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Seroprevalence of Chikungunya virus within arthritis patients in Khartoum, Sudan

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Abstract:

Background: *Chikungunya virus is an arbovirus belonging to the Togaviridae family. Its symptoms mimic rheumatoid arthritis (RA) and should be suspected in patients with RA-like features in endemic areas.*

Method: *This is a descriptive, cross sectional study was performed to detect seroprevalence of CHIKV in patients with rheumatoid arthritis symptoms attended Khartoum teaching Hospital*

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and Military Hospital in Khartoum, Sudan during December 2015 to March 2016. CHIKV antibodies IgM and IgG were estimated in 92 patients using ELISA.

Results: *out of 92 patients with arthritis symptoms; Rheumatoid factor and blood film for malaria were negative, 58.7 % have high Erythrocyte sedimentation rate (ESR), 65.2% were complaining of fever, 50% have skin rash, all patients were complaining of arthralgia 15.2% in Knees, 23.9% in Ankles,9.8% in Elbows, 22.8% in Wrists, 17.4% in Fingers and 10.9% in Shoulders. All samples were negative for CHIKV IgM, and three were positive for CHIKV IgG (3.3%).*

Conclusions: *In conclusion this study demonstrates that the seroprevalence of CHIKV infections within patients with arthritis in Khartoum, Sudan, was 3.3%.*

Key words: Chikungunya virus, ELISA, Seroprevalence, rheumatoid arthritis

INTRODUCTION

Chikungunya virus (CHIKV) is one of the emergent viruses worldwide and it's making serious outbreaks in many countries. Chronic arthralgia is a frequent complication of acute Chikungunya disease; it mimics rheumatoid arthritis (RA) by joint distribution and possible chronicity. It should be suspected in patients with RA-like features in endemic areas.

Chikungunya virus (CHIKV) is a mosquito-transmitted. It's an alphavirus belonging to the Togaviridae family. Alphaviruses are small spherical enveloped viruses, with a (65–70 nm) in diameter. Its genome is a single strand RNA molecule of positive polarity, encoding four nonstructural proteins involved in virus replication and pathogenesis and five structural proteins that compose the virion [(1)].

It was first isolated in Tanzania in 1952[(2)], in the last two decades, Chikungunya virus (CHIKV) has rapidly

expanded to several geographical areas, causing frequent outbreaks in sub-Saharan Africa, South East Asia, South America, and Europe [3].

CHIKV transmitted primarily by the *Aedes aegyptus* and *Aedes albopictus* species. The illness is characterized by 3–7 days of high fever, headache, rash, myalgia [4], and severe arthralgia/arthritis, the hallmark of the disease.

Arthropathy can be debilitating, accounting for the name "Chikungunya," which, in the language of the Makonde (northern Mozambique), means "that which bends up." It is mainly distal and symmetric, and can be persistent [5].

Chikungunya fever affects all age groups, and both genders are equally affected. The incubation period ranges from 3 to 12 days (usually 3-7 days) [6]. In susceptible populations, the attack rates can be as high as 40-85%.

Prodromal symptoms are rare. In the acute stage, the onset is usually abrupt and sudden with high-grade fever (usually 102-105 °F), severe arthralgia, myalgia, and skin rash [7]. Headache, throat discomfort, abdominal pain, and constipation may also be evident. Conjunctival suffusion, persistent conjunctivitis, cervical, or sometimes generalized lymphadenopathy may be present.

There is no specific therapy, and prevention is the main countermeasure. Prevention is based on insect control and in avoiding mosquito bites in endemic countries. Diagnosis is based on the detection of virus by molecular methods or by virus culture on the first days of infection, and by detection of an immune response in later stages. CHIKV infection must be suspected in patients with compatible clinical symptoms returning from epidemic/endemic areas. Differential diagnosis should take into account the cross-reactivity with other viruses from the same antigenic complex (i.e. O'nyong-nyong virus) [8].

In study done by Bacci*et.al* 2015, seroprevalence of anti-CHIKV antibodies in pregnant women living in an urban area

of Benin (West Africa) was investigated, Results were obtained by screening sera collected in 2006 and2007 with enzyme-linked immunosorbent assay (ELISA) for anti-CHIKV immunoglobulin G (IgG) and IgM. Positive results were confirmed by indirect immunofluorescence test and microneutralization assay, they founded that a large proportion (36.1%) of pregnant women living in Cotonou had specific IgG against CHIKV, indicating a high seroprevalence of the infection in urban southern Benin, whereas no active cases of CHIKV infection were detected [(9)].

In study done to determine the clinical and biological features of patients at the acute phase of CHIKV infection. A prospective study enrolled 274 consecutive patients with febrile arthralgia recorded at the Emergency Department of the Groupe Hospitalier Sud-Reunion between March and May 2006. Bivariate analyses of clinical and biological features between groups were performed. Patients with CHIKV viremia presented typically with asymmetrical bilateral polyarthralgia (96.5%) affecting the lower (98%) and small joints (74.8%), as well as asthenia (88.6%), headache (70%), digestive trouble (63.3%), myalgia (59%), exanthems (47.8%), conjunctival hyperhemia (23%) and adenopathy (8.9%). Vertigo, cutaneous dysesthesia, pharyngitis and haemorrhages were seldom observed. So far unreported symptoms such as chondrocostal arthralgia (20%), entesopathies (1.6%), talalgia (14%) were also noted. Prurit was less frequent during the viremic than post-viremic phase (13.9% vs. 41.2%; $p<0.001$), whereas lymphopenia was more frequent (87.6% vs. 39.4%; $p<0.001$). Others biological abnormalities included leukopenia (38.3%), thrombocytopenia (37.3%), increased ASAT and ALAT blood levels (31.6 and 7.3%, respectively) and hypocalcemia (38.7%). Lymphopenia <1,000/mm was very closely associated with viremic patients. Age under 65 was associated with a benign course, as no patients younger than 65 had to be hospitalized [(7)].

In study 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 aegyptus is endemic in this area, the population is at risk for a CHIKV epidemic[10].

MATERIALS AND METHODS:

This was hospital-based cross sectional study, carried out to determine serological status for CHIKV antibodies within patients complaining of rheumatoid arthritis symptoms attending Khartoum teaching Hospital and Military Hospital during December 2015 to March 2016.

A total of 92 Patients (50 Males and 42 Females) were included in this study. Their age range was from 18 up to 80 year. Rheumatoid factor test and blood film for malaria were done for each sample to exclude rheumatoid arthritis and malaria. Five ml of blood sample was collected from each patient in plain container, and then serum was separated and tested for CHIKV IgM and IgG by using ELISA.

Erythrocyte sedimentation rate was done for each patient and classified as normal or high.

Sociodemographic and clinical data were collected using instructed questionnaire: age, gender, fever, skin rash, and site of arthritis were included.

ELISA Procedure:

CHIKV IgM and IgG were tested by Anti-Chikungunya Virus EISA (IgM) EUROIMMUN (Germany), and Anti-Chikungunya Virus EISA (IgG) EUROIMMUN (Germany), semiquantitative analysis was done according to manufacturer instruction as:

1- 100µl of the calibrators, positive control, negative controls and diluted patients samples were transferred to microplate wells and incubated for 60 min at 37°C.
2- 450µl of working strength wash buffer was used to Wash microplate wells. And this process repeated three times.
3- 100µl of enzyme conjugate was pipetted into each of the microplate wells and incubated for 30 min at room temperature.
4- The microplate wells were washed again as previously described in step two.
5- 100µl of chromogen / substrate was pipetted into each microplate wells and incubated for 15 min at room temperature.
6- 100µl of stop solution was pipetted into each microplate wells.
7- Results was recorded at 450 nm wavelength filter's and also at 650 nm for reference reading by ELISA reader.
8- Calibrator 2 was measured to evaluate the results according to following formula:

$$\frac{\text{Extinction of the control or patient sample}}{\text{Extinction of calibrator 2}} = \text{Ratio}$$

Specimens with absorbance >1.1 standard-deviations above the mean absorbance of negative controls were considered as positive.

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

Ethical considerations: This study was approved by Ethical committee of faculty of medical laboratory science, Al Neelain

University. Ethical consents were obtained from each patient prior to sample and data collection.

RESULT

A total of 92 patients complaining of arthritis symptoms attending Khartoum teaching Hospital and Military Hospital during December 2015 to March 2016 were enrolled in this study, 50 of them were Males (54.3%) and 42 were Females (45.7%). Their age was ranged from 18 up to 80 years, they were classified into four age groups; less than 20 years, from 21 to 40 years, from 41 to 60 years and from 61 to 80 years and their distribution were 21(22.8%), 41(44.6%), 28 (30.4%), and 02 (2.2%) respectively (Table 1).

Clinical and laboratory data were obtained from each patient, all patients were negative for Rheumatoid factor and blood film for malaria, 54 patients have high ESR (58.7%), 60 of them complaining of fever (65.2%), 46 patients (50.0%) have skin rash, all patients were complaining of arthralgia distributed as 14 (15.2%), 22 (23.9%), 09 (9.8%), 21 (22.8%),16(17.4%) and 10 (10.9%) in Knees, Ankles, Elbows, Wrists, Fingers, and Shoulders respectively (Table 2).

Seroprevalence of CHIKV was determined by examination of blood samples collected from each patient for CHIKV IgM and CHIKV IgG, all samples were negative for CHIKV IgM, and three were positive for CHIKV IgG (3.3%).

Out of three positive patients two were female, two within age group from 21 to 40 year (Table1), two of them were febrile, skin rash was found in one patient, and two of them have knees arthralgia and one have wrists arthralgia (Table 2).

Table1: Distribution of Gender, Age, and CHIKV IgG results.

		IgG Positive		IgG Negative		Total
Gender	Male	01	2.0 %	49	98 %	50 (54.3%)
	Female	02	4.7 %	40	95.3 %	42 (45.7%)
Age group	Less than 20 years	01	4.7 %	20	95.3 %	21 (22.8%)
	21- 40 years	02	4.8 %	39	95.2 %	41 (44.6%)
	41- 60 years	0	0.0 %	28	100 %	28 (30.4%)
	61- 80 years	0	0.0 %	02	100 %	02 (2.2%)

Table 2: Distribution of Clinical data and CHIKV IgG results.

Clinical data		IgG Positive		IgG Negative		Total
Arthralgia	Knees	2	14 %	12	86 %	14 (15.2%)
	Ankles	0	0.0 %	22	100 %	22 (23.9%)
	Elbows	0	0.0 %	9	100 %	09 (9.8%)
	Wrists	1	4.7 %	20	95.3 %	21 (22.8%)
	Fingers	0	0.0 %	16	100 %	16 (17.4%)
	Shoulders	0	0.0 %	10	100 %	10 (10.9%)
Fever	Febrile patient	2	3.3 %	58	96.7 %	60 (65.2%)
	Non febrile patient	1	3.1 %	31	96.9 %	32 (34.8%)
Skin rash	Present	1	2.17 %	45	97.83 %	46 (50%)
	Absent	2	4.34 %	44	95.66 %	46 (50%)
ESR	Normal	1	2.6 %	37	58.7 %	38 (41.3%)
	High	2	3.7%	52	41.4 %	54 (58.7%)

DISCUSSION

Chikungunya virus is an arbovirus belonging to the Togaviridae family. It mimics rheumatoid arthritis (RA) and should be suspected in patients with RA-like features in endemic areas.

The objective of this descriptive, cross sectional study was to detect seroprevalence of CHIKV in patients with rheumatoid arthritis symptoms in Khartoum, Sudan. In Sudan and other malaria endemic countries, the lack of epidemiologic information concerning the etiology of acute febrile illness results in over-diagnosis and over-treatment of malaria [(11)]. Although available evidence from sub-Saharan countries,

strongly suggests that CHIKV would be among the main causes of acute febrile illness [12], this virus is neglected in Sudan and only two studies has been published, one reported CHIKV in western and central of Sudan [10] while the other one reported CHIKV along with yellow fever virus (YF) in South Kordofan [12].

The seroprevalence of anti CHIK IgG positive was (3.3%) one male and two females and no IgM positive cases were found of the total 92 rheumatoid arthritis patients. The seroprevalence was higher than study done by Adam *et al* in 2016 in central and eastern of Sudan in which they reported that the seroprevalence was 1.8% [10].

Regarding screening of IgM for CHIKVit was negative for all studied patients and this indicate there is no active infection.

The seroprevalence of CHIK antibodies among patients with arthritis was found to be higher in 20-40 years age group,this in agreement with study performedin Island of La Reunion in period between 2005-2006 [7].

Symptoms associated with CHIKV infection such as fever, joint pains, and myalgia are non-specific and could be mistakenly identified with a variety of other diseases including dengue, malaria, Rift Valley fever, and influenza. However, pronounced persistent severe joint pains that affect wrists, elbows, fingers, and knees in some patients should raise the suspicion of alphavirus infection, especially chikungunya disease or O'nyong nyong fever [13,14,15], regarding arthralgia in current study the lower joints were affected in 39.1 % of patients, and small joints were affected in 40.2%.

In conclusion this study demonstrates that the seroprevalence of CHIKV infections within patients with arthritis is 3.3% which is more than thought in central Sudan and thus we recommend further entomological and virological

studies to better understand the distribution of the CHIKV and vector in this area.

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