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Prevalence of IgG and IgM antibodies to Chikungunya virus among outpatients with febrile illness attending University of Maiduguri Teaching Hospital, Maiduguri, Borno State, Nigeria

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In Nigeria, there is paucity of information on the epidemiology of infections due to Chikungunya virus (CHIKV) especially among patients with febrile illness. Cases of febrile illness are usually associated with malaria and typhoid fever without considering the possibility of viral aetiology. This study was designed to determine the prevalence and identify other epidemiological parameters of CHIKV infections among outpatients with febrile illness attending University of Maiduguri Teaching Hospital, Nigeria. Sera from 370 patients were tested for presence of CHIKV immunoglobin (Ig) IgM and IgG antibodies using the enzyme-linked immunosorbent assay (ELISA). Of the 370 sera tested, 39 (10.5%) were positive for presence of CHIKV antibodies. A total of 24 (6.5%) tested positive for CHIKV IgM only, while none (0.0%) was positive for the presence of CHIKV IgG only. Fifteen (4.1%) of the serum samples simultaneously reacted to both IgG and IgM antibodies. A significant difference (p<0.0001) was observed in the distribution of CHIKV antibodies in relation to gender. The males had prevalence of 8.5% IgM antibodies as against 4.6% in females, 4.6% of females were positive for both CHIKV IgG and IgM antibodies, compared to 3.4% in males. The age group ≤60 years and the undisclosed age group were positive for the presence of CHIKV IgG and/or IgM antibodies. No significant difference was observed in the seasonal prevalence of CHIKV antibodies among the study subjects. Analysis of the prevalence of CHIKV antibodies in relation to clinical presentation in the patients revealed that headache and fever were the most frequently encountered ailments.

Key words: Chikunguya, antibodies, Borno.

INTRODUCTION

Chikungunya is derived from the root verb "kungunyala", meaning "to dry up" or "that which bends up" in reference to the stooped posture developed due to the rheumatological manifestations of the disease (Mohan et al., 2010). The disease is called "buka-buka" in Congo, meaning "broken-broken" reflecting the incapacitating arthralgias or to become contorted (Mohan et al., 2010). Chikungunya (CHIK) is caused by chikungunya virus

(CHIKV), formerly occurs only as an episodic arbovirus, is now a worldwide public health problem (Peyrefitte et al., 2007). This disease is gradually assuming a major health problem with potentially life-threatening and debilitating arthritis (Rashad et al., 2013). CHIKV is responsible for an acute infection with an abrupt onset of high fever, arthralgia, myalgia, headache and rash (Niyas et al., 2010; Yusof et al., 2011). Mothers afflicted with Chikungunya fever in the perinatal period can vertically transmit Chikungunya fever to neonates (Sebastian et al., 2009). Intrapartum transmission also contributes, while caesarean section does not appear to prevent the transmission. Neonatal Chikungunya fever is associated with fever, poor feeding, pain, distal edema, various skin manifestations, seizures, meningoencephalitis, and echocardiographic abnormalities in the newborn (Sebastian et al., 2009). The menace of this disease cuts across all ages and both sexes (World Health Organization, 2009). *Aedes* species (*Aedes aegypti, Aedes albopictus, Aedes africanus, Aedes furcifer, Aedes taylori* and *Aedes cordelliri*) are the principal vectors of CHIKV (Centre for Disease Control, 2012). Transmission cycle is from mosquito-to-man and man-to-mosquito; and such mosquitoes become infective approximately 10 days after feeding and remain infective for life (Centre for Disease Control, 2012). It is closely related to O'nyong-nyong (ONN) virus and believed to be enzootic throughout most of Africa and historical evidence indicates that it spreads to other parts of the world from this region (Powers et al., 2000). According to Weaver et al. (2012), monkeys are also involved in enzootic transmission cycles, where enzootic vector for example *A. furcifer* transmit CHIKV from monkeys to humans.

CHIKV is an emerging arbovirus that is widespread in tropical regions and is spreading rapidly to temperate climates with recent epidemics in Africa and Asia and also documented outbreaks in Europe and America (Rashad et al., 2013). In Nigeria, series of studies carried out gave rise to the evidence of CHIKV existence in the country (Moore et al., 1974; Fagbami and Fabiyi, 1975; Fagbami, 1977; Fagbami, 1978; Tomori et al., 1981; Adekolu-John and Fagbami, 1983; Adesina and Odelola, 1991; Baba et al., 2013; Ayorinde et al., 2016). Most of these previous studies on CHIKV infection conducted in Nigeria were carried out in South Western Nigeria. CHIKV has caused massive outbreaks in Africa and Asia and its magnitude and circulation especially in Nigeria remained poorly documented. Like most arboviruses, there is no specific surveillance carried out for CHIKV or viral screening for pyrexias of unknown origin in Nigeria. Therefore, there is paucity of information on the epidemiology of infections due to CHIKV especially among patients with febrile illness in Nigeria. Cases of febrile illness are usually associated with malaria and typhoid fever without considering the possibility of viral aetiology. This manuscript was designed to determine the prevalence and identify other epidemiological parameters of CHIKV infections among outpatients with febrile illness attending University of Maiduguri Teaching Hospital (UMTH).

MATERIALS AND METHODS

Study area

The study was conducted at the UMTH, Borno State, Nigeria. UMTH, a tertiary health institution designated as "Center of Excellence" in immunology and infectious diseases by the Federal Government of Nigeria. This institution has a 530 bed facility which is spread over 17 wards and serves a population of over 20 million in the North-Eastern sub-region of Nigeria as well as sizeable number across the borders of Cameroun, Chad and Niger Republics (Garba et al., 2011).

Study population

The target population were outpatients with febrile illness attending UMTH. Consent of the patients was sought orally and obtained before inclusion in the study. The ethical clearance for the study on patients was obtained from the Ethical Committee of UMTH, Borno State, Nigeria.

Sample collection

Blood samples were collected from a total of 370 febrile outpatients comprising 205 samples obtained during the rainy season (July, August and September 2012) and 165 samples collected during the dry harmattan season (November, December 2012 and January 2013). Using sterile syringes with needles, 5 ml of blood was collected from each patient into appropriately labeled sterile plain vacutainer tubes and kept at room temperature to clot. The sera from the clotted blood samples were separated by centrifugation at 134×g for 20 min. The harvested sera were stored in cryotubes at -20°C until tested. Socio-demographic information such as age, sex and clinical diagnosis were obtained from patients before blood sample collection.

Assay of serum samples for CHIKV antibody

Sera were analyzed for presence of IgM and IgG antibodies against CHIKV using the ELISA kits (IgM and IgG for human Chikungunya), manufactured by WKEA MED SUPPLIES CORP, Changchun 130012, China with LOT number 20130228. It is an indirect ELISA for specific detection of CHIKV IgM and IgG antibodies in human serum. Each serum sample was tested separately using the indirect ELISA employing secondary antibodies specific for IgM or IgG antibodies. In the indirect ELISA test, the sample antibody is sandwiched between the antigen coated on the plate and an enzyme-labeled, anti-species globulin conjugate. The addition of an enzyme substrate-chromogen reagent causes colour to develop.

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Table 1. Gender distribution of Chikungunya virus antibodies in human sera among outpatients with febrile illness attending UMTH, Borno State.

Total No. tested		Total No. positive	Distribution of antibodies in positive samples [No. (%) positive]		
			IgG only	IgM only	IgG + IgM
Female	194	18	0 (0.0)	9 (4.6)	9 (4.6)
Male	176	21	0 (0.0)	15 (8.5)	6 (3.4)
Total	370	39	0 (0.0)	24 (6.5)	15 (4.1)

χ^2=27.13, P<0.0001 (s).

Table 2. Age distribution of Chikungunya virus antibodies in human sera among outpatients with febrile illness attending UMTH, Borno State.

Age group	Total No. tested	Total No. (%) positive		
		IgG only	IgM only	IgG + IgM
0 – <15	63	0 (0.0)	5 (7.9)	4 (6.3)
15 – <30	136	0 (0.0)	5 (3.7)	7 (5.1)
30 – <45	55	0 (0.0)	4 (7.3)	0 (0.0)
45 – <60	28	0 (0.0)	1 (3.6)	1 (3.6)
60 – <75	3	0 (0.0)	0 (0.0)	0 (0.0)
75 – 90	2	0 (0.0)	0 (0.0)	0 (0.0)
Undisclosed age	83	0 (0.0)	9 (10.8)	3 (3.6)
Total	370	0 (0.0)	24 (6.5)	15 (4.1)

IgM only, χ^2= 5.042, df = 6, p= 0.5385 (ns); IgG+IgM, χ^2= 3.864, df = 6, p=0.6951 (ns).

This colour is directly proportional to the amount of bound sample antibody.

Statistical analysis

GraphPad Prism software was used for statistical analysis. Chi-square test was used to compare IgM and IgG antibodies to CHIKV in the study population. Fisher's exact test was used to compare the variables where appropriate.

RESULTS

Table 1 shows the gender distribution of CHIKV antibodies among outpatients with febrile illnesses attending UMTH, Maiduguri, Borno State. Out of the 370 sera tested, 39 (10.5%) were positive for presence of CHIKV antibodies. A total of 24 (6.5%) tested positive for CHIKV IgM only, while none (0.0%) was positive for presence of CHIKV IgG only. Fifteen (4.1%) of the serum samples simultaneously reacted to both CHIKV IgG and IgM and there was significant difference in the prevalence of IgG and IgM antibodies. A significant difference (p<0.0001) was observed in the distribution of CHIKV antibodies in relation to gender among outpatients with febrile illnesses attending UMTH, Maiduguri, Borno State. Males have significantly higher CHIKV antibodies than females in the study.

The distribution of CHIKV IgG and/or IgM antibodies among the age groups tested in the population under study, showed no significant difference (p>0.05). The age groups ≤60 years and the unclassified age group were positive for CHIKV IgG and/or IgM antibodies (Table 2). None of the samples from the age group >60 years was positive for CHIKV antibodies. This age group represents a very small minority of the overall population presenting with febrile illnesses in the study area.

The results of the present study showed no significant difference (p>0.05) in the prevalence of CHIKV antibodies between the rainy and dry seasons. The rainy season had prevalence of 16/205 (7.8%) for IgM only and 8/205 (3.9%) for IgG and IgM when compared with the dry season 8/165 (4.8%) for IgM only and 7/165 (4.2%) for IgG and IgM (Table 3).

No significant difference (p>0.05) was observed in the distribution of CHIKV antibodies in relation to clinical presentations (as observed by Clinicians) of the patients (Table 4).

However, considerable prevalence of antibodies was observed in patients with different ailments. For instance, head ache had 3/18 (16.7%) and 2/18 (11.1%) for IgM only and IgG and IgM, enteric fever/fever had 18/284 (6.3%) and 11/283 (3.9%) for IgM only and IgG and IgM, this is followed by malaria 2/9 (22.2%) and typhoid fever 1/36 (2.8%) for IgM only, abdominal pain ¼ (25.0%) and

Table 3. Seasonal distribution of Chikungunya virus antibodies in human sera among outpatients with febrile illness attending UMTH, Borno State.

Season	Total No. tested	Total No. (%) positive		
		IgG only	IgM only	IgG + IgM
Rainy [July – September]	205	0 (0.0)	16 (7.8)	8 (3.9)
Dry (Harmattan) [November to January]	165	0 (0.0)	8 (4.8)	7 (4.2)
Total	370	0 (0.0)	24 (6.5)	15 (4.1)

IgM only, p=0.2929 (ns); IgG + IgM, p=1.000 (ns).

Table 4. Distribution of Chikungunya virus antibodies from human sera in relation to clinical presentation (as observed by Clinicians) in patients attending UMTH, Borno State.

Clinical presentations	Total No. tested	Distribution of antibodies in positive sera		
		IgG only	IgM only	IgG + IgM
		No. (%) positive	No. (%) positive	No. (%) positive
Abdominal pain	4	0 (0.0)	0 (0.0)	1 (25)
Body/Joint pain	9	0 (0.0)	0 (0.0)	1 (11.1)
Dysentery	1	0 (0.0)	0 (0.0)	0 (0.0)
Enteric fever/fever	284	0 (0.0)	18 (6.3)	11 (3.9)
Fever + Headache	3	0 (0.0)	0 (0.0)	0 (0.0)
Fever + Abdominal pain	2	0 (0.0)	0 (0.0)	0 (0.0)
Fever + Joint pain	1	0 (0.0)	0 (0.0)	0 (0.0)
Headache	18	0 (0.0)	3 (16.7)	2(11.1)
Loss of stamina	2	0 (0.0)	0 (0.0)	0 (0.0)
Malaria	4	0 (0.0)	2 (50.0)	0 (0.0)
Weakness	6	0 (0.0)	0 (0.0)	0 (0.0)
Typhoid fever	36	0 (0.0)	1 (2.8)	0 (0.0)
Total	370	0 (0.0)	24 (6.5)	15 (4.1)

IgM only, χ^2=18.4, df=14, P=0.1893; IgG +IgM, χ^2 = 10.36, df=14, P=0.7358.

body/joint pains 1/9(11.1%) for IgG and IgM (Table 4).

DISCUSSION

Chikungunya is specifically a tropical disease that is relatively uncommon and poorly documented (Pialoux et al., 2007). The CHIKV, an emerging arthropod borne virus is widespread in tropical regions (Africa and Asia) and is spreading rapidly to temperate climates with recent outbreaks in Europe and the Americas (Rashad et al., 2013). The virus has increasingly great impact on man with potentially life-threatening and debilitating arthritis (Rashad et al., 2013). The 10.5% prevalence of CHIKV infections observed among outpatients with febrile illness, attending University of Maiduguri Teaching Hospital is similar to the reports previously obtained in Nigeria (Fagbami and Fabiyi, 1975; Fagbami, 1977; Adesina and Odelola, 1991). It was observed in this study that none of the sera tested was positive for IgG antibody only, but 4.1% were positive for both IgG and IgM, and 6.5% were positive for IgM only. IgM ELISA is the diagnostic test of choice for detecting recent infection and may be applied to single serum sample in some instances. The presence of IgG antibody indicates past infection. The prevalence of IgM antibody observed in this study is therefore an indication of a recent and active infection of CHIKV among the study population as vaccination against the disease has never been carried out in the study area. The absence of CHIK IgG only when compared with a relatively high prevalence of IgM only observed in this study could be an indication of the sporadic nature of CHIKV infection in the study area during the study period. In addition, the absence of CHIK IgG only is an indication that the CHIKV infection is not endemic during the study period. However, these observations contrasted with other studies (Sergon et al., 2008; Gerardin et al., 2008; Kumar et al., 2010; Sissoko et al., 2008 and Mohanty et al., 2013). The difference could be attributed to difference in geographical locations and periods of studies and could be attributed to the exposure of the population engaged in the rubber plantation to the infective biting of

Aedes albopictus as observed in previous studies. The present study was carried out in the Sudano-Sahelian vegetational zone which is a semi-arid area in north-eastern part of Nigeria where the vectoral activities of *A. albopictus* could be relatively low when compared with areas where the previous studies were carried out. The results of this study represent the first report on the prevalence of CHIKV in Sudano-Sahelian zone of Nigeria. The relatively high prevalence observed in study could be associated with low socio-economic condition, insurgency/terrorism and poor sanitation of the study area that could facilitate the abundance of *Aedes* vector species in the study area. A difference in gender prevalence of CHIKV infection was observed in the present study with the males showing higher prevalence rate than the females which is consistent with the observations made in previous studies (Suryawanshi et al., 2009; Kumar et al., 2010, 2011; Patil et al., 2013). This may be associated with the occupational risks engaged by the males. The males are usually engaged in occupations that may expose them to bites by the vectors. However, the present finding disagrees with the earlier reports (Balasubramaniam et al., 2011; Dwibedi et al., 2011; Mohanty et al., 2013). The difference may probably be as a result of difference in geographical location and presence of socio-economic factors that facilitated the breeding of *Aedes* vector in their study area.

It was observed in this study that the age group ≤60 years of age were found to be more susceptible to CHIKV infection which is in consonance with previous reports (Mohanty et al., 2013; Patil et al., 2013). This is the most active age group and coupled with movement of people outdoors during the day time when the activity of *A. albopictus* is at its peak, lesser personal protection and individual difference in immune response to diseases are some of the speculative reasons for increased susceptibility to CHIKV infection in this age group.

Seasonal prevalence of CHIKV infection among subjects in the present study revealed that CHIKV infection is more during rainy season (July to September) than dry season (November to January). This could be attributed to the increased vector abundance and activity during rainy season as observed in study and in previous investigations (Mavalankar et al., 2008; Suryawanshi et al., 2009; Balasubramaniam et al., 2011; Dwibed et al., 2011; Mohanty et al., 2013). The patients in the present study that exhibited fever and headache were observed to show the highest prevalence of CHIKV antibody which could be the major symptoms of CHIKV infection in this environment.

Conclusion

The results of this study on the prevalence of IgG and IgM antibodies to CHIKV among outpatients with febrile illnesses attending University of Teaching Hospital, Maiduguri, Borno State, has shown that out of the 370 sera tested, 39 (10.5%) were positive for the presence of CHIKV antibodies and 6.5 and 4.1% of the patients had only IgM and both IgG and IgM antibodies, respectively. This indicates recent infection and high prevalence of antibodies to CHIKV among the study population. The absence of IgG only antibody in the study population showed that the infection is not endemic but sporadic. It is possible to suggest that CHIKV infection is not endemic in the study area prior to this current study, due to the absence of CHIKV antibodies in the older age groups (≥60 years). The results of the study also showed that the gender, season and age as well as fever and headache are important factors influencing the prevalence of CHIKV antibodies in the study area. Comprehensive studies are needed to determine the seasonal distribution of CHIKV infection vis-à-vis vector dynamics and distribution. There is need to institute specific CHIKV/antibody surveillance and routine screening for the virus especially among patients with pyrexia and headache.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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