

Original Research Article

SEROLOGICAL EVIDENCE OF ACUTE CHIKUNGUNYA VIRUS INFECTION AMONG OUTPATIENTS WITH FEBRILE ILLNESS ATTENDING GENERAL HOSPITAL IN KALTUNGO, GOMBE STATE NIGERIA

ABSTRACT

Chikungunya is a re-emerging arthropod-borne viral disease that displays a large cell organ tropism, and causes a broad range of clinical symptoms in humans. The virus is listed on the WHO blueprint priority pathogens. However, Chikungunya is a neglected tropical disease in Nigeria and has never been investigated in Kaltungo. Chikungunya virus resembles malaria/typhoid fever in clinical syndrome; misdiagnosis is often common among clinicians. A descriptive cross-sectional hospital-based study was carried out aimed at “serological evidence of acute chikungunya virus infection among outpatients with febrile illness attending general hospital kaltungo, gombe state Nigeria”. A well-structured pre-tested questionnaire along with the consent forms was used to collect both demographic and clinical information of the study participants. Sera of 200 consented patients from all age groups, 112(56%) females and 88(44%) males were collected. Chi-square test was used for the analysis. Of the Sera tested, 14(7.0%) and 18(9.0%) were found to be positive for CHIKV IgM antibody using RDT & ELISA. There were statistical significant associations between gender, age and CHIKV infection ($P \leq 0.05$); males had 9(4.5%) IgM while females had 5(2.5%), age group 31-40 years had the highest positivity of 3.5% IgM and was found to be more infected with the virus. IgM antibody was detected higher 16(8.0%) among samples collected from 1-7 days while only 2(1.0%) were obtained on samples collected from 7-10 days. Antimalarial/antibiotic intake and recent travelling had not shown any statistical significant association ($P > 0.05$). The techniques used in this study revealed the presence of CHIKV IgM antibody among febrile patients evidencing the existence of CHIKV in Kaltungo. Therefore, there is need for the inclusion of CHIKV in routine differential diagnosis of febrile patients to avoid misdiagnosis of febrile condition.

Keywords: Chikungunya, Antibody, Serological, Febrile illness, Kaltungo, Nigeria

1.0 INTRODUCTION

Chikungunya (CHIK), a mosquito-borne disease endemic to tropical regions, has emerged as an epidemic threat over the past 15 years. It infects over 1 million people per year and cause debilitating joint pain (Yactayo *et al.*, 2016). Chikungunya fever (CHIKF) is caused by Chikungunya virus (CHIKV), an arthropod-borne virus (arbovirus) of the genus Alphavirus and the family *Togaviridae* (Mohan *et al.*, 2010). Within the genus, there are 30 species of arthropod-carried alphaviruses all sharing seven specific antigenic complexes (Caglioti *et al.*, 2013). CHIKV is closely related to several other alphaviruses, including Ross river virus (RRV), Borna forest virus (BFV), O'nyong-nyong virus, the Sindbis group of virus and the Mayaro virus all of which are known to cause arthritis (Suhrieb *et al.*, 2012). The virus itself is a positive sense, single-stranded RNA virus approximately 11.8kb long (Rougeron *et al.*, 2015), it has an icosahedral capsid which is covered by a lipid layer, a diameter of approximately 65nm, and is sensitive to temperature $>58^{\circ}\text{C}$ (Thiberville *et al.*, 2013). It contain two open reading frame (ORFs), one on the 5' end and the other on the 3' end (Kumar *et al.*, 2015), with the ORF at the 5' end producing 4 non-structural proteins (nsP1-4) and the ORF on the 3' end producing the structural proteins, which are composed of a Capsid protein, 2-envelope glycoproteins (E1 and E2), and 2 small cleavage products (E3 and 6k) (Petitdémange *et al.*, 2015). CHIKV has three genotypes: Asian, West African, and East Central South Africa, all named after their geographical distributions, the Indian Ocean lineage (IOL) was identified in 2004 as a descendant of the ECSA lineage (Nunes *et al.*, 2015).

Specifically, the virus is primarily transmitted by *Aedes aegypti* and *Aedes albopictus*. The principal vector in CHIKV transmission has historically been *Aedes aegypti*, but *Aedes albopictus* acted as the major

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vector in several recent outbreaks in Reunion, Europe and Gabon(.....), although *Ae. Aegypti* continue to be an important viral vector as seen during the Caribbean outbreak in 2013 (Mowatt and Jackson, 2014). *Ae. aegypti* is found in tropical and subtropical regions, whereas *Ae. albopictus* has a wider distribution and is found in temperate regions(.....). Less common modes of transmission include vertical transmission, where a mother afflicted with CHIKF in the perinatal period can vertically transmit CHIKF to neonates, intrapartum transmission also contribute, while caesarean section does not appear to prevent the transmission (Sebastian *et al.*, 2009), transmission via infected blood products and through organ donation is also theoretically possible during times of outbreak, though no cases have yet been documented (Burt *et al.*, 2012). The menace of this disease cuts across all ages and both sexes (W.H.O. 2009). CHIK is part of the group of neglected tropical disease (W.H.O. 2016).

Comment [DI10]: citation/references needed for these recent outbreaks

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Febrile illnesses in developing countries are often misdiagnosed as malaria or typhoid fever and Nigeria is not an exception(.....). Although arboviral infections have similar clinical symptoms, they are usually not screened because of limited resources and the fact that there are several viruses in this group. CHIK is part of the group of neglected tropical disease (W.H.O. 2016). Chikungunya virus 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 (Akinola *et al.*, 2017). Therefore, there is dearth of information on the epidemiology of infection due to CHIKV especially among febrile illness in Kaltungo Gombe State, Nigeria.

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In Nigeria, data defining the burden of CHIKV infections in the general population and febrile illness sub-population are scanty; most of these studies on the seroprevalence of anti-CHIKV antibody among febrile illnesses were from the south-western region of the country (Akinola *et al.*, 2017). However, the present study aimed at serological detection of Chikungunya virus infection among outpatients with febrile illness seeking for medical care at General Hospital Kaltungo with a view to generating data that will aid formulation of prevention and control policies before the disease assumes epidemic proportions. Similarly, this study employs the use of two (2) diagnostic methods viz; immunochromatography and enzyme-linked immunosorbent assays to demonstrate the presence of acute infection due to CHIKV in a cohort of 200 febrile patients.

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2.0 MATERIALS AND METHODS

2.1 Study Area

The study was carried out at the Department of Science Laboratory Technology, Federal Polytechnic Kaltungo, located along Yola road in Kaltungo, Gombe State Nigeria. The institution is one of the newly established Federal Polytechnics in the year 2020. Kaltungo is a Local Government Area of Gombe State, Nigeria. Its headquarters are in the town of Kaltungo in the west of the area on the A345 highway at 9°48'51"N 11°18'32"E. It has an area of 881 km² and a population of 149,805 at the 2006 census (Nipost, 2009).

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2.2 Study Design and Population

This is a descriptive cross-sectional hospital based study and the target population was outpatients with febrile illness seeking medical care at General Hospital Kaltungo, Gombe State Nigeria.

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2.3 Inclusion Criteria and Exclusion Criteria

Patients presenting to the general outpatient department (GOPD) with some signs and symptoms compatible with the diagnosis of CHIK (Fever which can be recent or in evidence during previous 2-4 days or/and other symptoms of febrile diseases such as chills, headache, joint pain, muscle and body pains etc.) were recruited for this study. Exclusion criteria included patient unwillingness to participate in the study, patients with good health status and those with chronic or underlying diseases.

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2.4 Ethical Consideration

The ethical approval was sought and collected from the Research and Ethical Committee of Gombe State Ministry of Health (Reference number: Our Ref: MOH/ADM/621/V.1/319). Informed Consent Form was provided and signed by each patients/defenders that participated in the study, demographic and clinical information were also obtained using a structured pre-tested questionnaire.

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2.5 Sample Size Determination

Based on 13% prevalence rate of CHIKV antibody from a previous study in Ilorin, Kwara State Nigeria by Kolawole *et al.* (2017), the minimum sample size was determined using the formula;

$$n = \frac{Z^2 PQ}{d^2}$$
 (Sarmukaddam and Garad, 2006),

$n = 174$. To minimized error, sample of 200 patients were collected for this study.

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2.6 Sample Collection

Samples were collected from 6th June to 7th July, 2023 at General Hospital Kaltungo, Gombe State Nigeria. Using sterile syringes with needles, 5ml of whole blood was drawn aseptically from each patient by venipuncture and carefully transferred into appropriate labeled sterile plain vacutainer tubes and allowed to clot at room temperature (Akinola *et al.*, 2017). The resultant sera from the clotted blood samples were separated by centrifugation at 8,000 rpm for 5minutes (Baba *et al.*, 2013). The harvested sera were stored in cryotubes at -20°C until used.

2.7 Laboratory Investigations:

2.7.1 Chikungunya IgM rapid test device (RDT)

Serum samples were tested for anti-CHIK IgM antibodies using Chikungunya IgM Rapid Test Device (D-CHIKMD20) (Rapid Labs Ltd, United Kingdom). Standard protocols were followed according to the manufacturer's instruction. The CHIK IgM EIA kit has a relative sensitivity: 90.3% (95%CI: 81.0%-96.0%), relative specificity: >99.9% (95%CI: 86.7%-100%), accuracy: 92.5% (95%CI: 85.1%-96.9%) and showed no cross-reactivity.

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2.7.2 Method:

The test was carried out according to the manufacturer's instruction. The test device and the serum samples were brought to room temperature. Using a sterile pipette, one drop of serum sample (approximately 30µl) was transferred to the specimen well of the test device followed by one drop (approximately 40µl) of buffer and allowed to stand at room temperature for 5 minutes. Results were read in 15 minutes (Rapid Labs Ltd, U.K).

2.7.3 Enzyme-linked immunosorbent assay (ELISA)

Antibodies against CHIKV were targeted using the AccuDiag™ Chikungunya IgM ELISA Kit (Diagnostic Automation, California, USA).

2.7.4 Method:

The assay was carried out according to the manufacturer's instruction. Serum samples and controls (positive, cut-off and negative) were diluted 1/100 in the sample dilution buffer for Chikungunya into a clean microtitre plate wells (4µl of sample into 396µl of sample dilution buffer). Controls were assayed in duplicate and each dilution was mixed well by pipetting up and down several times. Fifty microliter (50µl) of the 1/100 diluted controls and serum samples were added to the appropriate ELISA plate wells, covered with parafilm and incubated at 37°C for 30 minutes in an incubator. After incubation, the plate is

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washed six (6) times with an automatic plate washer using 1x wash buffer (300µl per well in each wash cycle was used). To the ELISA plate well, 50µl of the Chikungunya antigen was added to each well; the plate was covered with parafilm and incubated again at 37°C for 30 minutes in an incubator. After the incubation, second washing was done as described above. Then, 50µl of the ready-to-use enzyme conjugate was added to each ELISA plate well, covered with parafilm and incubated at 37°C for 30 minutes. After subsequent incubation, the plate is washed again as above; followed by the application of 75µl of liquid TMB (tetramethylbenzidine) substrate into all wells using a multi-channel pipette and the plate was incubated in the dark, at room temperature for 10 minutes. All wells were covered with 50µl of stop solution using multi-channel pipette and allowed the plate stand, uncovered at room temperature for 1 minute. The optical density value was read at 450nm (OD₄₅₀) with a Microplate reader (AccuDiag IgM ELISA Kit, USA).

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2.8 Statistical Analysis

Comment [DI32]: Data

Data obtained were entered into Excel software and analyzed using IBM SPSS advanced statistics version 20.0 (SPSS Inc., Chicago, IL, U.S.A). A Non-Parametric Chi-square test was used and established a statistical difference or association between participant's variables and prevalence rates. Data were presented on tables at 95% Confidence Interval (C.I) with $P \leq 0.05$ were considered statistically significant.

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3.0 RESULT AND DISCUSSION

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3.1 Demographic and clinical profile of the study participants

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Demographic profile of the participants revealed that the proportion of females 112(56.0%) was significantly higher than that of males 88(44.0%) (Table.1), this is consistent with previous reports that women and girls exhibit greater health-seeking behaviors (Omatola *et al.*, 2020).

Table 1: Demographic and clinical profile of the study participants

Demographics	Variables	Frequency (n)	Percentage (%)
Gender	Male	88	44.0
	Female	112	56.0
Age (years)	0 – 10	5	2.5
	11 – 20	35	17.5
	21 – 30	43	21.5
	31 – 40	67	33.5
	41 – 50	31	15.5
	≥ 51	19	9.5
Onset of fever (days)	1 – 7	159	79.5
	7 – 10	41	20.5
Treat fever before test	Yes	132	66.0
	No	68	34.0
Antimalarial/ Antibiotic Intake	Yes	122	61.0
	No	78	39.0
Recent travelling	Yes	44	22.0
	No	156	78.0
Total		200	100.0

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The study participants were 112(56%) females and 88(44%) males. Patients of all age groups were recruited for the study, 159(79.5%) of them had the onset of fever within 1-7 days of infection while 41(20.5%) of them had fever onset within 7-10 days of infection. Among the study subjects, 132(66.0%) treated fever before going to the hospital while 68(34.0%) did not. Most of the patients, 122(61.0%) were already on anti-malarial/antibiotic drugs while 78(39.0%) of them were not on medication at the time of sample collection. Less than half of the study participants 44(22.0%) were reported to have travelled out of the study area for more than a week, while most of them 156(78.0%) have not travelled (Table 1).

3.2 Prevalence of Chikungunya virus infection using rapid diagnostic technique and enzyme-linked immunosorbent assay

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Out of the 200 samples tested for this study, 14(7.0%) were positive for the presence of CHIKV antibody using rapid diagnostic technique (RDT) and 18(9.0%) of the patients were positive for CHIKV IgM using enzyme-linked immunosorbent assay (ELISA) (Table.2).

Table 2: Prevalence of Chikungunya virus infection using rapid diagnostic technique and enzyme-linked immunosorbent assay

Technique		Frequency (n)	Percentage (%)
Rapid diagnostic technique	Negative	186	93.0
	Positive	14	7.0
Enzyme-linked immunosorbent assay	Negative	182	91.0
	Positive	18	9.0
Total		200	100.0

This study reveals a low infectivity of 7.0% CHIKV antibodies among patients with febrile illness using RDT. However, this observation contrasted with Omatola *et al.*, (2020) were a seroprevalence of 5.8% was reported using the same technique in Kogi State Nigeria. The difference could be attributed to the sensitivity of the rapid kits used. The ELISA-based preliminary assessment revealed a seropositivity of 9.0% IgM antibodies against CHIKV among the study population, this report contradicted Akinola *et al.*, (2017) were a prevalence of 6.5% CHIKV IgM antibodies was reported. The disparity could be as a result of exposure of the population in question or the time of sample collection. This study has shown the existence and distribution of CHIKV infection among outpatients with febrile illness in Kaltungo town, Gombe State, Nigeria and has clearly confirmed the circulation of the virus among the populace. However, the 9.0% seropositivity of CHIKV infections observed in this study was lower to the reports obtained by both Ayorinde *et al.*, (2015) and Kolawole *et al.*, (2017) were a seroprevalence of 11.0% and 13.0% were reported in Ogun and Ilorin, Kwara States Nigeria. The difference could be attributed to the geographical location or the cross-reactivity of CHIKV with other alphaviruses that belongs to the Semliki forest antigenic complex like Semliki Forest virus, Ross River virus that cross-react in ELISA test. Furthermore, this report also disagreed with Olajiga *et al.*, (2017) where a seroprevalence of 38% CHIKV IgM antibodies was reported in both Lagos and Osun States Nigeria and the difference might be attributed to the geographical location, socio-economic factors that facilitate the breeding site of *Aedes* vector in their study area.

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There are several methods currently employed for the diagnosis of CHIKV infections including tissue culture, RT-PCR and serological tests. Immunofluorescence antibody technique (IF) enables definitive identification of the CHIKV infection. Although virus isolation, is still considered as the "gold standard" for the diagnosis of CHIKV infections, it is time consuming and laborious. Serological techniques are being used exclusively in clinical laboratories. These serological techniques include the plaque reduction neutralization test (PRNT) and haemagglutination inhibition (HI) assay both of which can distinguish between primary and secondary infection. However, these methods are time consuming and in addition, HI requires paired serum samples. Most recently, ELISA methods are commonly used for the detection of IgM and IgG antibodies. These methods have been shown to be useful in the diagnosis of CHIKV

infections in the early onset of the disease. The use of rapid diagnostic test was shown to detect the IgM antibodies in acute phase of the CHIKV infections.

3.3 Relationship between the CHIKV seropositivity and participant's variables using rapid diagnostic technique (RDT)

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The relationship between the Chikungunya virus seropositivity and variables of the study participants was established using rapid diagnostic technique (RDT). Among the 200 sera tested for this study, fourteen samples 14(7.0%) were found to be positive for CHIKV infection comprising 9(4.5%) males while females had 5(2.5%) positivity. Eleven, 11(5.5%) of the patients had fever onset within 1-7 days of infection. There was a statistical significant association ($P=0.05$) between gender and distribution of CHIKV antibody. Also men were more exposed and had greater chance of contracting CHIKV infection as compared to females despite having higher population of females visiting the health facility (Table.3). This is consistent with the previous reports by Akinola *et al.*, (2017) and Omatola *et al.*, (2020). This gender bias has been previously reported and might be associated with occupational risks engaged by the males, however, the present report disagrees with Ayorinde *et al.* (2015) and the difference might be attributed to the socio-economic factors, cultural habits and behaviors which predisposed males to mosquito bites(.....). Men are usually more industrious and adventurous; they engaged in so many activities in search for food, irrespective of the place and time and may not protect themselves from *Aedes* species bites.

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Table 3: Relationship between the CHIKV seropositivity and participant's variables using rapid diagnostic technique (RDT)

Demographics	Variables	Number tested	Negative (%)	CHIKV-Positive (%)	P-value
Gender	Male	88	79 (39.5)	9 (4.5)	0.05*
	Female	112	107 (53.5)	5 (2.5)	
Age (years)	0 – 10	5	5 (2.5)	0 (0.0)	0.69
	11 – 20	35	33 (16.5)	2 (1.0)	
	21 – 30	43	40 (20.0)	3 (1.5)	
	31 – 40	67	61 (30.5)	6 (3.0)	
	41 – 50	31	29 (14.5)	2 (1.0)	
	≥ 51	19	18 (9.0)	1 (0.5)	
Onset of fever (days)	1 – 7	159	148 (74.0)	11 (5.5)	0.67
	7 – 10	41	38 (19.0)	3 (1.5)	
Treat fever before test	Yes	132	123 (61.5)	9 (4.5)	0.42
	No	68	63 (31.5)	5 (2.5)	
Antimalarial/ Antibiotic Intake	Yes	122	113 (56.5)	9 (4.5)	0.26
	No	78	73 (36.5)	5 (2.5)	
Recent travelling	Yes	44	41 (20.5)	3 (1.5)	0.65
	No	156	145 (72.5)	11 (5.5)	
Total		200	186 (93.0)	14 (7.0)	

*Significant difference, $P=0.05$

3.4 Relationship between the infectivity with CHIKV and participant's variables using enzyme-linked immunosorbent assay (ELISA)

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The relationship and distribution of CHIKV antibodies across the age, gender and clinical information of the study participants was established using enzyme-linked immunosorbent assay (ELISA). Out of the 200 samples tested, 18(9.0%) were positive for the presence of CHIKV antibodies, which are made of 12(6.0%) males and females account for 6(3.0%) in the study population. A significant association was observed ($P=0.05$) in the distribution of CHIKV antibodies in relation to age among patients with febrile illness, age group 31-40 years had the highest sero-positivity of 7(3.5%) antibodies against CHIKV. The distribution of CHIKV IgM antibodies among outpatients with febrile illness with respect to gender tested in the population under study showed no significant association ($P>0.05$). There was a distribution of CHIKV antibodies across most of the age groups, none of the sample from age group 0-10 years was positive for CHIKV. This age group represents a very small minority of the overall population presenting with febrile illness (Table.4).

Table 4: Relationship between the infectivity with CHIKV and participant's variables using enzyme-linked immunosorbent assay (ELISA)

Demographics	Variables	Number tested	Negative (%)	CHIKV-Positive (%)	P-value
Gender	Male	88	76 (38.0)	12 (6.0)	0.28
	Female	112	106 (53.0)	6 (3.0)	
Age (years)	0 – 10	5	5 (2.5)	0 (0.0)	0.03*
	11 – 20	35	32 (16.0)	3 (1.5)	
	21 – 30	43	39 (19.5)	4 (2.0)	
	31 – 40	67	60 (30.0)	7 (3.5)	
	41 – 50	31	29 (14.5)	2 (1.0)	
	≥ 51	19	17 (8.5)	2 (1.0)	
Onset of fever (days)	1 – 7	159	143 (71.5)	16 (8.0)	0.38
	7 – 10	41	39 (19.5)	2 (1.0)	
Treat fever before test	Yes	132	121 (60.5)	11 (5.5)	0.64
	No	68	61 (30.0)	7 (3.5)	
Antimalarial/ Antibiotic Intake	Yes	122	111 (55.5)	11 (5.5)	0.96
	No	78	71 (35.5)	7 (3.5)	
Recent travelling	Yes	44	40 (2.0)	4 (2.0)	0.96
	No	156	142 (71.0)	14 (7.0)	
Total		200	182 (91.0)	18 (9.0)	

*Significant difference, $P=0.03$

This study also highlights the importance of the kinetics of immune response in the choice of a diagnostic test. In this study, IgM antibody which is a hallmark of recent acute CHIKV infections was detected higher 16(8.0%) among samples collected from 1-7 days while only 2(1.0%) was obtained on samples collected from 7-10 days post onset of symptoms. According to [CDC \(2012\)](#), CHIKV-specific IgM is detectable in serum samples for 5 to 7 days after symptoms onset. This study shows that the possibility of detecting CHIKV IgM is higher if samples are collected 1-7 days post onset of symptoms than later (Table 4.). Though the study has limited number of samples, it confirms the circulation of CHIKV infections in Kaltungo town and likely misdiagnosed as malaria or typhoid fever as reported by other studies (Ayorinde *et al.*, 2016)..

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A significant statistical association was observed in the distribution of CHIKV antibodies in relation to the ages of the study participants ($P=0.03$) (Table.4). Active CHIKV infection was found in almost all age groups but more common in age group ... 31-40 years. Lowest CHIKV infection was found in age group ... 41-50 years and in people older than 51 years. zero CHIKV infection was not recorded in age group ... 0-10 years (Table.4).

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Higher rate of CHIKV infectivity was observed among febrile patients aged 31-40 years (3.5%) after which it decreased with increased age. The plausible reason for this is the fact that this 31-40 years old participants, constitute the active work-force which stays more outdoors where they engaged in various occupations or leisure activities. It was observed in this study that the age group 31-40 years was found to be more infected with CHIKV and this is in agreement with Olajiga *et al.*, (2017). This is the most active age group and couple with movement of people outdoors during the daytime when the activity of the *Aedes* mosquitoes is at its peak. Lesser personal protection and individual difference in immune response to diseases are some of the speculative reasons for increased infectivity to CHIKV in this age group.

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A considerable number of patients who had CHIKV infections had treatment with antibiotics and/or anti-malarial medications without a laboratory investigation. This signals evidence of poor health seeking behavior of the patients and possible self-medication on assumption that the febrile illness was caused by either malaria parasite/and or bacteria. Since these medications had no effect on CHIKV, the symptoms would have remitted after it fulfilled its self-limited course. Also, wrong treatment could also lead to drug abuse, complications which may eventually culminate into anti-microbial resistance. So, there was no statistical association between the infectivity with CHIKV and anti-malarial or antibiotic intake ($P>0.05$) (Table.4).

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A relatively high seropositivity of 14(7.0%) and 10(5.5%) was obtained among patients that did not travelled out of the study area as compared to 4(2.0%) and 4(1.5%) using ELISA and RDT respectively, this implies, most likely CHIKV-autochthonous urban transmission cycle.

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The present study was carried out at Kaltungo town and to the best of our knowledge, this is the first scientific work/research to be conducted in the study area on CHIKV infectivity and the results of this study represents the 1st scientific report on the serological evidence of CHIKV infection in Kaltungo town. This serves as a baseline data for further investigations and could aid formulation of prevention and control policies before the disease assumes epidemic proportions.

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The limitation of this study lies in the facts that only symptomatic patients were considered and CHIKV antibodies were tested only from the acute phase of the disease. However, many arbovirus infections are asymptomatic, and high levels of antibodies could potentially be detected in apparently healthy populations. Furthermore, the two (2) diagnostic assays used in this work (RDT and ELISA) test antibodies from acute phase and do revealed the existence and ongoing transmission of this virus.

4.0 CONCLUSION AND RECOMMENDATION

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RECOMMENDATIONS

4.1 Conclusion

In conclusion, This study shows CHIKV evidence of existence with 9.0% IgM seropositivity among outpatients with febrile illness in Kaltungo town. This indicated recent acute CHIKV infection and hence the need to rule out different causes of arboviral infections. This study has clearly revealed the circulation of CHIKV infection in the study area and has been misdiagnosed for malaria and/or typhoid fever, improperly treated and mismanaged. Routine differential diagnosis of febrile illness for CHIKV infection is crucial for proper management/treatment and diagnosis of this viral infection. This study shows that age and gender were the main factors associated with CHIKV seropositivities. Furthermore, the present study was carried out at Kaltungo town and To the best of our knowledge, this result represents the 1st scientific report on the serological evidence of CHIKV infection in Kaltungo town. This will serves as a baseline data for further investigations and could aid in formulation of prevention and control policies before the disease assumes epidemic proportions.

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4.2 Recommendations(s)

Based on the findings of this research work, it is recommended that;

1. There is need for awareness campaigns on CHIKV infections as possible etiological agents of febrile illness; this will reduce over diagnosis of malaria/typhoid and indiscriminate use of anti-malaria/antibiotic drugs.
2. There is need for the inclusion of CHIKV in routine differential diagnosis of febrile conditions to avoid misdiagnosis.
3. Governments should set-up arbovirus surveillance programmes and establish a standard virology laboratory for the diagnosis of viral infections including Chikungunya.
4. Molecular studies should be carried out to determine and characterize the genotypes circulating in the study area.

Abbreviations:

- **CHIK**- Chikungunya
- **CHIKV**- Chikungunya Virus
- **CHIKF**- Chikungunya Fever
- **RRV**- Ross river virus
- **BFV**- Barma Forest Virus
- **IgM**- Immunoglobulin M
- **RDT**- Rapid Diagnostic Test
- **ELISA**- Enzyme-linked Immunosorbent Assay
- **WHO**- World Health Organisation

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[The study participants were 112(56%) females and 88(44%) males. Patients of all age groups were recruited for the study, 159(79.5%) of them had the onset of fever within 1-7 days of infection while 41(20.5%) of them had fever onset within 7-10 days of infection. Among the study subjects, 132(66.0%)

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treated fever before going to the hospital while 68(34.0%) did not. Most of the patients, 122(61.0%) were already on anti-malarial/antibiotic drugs while 78(39.0%) of them were not on medication at the time of sample collection. Less than half of the study participants 44(22.0%) were reported to have travelled out of the study area for more than a week, while most of them 156(78.0%) have not travelled (Table 2).

The relationship between the Chikungunya virus seropositivity and variables of the study participants was established using rapid diagnostic technique (RDT). Among the 200 sera tested for this study, fourteen samples 14(7.0%) were found to be positive for CHIKV infection comprising 9(4.5%) males while females had 5(2.5%) positivity. Eleven, 11(5.5%) of the patients had fever onset within 1-7 days of infection. There was a statistical significant association ($P=0.05$) between gender and distribution of CHIKV antibody (Table 3).

The relationship and distribution of CHIKV antibodies across the age, gender and clinical information of the study participants was established using enzyme-linked immunosorbent assay (ELISA). Out of the 200 samples tested, 18(9.0%) were positive for the presence of CHIKV antibodies, which are made of 12(6.0%) males and females account for 6(3.0%) in the study population. A significant association was observed ($P=0.05$) in the distribution of CHIKV antibodies in relation to age among patients with febrile illness, age group 31-40 years had the highest sero-positivity of 7(3.5%) antibodies against CHIKV. The distribution of CHIKV IgM antibodies among outpatients with febrile illness with respect to gender tested in the population under study showed no significant association ($P>0.05$). There was a distribution of CHIKV antibodies across most of the age groups, none of the sample from age group 0-10 years was positive for CHIKV. This age group represents a very small minority of the overall population presenting with febrile illness (Table.4).

3.2 Discussion:

There are several methods currently employed for the diagnosis of CHIKV infections including tissue culture, RT-PCR and serological tests. Immunofluorescence antibody technique (IF) enables definitive identification of the CHIKV infection. Although virus isolation, is still considered as the "gold standard" for the diagnosis of CHIKV infections, it is time consuming and laborious. Serological techniques are being used exclusively in clinical laboratories. These serological techniques include the plaque reduction neutralization test (PRNT) and haemagglutination inhibition (HI) assay both of which can distinguish between primary and secondary infection. However, these methods are time consuming and in addition, HI requires paired serum samples. Most recently, ELISA methods are commonly used for the detection of IgM and IgG antibodies. These methods have been shown to be useful in the diagnosis of CHIKV infections in the early onset of the disease. The use of rapid diagnostic test was shown to detect the IgM antibodies in acute phase of the CHIKV infections.

The current study was conducted to investigate the evidence of CHIKV infection among outpatients with febrile illness attending Kaltungo General Hospital, Gombe State Nigeria and to identify any risk factor(s) associated with the disease. Two hundred (200) febrile patients were recruited for the study and two (2) serological techniques were employed for the detection of antibodies against chikungunya virus.

Among the analyzed 200 samples, 14(7.0%) were positive by RDT and 18(9.0%) of the patients were tested positive on IgM ELISA (Table 1). This study reveals a low infectivity of 7.0% CHIKV antibodies among patients with febrile illness using RDT. However, this observation contrasted with Omatola *et al.*, (2020) where a seroprevalence of 5.8% was reported using the same technique in Kogi State Nigeria. The difference could be attributed to the sensitivity of the rapid kits used. The ELISA-based preliminary assessment revealed a seropositivity of 9.0% IgM antibodies against CHIKV among the study population, this report contradicted Akinola *et al.*, (2017) where a prevalence of 6.5% CHIKV IgM antibodies was reported. The disparity could be as a result of exposure of the population in question or the time of sample collection. This study has shown the existence and distribution of CHIKV infection among outpatients with febrile illness in Kaltungo town, Gombe State, Nigeria and has clearly confirmed the circulation of the virus among the populace. However, the 9.0% seropositivity of CHIKV infections observed in this study was lower to the reports obtained by both Ayorinde *et al.*, (2015) and Kolawole *et al.*, (2017) where a seroprevalence of 11.0% and 13.0% were reported in Ogun and Ilorin, Kwara States Nigeria. The difference could be attributed to the geographical location or the cross-reactivity of CHIKV

with other alphaviruses that belongs to the Semliki forest antigenic complex like Semliki Forest virus, Ross River virus that cross-react in ELISA test. Furthermore, this report also disagreed with Olajiga *et al.*, (2017) where a seroprevalence of 38% CHIKV IgM antibodies was reported in both Lagos and Osun States Nigeria and the difference might be attributed to the geographical location, socio-economic factors that facilitate the breeding site of *Aedes* vector in their study area.

This study also highlights the importance of the kinetics of immune response in the choice of a diagnostic test. In this study, IgM antibody which is a hallmark of recent acute CHIKV infections was detected higher 16(8.0%) among samples collected from 1-7 days while only 2(1.0%) was obtained on samples collected from 7-10 days post onset of symptoms. According to CDC (2012), CHIKV-specific IgM is detectable in serum samples for 5 to 7 days after symptoms onset. This study shows that the possibility of detecting CHIKV IgM is higher if samples are collected 1-7 days post onset of symptoms than later (Table 4.). Though the study has limited number of samples, it confirms the circulation of CHIKV infections in Kaltungo town and likely misdiagnosed as malaria or typhoid fever as reported by other studies (Ayorinde *et al.*, 2015). Analysis of demographic profile of the participants revealed that the proportion of females 112(56.0%) was significantly higher than that of males 88(44.0%) (Table.2), this is consistent with previous reports that women and girls exhibit greater health-seeking behaviors (Omatola *et al.*, 2020).

In the present study, a statistical association was observed ($P=0.05$) in gender infectivity with CHIKV, males 9(4.5%) were more infected with CHIKV than females 5(2.5%), and also men were more exposed and had greater chance of contracting CHIKV infection as compared to females despite having higher population of females visiting the health facility (Table.3). This is consistent with the previous reports by Akinola *et al.*, (2017) and Omatola *et al.*, (2020). This gender bias has been previously reported and might be associated with occupational risks engaged by the males, however, the present report disagrees with Ayorinde *et al.* (2015) and the difference might be attributed to the socio-economic factors, cultural habits and behaviors which predisposed males to mosquito bites (.....). Men are usually more industrious and adventurous; they engaged in so many activities in search for food, irrespective of the place and time and may not protect themselves from *Aedes* specie bites.

A significant statistical association was observed in the distribution of CHIKV antibodies in relation to the ages of the study participants ($P=0.03$) (Table.4). Active CHIKV infection was found in almost all age groups but more common in age group ... 31-40 years. Lowest CHIKV infection was found in age group ... 41-50 years and in people older than 51 years. zero CHIKV infection was not recorded in age group ... 0-10 years (Table.4).

Higher rate of CHIKV infectivity was observed among febrile patients aged 31-40 years (3.5%) after which it decreased with increased in age. The plausible reason for this is the fact that this 31-40 years old participants, constitute the active work-force which stays more outdoors where they engaged in various occupations or leisure activities. It was observed in this study that the age group 31-40 years was found to be more infected with CHIKV and this is in agreement with Olajiga *et al.*, (2017). This is the most active age group and couple with movement of people outdoors during the daytime when the activity of the *Aedes* mosquitoes is at its peak. Lesser personal protection and individual difference in immune response to diseases are some of the speculative reasons for increased infectivity to CHIKV in this age group.

A considerable number of patients who had CHIKV infections had treatment with antibiotics and/or anti-malarial medications without a laboratory investigation. This signals evidence of poor health seeking behavior of the patients and possible self-medication on assumption that the febrile illness was caused by either malaria parasite/and or bacteria. Since these medications had no effect on CHIKV, the symptoms would have remitted after it fulfilled its self-limited course. Also, wrong treatment could also lead to drug abuse, complications which may eventually culminate into anti-microbial resistance. So, there was no statistical association between the infectivity with CHIKV and anti-malarial or antibiotic intake ($P>0.05$) (Table.4).

A relatively high seropositivity of 14(7.0%) and 10(5.5%) was obtained among patients that did not travelled out of the study area as compared to 4(2.0%) and 4(1.5%) using ELISA and RDT respectively, this implies, most likely CHIKV-autochthonous urban transmission cycle.

The present study was carried out at Kaltungo town and to the best of our knowledge, this is the first scientific work/research to be conducted in the study area on CHIKV infectivity and the results of this study represents the 1st scientific report on the serological evidence of CHIKV infection in Kaltungo town. This serves as a baseline data for further investigations and could aid formulation of prevention and control policies before the disease assumes epidemic proportions.

The limitation of this study lies in the facts that only symptomatic patients were considered and CHIKV antibodies were tested only from the acute phase of the disease. However, many arbovirus infections are asymptomatic, and high levels of antibodies could potentially be detected in apparently healthy populations. Furthermore, the two (2) diagnostic assays used in this work (RDT and ELISA) test antibodies from acute phase and do revealed the existence and ongoing transmission of this virus.

Type of Technique	Frequency (n)	Percentage (%)
RDT		
Negative	186	93.0
Positive	14	7.0
Total	200	100.0
ELISA		
Negative	182	91.0
Positive	18	9.0
Total	200	100.0

Table 1: Prevalence of Chikungunya virus infection using rapid diagnostic technique and enzyme-linked immunosorbent assay

Key: *RDT –Rapid diagnostic technique
 *ELISA –Enzyme-linked immunosorbent assay

Table 2: Demographic and clinical profile of the study participants

Variables	Frequency (n)	Percentage (%)
Gender		
Male	88	44.0
Female	112	56.0
Total	200	100.0
Age (years)		
0 – 10	5	2.5
11 – 20	35	17.5
21 – 30	43	21.5
31 – 40	67	33.5
41 – 50	31	15.5
≥ 51	19	9.5
Total	200	100.0
Onset of fever (days)		
1 – 7	159	79.5

7 – 10	41	20.5
Total	200	100.0
Treat fever before test		
Yes	132	66.0
No	68	34.0
Total	200	100.0
Antimalarial/ Antibiotic Intake		
Yes	122	61.0
No	78	39.0
Total	200	100.0
Recent travelling		
Yes	44	22.0
No	156	78.0
Total	200	100.0

Table 3: Relationship between the CHIKV seropositivity and participant's variables using rapid diagnostic technique (RDT)

Variables	Total No. tested	Negative (%)	CHIKV-Positive (%)	P-value
Gender				
Male	88	79 (39.5)	9 (4.5)	0.05*
Female	112	107 (53.5)	5 (2.5)	
Total	200	186 (93.0)	14 (7.0)	
Age (years)				
0 – 10	5	5 (2.5)	0 (0.0)	0.69
11 – 20	35	33 (16.5)	2 (1.0)	
21 – 30	43	40 (20.0)	3 (1.5)	
31 – 40	67	61 (30.5)	6 (3.0)	
41 – 50	31	29 (14.5)	2 (1.0)	
≥ 51	19	18 (9.0)	1 (0.5)	
Total	200	186 (93.0)	14 (7.0)	
Onset of fever (days)				
1 – 7	159	148 (74.0)	11 (5.5)	0.67
7 – 10	41	38 (19.0)	3 (1.5)	
Total	200	186 (93.0)	14 (7.0)	
Treat fever before test				
Yes	132	123 (61.5)	9 (4.5)	0.42
No	68	63 (31.5)	5 (2.5)	
Total	200	186 (93.0)	14 (7.0)	
Antimalarial/ Antibiotic Intake				
Yes	122	113 (56.5)	9 (4.5)	0.26
No	78	73 (36.5)	5 (2.5)	
Total	200	186 (93.0)	14 (7.0)	
Recent travelling				
Yes	44	41 (20.5)	3 (1.5)	0.65
No	156	145 (72.5)	11 (5.5)	
Total	200	186 (93.0)	14 (7.0)	

*=Significant difference, P=0.05

Table 4: Relationship between the infectivity with CHIKV and participant's variables using enzyme-linked immunosorbent assay (ELISA)

Variables	Total No. tested	Negative (%)	CHIKV-Positive (%)	P-value
Gender				
Male	88	76 (38.0)	12 (6.0)	0.28
Female	112	106 (53.0)	6 (3.0)	
Total	200	182 (91.0)	18 (9.0)	
Age (years)				
0 – 10	5	5 (2.5)	0 (0.0)	0.03*
11 – 20	35	32 (16.0)	3 (1.5)	
21 – 30	43	39 (19.5)	4 (2.0)	
31 – 40	67	60 (30.0)	7 (3.5)	
41 – 50	31	29 (14.5)	2 (1.0)	
≥ 51	19	17 (8.5)	2 (1.0)	
Total	200	182 (91.0)	18 (9.0)	
Onset of fever (days)				
1 – 7	159	143 (71.5)	16 (8.0)	0.38
7 – 10	41	39 (19.5)	2 (1.0)	
Total	200	182 (91.0)	18 (9.0)	
Treat fever before test				
Yes	132	121 (60.5)	11 (5.5)	0.64
No	68	61 (30.0)	7 (3.5)	
Total	200	182 (91.0)	18 (9.0)	
Antimalarial/ Antibiotic Intake				
Yes	122	111 (55.5)	11 (5.5)	0.96
No	78	71 (35.5)	7 (3.5)	
Total	200	182 (91.0)	18 (9.0)	
Recent travelling				
Yes	44	40 (2.0)	4 (2.0)	0.96
No	156	142 (71.0)	14 (7.0)	
Total	200	182 (91.0)	18 (9.0)	

*=Significant difference, $P=0.03$

5.0 CONCLUSION AND RECOMMENDATION

4.1 Conclusion

In conclusion, this study shows CHIKV evidence of existence with 9.0% IgM seropositivity among outpatients with febrile illness in Kaltungo town. This indicated recent acute CHIKV infection and hence the need to rule out different causes of arboviral infections. This study has clearly revealed the circulation of CHIKV infection in the study area and has been misdiagnosed for malaria and/or typhoid fever, improperly treated and mismanaged. Routine differential diagnosis of febrile illness for CHIKV infection is crucial for proper management/treatment and diagnosis of this viral infection. This study shows that age and gender were the main factors associated with CHIKV seropositivities. Furthermore, the present study was carried out at Kaltungo town and to the best of our knowledge, the results of this study represents the 1st scientific report on the serological evidence of CHIKV infection in Kaltungo town. This serves as a baseline data for further investigations and could aid formulation of prevention and control policies before the disease assumes epidemic proportions.

4.2 Recommendations(s)

Based on the findings of this research work, it is recommended that;

5. There is need for awareness campaigns on CHIKV infections as possible etiological agents of febrile illness; this will reduce over diagnosis of malaria/typhoid and indiscriminate use of anti-malaria/antibiotic drugs.
6. There is need for the inclusion of CHIKV in routine differential diagnosis of febrile condition to avoid misdiagnosis.
7. Governments should set-up arbovirus surveillance programs and establish a standard virology laboratory for the diagnosis of viral infections including Chikungunya.
8. Molecular studies should be carried out to determine and characterize the genotypes circulating in the study area.

Abbreviations:

- **CHIK**- Chikungunya
- **CHIKV**- Chikungunya Virus
- **CHIKF**- Chikungunya Fever
- **RRV**- Ross river virus
- **BFV**- Barma Forest Virus
- **IgM**- Immunoglobulin M
- **RDT**- Rapid Diagnostic Test
- **ELISA**- Enzyme-linked Immunosorbent Assay
- **WHO**- World Health Organisation

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