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First Serological Evidence of West Nile Virus among Individuals with Febrile Illness in a Tertiary Hospital in Port Harcourt, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: West Nile Virus (WNV) infection can cause severe illness. Very little is known about the seroepidemiology of WNV infection in individuals with febrile illness in Nigeria and many other developing countries. This study was carried out to determine the seroprevalence of WNV in

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individuals with febrile illness attending a tertiary hospital in Port Harcourt, Nigeria and to determine if there was an association between WNV infection with age and sex.
Study Design: Cross-Sectional Study
Place and Duration of Study: Port Harcourt in Rivers State, Nigeria from September 2019 to December 2019.
Methods: Human sera were obtained and WNV IgG was determined using the Enzyme-Linked Immunosorbent Assay (ELISA) technique.
Results: Of the 90 study subjects tested, WNV IgG antibodies were present in 27 (30.0%) study participants while 63 (70.0%) study participants were seronegative for WNV IgG antibody. With age, a higher prevalence of WNV occurred among 61-70-year-olds (31.3%, n= 5) compared to 41-60 (30.8%, n= 12) and 20-40 (28.6%, n= 10). A higher prevalence of WNV IgG antibodies occurred in males (34.3%, n=12) than their female counterparts (30.9%, n=17). This study indicated that there is no association between WNV infection with age and sex.
Conclusion and Recommendations: These results show that WNV is circulating in Rivers State and has accounted for malaria-like infection in the region. It is recommended that WNV serological testing for malaria-infected individuals should be included as a routine test since they are most likely to present similar symptoms of WNV fever. Also, proper hygiene which includes eliminating mosquito breeding sites is recommended to mitigate the spread of West Nile Virus infection.

Keywords: West Nile Virus (WNV); antibodies; IgG; seroprevalence; ELISA.

1. INTRODUCTION

"West Nile virus (WNV) is an emerging and re-emerging zoonotic flavivirus first identified in and endemic to Africa" [1]. "West Nile Virus (WNV) is a mosquito-borne viral pathogen that is the causative agent of West Nile fever and encephalitis" [2]. It is one of the most prevalent arboviruses in the world and has been documented as a pathogen that has an impact on both human and animal health [3].

"The virus is transmitted between birds by biting mosquitoes, with equids and humans being incidental hosts" [1]. "The majority of infected incidental hosts display no or only mild clinical signs, but a fraction develop encephalitis" [1].

"First discovered in 1937, the West Nile virus (WNV) was found in a patient who had a febrile illness and lived in the West Nile district of northern Uganda" [4,5]. The WNV has been isolated across several continents of the world including Africa, Asia, Europe, the Mediterranean region, the Middle East, Australia and the Americas [6], and in 1951, the first known WNV human epidemic was reported in Israel, with young children accounting for the majority of cases [7]. "It was the first time that the main clinical signs were properly described, and they primarily included fever, headache, anorexia, exanthema, myalgia, abdominal discomfort, and vomiting and the condition was minor, with no known fatalities and infections appeared to be sporadic with symptoms of lymphadenopathy, sore throat, and diarrhoea" [5].

The West Nile virus (WNV) is a neurovirulent, zoonotic, mosquito-borne virus that belongs to the family *Flaviviridae* in the genus *Flavivirus* and is well-known to cause encephalitis and meningitis outbreaks [8,9,10]. The virus can infect and cause disease in horses and humans while also maintaining an enzootic cycle between ornithophilic mosquitoes and birds [11]. Infection in humans is initiated following a bite from a female mosquito (e.g., *Culex* mosquito species) infected with the WNV virus [7,10].

Studies carried out in the Nile Delta region revealed that WNV was endemic along the Nile, with a 60% seroprevalence rate in people, and that it was contagious in a wide variety of animals, including birds and non-human mammals [4,5]. WNV's arthropod-borne nature was first hypothesized in 1943, and it has subsequently been identified as one of the most pervasive arboviruses since it can infect more than 65 different species of mosquitoes [12].

In Northern Nigeria, WNV has been linked to mosquitoes and specific antibodies have been isolated in patients with febrile illness [13]. Seroprevalence studies can be used to monitor WNV activity in any population. As a result, it has been reported that camels, goats, cattle, sheep, and horses in Nigeria and Romania have antibodies against WNV that prevent hemagglutination [14,15].

"Blood serves as a vehicle for transmission of blood-borne pathogens including hemiparasites" [16]. "Malaria is one of the most prevalent and deadly widespread of all parasitic diseases in the world. It is mosquito-borne and one of the killer diseases of the world" [17]. Malaria is a life-threatening disease caused by parasites that are transmitted to people through the bites of infected female Anopheles mosquitoes [18-22].

Malaria remains a significant health threat worldwide [21,23]. Almost half of the world's population is at risk of malaria [24,25], although malaria transmission is unstable throughout the year in these reports [26]. Over 50% of the people on earth are vulnerable to malaria infection and they have estimated over 300 million malaria cases annually in the tropics [25,27,28]. An estimated 300 million malaria cases occur annually in the tropics, with 90% of these in the sub-Sahara, a region that already suffers the tremendous burden of HIV-1 infection [20,22]. Co-infection with malaria and viruses such as HIV, West Nile virus, and Dengue virus, among others, have led to several deaths, especially in sub-Saharan Africa [29].

"Diagnostic tools for WNV infection in Nigeria are not well established hence the current prevalence rate of WNV infection in Nigeria is unknown" [2]. In a recent study reported by Sule and Oluwayelu [10], the study showed that there is no correlation between West Nile Virus (WNV) infection and febrile illness in people of Southwestern Nigeria. Although very few studies have shown the prevalence of WNV in Nigeria [30], none have shown its presence in the study area and there is no evidence of WNV exposure among humans in this area. In this study, we report the first serological evidence of West Nile Virus (WNV) among individuals with febrile illness attending a tertiary hospital in Port Harcourt, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

The study area is Port Harcourt located in the municipal area of Rivers State, Nigeria. Port Harcourt is the capital city of Rivers State, also known as the Garden City, it is located in the forest zone of South Southern Nigeria. Port Harcourt city lies on the longitude 7^{o} East of Greenwich meridians and latitude 4^{o}75$^{'}$ North of the Equator. Port Harcourt's heaviest precipitation occurs during September with an average of 370mm of rain. December on average is the driest month of the year; with an average rainfall of 20mm. Temperatures throughout the year in the city are relatively constant, showing little variation throughout the year. The average temperature is typically between 25^{o}C - 28^{o}C in the city. The city is an important trade majorly petroleum and educational centre and houses one of the largest and foremost teaching hospitals in Africa.

2.2 Study Population

The study population constituted 90 suspected malaria-infected individuals with apparent symptoms of fever in the University of Port Harcourt Teaching Hospital (UPTH).

2.3 Blood Sample Collection, Plasma Preparation and Storage

About 5ml of blood sample was aseptically collected by venipuncture from patients. Each blood sample was dispensed into an appropriately labelled EDTA-treated blood sample tube, screw-capped and left at room temperature for about 40 min, after which it was spun at 3,000 rpm for 10 min to separate plasma from the blood. The plasma was dispensed into labelled Eppendorf tubes and stored at -20^{o}C until analyzed for WNV antibodies. Samples were identified with codes. Haemolysed and visibly hyperlipemic samples, as well as those containing residues of fibrin or heavy particles, were discarded as they could generate false serologic results. Plasma samples were stored at +2^{o}C - 8^{o}C up to five days after collection. For longer storage periods, samples were stored frozen at -20^{o}C.

2.4 Serological Analysis of West Nile Virus Antibody

Microplates were coated with a highly purified immune-dominant West Nile virus (WNV) antigen. In the first incubation, the solid phase is treated with diluted samples and anti-WNV antibodies are captured, present by the antigens. After washing out all the components of the sample, in the second incubation bound, anti-WNV were detected by the addition of a mix of both anti-hlgG antibodies labelled with peroxidase (HRP). The enzyme captured on the solid phase, acting on the substrate/chromogen mixture generates an optical signal that is proportional to the amount of WNV antibodies present in the sample. Test results were

interpreted as ratio sample OD450nm (S) and the cut-off value (Co), mathematically S/Co ≤0.9 as Negative, 0.9-1.1 as Equivocal and >1.2 as Positive. The results were then used to evaluate the sociodemographic characters obtained from the questionnaires administered to participants who were enrolled in the study.

2.5 Data Analysis

Data analysis was carried out using Microsoft Excel 2016 version to calculate the international unit (IU) from optical density (OD). Values less than or equal to 0.9 were considered negative while values greater than or equal to 1.2 were considered positive. Results are expressed as numbers and percentages.

3. RESULTS

3.1 Patients Characteristics

The total number of patients with febrile illness included in this study was 90 with an age range of 20-70 years. The age group (41-60yrs) constituted the largest population making up 43.3%, followed by the age group (20-40yrs) (38.9%) while the age group (61-70yrs) were the least (17.8%). Characteristics taken into consideration were age and sex as shown in Table 1.

Table 1. Participants Characteristics

Characteristics	No. Tested (%)
Age groups (years)	
20-40	35 (38.9)
41-60	39 (43.3)
61 and above	16 (17.8)
Sex	
Males	35 (38.9)
Females	55 (61.1)
Total	**90 (100.0)**

3.2 Overall Seroprevalence of West Nile Virus (WNV)

A total number of 90 samples were tested, of which 27(30.0%) were seropositive and 63(70.0%) were seronegative for WNV as shown in Fig. 1.

3.3 Seroprevalence of West Nile Virus (WNV) IgG antibodies with Age

With age, higher prevalence of West Nile Virus (WNV) IgG antibodies was observed in the elderly (61-70yrs) (31.3%, n= 5) compared to middle-aged subjects (41-60yrs) (30.8%, n= 12) and the young adults (20-40yrs) (28.6%, n= 10) as presented in Fig. 2. This difference was not statistically associated (X^2 = 0.971947, df= 2, p>0.05).

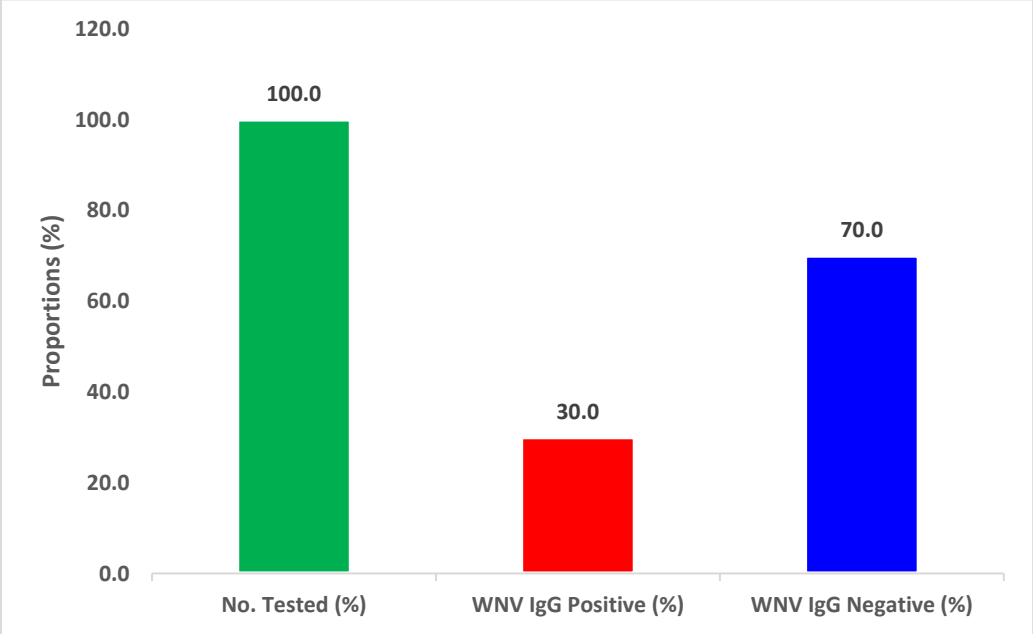

Fig. 1. Overall Seroprevalence of West Nile Virus (WNV) IgG antibodies

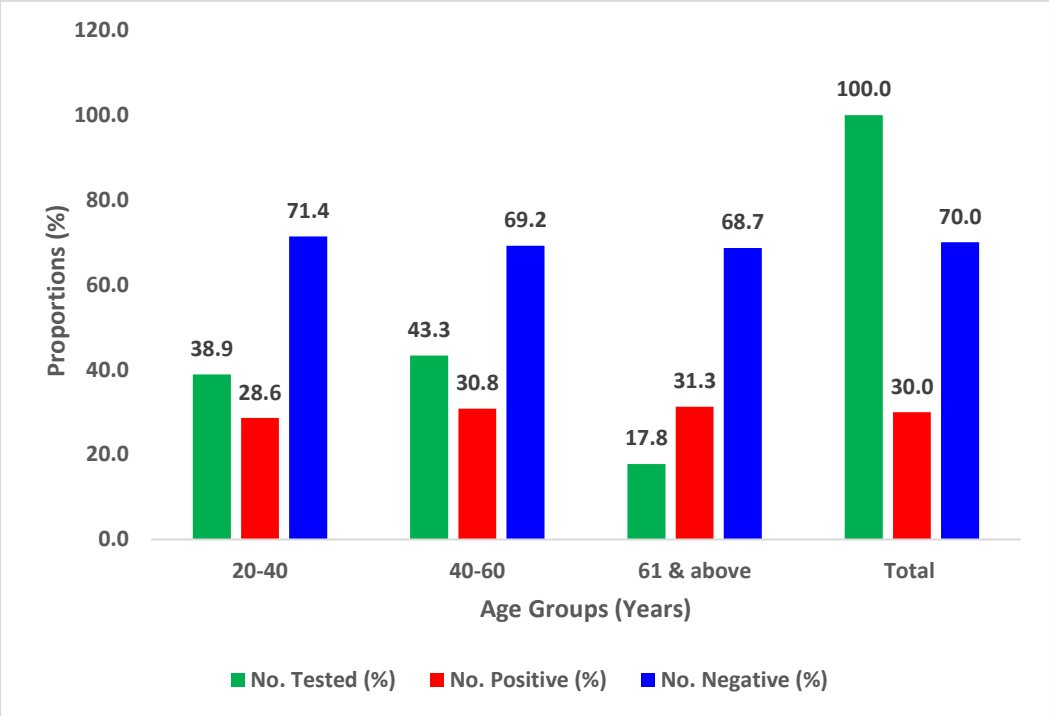

Fig. 2. Seroprevalence of West Nile Virus (WNV) IgG antibodies with age

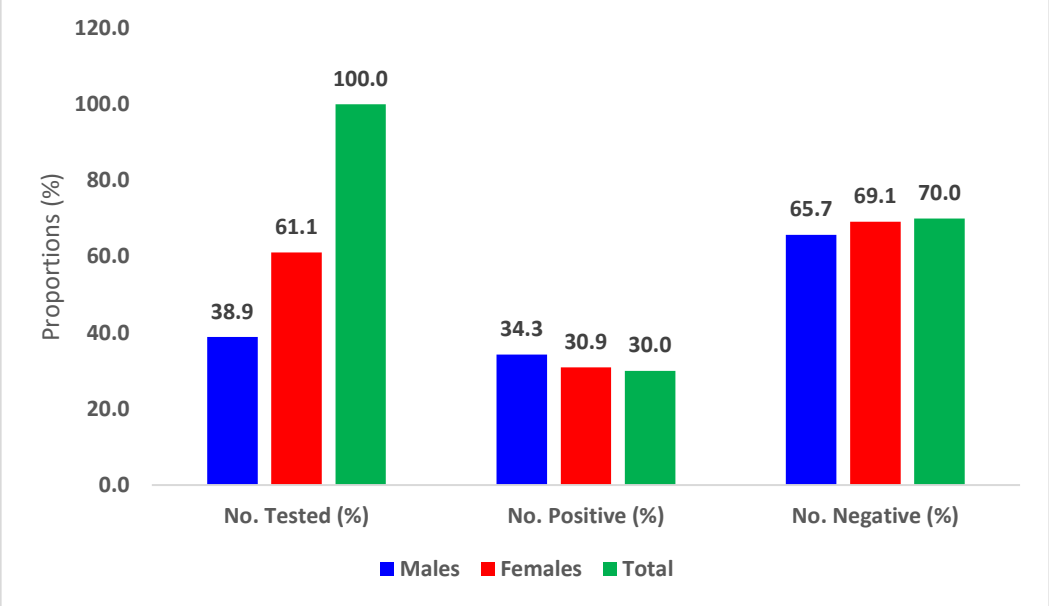

Fig. 3. Seroprevalence of West Nile Virus (WNV) IgG antibodies with sex

3.4 Seroprevalence of West Nile Virus (WNV) IgG antibodies with Sex

A higher prevalence of WNV IgG antibodies was detected in male subjects (34.3%, n=12) than in female subjects (30.9%, n= 17) as shown in Fig. 3. This difference was not statistically associated (X^2 = 0.738258, df= 1, p>0.05).

4. DISCUSSION

This study provides scientific data on the seroprevalence of West Nile Virus (WNV) IgG antibodies among patients who were recruited in the study with febrile illness in Port Harcourt, Rivers State, Nigeria. The seroprevalence of West Nile Virus in certain parts of Nigeria is well

documented [30,10]. In this study, we examined a total of 90 study participants who were accessing healthcare services in a tertiary teaching hospital in Port Harcourt who were recruited in this study with clinical presentation of febrile illness. Of this number, more females participated in the study. This observation agrees favorably with Ahmed and Hamedelnil [31], and Kolawole et al. [2] findings. This higher proportion of females shifted the population density in favour of the female subjects [2]. Also, higher proportions of the participants were in the age group 41-60 years age group. This observation differs from that of Kolawole et al. [2] who observed a higher proportion in the age group 21-30 years. These higher proportions in the 41-60 years age group shifted the population density in favour of this age group.

Of these 90 participants, 30.0% were positive for anti-WNV IgG antibodies. In recent years, more seroprevalence studies have been reported, similar to the findings of this study [30], however, this figure (30.0%) is higher than the 19.4% and 7.5% reported by Kolawole et al. [2], respectively, in Ilorin, Nigeria and 25.0% reported by Baba et al. [13] in Maiduguri, Nigeria. It is higher than the 9.5% and 13.2% obtained in Kenya and Sudan, respectively [32,31]; the 24.0% reported in another region of Kenya [33].

The 30.0% reported here is lower than the 66.0% reported in Democratic Republic of Congo [34], the 80.2% reported by Baba et al. [30] among patients with febrile illness suspected of malaria/typhoid fever in Maiduguri, Nigeria, the 35.0% and 85.0% reported by Oderinde et al. [35] in Maiduguri, Nigeria, and the 76.5% reported in Nigeria [36]. Baba et al. [30] concluded that their study area was endemic for flavivirus and that the peak of WNV activity in the semi-arid zone in Nigeria appeared to be in November.

In another study, it was reported that 45.0% of febrile participants had WNV antibodies but were negative for malaria parasite and Widal tests, thereby accounting for undifferentiated febrile illness [37]. Increasingly, surveys suggest a high seroprevalence of WNV antibodies in Nigerian populations. The differences in the results obtained from this study and other studies in Nigeria and overseas could be largely influenced by environmental conditions [30,2]. This in turn indicates that WNV is highly endemic within Nigeria and further investigations for the presence and distribution of virus in reservoir avian species and vector mosquito species are needed [37].

In this present study, the elderly (61 years and above) were more susceptible to WNV infection (suggesting underlying diseases or immunosuppressed state) than young adults (20-40 years) and middle-aged (41-60 years) but the difference was not statistically significant. This is different from what was reported by Ahmed and Hamedelnil [31] and Kolawole et al. [2]. They [30,31,2] reported that age is not a potential risk factor in the acquisition of WNV infection. The distribution of WNV concerning age in their studies was widespread and not limited to a particular age group.

Also, this study indicated that a higher prevalence of WNV IgG antibodies occurred in male subjects than in female subjects but the difference was not statistically significant. This showed that gender is not a risk factor important for infection with WNV. Hence, WNV infection and gender are independent or have no association. This is in agreement with the previous report on gender and acquisition of WNV infection [13,2]. However, our results differ from what was reported by Ahmed and Hamedelnil [31] and Kolawole et al. [2] who reported higher prevalences in females than males.

Factors which could influence the acquisition of WNV infection and its prevalence may include housing and roofing type, an abundance of surrounding bushes and trees which can become a favourable habitat for mosquitoes and the presence of stagnant water which is an established factor that contributes significantly as a breeding site for mosquitoes, thereby increasing the numbers of mosquitoes and consequently the potential risk for WNV infection.

5. CONCLUSION

In this study, a low prevalence of West Nile Virus (WNV) IgG antibodies was shown among febrile individuals in Rivers State, Nigeria. This study indicated that there is no association between WNV IgG antibodies with age and sex. Although, estimating the global burden of this infection is important for appreciating the scale of an epidemic, the distribution of resources to those most affected which includes identifying testing and counselling those at risk is strongly recommended. Also, local healthcare providers should encourage personal hygiene practices

and the correct use of mosquito-treated nets as this will significantly reduce exposure to mosquito bites and the risk for WNV infection in people living in mosquito endemic regions.

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ETHICAL APPROVAL AND CONSENT

Clearance from the health research ethical committee of the University of Port Teaching Hospital (UPTH) was obtained following the code of ethics for biomedical research involving human subjects. Signed informed consent was also obtained from each of the subjects after carefully explaining the concept of the study to them and questionnaires were distributed.

COMPETING INTERESTS

The authors have declared that no competing interests exist.

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