© 2018 IJSRSET | Volume 4 | Issue 9 | Print ISSN: 2395-1990 | Online ISSN : 2394-4099
Themed Section : Engineering and Technology

Serological and Molecular Detection of Chikungunya Virus among Arthritis Patient in Khartoum State, Sudan

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ABSTRACT

Background: Chikungunya virus is an arthropod-borne virus belonging to the Togaviridae family and the genus Alphavirus. Its symptoms imitate rheumatoid arthritis (RA) and should be suspected in patients with RA-like features in endemic areas.

Method: This is a descriptive, cross sectional study performed to detect IgM antibody and genome of CHIKV in patients with arthritis symptoms who attended the Military Hospital in Khartoum, Sudan during June 2017 to December 2017. CHIKV IgM antibodies and CHIKV RNA were estimated in 90 patients using ELISA and Reverse transcriptase Real time PCR respectively.

Results: out of 90 patients with arthritis symptoms; Rheumatoid factor and anti Cyclic Citrullinated Peptide were negative, 66.3% were complaining of fever 52.6 % have high Erythrocyte sedimentation rate (ESR), all patients were complaining of arthralgia, 22.9% in Fingers, 24.3% in Wrists 16.7% in Knees, 26% in Ankles, and 10.1% in Elbows, three samples were positive for CHIKV IgM (3.3%)., and six were positive for CHIKV RNA (6.7%).

Conclusions: CHIKV IgM antibodies and CHIKV RNA were detected among arthritis patients in Khartoum, Sudan. Further investigations are required to delineate the distribution and the importance of CHIKV in the etiology of arthritis and other disease syndromes in Sudan.

Keywords : Chikungunya virus, ELISA , anti ccp, Reverse transcriptase Real time PCR .

I. INTRODUCTION

Chikungunya virus, is an RNA virus that belongs to the family Togaviridae and genus Alphavirus, is an arthritogenic virus, first isolated in the southern province of Tanzania in 1952–1953 [(1)]. The disease is transmitted by the bite of Aedes aegypti and Aedes albopictus mosquitoes [(2)]. Initial infectious outbreaks were locally confined and were of low intensity. Ever since its re-emergence in 2000, the virus has evolved into a major public health concern globally. It has transcended international geographic boundaries with frequent epidemics, affecting millions of people mainly in countries within African and Asian subcontinents and Reunion islands in the Indian Ocean[(3)].

CHIKV cause Chikungunya fever in both male and female individual of any age . The disease is characterized by fever, headache, myalgia, rash, and polyarthritis. Generally, these episodes are self-limiting and resolve within a few weeks. However, in

10–60% of cases, musculoskeletal symptoms may persist for up to 3–5 years [4,5] .

CHIKV can cause acute, subacute, and chronic disease. Acute disease is most often characterized by sudden onset of high fever (typically greater than 102°F (39°C) and severe joint pain.[6,7] Other signs and symptoms may include headache, diffuse back pain, myalgias, nausea, vomiting, polyarthritis, rash, and conjunctivitis.[8]

CHIKV persist in synovial fibroblasts and osteoblasts of infected individuals. Upon induction, fibroblasts produce IL-6 and MCP-1. Monocytes or macrophages are recruited to the site of infection. Virus replicates in macrophages, which in turn secrete pro-inflammatory cytokines IL-1β, TNF-α, and IL-17. These pro-inflammatory cytokines and RANKL induce osteoclastogenesis, bone loss, and erosions in chronically infected individuals[9]. These changes are similar to the pathogenesis of rheumatoid arthritis.

On study was done in Sudan to investigate the seroprevalence of CHIKV infection in 379 serum samples from patients with fever in the outpatient clinics of three hospitals in eastern and central Sudan. The seroprevalence was 1.8%, indicating that CHIKV infections are rare in these parts of Sudan. As the vector Aedes aegypti is endemic in this area, the population is at risk for a CHIKV epidemic[10]. Also other study done in Sudan to investigate the seroprevalence of CHIKV among Blood Donors in Singa City Sinnar, 90 serum samples were collected from blood donors who attended Singa Hospital blood bank. The seroprevalence was 5.5%, indicating that CHIKV infection was frequent among apparently healthy blood donors in Singa city, Sinnar State-Sudan [11] .

CHIKV infection may cause an algoeruptive syndrome with disabling joint pain and recurrent rheumatic manifestations [12,13] , Fragmentary information is available on GHIKV infection and their risk in arthritis patients in sudan . as only limited capacity for GHIKV diagnosis exists in the country.

This study was conducted to detect the presence of GHIKV IgM antibody and GHIKV RNA among arthritis Patient in Khartoum State.

II. MATERIALS AND METHODS

This was descriptive cross-sectional, hospital and laboratory _based study, The study included 90 arthritis patients (47 Males and 43 Females) recruited from Military Hospital in Khartoum, Sudan.. Their age range was from 18 up to 70 year. Rheumatoid factor test and anti ccp were done for each sample to exclude rheumatoid arthritis. Five milliliter (ml) of venous blood were collected from each patient in plain container, and then serum was separated and tested for CHIKV IgM and RNA by using ELISA and Real time PCR respectively .

CHIKV IgM were tested using Anti-Chikungunya Virus ELISA (IgM) EUROIMMUN kit (Germany), the analysis was done according to manufacturer instruction .

RAN extraction:

Total RNA was extracted by using the QIAampViralRNA Mini spin according to the protocol of the manufacturer (Qiagen, Germany).

reverse transcriptase polymerase chain reaction (qRT-PCR):

The reaction was performed in a final volume of 25 µl using Chikungunya virus Real time RT-PCR kit (Shanghai ZJ bio –Tech Co.,Ltd China) which contained 18 µl super Mix, 1 µl Enzyme Mix , 1 µl Intrnal Control , and 5 µl of target RNA . The mixture was incubated in real time PCR (Rotor Gene) at 45ºC for 10 min (1 cycle), 95ºC for 15 min (1cycle) and at 95ºC for 15 sec, 60ºC for 1min (40cycles) and fluorescence measured at 60 ºC using FAM channel for Target Nucleic Acid and HEX/VIC/LOE for internal control.

Data analysis: Data collected were analyzed by using statistical package for social science (SPSS) computer program version 21.

Ethical considerations: This study was approved by Ethical committee of the Faculty of Medical Laboratory Science, Al Neelain University. Written consents were obtained from each patient prior to sample and data collection.

III. RESULT

This study included 90 patients complaining of arthritis symptoms, 47 of them were Males (52.2%) and 43 were Females (47.8%). Their age ranged from 18 up to 70 years, they were classified into four age groups; less than 20 years, from 21 to 30 years, from 31 to 50 years and from 51 to 70 years and their distribution were 18 (20%) , 38 (42.2%), 32 (53.6%), and 2 (2.2%) respectively (table 1).

Clinical and laboratory data were obtained from each patient, all patients were negative for Rheumatoid factor and anti ccp , 59 patients w ere complaining of fever (66.3%) , 47 patients have high Erythrocyte sedimentation rate (ESR) (52.6 %), all patients were complaining of arthralgia ,21 (22.9%) in fingers, 22 (24.3)% in wrists,15(16.7%) in knees, 23 (26%) in ankles, and 9 (10.1%) in elbows, CHIKV IgM and RNA was determined by examination of blood samples collected from each patient , three samples were positive for CHIKV IgM (3.3%)., and six were positive for CHIKV RNA (6.7%) .

Table (2) shows that three of the patients (2 females and 1 male) were positive for CHIKV IgM and six (4 females and 2 males) were positive for CHIKV RNA .

Table 1. Distribution of Age groups , CHIKV IgM and CHIKV RNA results

Age group in years	Number (%)	IgM positive	IgM negative	RNA positive	RNA negative
Less than 20 years	18 (20%)	1 (5.6%)	17 (94.4%)	1 (5.6%)	17 (94.4%)
From 21 to 30 years	38 (42.2%)	2 (5.3%)	36 (94.7%)	3 (7.9%)	35 (92.1%0
From 31 to 50 years	32 (35.6%)	0 (0%)	32 (100%)	1 (3.1%)	31 (96.9%)
From 51 to 70 years	2 (2.2%)	0 (0%)	2 (100%)	1 (50%)	1 (50%)
Total	90 (100%)	3 (3.3%)	87 (96.7%)	6 (6.7%)	84 (93.3%)

Table 2. Distribution of Gender, CHIKV IgM and CHIKV RNA results:

Gender	Number (%)	IgM positive	IgM negative	RNA positive	RNA negative
Male	47 (52.2%)	1 (2.1%)	46 (97.9%)	2 (4.3%)	45 (95.7%)
Female	43 (47.8%)	2 (4.7%)	41 995.3)	4 9 (9.3%)	39 (90.7%)
Total	90 (100%)	3 (3.3%)	87 (96.7%)	6 (6.7%)	84 (93.3%)

IV. DISCUSSION

The objective of this study was to detect CHIKV IgM antibody and GHIKV RNA in patients with rheumatoid arthritis symptoms in Khartoum, Sudan. There is several studies has been published on the existence of CHIKV in Sudan, one of them reported the virus in western and central Sudan (14) while the other one reported CHIKV along with yellow fever virus (YF) in South Kordofan (15). Also there is recent study reported CHIKV IgM antibidy among blood donor in Singa City Sinnar State (11) .

Seroprevalence of CHIKV IgM positivity was (3.3%) where one male and two females were found positive out of 90 arthritis patients tested. This was higher than indicated by Ahmed et al (2016) in Khartoum State , these authors reported that no IgM antibodies was detected in arthritis patients (16). While this Seroprevalence was lower than indicated by Mohamed et al (2017) in Singa City Sinnar State these authors reported that out of 90 blood donors 5 (5.5%) were positive for CHIKV IgM antibodies (11) . On the other hand, CHIK RNA positive samples were (6.7%) were detected in four females and two males. Which is lower than that reported by Nanikaly et al in 2011 in Republic of Congo. (17)

V. CONCLUSION

In this study, the detection of both CHIKV aIgM antibodies and Viral RNA indicate active transmission of the virus in Khartoum State ,Sudan. Thus we recommended further investigations are required to delineate the distribution and the importance of CHIKV in the etiology of arthritis and other disease syndromes in Sudan.

VI. ACKNOWLEDGMENT

We would like to acknowledge for the staff at central laboratory for their good cooperation during practical, and great thanks to Dr. Kalid Enan for his price instruction and for my college Roua Ahmed .

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