Prevalence of Chikungunya Virus IgG and IgM Antibodies among Febrile Patients Presumptively Diagnosed with Malaria in Ogun State, Nigeria

Idowu FA[1], Enitan SS[1], Iduh MU[2], Makanjuola SO[3], Akele RY[4], Edafetanure-Ibeh OM[5], Mensah-Agyei GO[6], Itodo GE[7], Adetiloro EO[6], Effiong EJ[1].

[1]Department of Medical Laboratory Science, School of Public and Allied Health, Babcock University, Ilishan-Remo, Ogun State, Nigeria.

[2]Department of Medical Microbiology, School of Medical Laboratory Science, Usmanu Danfodiyo University Sokoto, Sokoto State, Nigeria.

[3]Department of Medical Microbiology and Parasitology, Olabisi Onabanjo University, Sagamu, Ogun State, Nigeria.

[4]Department of Biomedical Science, School of Applied Science, University of Brighton, Brighton, United Kingdom.

[5]Department of Environmental and Occupational Health, School of Public Health, Texas A&M University, Texas, United States.

[6]Department of Microbiology, School of Science and Technology, Babcock University, Ilishan-Remo, Ogun State Nigeria.

[7]Department of Microbiology, Federal Teaching Hospital Lokoja, Kogi State, Nigeria.

Corresponding Author
Seyi Samson Enitan
Email: enitans@babcock.edu.ng
ORCID: 0000 0001 5993 7920
Mob. +2348065483761
Postal address :

© 2024 The author (s). Published by Zagazig University. This is an open access article under the CC BY 4.0 license https://creativecommons.org/licenses/by/4.0./

Receive date:1/8/2024
Revise date:5/9/2024
Accept date:9/10/2024
Publish date:14/10/2024
Keywords: Chikungunya Virus, IgG and IgM Antibodies, Febrile Patients, Malaria, Ogun State, Nigeria

Background and study aim: 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. The aim of the study was to determine the prevalence and associated risk factors of CHIKV infections among febrile illness presumptively diagnosed of malaria in Ogun State, Nigeria. **Patients and Methods:** A total of 165 patients (94 females and 71 males) were recruited for the study. The presence of malarial parasite was determined by microscopic examination of blood films. Sera were tested for presence of CHIKV immunoglobin (Ig) IgM and IgG antibodies using CHIKV rapid diagnostic test kit. **Results:** Out of the 165 patients screened, 102 (61.8%) were positive for malarial parasite, 18 (10.9%), 5 (3.0%) and 4 (2.4%) were positive for the presence of CHIKV IgM Ab only, CHIKV IgG Ab only, and both, respectively. A statistically significant association was observed between CHIKV IgM antibody positivity and malaria. Participants who tested positive for CHIKV IgG antibody only were all negative for malaria. Meanwhile, participants positive for both CHIKV IgM and IgG antibodies did not test positive for malaria. Lack of knowledge of CHIKV, presence of mosquitos in the environment, presence of stagnant water sources around, lack of routine medical check-ups and self-medication practices are some of the risk factors identified in this study. **Conclusion:** The findings underscore the importance of considering CHIKV infection alongside malaria in febrile patients, particularly in malaria-endemic regions.

INTRODUCTION

Chikungunya virus (CHIKV), an arthropod-borne virus primarily transmitted by Aedes mosquitoes (first identified in 1952 in the United Republic of Tanzania), has emerged as a significant public health concern in tropical and subtropical regions of the world with over 5 million cases in the past 15 years, though severe illness and fatalities from CHIKV infections remain relatively uncommon [1-6]. Nonhuman primates, small animals, and Aedes mosquitoes play pivotal roles in the enzootic sylvatic transmission cycle of CHIKV, particularly in Africa [7] However; outbreaks of CHIKV can also occur without the involvement of animal reservoirs [8]. In populations lacking pre-existing immunity, such outbreaks can be explosive, with confirmed attack rates reaching up to 70% [9].

The introduction of CHIKV into Asia occurred in the 1950s, sparking outbreaks in Southeast Asia and India. The significant epidemics in India from 2005 to 2006 and in the Indian Ocean islands, such as the Comoros Islands, La Reunion, and Mauritius, resulted from the re-emergence of CHIKV from Africa in 2004 [10]. Concurrently, CHIKV was introduced to temperate regions, leading to autochthonous transmission in France and Italy. In 2013, localized transmission of CHIKV was initially reported in Saint Martin, subsequently spreading to over 40 nations and territories in North, Central, and South America [9]. Several factors, such as increasing urbanization, international travel, and a series of adaptive changes in the virus that enhance transmission by Aedes mosquitoes, likely contribute to the widespread magnitude of CHIKV outbreaks in recent years [4, 7, 9].

The incubation period for chikungunya usually falls within the range of 2 to 4 days (with a span of 1 to 14 days).3% to 28% of individuals infected with CHIKV show no symptoms, those who develop Chikungunya can experience fever, headache, and a skin rash. Additionally, approximately 20-30% of patients suffer from severe, long-lasting joint and muscle pain (polyarthralgia and myalgia) [11, 12], which is why the disease is named Chikungunya, meaning "bending over in pain" in the Makonde dialect of Africa. Over the past sixteen years, the majority of Chikungunya infections have led to symptoms, with more than 85% of individuals who show serologic evidence of infection also reporting the experience of symptoms. Chikungunya virus disease has been linked to a heightened risk of death for up to 84 days following the onset of symptoms, with fatalities including those from cerebrovascular diseases, ischemic heart diseases, diabetes and neurological disorders [11, 13, 14]. There is no specific treatment for Chikungunya, only supportive care, and it can be debilitating and even fatal for newborns. Preventative measures focus on avoiding mosquito bites and eliminating mosquito breeding sites, as there are no specific vaccines or therapeutic drugs currently available [15, 16]. However, several vaccines are in Phase II and III clinical trials. Notably, Valneva's single-dose live-attenuated Ixchiq (VLA1553) vaccine has recently received approval from the Food and Drug Administration (FDA) for use [5, 17]. The clinical presentation of Chikungunya fever can overlap with other febrile illnesses such as malaria, particularly in endemic areas [1,2,9,18]. This overlap complicates the accurate diagnosis and treatment of these infections, highlighting the need for comprehensive epidemiological studies to delineate their prevalence and guide effective healthcare strategies. Ogun State, situated in southwestern Nigeria, is characterized by a tropical climate conducive to the proliferation of Aedes mosquitoes, the primary vectors of CHIKV. The region is also endemic for malaria, predominantly caused by Plasmodium falciparum, which presents with similar clinical features to Chikungunya fever. Consequently, febrile patients in this region are often presumptively diagnosed with malaria based on clinical presentation and limited diagnostic resources. This presumptive diagnosis, while pragmatic in resource-limited settings, risks the misdiagnosis and mistreatment of patients potentially infected with CHIKV, leading to prolonged morbidity and the further spread of the virus [19-23].

Chikungunya and malaria often share common symptoms, including fever, headache, and muscle pain. Relying on clinical symptoms alone for presumptive diagnosis may lead to misdiagnosis. Hence, there is a need for specific and sensitive rapid diagnostic test kits that can accurately identify both malaria and other prevalent febrile illnesses, such as Chikungunya, at the point of care [24]. Serological testing for CHIKV-specific IgG and IgM antibodies provides a valuable tool for distinguishing between current and past infections, offering insight into the true prevalence of the virus among febrile patients [25]. IgM antibodies typically appear within days of infection and indicate recent exposure, while IgG antibodies can persist for years, reflecting previous infection. Understanding the prevalence of these antibodies among febrile patients presumptively diagnosed with malaria can illuminate the burden of CHIKV in Ogun State and inform public health interventions.

There is a potential for super- or co-infection, as Chikungunya and malaria can coexist in regions where both vectors are present, as indicated by studies conducted by Baba et al. [19], Metz et al. [20], Olajiga et al. [21], Fgbami et al. [22], and Ingoba et al. [23]. In Nigeria, diseases

transmitted by mosquitoes, such as dengue/chikungunya and malaria/dengue/chikungunya, are widespread. Research highlights that the occurrence of co-infections involving acute febrile illnesses (AFIs), including chikungunya and malaria, can result in severe illness and mortality, carrying significant implications for public health [19, 26-28]. Previous studies in Nigeria have documented the presence of CHIKV [19, 20, 26-32], but data specific to Ogun State remain sparse. The current lack of information on the percentage occurrence of Chikungunya virus IgG and IgM among febrile patients presumptively diagnosed with malaria in Abeokuta, Ogun State, Nigeria, highlights the necessity for this study. The aim of this study is therefore to assess the prevalence of Chikungunya virus IgG and IgM antibodies and associated risk factors among febrile patients presumptively diagnosed with malaria in Ogun State, Nigeria. The investigation will contribute to a better understanding of the epidemiological landscape of febrile illnesses. This research is critical for optimizing diagnostic protocols, improving patient management, and devising targeted vector control strategies to reduce the transmission of both CHIKV and malaria.

PATIENTS/MATERIALS AND METHODS

Research Design

The chosen research design for this investigation was a cross-sectional epidemiological study.

Study Location

The epidemiological study was carried out at Ijaiye State Hospital, Abeokuta, Ogun State. The participants involved were febrile male and female patients who have received a presumptive diagnosis of malaria at the aforementioned medical facility. Ijaiye State Hospital is a government-owned general hospital situated in Ogun State, Nigeria, with geographical coordinates: 7.1475° N, 3.3619° E.

Study Duration

The research activities lasted over a period of three months (January-March, 2024).

Participant Demographics

This cross-sectional, institution-based study encompassed febrile male and female patients who have been presumptively diagnosed with malaria. The inclusion criteria involved individuals presented with elevated body temperature attending Ijaiye State Hospital, Abeokuta, Ogun State.

Sample Size Determination

The sample size for the study was computed utilizing the formula established by Pourhoseingholi et al. [33]: $N = Z^2 \times P (1- P)/ D^2$

Where: N= minimum sample size required, Z= confidence interval (1.96), P= proportion of the population with Chikungunya virus infection from previous study, D= desired level of significance (0.05)

For the calculation, a 95% confidence interval, a P value of 0.11, i.e., a prevalence rate of 11% from previous study by Ayorinde et al. [26], and margin of error (d) set at 0.05 was utilized. 10% of the sample size was added to reduce errors that could result from likely non- compliance.

$N= Z^2 \times P (1- P)/D^2$

Z= 1.96, P= 11% (Ayorinde et al. [26]), D= 0.05

$$N= \frac{1.962 \times 0.11 (1- 0.11)}{(0.05)^2}$$

$$N= \frac{3.8416 \times 0.11 \times 0.89}{0.0025}$$

$$N= \frac{0.37609264}{0.0025}$$

N=150.4

N= 150

10% 150:10/ 100 X 150 = 15

Sample size is therefore 150 + 15 = 165

Therefore, a total of 165 blood samples were used for this study.

The sampling technique employed systematic random sampling, ensuring a representative selection of participants from the pool of febrile patients at Ijaiye State Hospital. This approach aimed to eliminate biases and enhance the generalizability of the findings.

Sample Size

A total of 165 blood specimens were collected from consenting 165 febrile patients at Ijaiye state hospital, Abeokuta, Ogun State.

Eligibility of Subjects

Inclusion Criteria: All patients, irrespective of age, who provide informed consent and present with symptoms of fever (>38.0°C) was included in the study. These individuals were clinically diagnosed or suspected to have malaria infection while attending Ijaiye State Hospital, Abeokuta, Ogun State, Nigeria. In the case of children, inclusion also required parental consent, and the children presented with fever, clinically diagnosed or suspected to have malaria infection.

Exclusion Criteria: Patients of all ages who have provided consent but do not present with symptoms of fever (>38.0°C) or are not clinically diagnosed or suspected to have malaria infection were excluded from the study. In the case of children, exclusion applied to those whose parents did not provide consent or children without fever or clinical suspicion of malaria infection. These criteria were established to ensure the relevance and accuracy of data collected for the study.

Data Collection

Before specimen collection, detailed demographic and clinical information were gathered from participants using meticulously prepared questionnaires. These questionnaires were assigned unique participant identification numbers (PIDN). The data collection process was spanned at an average of twenty-one (14) days at the study location. This duration encompassed subject selection, questionnaire distribution and retrieval, and sample collection. The pre-test questionnaires were directly administered to participants, consisting of two sections. The first section captured participants' biodata, including age, religion, tribe, educational level, and gender. The second section focused on clinical data, soliciting brief histories of symptoms related to Chikungunya virus fever, such as headache, fever, back pain, rash, nausea, vomiting, conjunctivitis, etc. All completed questionnaires went through meticulous accuracy checks and was securely stored daily. To maintain confidentiality, only the PIDN was recorded on specimen bottles and result sheets.

Measurement of Body Temperature

A battery-powered digital clinical thermometer was utilized to accurately measure the body temperature of the participants.

Venous Blood Collection

Five (5) milliliters of each participant's venous blood was drawn through venous puncture and collected into an EDTA sample bottle labeled with each PIDN.

Sample transportation and Storage

The blood samples was promptly transported to the laboratory unit of the Department of Medical Laboratory Science at Babcock University and analyzed within 2 hours of collection. Each sample was transported expeditiously to the laboratory, ensuring processing on the same day as collection. Specimens from each participant were labeled with their unique identification number on the specimen container. Due to the requirement for immediate processing, specimens was being stored. However, in cases where a delay is anticipated, sera was kept at 2-8℃ for up to 3 days. For extended periods, specimens were stored below -20℃. Frozen samples was fully thawed and thoroughly mixed prior to testing. Repeated freezing and thawing of sera was avoided. Samples displaying turbidity, gross lipemia, or gross hemolysis was not used to prevent interference with result interpretation.

Laboratory Analysis

Detection of Malaria Parasite

The presence of the malaria parasite was determined using the gold standard (Microscopy). Briefly; for each sample, thin and thick blood films were made on a labeled, clean, grease-free slide. The slide was placed horizontally on a staining rack. A small drop of absolute methanol was applied to the thin film, ensuring it does not touch the thick film to prevent lysis of red cells. The thin film was allowed to fix for 1 minute. The thick film was allowed to air dry. Blood films was stained for 10 minutes using 10% Giemsa stain solution. The stain was flushed from the slide using buffered water with a pH of 7.2 to avoid fine deposit coverage on the films. The back of the slide was wiped clean with cotton wool and placed in a draining rack for air-drying preparation. Films were viewed under the microscope using immersion oil and x100 (oil immersion) objective lenses with light microscopy. The diagnosis of malaria was based on the identification of asexual stages of Plasmodium on thick blood smears. Thin blood smears were used to identify Plasmodium

species. If no parasite is seen, blood films were declared negative. Each slide was independently read. In case of discordant results, the slide was examined by two microscopists independently.

Detection of Chikungunya Virus

The detection of Chikungunya Virus IgG and IgM was conducted using the standard Q Chikungunya IgG and IgM rapid antibody test cassette following the manufacturer's instructions.

Principle of the RDT KIT

The standard Q IgG and IgM combo test kit has 'M', 'G' test lines and 'C' control line. Monoclonal anti- human IgM and monoclonal anti- human IgG are immobilized at two individual test lines respectively (M, G line) on the nitrocellulose membrane. The IgM line in the result window is closer to the specimen well and followed by IgG line. Inactivated Chikungunya virus in the antigen pad and monoclonal anti-chikungunya E1- gold in the conjugate pad release by adding buffer and react with anti-Chikungunya IgM or IgG in patient specimen. If human anti-Chikungunya IgM or IgG exist in patient serum, the individual test line appear visible band respectively forming the complex with anti-human IgM/IgG, human IgM/IgG, inactivated Chikungunya virus, and anti-Chikungunya E1- gold, which means a positive test results. The violet line at the control region should always appear if the assay is performed correctly.

Procedure

Briefly; whole blood was centrifuged at 1500g for five (5) minutes to obtain plasma. The test cassette was retrieved from the pack, open it, and place it on a clean workbench. The specimen's unique identification number was labelled accurately on the test cassette. 30 microliters - 45 microliters (1 drop) of plasma was collected and added to the sample well on the cassette. One drop of buffer was added (sample diluent). Test samples were ran alongside external positive and negative controls. The results were read after 15 minutes using a timer.

Interpretation of results: Positive Result

- Presence of 'M' line for IgM confirmed IgM anti-CHIKV in the specimen, along with the 'C' line. The outcome was positive or negative.
- Presence of IgG "G" line confirmed IgG anti-CHIKV in the specimen, along with the C line. The outcome was negative or positive.
- Presence of both "M" and "G" lines confirmed both IgG and IgM anti-CHIKV in the samples. The outcome was positive or negative.

Negative Result

- The presence of only one color band at the control region of the result window indicated a negative result.

Invalid Result

- The assay was inaccurate if no control "C" line develops, irrespective of any pink hue in the test bands. A complete lack of color or the appearance of only one color band suggested a technique error or reagent deterioration. In such cases, the assay was redone using a different kit.

General Precautions

All blood samples were treated as potentially contagious. Protective clothing and disposable gloves were worn when handling blood specimens and kit reagents. The testing procedures followed US-CDC Universal Precautions for preventing the transmission of blood-borne viruses. Used Chikungunya test kits and clinical specimens were discarded as biohazardous waste. Autoclaving at 121°C for 15 minutes at 15psi was performed first, followed by appropriate incineration at the conclusion of the screening process.

Data Analysis

Data from serum antibody testing and questionnaires was entered into Microsoft Excel. Statistical analysis was performed using the SPSS-18.0 statistical tool. One-way analysis of variance (ANOVA) and Turkey-Kramer Multiple Comparisons Test was used to test for significant differences between the sero-positive rates of CHIKV IgG and IgM antibodies, as well as the percentage occurrence of past and present CHIKV infection. Data analysis outputs were presented using tables and charts.

RESULTS

A comprehensive overview of the socio-demographic characteristics of the study participants reveals that the majority of the study participants were female (57.0%) compared to male study participants (43.0%). In terms of age distribution, the largest proportion falls within the 26-33 years age range (43.0%), followed by the 34-41 years (24.8%), 18-25 years 17.6%), >50 years (4.8%) and <18 years (4.8%). Christianity is the predominant religion among the study participants (84.8%), with Islam representing a smaller percentage (14.5%). Yoruba is the most prevalent tribe (83.0%), followed by Igbo (15.8%). Married individuals constitute the majority (55.2%) in terms of marital status. In terms of educational status, study participants with tertiary education are the most prevalent (43.6%), followed by those with no formal education (50.3%). The majority of study participants reside in urban areas (95.8%), and the most common occupation reported is self-employment (44.8%), followed by civil servants (18.8%) and students (18.2%) (table 1).

The prevalence of malaria infection and Chikungunya Virus (CHIKV) antibodies among the study population is presented in Figure 1. The majority of study participants were positive for malaria parasites (102, 61.8%), while 63 (38.2%) tested negative. Regarding CHIKV antibodies, a small proportion of study participants tested positive for CHIKV IgM antibodies only (10.9%), CHIKV IgG antibodies only (3.0%), and both CHIKV IgM and IgG antibodies (2.4%). The prevalence of CHIKV antibodies suggests some exposure to the virus within the population, albeit at a lower rate compared to malaria.

Table 2 presents the association between socio-demographic factors and the malaria status of the participants, focusing on CHIKV (Chikungunya virus) IgM and IgG antibodies. The table displays the frequencies and percentages of participants testing negative and positive for CHIKV IgM antibody only, CHIKV IgG antibody only, and both CHIKV IgM & IgG antibodies, along with the results of the Pearson Chi-Square test assessing the association between each antibody status and malaria. Among participants testing negative for CHIKV IgM antibody only, 30.9% were negative for malaria, while 58.2% were positive. For those testing positive for CHIKV IgM antibody only, 7.3% were negative for malaria, and 3.6% were positive. The Pearson Chi-Square test revealed a statistically significant association between CHIKV IgM antibody status and malaria (χ^2 = 6.945, df = 1, p = 0.008). Participants negative for CHIKV IgG antibody only had 35.2% negativity and 61.8% positivity for malaria. However, among those positive for CHIKV IgG antibody only, 3.0% were negative for malaria, while none were positive. This significant difference was indicated by the Pearson Chi-Square test (χ^2 = 8.348, df = 1, p = 0.004). For participants negative for both CHIKV IgM & IgG antibodies, 35.8% were negative for malaria, and 61.8% were positive. No participant positive for both antibodies tested positive for malaria, and only 2.4% tested negative. This difference was statistically significant (χ^2 = 6.637, df = 1, p = 0.010).

Table 3 shows the patterns of preventive practices associated with the occurrence of Chikungunya virus antibodies across different demographic variables. In terms of gender, the data showcases that among females, 40.0% exhibited poor preventive practices, whereas 17.0% demonstrated good preventive practices. Similarly, among males, 35.2% displayed poor preventive practices, and 7.9% had good preventive practices. Although the Pearson Chi-Square test indicated a slight association between gender and preventive practices, the difference didn't attain statistical significance (χ^2 = 2.853, df = 1, p = 0.091), suggesting that gender might not be a significant determinant of preventive practices within this population. Regarding age range, no notable association was observed between different age groups and preventive practices (χ^2 = 7.254, df = 5, p = 0.202), implying that age might not significantly influence the adoption of preventive measures against Chikungunya virus. The data also explored the relationship between religious affiliation and preventive practices, showing some variation across different religious groups, yet the difference wasn't statistically significant (χ^2 = 2.685, df = 2, p = 0.261). Likewise, no significant association was found between tribe and preventive practices (χ^2 = 1.250, df = 2, p = 0.535).Similarly, while some variance in preventive practices was observed among different marital statuses, the disparity wasn't statistically significant (χ^2 = 4.149, df = 2, p = 0.126). The distribution of preventive practices across different educational levels also didn't

yield statistically significant associations (□² = 4.101, df = 3, p = 0.251). Residential location (urban or rural) showed some difference in preventive practices, but again, this distinction wasn't statistically significant (□² = 2.417, df = 1, p = 0.120). Similarly, no significant association was found between occupation and preventive practices (□² = 5.448, df = 5, p = 0.364). These findings suggest a need for further investigation to understand the underlying factors influencing preventive practices against Chikungunya virus. Table 4 provides an analysis of knowledge and risk factors associated with the occurrence of CHIKV IgM antibody among the study participants. Each variable category is evaluated, presenting frequencies for both negative and positive CHIKV IgM antibody results, along with Pearson Chi-Square statistic (χ^2), degrees of freedom (df), and p-values to assess significance. No significant associations are found between travel history and CHIKV IgM antibody positivity (χ^2 = 0.248, p = 0.619), awareness of the Chikungunya virus (χ^2 = 0.123, p = 0.726), understanding of its transmission (χ^2 = 0.123, p = 0.726), or history of Chikungunya virus infection. Additionally, symptoms related to Chikungunya virus such as eye pain, fever (>40°C), headache, muscle pain, and vomiting do not exhibit significant associations with CHIKV IgM antibody positivity (p > 0.05 for all variables). Similarly, the presence of mosquitoes in the environment does not show a significant association with CHIKV IgM antibody positivity (χ^2 = 0.000, df = 1, p = 1.000). However, a history of mosquito bites exhibits a significant association with CHIKV IgM antibody positivity. The presence of stagnant water sources around individuals also demonstrates a significant association with CHIKV IgM antibody positivity (χ^2 = 0.003, df = 1, p = 0.954), suggesting a potential environmental risk factor. Interestingly, the frequency of medical check-ups/laboratory tests and self-medication practices do not show significant associations with CHIKV IgM antibody positivity (p > 0.05 for both variables).

Table 5 shows the knowledge and risk factors related to the positivity of CHIKV IgG antibody. Each category within the variables is examined, presenting frequencies for both negative and positive CHIKV IgG antibody results. Pearson Chi-Square statistic (χ^2), degrees of freedom (df), and p-values are provided to assess significance. Regarding travel history, there's no significant association found between travel history and CHIKV IgG antibody positivity (χ^2 = 0.063, p = 0.801). Similarly, awareness of the Chikungunya virus, including its transmission by Aedes spp mosquito, does not exhibit a significant relationship with CHIKV IgG antibody positivity (p > 0.05 for all variables). Individuals with a history of Chikungunya virus infection also do not show a significant association with CHIKV IgG antibody positivity. Similarly, the presence of mosquitoes in the environment (χ^2 = 0.103, df = 1, p = 0.748) and history of mosquito bites (χ^2 = 0.379, df = 1, p = 0.538) do not exhibit significant associations with CHIKV IgG antibody positivity. Regarding environmental factors, the presence of stagnant water sources around individuals also does not demonstrate a significant association with CHIKV IgG antibody positivity (χ^2 = 0.379, df = 1, p = 0.538). Furthermore, the frequency of medical check-ups/laboratory tests and self-medication practices do not show significant associations with CHIKV IgG antibody positivity (p > 0.05 for both variables).

Figure 2 shows the symptoms profile observed in suspected cases of Chikungunya virus infection. Among the reported symptoms, fever (>40°C) is prevalent, with 60.6% of respondents experiencing it, followed closely by headache, also reported by 60.6% of respondents. Muscle pain is another common symptom, reported by 36.4% of respondents. Additionally, vomiting is noted in 46.1% of cases. Eye pain, while less common, is still present in 9.1% of cases. Table 6 shows the occurrence of Chikungunya virus) IgM antibodies according to the socio-demographic characteristics of the study participants. The analysis of the data reveals no significant association between gender and CHIKV IgM antibody positivity (χ^2 = 1.293, df = 1, p = 0.255). Similarly, age range does not exhibit a significant association with antibody positivity (χ^2 = 6.098, df = 5, p = 0.297), indicating that age group alone may not be a determining factor for susceptibility to Chikungunya virus. Religious affiliation also does not demonstrate a significant association with antibody positivity (χ^2 = 0.325, df = 2, p = 0.850). Moreover, tribal identity (χ^2 = 0.605, df = 2, p = 0.739), marital status (χ^2 = 1.323, df = 2, p = 0.516), educational status (χ^2 = 2.020, df = 3, p = 0.568), residential location (χ^2 = 2.346, df = 1, p = 0.126), and occupation (χ^2 = 4.813, df = 5, p = 0.439) do not demonstrate significant

associations with CHIKV IgM antibody positivity. These results suggest that none of the socio-demographic factors examined in this study have a statistically significant impact on the occurrence of Chikungunya virus antibodies.

Analysis of Table 7 indicates no significant association between gender and CHIKV IgG antibody positivity (χ^2 = 0.606, df = 1, p = 0.436). Similarly, age range does not show a significant association with antibody positivity (χ^2 = 3.686, df = 5, p = 0.595), indicating that age group alone may not be a determining factor for susceptibility to Chikungunya virus. Religious affiliation also does not demonstrate a significant association with antibody positivity (χ^2 = 2.703, df = 2, p = 0.259). Moreover, tribal identity (χ^2 = 1.054, df = 2, p = 0.590), marital status (χ^2 = 2.780, df = 2, p = 0.249), educational status (χ^2 = 2.816, df = 3, p = 0.421), residential location (χ^2 = 0.228, df = 1, p = 0.633), and occupation (χ^2 = 5.002, df = 5, p = 0.416) do not exhibit significant associations with CHIKV IgG antibody positivity. These results suggest that none of the socio-demographic factors examined in this study have a statistically significant impact on the occurrence of Chikungunya virus IgG antibodies.

Table 8 showing the occurrence of Chikungunya virus) IgM and IgG antibodies according to the socio-demographic characteristics of the study participants.Analysis of the data indicates no significant association between gender and CHIKV IgM and IgG antibody positivity (χ^2 = 0.544, df = 1, p = 0.461). Similarly, age range does not show a significant association with antibody positivity (χ^2 = 5.427, df = 5, p = 0.366), indicating that age group alone may not be a determining factor for susceptibility to Chikungunya virus. Religious affiliation also does not demonstrate a significant association with antibody positivity (χ^2 = 0.732, df = 2, p = 0.693). Moreover, tribal identity (χ^2 = 0.838, df = 2, p = 0.658), marital status (χ^2 = 3.334, df = 2, p = 0.189), educational status (χ^2 = 0.287, df = 3, p = 0.963), residential location (χ^2 = 0.182, df = 1, p = 0.670), and occupation (χ^2 = 3.267, df = 5, p = 0.659) do not exhibit significant associations with CHIKV IgM and IgG antibody positivity. These results suggest that none of the socio-demographic factors examined in this study have a statistically significant impact on the occurrence of Chikungunya virus antibodies.

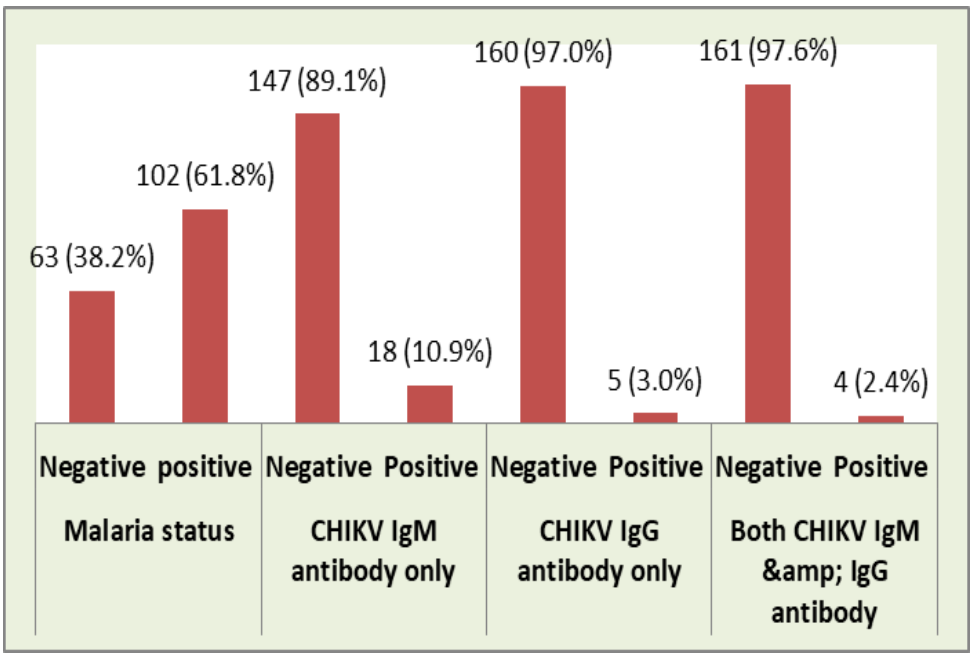

Figure 1: The prevalence of malaria infection and Chikungunya Virus (CHIKV) antibodies among the study participants

Table 1: Demographic Profile and Socioeconomic Characteristics of Study participants

Variable	Categories	Frequency	Percent
Gender	Female	94	57.0
	Male	71	43.0
Age Range	<18 YRS	8	4.8
	>50 YRS	8	4.8
	18-25 YRS	29	17.6
	26-33 YRS	71	43.0
	34-41 YRS	41	24.8
	42-49 YRS	8	4.8
Religion	Christianity	140	84.8
	Islam	24	14.5
	Others	1	0.6
Tribe	Hausa	2	1.2
	Igbo	26	15.8
	Yoruba	137	83.0
Marital Status	Married	91	55.2
	Single	72	43.6
	Widow	2	1.2
Educational Status	None	83	50.3
	Primary	4	2.4
	Secondary	6	3.6
	Tertiary	72	43.6
Residential location	Rural	7	4.2
	Urban	158	95.8
Occupation	Civil servant	31	18.8
	Farmer	2	1.2
	Public servant	9	5.5
	Self employed	74	44.8
	Student	30	18.2
	Unemployed	19	11.5

Table 2: Association between the malaria status and occurrence of Chikungunya virus antibodies among the study participants

Variable	**Categories**	**Malaria status**		**Total** N (%)	**Pearson** Chi-Square ($\square^2$)	**df**	**P-value**
		Negative N (%)	**Positive** N (%)				
CHIKV IgM antibody only	**Negative**	**51(30.9)**	**96(58.2)**	**147(89.1)**	**6.945**	**1**	**0.008**
	Positive	**12(7.3)**	**6(3.6)**	**18(10.9)**			
CHIKV IgG antibody only	**Negative**	**58(35.2)**	**102(61.8)**	**160(97.0)**	**8.348[a]**	**1**	**0.004**
	Positive	**5(3.0)**	**0(0.0)**	**5(3.0)**			
Both CHIKV IgM & IgG antibody	**Negative**	**59(35.8)**	**102(61.8)**	**161(97.6)**	**6.637[a]**	**1**	**0.010**
	Positive	**4(2.4)**	**0(0.0)**	**4(2.4)**			

Table 3: Relationship between socio-demographic and preventive practice associated with occurrence of Chikungunya virus among the study participants

Variable	Categories	Poor practice N (%)	Good practice N (%)	Total N (%)	Pearson Chi-Square (χ^2)	df	P-value
Gender	Female	66(40.0)	28(17.0)	94(57.0)	2.853	1	0.091
	Male	58(35.2)	13(7.9)	71(43.0)			
Age Range	<18 YRS	8(4.8)	0(0.0)	8(4.8)	7.254	5	0.202
	>50 YRS	7(4.2)	1(0.6)	8(4.8)			
	18-25 YRS	23(13.9)	6(3.6)	29(17.6)			
	26-33 YRS	53(32.1)	18(10.9)	71(43.0)			
	34-41 YRS	26(15.8)	15(9.1)	41(24.8)			
	42-49 YRS	7(4.2)	1(0.6)	8(4.8)			
Religion	Christianity	108(65.5)	32(19.4)	140(84.8)	2.685	2	0.261
	Islam	15(9.1)	9(5.5)	24(14.5)			
	others	1(0.6)	0(0.0)	1(0.6)			
Tribe	Hausa	2(1.2)	0(0.0)	2(1.2)	1.250	2	0.535
	Igbo	21(12.7)	5(3.0)	26(15.8)			
	Yoruba	101(61.2)	36(21.8)	137(83.0)			
Marital Status	Married	63(38.2)	28(17.0)	91(55.2)	4.149	2	0.126
	Single	59(35.8)	13(7.9)	72(43.6)			
	widow	2(1.2)	0(0.0)	2(1.2)			
Educational Status	None	59(35.8)	24(14.5)	83(50.3)	4.101	3	0.251
	Primary	4(2.4)	0(0.0)	4(2.4)			
	Secondary	6(3.6)	0(0.0)	6(3.6)			
	Tertiary	55(33.3)	17(	72(43.6)			
Residential location	Rural	7(4.2)	0(0.0)	7(4.2)	2.417	1	0.120
	Urban	117(70.9)	41(24.8)	158(95.8)			
Occupation	Civil servant	25(15.2)	6(3.6)	31(18.8)	5.448	5	0.364
	farmer	2(1.2)	0(0.0)	2(1.2)			
	Public servant	8(4.8)	1(0.6)	9(5.5)			
	Self employed	50(30.3)	24(14.5)	74(44.8)			
	Student	25(15.2)	5(3.0)	30(18.2)			
	Unemployed	14(8.5)	5(3.0)	19(11.5)			

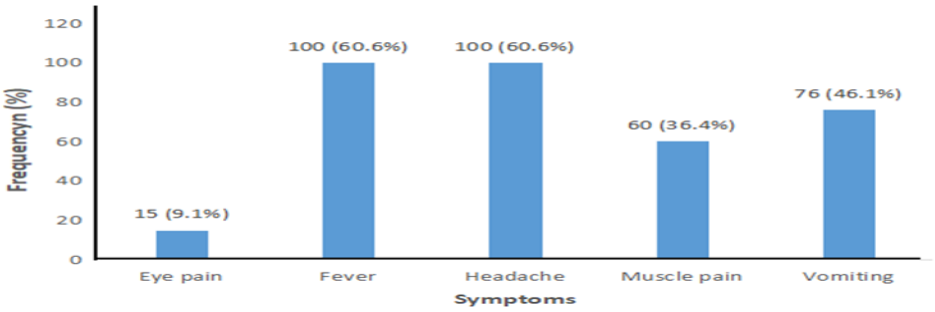

Figure 2: Symptoms profile observed in suspected cases of Chikungunya virus

Table 4: Knowledge and risk factors associated with occurrence of CHIKV IgM antibody among the study participants

Enquires	Categories	CHIKV IgM antibody		Pearson Chi-Square (χ^2)	df	P-value
		Negative	Positive			
Have you heard of Chikungunya virus?	No	146(88.5%)	18(10.9%)	.123[a]	1	0.726
	Yes	1(0.6%)	0(0.0%)			
Are you aware that Chikungunya Virus is transmitted by Aedes spp mosquito?	No	147(89.1%)	18(10.9%)			
What is the mode of transmission of Chikungunya Virus?	Mosquito bite	1(0.6%)	0(0.0%)	.123[a]	1	0.726
	No idea	146(88.5%)	18(10.9%)			
Travel history	Ever lived abroad	2(1.2%)	0(0.0%)	.248[a]	1	0.619
	Never lived abroad	145(87.9%)	18(10.9%)			
Do you have any history of Chikungunya Virus?	No	147(89.1%)	18(10.9%)			
Do you have mosquitoes in your environment?	No	49(29.7%)	6(3.6%)	.000[a]	1	1.000
	Yes	98(59.4%)	12(7.3%)			
Have you ever been bitten by mosquito before?	Yes	147(89.1%)	18(10.9%)			
Any source of stagnant water around you?	No	99(60.0%)	12(7.3%)	.003[a]	1	0.954
	Yes	48(29.1%)	6(3.6%)			
How often do you go for medical check-up/laboratory test?	Less often	30(18.2%)	1(0.6%)	4.703[a]	3	0.195
	Much often	8(4.8%)	0(0.0%)			
	Never	104(63.0%)	17(10.3%)			
	Often	5(3.0%)	0(0.0%)			
How often do you practice self-medication?	Less often	29(17.6%)	4(2.4%)	.352[a]	2	0.839
	Much often	68(41.2%)	7(4.2%)			
	Never	50(30.3%)	7(4.2%)			

Table 5: Knowledge and risk factors associated with occurrence of CHIKV IgG antibody among the study participants

Enquires	Categories	CHIKV IgG antibody		Pearson Chi-Square (χ^2)	Df	P-value
		Negative N (%)	Positive N (%)			
Have you heard of Chikungunya virus?	No	159	5	.031[a]	1	0.859
	Yes	1	0			
Are you aware that Chikungunya Virus is transmitted by Aedes spp mosquito?	No	160	5			
	Yes	0	0			
What is the mode of transmission of Chikungunya Virus?	Mosquito bite	1	0	.031[a]	1	0.859
	No idea	159	5			
Travel history	Ever lived abroad	2	0	.063[a]	1	0.801
	Never lived abroad	158	5			
Do you have any history of Chikungunya Virus?	No	160	5			
	Yes	0	0			
Do you have mosquitoes in your environment?	No	53	2	.103[a]	1	0.748
	Yes	107	3			
Have you ever been bitten by mosquito before?	Yes	160	5			
	No	0	0			
Any source of stagnant water around you?	No	107	4	.379[a]	1	0.538
	Yes	53	1			
How often do you go for medical check-up/laboratory test?	Less often	31	0	3.598[a]	3	0.308
	Much often	7	1			
	Never	117	4			
	Often	5	0			
How often do you practice self-medication?	Less often	32	1	.556[a]	2	0.757
	Much often	72	3			
	Never	56	1			

Table 6: The occurrence of Chikungunya virus IgM antibodies according to the socio-demographic characteristics of the study participants

Variables	Category	CHIKV IgM antibody		Total	Pearson Chi-Square (□2)	df	P-value
		Negative	Positive				
Gender	Female	86(52.1%)	8(4.8%)	94(57.0%)	1.293a	1	0.255
	Male	61(37.0%)	10(6.1%)	71(43.0%)			
Age Range	<18 YRS	8(4.8%)	0(0.0%)	8(4.8%)	6.098a	5	0.297
	>50 YRS	8(4.8%)	0(0.0%)	8(4.8%)			
	18-25 YRS	27(16.4%)	2(1.2%)	29(17.6%)			
	26-33 YRS	59(35.8%)	12(7.3%)	71(43.0%)			
	34-41 YRS	37(22.4%)	4(2.4%)	41(24.8%)			
	42-49 YRS	8(4.8%)	0(0.0%)	8(4.8%)			

Religion	Christianity	124(75.2%)	16(9.7%)	140(84.8%)	.325a	2	0.850
	Islam	22(13.3%)	2(1.2%)	24(14.5%)			
	others	1(0.6%)	0(0.0%)	1(0.6%)			
Tribe	Hausa	2(1.2%)	0(0.0%)	2(1.2%)	.605a	2	0.739
	Igbo	24(14.5%)	2(1.2%)	26(15.8%)			
	Yoruba	121(73.3%)	16(9.7%)	137(			
Marital Status	Married	83(50.3%)	8(4.8%)	91(55.2%)	1.323a	2	0.516
	Single	62(37.6%)	10(6.1%)	72(43.6%)			
	widow	2(1.2%)	0(0.0%)	2(1.2%)			
Educational Status	None	75(45.5%)	8(4.8%)	83(50.3%)	2.020a	3	0.568
	Primary	4(2.4%)	0(0.0%)	4(2.4%)			
	Secondary	6(3.6%)	0(0.0%)	6(3.6%)			
	Tertiary	62(37.6%)	10(6.1%)	72(43.6%)			
Residential location	Rural	5(3.0%)	2(1.2%)	7(4.2%)	2.346a	1	0.126
	Urban	142(86.1%)	16(9.7%)	158(95.8%)			
Occupation	Civil servant	26(15.8%)	5(3.0%)	31(18.8%)	4.813a	5	0.439
	farmer	2(1.2%)	0(0.0%)	2(1.2%)			
	Public servant	7(4.2%)	2(1.2%)	9(5.5%)			
	Self employed	65(39.4%)	9(5.5%)	74(44.8%)			
	Student	29(17.6%)	1(0.6%)	30(18.2%)			
	Unemployed	18(10.9%)	1(0.6%)	19(11.5%)			

Table 7: Theoccurrence of Chikungunya virus IgG antibodies according to the socio-demographic characteristics of the study participants

Variables	**Category**	**CHIKV IgG antibody only**		**Total**	**Pearson Chi-Square (□2)**	**df**	**P-value**
		Negative	**Positive**				
Gender	Female	92(55.8%)	2(1.2%)	94(57.0%)	.606a	1	0.436
	Male	68(41.2%)	3(1.8%)	71(43.0%)			
Age Range	<18 YRS	8(4.8%)	0(0.0%)	8(4.8%)	3.686a	5	0.595
	>50 YRS	8(4.8%)	0(0.0%)	8(4.8%)			
	18-25 YRS	28(17.0%)	1(0.6%)	29(17.6%)			
	26-33 YRS	67(40.6%)	4(2.4%)	71(43.0%)			
	34-41 YRS	41(24.8%)	0(0.0%)	41(24.8%)			
	42-49 YRS	8(4.8%)	0(0.0%)	8(4.8%)			
Religion	Christianity	137(83.0%)	3(1.8%)	140(84.8%)	2.703a	2	0.259
	Islam	22(13.3%)	2(1.2%)	24(14.5%)			
	others	1(0.6%)	0(0.0%)	1(0.6%)			
Tribe	Hausa	2(1.2%)	0(0.0%)	2(1.2%)	1.054a	2	0.590

	Igbo	26(15.8%)	0(0.0%)	26(15.8%)			
	Yoruba	132(80.0%)	5(3.0%)	137(83.0%)			
Marital Status	Married	90(54.5%)	1(0.6%)	91(55.2%)	2.780a	2	0.249
	Single	68(41.2%)	4(2.4%)	72(43.6%)			
	widow	2(1.2%)	0(0.0%)	2(1.2%)			
Educational Status	None	82(49.7%)	1(0.6%)	83(50.3%)	2.816a	3	0.421
	Primary	4(2.4%)	0(0.0%)	4(2.4%)			
	Secondary	6(3.6%)	0(0.0%)	6(3.6%)			
	Tertiary	68(41.2%)	4(2.4%)	72(43.6%)			
Residential location	Rural	7(4.2%)	0(0.0%)	7(4.2%)	.228a	1	0.633
	Urban	153(92.7%)	5(3.0%)	158(95.8%)			
Occupation	Civil servant	31(18.8%)	0(0.0%)	31(18.8%)	5.002a	5	0.416
	farmer	2(1.2%)	0(0.0%)	2(1.2%)			
	Public servant	8(4.8%)	1(0.6%)	9(5.5%)			
	Self employed	72(43.6%)	2(1.2%)	74(44.8%)			
	Student	28(17.0%)	2(1.2%)	30(18.2%)			
	Unemployed	19(11.5%)	0(0.0%)	19(11.5%)			

Table 8: The co-occurrence of Chikungunya virus IgM and IgG antibodies according to the socio-demographic characteristics of the study participants

Variables	**Category**	**CHIKV IgM & IgG antibody**		**Total**	**Pearson Chi-Square (□2)**	**df**	**P-value**
		Negative	**Positive**				
Gender	Female	91(55.2%)	3(1.8%)	94(57.0%)	.544a	1	0.461
	Male	70(42.4%)	1(0.6%)	71(43.0%)			
Age Range	<18 YRS	8(4.8%)	0(0.0%)	8(4.8%)	5.427a	5	0.366
	>50 YRS	8(4.8%)	0(0.0%)	8(4.8%)			
	18-25 YRS	29(17.6%)	0(0.0%)	29(17.6%)			
	26-33 YRS	67(40.6%)	4(2.4%)	71(43.0%)			
	34-41 YRS	41(24.8%)	0(0.0%)	41(24.8%)			
	42-49 YRS	8(4.8%)	0(0.0%)	8(4.8%)			
Religion	Christianity	136(82.4%)	4(2.4%)	140(84.8%)	.732a	2	0.693
	Islam	24(14.5%)	0(0.0%)	24(14.5%)			
	others	1(0.6%)	0(0.0%)	1(0.6%)			
Tribe	Hausa	2(1.2%)	0(0.0%)	2(1.2%)	.838a	2	0.658
	Igbo	26(15.8%)	0(0.0%)	26(15.8%)			
	Yoruba	133(80.6%)	4(2.4%)	137(83.0%)			
Marital Status	Married	87(52.7%)	4(2.4%)	91(55.2%)	3.334a	2	0.189

	Single	72(43.6%)	0(0.0%)	72(43.6%)			
	widow	2(1.2%)	0(0.0%)	2(1.2%)			
Educational Status	None	81(49.1%)	2(1.2%)	83(50.3%)	.287a	3	0.963
	Primary	4(2.4%)	0(0.0%)	4(2.4%)			
	Secondary	6(3.6%)	0(0.0%)	6(3.6%)			
	Tertiary	70(42.4%)	2(1.2%)	72(43.6%)			
Residential location	Rural	7(4.2%)	0(0.0%)	7(4.2%)	.182a	1	0.670
	Urban	154(93.3%)	4(2.4%)	158(95.8%)			
Occupation	Civil servant	31(18.8%)	0(0.0%)	31(18.8%)	3.267a	5	0.659
	farmer	2(1.2%)	0(0.0%)	2(1.2%)			
	Public servant	9(5.5%)	0(0.0%)	9(5.5%)			
	Self employed	71(43.0%)	3(1.8%)	74(44.8%)			
	Student	30(18.2%)	0(0.0%)	30(18.2%)			
	Unemployed	18(10.9%)	1(0.6%)	19(11.5%)			

DISCUSSION

In malaria-endemic regions like Ogun State, Nigeria, febrile illnesses pose a significant health burden, with malaria being a primary concern. However, the co-circulation of other pathogens, such as Chikungunya virus (CHIKV), complicates febrile illness diagnoses. Chikungunya is specifically a tropical disease that is relatively uncommon and poorly documented [34]. 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. The virus has increasingly great impact on man with potentially life-threatening and debilitating arthritis [35]. Understanding the epidemiological dynamics of CHIKV alongside malaria is crucial for effective disease management. This study determines the prevalence of Chikungunya virus IgG and IgM antibodies among febrile patients presumptively diagnosed of malaria in Ogun State, Nigeria.

A total of 165 samples were screened using rapid diagnostic test kits in this study. Out of the 165 participants examined, 18 (10.9%) were positive for Chikungunya virus IgM antibody only, 5 (3.0%) were positive for Chikungunya virus IgG antibodies only, while 4 (2.4%) were positive for both Chikungunya virus IgG and IgM antibodies. The 3.0% prevalence of IgG CHIKV recorded in this research is similar to the work of Olajiga et al. [21], who reported a prevalence of 3.5% in a study conducted in Karagwe district, Tanzania. On one hand, the prevalence of IgG CHIKV recorded in this study was found to be lower than that of previous studies for instance, Akinola et al. [36], reported 10.5%, while Adusei et al. [30] reported 4.2%. Also, a more recent study carried out by Inziani et al. [37] in Teso South Sub County, Western Kenya, reported a prevalence of 5.6%.

On the other hand, the prevalence of IgG CHIKV recorded in this study was higher than that of previous studies [21, 38]. The difference in prevalence might be due to differences in sample sizes, study duration, diagnostic technique, geographical location, socio-economic status, cultural or environmental status. The prevalence of IgM CHIKV recorded in this study (10.9%) was higher than that of Adusei et al. [30] who reported a prevalence rate of 1.8% among febrile patients suspected of having malaria, while Akintola et al. [36] reported a prevalence rate of 4.1%. Also, the IgM CHIKV prevalence recorded in this study was lower than previous studies, For instance, Adusei et al. [30] (2021) reported 1.8%; while Olajiga et al. [21] reported 3.0% in Osogbo, Osun State, Nigeria. As previously stated, the variations in prevalence rates could be attributed to differences in the geographical location, sample size or study duration. It could also be due to lack of exposure of the participants to the virus.

The prevalence of co-occurrences of IgG and IgM CHIKV observed in this study was 2.45%.,

which was found to be lower than that of some previous studies like Olajiga et al. [21] and Akinola et al. [36]. On the other hand, it was higher than of Adusei et al. [30]. The variations in prevalence rates could be attributed to study duration, sample size, period of study. It could also be attributed to abundance of Aedes mosquito in the study area. Some participants were co-infected with malaria and CHIKV infection. Both are mosquito borne infections and not mutually exclusive. This is why it is very difficult to distinguish clinically between malaria and arboviral infections, leading to misdiagnoses. The results of this study emphasize the importance of considering multiple infectious etiologies in febrile illness diagnoses, especially in regions where both malaria and arboviruses like CHIKV are endemic .

The significant association observed between CHIKV IgM antibody positivity and malaria suggests a potential overlap in clinical presentation or shared epidemiological factors between the two diseases [23, 39]. It is notable that a substantial proportion of participants testing positive for CHIKV IgM antibody only were also positive for malaria. This highlights the necessity for healthcare practitioners to consider CHIKV as a differential diagnosis in febrile patients presumptively diagnosed with malaria, particularly in areas where both diseases are prevalent.

Similarly, the presence of CHIKV IgG antibodies among febrile patients exhibited an intriguing pattern. Notably, participants testing positive for CHIKV IgG antibody only were all negative for malaria, suggesting a potential protective role of previous CHIKV infection against malaria. However, further studies are warranted to elucidate the underlying mechanisms responsible for this observation.

Moreover, the absence of malaria positivity among participants positive for both CHIKV IgM and IgG antibodies raises questions regarding the potential interference of CHIKV infection or immunity with malaria infection. This finding underscores the complexity of immune responses and cross-reactivities among different pathogens, warranting comprehensive investigations into the immunological interactions between CHIKV and malaria.

With regard to the clinical presentation of the study participants, fever, headache, vomiting and muscle pain are the predominant acute symptoms associated with CHIKV in this study. Similar symptoms have been reported by Omatola et al. [28] and Danis-Lozano et al. [40] among people living with CHIKV. Lack of knowledge of CHIKV, presence of mosquitos in the environment, presence of stagnant water sources around, low frequency of medical check-ups and self-medication practices are some of the risk factors identified in this study. However, their association with CHIKV IgG/IgM antibodies are not significant. A recent study by Inziani et al. [37] indicated that design of house, type of mosquito nets used and other mosquito control methods were some of the risk factors associated with the infection.

CONCLUSION:

The Chikungunya virus (CHIKV) could be an under-recognized cause of febrile illness in Ogun State, Nigeria, where malaria is often the presumptive diagnosis. The febrile patients may be misdiagnosed and treated for malaria, overlooking potential CHIKV infections. Therefore, integrating CHIKV testing into the diagnostic process for febrile illnesses is essential to avoid misdiagnosis and improve patient outcomes.

Ethical Consideration

Prior to the commencement of the study, ethical clearance was obtained from the Babcock University Health Research Ethic Committee (BUHREC), with ethical approval registration number: BUHREC 903/23.

Competing Interests

The authors declare no competing interests.

Data Availability

Data supporting the findings of this study are available on reasonable request from the corresponding author [Seyi Samson Enitan], exclusively for non-commercial use and under a Data Usage Agreement.

Conflict of Interest

There is no conflict of interest reported by the authors.

Funding

None

Author contribution

Study concept and design: FAI & SSE; Literature search: FAI, SSE, MUI, SOM, RYA, OME, GOM, GEI, EOA & EJE; Acquisition of

data: FAI, SSE, MUI & SOM; Analysis and interpretation of data: POA, SSE, MOD &, ARA; Statistical analysis: RYA, OME & GOM; Study supervision: SSE & SOM; Drafting of the manuscript: FAI, SSE, MUI, SOM, RYA & OME; Critical revision of the manuscript for important intellectual content: SSE, MUI, SOM, RYA, GEI & EJE; Final approval of the manuscript: FAI, SSE, MUI, SOM, RYA, OME, GOM, GEI, EOA & EJE.

HIGHLIGHTS:

- Chikungunya virus (CHIKV) and malaria are prevalent in tropical regions, including Ogun State, Nigeria, where both can present with similar febrile symptoms. Differentiating between these infections is crucial for appropriate treatment and management.
- The presence of CHIKV antibodies will clarify the burden of co-infection and inform diagnostic and therapeutic strategies.

Limitations of the study

- The study relies on serological assays for the detection of CHIKV antibodies, which may have limitations such as potential cross-reactivity with other related viruses. While serological testing is commonly used in epidemiological studies, molecular techniques could provide more specific and sensitive detection of CHIKV infection.
- The findings of the study may have limited generalizability beyond the study population in Ogun State, Nigeria. Factors such as local variations in CHIKV and malaria prevalence, as well as healthcare infrastructure, could influence the applicability of the results to other settings.
- The study may not have accounted for all potential confounding factors that could influence the association between CHIKV antibodies and malaria. Variables such as socioeconomic status, travel history, and concurrent infections could impact the outcomes but were not fully explored in the analysis.

Recommendations for future study

Based on the limitations identified in the study, the following recommendations are suggested to address these issues and enhance the validity and generalizability of future research:

- Future researchers should conduct longitudinal studies to establish temporal relationships between CHIKV antibodies and malaria. Longitudinal designs allow for the assessment of changes in antibody status over time and provide insights into the dynamics of infection and immunity.
- Where funding is available, more sensitive methods like polymerase chain reaction (PCR) and enzyme linked immunosorbent assay (ELISA) should be used to determine the prevalence of Chikungunya virus among the study population. Molecular methods like PCR offer greater specificity and sensitivity, minimizing the potential for cross-reactivity and providing more accurate diagnosis.
- Consider employing controlled study designs, such as case-control or cohort studies, to control for potential confounding factors and improve causal inference. Matching cases and controls on relevant variables and adjusting for confounders in the analysis can enhance the reliability of the findings.
- Expand the study to include diverse geographic locations and populations to improve the generalizability of the findings. Investigating CHIKV and malaria co-infection in different epidemiological settings can provide insights into regional variations and facilitate broader applicability of the results.
- Collect comprehensive data on potential confounding factors, including socioeconomic status, travel history, and concurrent infections, to better understand their influence on the association between CHIKV antibodies and malaria. Integrating multivariable analyses can help identify and account for these confounders.

List of Abbreviations

Aedes spp - Aedes species
AFIs - Acute febrile illnesses
BUHREC - Babcock University Health Research Ethics Committee
CHIKV - Chikungunya virus
df - Degrees of Freedom
EDTA - Ethylenediaminetetraacetic acid
Ig - Immunoglobulin
IgG - Immunoglobulin G
IgM - Immunoglobulin M
IRB - Institutional Review Board
p - p-value
PIDN - Participant Identification Number
RDT - Rapid Diagnostic Test
SPSS - Statistical Package for the Social Sciences
US-CDC - United States Centers for Disease Control and Prevention
χ^2 - Chi-Square Statistic

REFERENCES

1. Simon F, Caumes E, Jelinek T, Lopez-Velez R, Steffen R, Chen LH. Chikungunya: risks for travellers. *Journal of Travel Medicine*. 2023, Mar; 30(2): taad008. https://doi.org/10.1093/jtm/taad008
2. Mourad O, Makhani L, Chen LH. Chikungunya: An Emerging Public Health Concern. *Curr Infect Dis Rep*. 2022; 24 (12):217-228. https://doi.org/10.1007/s11908-022-00789-y
3. Adu EA, John JH, Alani H, Wiredu AP, Boakye AO, Adobasom-Anane AG, et al. (Sero) Prevalence of (Re) Emerging Major Arbovirus Infections in Africa: A Systematic Review and Meta-Analysis Protocol. https://doi.org/10.21203/rs.3.rs-3906474/v1
4. Athukorala N, Wickramasinghe S, Yatawara L, Weerakoon K. A Systematic Review of Zoonotic Disease Prevalence in Sri Lanka (2000–2022). *Vector-Borne and Zoonotic Diseases.* 2024 May 22. https://doi.org/10.1089/vbz.2023.014
5. Ly H. Ixchiq (VLA1553): The first FDA-approved vaccine to prevent disease caused by Chikungunya virus infection. *Virulence.* 2024 Dec 31;15(1):2301573. https://doi.org/10.1080/21505594.2023.2301573
6. Krambrich J, Mihalič F, Gaunt MW, Bohlin J, Hesson JC, Lundkvist Å. et al. The evolutionary and molecular history of a chikungunya virus outbreak lineage. *PLOS Neglected Tropical Diseases*. 2024 Jul 26;18(7):e0012349. https://doi.org/10.1371/journal.pntd.0012349
7. Ramphal Y, Tegally H, San JE, Reichmuth ML, Hofstra M, Wilkinson E. et al. CLIMADE Consortium, de Oliveira T, Moir M. Understanding the Transmission Dynamics of the Chikungunya Virus in Africa. *Pathogens.* 2024 Jul 22;13(7):605. https://doi.org/10.3390/pathogens13070605
8. Msellemu D, Tanner M, Yadav R, Moore SJ. Occupational exposure to malaria, leishmaniasis and arbovirus vectors in endemic regions: A systematic review. *Current Research in Parasitology & Vector-Borne Diseases*. 2024 Jun 1:100185. https://doi.org/10.1016/j.crpvbd.2024.100185
9. Yactayo, S.; Staples,J.E.; Millot, V.; Cibrelus, L. Ramon-Pardo, P. Epidemiology of Chikungunya in Americas. *J. Infect. Dis*, 2016; 214, S441–S445. https://doi.org/10.1093/infdis/jiw390
10. Weaver SC, Lecuit M. Chikungunya virus infections, *NEngl J Med*, 2015; 373(1): 94-95. https://doi.org/10.1056/NEJMc150550
11. de Souza WM, Fumagalli MJ, de Lima ST, Parise PL, Carvalho DC, Hernandez C. et al. Pathophysiology of chikungunya virus infection associated with fatal outcomes. *Cell Host & Microbe*. 2024 Apr 10;32(4):606-22. https://doi.org/10.1016/j.chom.2024.02.011
12. Sagar R, Raghavendhar S, Jain V, Khan N, Chandele A, Patel AK. et al. Viremia and clinical manifestations in acute febrile patients of Chikungunya infection during the 2016 CHIKV outbreak in Delhi, India. *Infectious Medicine.* 2024 Mar 1;3(1):100088. https://doi.org/10.1016/j.imj.2024.100088
13. Cerqueira-Silva T, Pescarini JM, Cardim LL, Leyrat C, Whitaker H, de Brito CA. et al. Risk of death following chikungunya virus disease in the 100 Million Brazilian Cohort, 2015–18: a matched cohort study and self-controlled case series. The Lancet Infectious Diseases. 2024 May 1; 24(5):504-13. https://doi.org/10.1016/S1473-3099(23)00739-9
14. da Costa VG, Saivish MV, Sinhorini PF, Nogueira ML, Rahal P. A meta-analysis of Chikungunya virus in neurological disorders. *Infectious Diseases Now*. 2024 Jun 15:104938. https://doi.org/10.1016/j.idnow.2024.104938

15. Ghildiyal R, Gabrani R. Antiviral therapeutics for chikungunya virus. *Expert opinion on therapeutic patents.* 2020 Jun 2;30(6):467-480. https://doi.org/10.1080/13543776.2020.1751817

16. Shaikh MS, Faiyazuddin M, Khan MS, Pathan SK, Syed IJ, Gholap AD. et. al. Chikungunya virus vaccine: a decade of progress solving epidemiological dilemma, emerging concepts, and immunological interventions. *Frontiers in Microbiology*. 2024 Jul 22;15:1413250. https://doi.org/10.3389/fmicb.2024.1413250

17. Lisa F.P., Laurent-Rénia Ng. Live-attenuated chikungunya virus vaccine. 2024; 87(4): 813-813.E1. https://doi.org/10.1016/j.cell.2024.01.033

18. Weaver SC. Arrival of chikungunya virus in the new world: prospects for spread and impact on public health. *PLoS neglected tropical diseases.* 2014 Jun 26;8(6):e2921. https://doi.org/10.1371/journal.pntd.0002921

19. Baba, M.; Logue, C.H.; Oderinde, B.; Abdulmaleek, H.; Williams, J.; Lewis, J. et al. Evidence of arbovirus coinfection in suspected febrile malaria and typhoid patients in Nigeria. J. Infect. Dev. *Ctries.* 2013; 7, 51–59. https://doi.org/10.3855/jidc.2411

20. Metz SW, Gardner J, Geertsema C, Le TT, Goh L, Vlak JM, et al. Effective chikungunya virus-like particle vaccine produced in insect cells. *PLoS neglected tropical diseases.* 2013 Mar 14;7(3):e2124. https://doi.org/10.1371/journal.pntd.0002124

21. Olajiga OM, Adesoye OE, Emilolorun AP, Adeyemi AJ, Adeyefa EO, Aderibigbe IA, et al. Chikungunya virus seroprevalence and associated factors among hospital attendees in two states of Southwest Nigeria: a preliminary assessment. *Immunological Investigations.* 2017 Aug 18;46(6):552-565. https://doi.org/10.1080/08820139.2017.1319383

22. Fagbami AH, Onoja AB. Dengue haemorrhagic fever: An emerging disease in Nigeria, West Africa. *Journal of infection and public health*. 2018 Nov 1; 11(6):757-762. https://doi.org/10.1016/j.jiph.2018.04.014

23. Ingoba, L.L.; Adedoja, A.; Peko, S.M.; Vairo, F.; Haider, N.; Kock, R. et al. Diagnosis of Chikungunya Virus in Febrile Patients from a Malaria Holoendemic Area. *Int. J. Infect*. Dis. 2021; 109, 247–252. https://doi.org/10.1016/j.ijid.2021.06.043

24. Bhardwaj, P., Sah, K., Yadav, V., Gulafshan, S., Dhangur, P., Srivastava, U. et al.. Molecular and serological evidence of chikungunya virus infection with high case fatality among pediatric population with acute encephalitis syndrome: first report from Eastern Uttar Pradesh, India. *European Journal of Clinical Microbiology & Infectious Diseases*, 2024; *43*(6), pp.1205-1212. https://doi.org/10.1007/s10096-024-04817-8

25. Okonko IO, Innocent-Adiele HC, Njoku OV, Eugene EA. First serological prevalence of dengue virus IgG antibody among pregnant women in Port Harcourt, Nigeria. *Scientia Africana.* 2023 Oct 10; 22(2):239-48. https://doi.org/10.4314/sa.v22i2.21

26. Ayorinde AF, Oyeyiga AM, Nosegbe NO, Folarin OA. A survey of malaria and some arboviral infections among suspected febrile patients visiting a health centre in Simawa, Ogun State, Nigeria. Journal of Infection and Public Health. 2016 Jan 1;9(1):52-59. https://doi.org/10.1016/j.jiph.2015.06.009

27. Abdullahi IN, Akande AO, Muhammed Y, Rogo LD, Oderinde BS. Prevalence pattern of chikungunya virus infection in Nigeria: a four decade systematic review and meta-analysis. *Pathogens and global health*. 2020; Apr 2; 114(3):120-125. https://doi.org/10.1080/20477724.2020.1743087

28. Omatola CA, Onoja BA, Fassan PK, Osaruyi SA, Iyeh M, Samuel MA. et al.. Seroprevalence of chikungunya virus infection in five hospitals within Anyigba, Kogi State of Nigeria. *The Brazilian Journal of Infectious Diseases.* 2020; Jan 1;24(1):1-6. https://doi.org/10.1016/j.bjid.2020.01.001

29. Robinson ML, Kadam D, Khadse S, Balasubramanian U, Raichur P, Valvi C. et al. Vector-borne disease is a common cause of hospitalized febrile illness in India. The American journal of tropical medicine and hygiene. 2018 May; 98(5):1526-1533. https://doi.org/10.4269/ajtmh.17-0571

30. Adusei, J.A.; Narkwa, P.W.; Owusu, M.; Domfeh, S.A.; Alhassan, M.; Appau, E. et al. Evidence of chikungunya virus infections among febrile patients at three secondary health facilities in the Ashanti and the Bono Regions of Ghana. PLoSNegl. *Trop. Dis*. 2021, 15, e0009735. https://doi.org/10.1371/journal.pntd.0009735

31. Kotepui M, Masangkay FR, Kotepui KU, Milanez GD. Preliminary review on the prevalence, proportion, geographical distribution, and characteristics of naturally acquired Plasmodium cynomolgi infection in mosquitoes, macaques, and humans: a systematic review and meta-analysis. *BMC Infectious Diseases*. 2021 Dec; 21, 259. https://doi.org/10.1186/s12879-021-05941-y

32. Mala W, Wilairatana P, Kotepui KU, Kotepui M. Prevalence of malaria and chikungunya co-infection in febrile patients: A systematic review and meta-analysis. *Tropical Medicine and Infectious Disease.* 2021 Jun 30;6(3):119. https://doi.org/10.3390/tropicalmed6030119

33. Pourhoseingholi MA, Vahedi M, Rahimzadeh M. Sample size calculation in medical studies, Gastroenterology and Hepatology from bed to bench, 6. *Hepatol Bed Bench.* 2013; 6(1), 14-17. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4017493/

34. Pialoux G, Gaüzère BA, Jauréguiberry S, Strobel M. Chikungunya, an epidemic arbovirosis. *The Lancet infectious diseases.* 2007 May 1;7(5):319-327. https://doi.org/10.1016/S1473-3099(07)70107-X

35. Rashad AA, Mahalingam S, Keller PA. Chikungunya virus: emerging targets and new opportunities for medicinal chemistry. *Journal of Medicinal Chemistry*. 2014 Feb 27;57(4):1147-1166. https://doi.org/10.1021/jm400460d

36. Akinola MT, El-Yuguda AD, Bukbuk DN, Baba SS. Prevalence of IgG and IgM antibodies to Chikungunya virus among outpatients with febrile illness attending University of Maiduguri Teaching Hospital, Maiduguri, Borno State, Nigeria. African *Journal of Microbiology Research*. 2017 Feb 21;11(7):306-11. https://doi.org/10.5897/AJMR2016.8356

37. Inziani, M., Kilonzo, J., Kerubo, M., Mango, S., Kavurani, M., Ndirangu, A. et al. Risk factors for arbo viral seropositivity in children in Teso South Sub County, Western Kenya. MedRxiv(ColdSpringHarbor Laboratory). 2023; https://doi.org/10.1101/2023.04.06.23288220

38. Udeze, A., Odebisi-Omokanye, B., Onoja, A., Daodu, D. & Olasomi, O. Screening of immunoglobulin g antibodies against Chikungunya virus among urban population in Ilorin Nigeria. *Nigerian Veterinary Journal*, 2019; 40(3), 227. https://doi.org/10.4314/nvj.v40i3.7

39. Ali S, Inlamea O, Muianga A, Maholela P, Oludele J, Melchior B, Antonio V. et al. Investigation of antibodies against Chikungunya, Dengue and Zika virus in serum samples from febrile patients and its co-occurrence with malaria in six districts highly endemic for malaria in Mozambique between 2017–2018. *medRxiv*. 2022 Jun 14:2022-06. https://doi.org/10.1101/2022.06.10.22276235

40. Danis-Lozano R, Díaz-González EE, Trujillo-Murillo KD, Caballero-Sosa S, Sepúlveda-Delgado J, Malo-García IR. et al. Clinical characterization of acute and convalescent illness of confirmed chikungunya cases from Chiapas, S. Mexico: A cross sectional study. *PLoS One*. 2017 Oct 24;12(10):e0186923. https://doi.org/10.1371/journal.pone.0186923

Cite as: Idowu, F., Enitan, S., Iduh, M., Makanjuola, S., Akele, R., Edafetanure-Ibeh, O., Mensah-Agyei, G., Itodo, G., Adetiloro, E., Effiong, E. Prevalence of Chikungunya Virus IgG and IgM Antibodies among Febrile Patients Presumptively Diagnosed with Malaria in Ogun State, Nigeria. *Afro-Egyptian Journal of Infectious and Endemic Diseases*, 2024;14(4):440-459. doi: 10.21608/aeji.2024.308937.1403