV IgG were higher in percentage (32%) to DENV IgG and (18%) indicating past infection for both viruses. Among all patients, only 1 patient was positive CHIKV IgM and DENV IgM, thus possibility of co-infection figure (5).

Molecular tests such as the reverse transcriptase – polymerase chain reaction (RT-PCR) are useful for the diagnosis of dengue infection in the early phase (< 5 days

of illness). It was shown to have a sensitivity of 100% in the first 5 days of disease, but reduced to about 70% by day 6, following the disappearance of the viremia<sup>18</sup>. In this study, serologically positive Chikungunya infections were not detected by RT-PCR. Regarding detection of Chikungunya virus by molecular method<sup>19</sup> mentioned that RT-PCR technique was useful in detecting the viral infection only in the acute phase and also indicated the usefulness of molecular techniques in an outbreak scenario. This current study blood samples probably were not collected during acute phase of infection, for that negative results have been observed by RT-PCR figure (6).

This study was carried out in an area where dengue virus infection was endemic. Hence, co-infection with CHIKV infection was also very likely. Patients with serology-positive dengue were included, co-infection if any could still be detected by PCR.

### CONCLUSION

In conclusion, the present study highlighted important clinical observations of Chikungunya and dengue fevers, the serological and molecular techniques used revealed the co-circulation of both viruses as 5 patients were CHIKV IgM positive and 21 patients were DENV IgM positive and 1 patient had positive results for both CHIKV IgM and DENV IgM, thus coinfection.

### Recommendations:

Regarding proper diagnosis, blood samples should be collected within the first week of infection, and symptoms should not be neglected, serological and molecular investigations should be conducted together to rule out the present confusion and might be guidelines in early detection of the viral diseases so that appropriate management may be undertaken to reduce long-lasting consequences in health. Further studies

should be conducted for the emerging of Chikungunya fever is quite a trend, In Sudan.

### Conflict of interest:

None declared.

### REFERENCES

1. Felicity J Burt, Micheal S Rolph, Nestor E Rulli, Suresh Mahalingam, Mark T Heise. Chikungunya: a re-emerging virus; *Lancet* 2012; 379: 662–71.
2. Shu-Fen Chang, Chien-Ling Su, Pei-Yun Shu, Cheng-Fen Yang, Tsai-Ling Liao, Chia-Hsin Cheng, Huai-Chin Hu, and Jyh-Hsiung Huang. Concurrent Isolation of Chikungunya Virus and Dengue Virus from a Patient with Coinfection Resulting from a Trip to Singapore. *JOURNAL OF CLINICAL MICROBIOLOGY*, Dec. 2010; Vol. 48: p. 4586–4589, No. 12
3. WHO, 2006. Guidelines for prevention and control of chikungunya fever. World Health Organization.
4. Amarasinghe A, Kuritsk JN, Letson GW, Margolis HS. Dengue virus infection in Africa. *Emerg Infect Dis*. 2011;17(8):1349-54.
5. Mélanie Caron, Christophe Paupy, Gilda Grard, Pierre Becquart, Illich Mombo, Branly Bikie Bi Nso, Fabrice Kassa Kassa, Dieudonné Nkoghe, and Eric Maurice Leroy. Recent Introduction and Rapid Dissemination of Chikungunya Virus and Dengue Virus Serotype 2 Associated With Human and Mosquito Coinfections in Gabon, Central Africa. *Oxford journal – Clinical Infectious Diseases* 2012; 55 (6) :e45–e53.
6. Parreira R, Centeno-Lima S, Lopes A, Portugal-Calisto D, Constantino A, Nina J. Dengue virus serotype 4 and chikungunya virus coinfection in a traveller returning from Luanda, Angola, January 2014. *Euro Surveill*. 2014;19(10):pii=20730. Available online.

7. Gould LH, Osman MS, Farnon EC, et al. An outbreak of yellow fever concurrent with chikungunya virus transmission in South Kordofan, Sudan, 2005. *Trans R Soc Trop Med Hyg* 2008; 102: 1247–54.
8. Humphrey JM, Cleton NB, Reusken CBEM, Glesby MJ, Koopmans MPG, Abu-Raddad LJ (2017) Urban Chikungunya in the Middle East and North Africa: A systematic review. *PLoS Negl Trop Dis* 11(6): e0005707. <https://doi.org/10.1371/journal.pntd.0005707>
9. Braira Wahida , Amjad Alia, \*, Shazia Rafiquea , Muhammad Idreesab, Global expansion of chikungunya virus: mapping the 64-year history, *International Journal of Infectious Diseases* 58 (2017) 69–76
10. Nick Golding<sup>1\*</sup>, Anne L. Wilson<sup>2</sup> , Catherine L. Moyes<sup>1</sup> , Jorge Cano<sup>3</sup> , David M. Pigott<sup>1</sup> , Raman Velayudhan<sup>4</sup> , Simon J. Brooker<sup>3</sup> , David L. Smith<sup>5,6,7</sup> , Simon I. Hay<sup>1,5,7</sup> and Steve W. Lindsay , Integrating vector control across diseases October 2015.
11. Gianandrea Borgherini, Patrice Poubeau, Frederik Staikowsky, Manuella Lory, Nathalie Le Moullec, Jean Philippe Becquart, Catherine Wengling, Alain Michault, and Fabrice Paganin, Outbreak of Chikungunya on Reunion Island: Early Clinical and Laboratory Features in 157 Adult Patients; *Clinical Infectious Diseases* 2007; 44:1401–7.
12. Frederik Staikowsky ,François Talarmin, Philippe Grivard, Abdel Souab, Isabelle Schuffenecker, Karin Le Roux, Marc Lecuit equal contributor, Alain Michault equal contributor, Prospective Study of Chikungunya Virus Acute Infection in the Island of La Réunion during the 2005–2006 Outbreak; 2009:10.1371.
13. Pialoux G<sup>1</sup>, Gaüzère BA, Jauréguiberry S, Strobel M, Chikungunya, an epidemic arbovirosis; *Lancet Infect Dis.* 2007 May;7(5):319-27.
14. Robin, S., D. Ramful, F. Le Seach, M.C. Jaffar-Bandjee, G. Rigou and J.L. Alessandri, 2008. Neurologic manifestations of pediatric Chikungunya infection. *J. Child Neurol.* 23 1028–1035 doi: 10.1177/0883073808314151 18287573.
15. Pardigon, N., 2008. The biology of chikungunya: A brief review of what we still do not know. *Pathologie Biologie*, 57: 127–132. doi:10.1016/j.patbio.2008.02.016 18456435 r-16. Powers, A.M. and C.H. Logue, 2007. Changing patterns of chikungunya virus: re-emergence of a zoonotic arbovirus. *J. Gen. Virol.* 88: 2363–2377 DOI 10.1099/vir.0.82858-0 17698645.
16. Mavalankar, D., P. Shastri and P. Raman, 2007. Chikungunya epidemic in India: A major public health disaster. *Lancet Infect. Dis.*, 7: 306–307. doi:10.1016/S1473-3099(07)70091-9 17448932.
17. Chua, H.H., A.K. Rashid, W.C. Law, A. Hamizah, Y.K. Chem, A.H. Khairul and K.B. Chua, 2010. A fatal case of chikungunya virus infection with liver involvement. *Med. J. Malaysia* 65 (1): 83–84 21265260.
18. Yong, Y.K., R. Thayan, H.T. Chong, C.T. Tan and S.D. Sekaran, 2007. Rapid detection and serotyping of dengue virus by multiplex RT-PCR and real-time SYBR green RT-PCR. *Singapore Med. J.*, 48(7):662-668 17609830 <http://smj.sma.org.sg/4807/4807a11>.
19. Srikanth, P., G. Sarangan, K. Mallilankaraman, S.A. Nayar, R. Barani, T. Mattew, G.F Selvaraj, K.A. Sheriff, G. Palani, K. Muthumani, 2010 Molecular characterization of Chikungunya virus during an outbreak in South India. *Indian J Med Microbiol.*28:299-302.