Serological Evidence of West Nile Virus(WNV)among individuals with febrile illnessin a Tertiary Hospital in Port Harcourt, Nigeria.

ABSTRACT

WNV infection can cause severe illness. Very little is known about the seroepidemiology of WNV infection in patients with febrile illness in Nigeria and many other developing countries. This study was carried out to determine the seroprevalence of WNV in patients with febrile illness attending the University of Port Harcourt Teaching Hospital (UPTH), Rivers State, Nigeria and to determine if there was an association between WNV infection in relation to age and sex. Human sera were obtained and WNV IgG was determined using Enzyme Linked Immunosorbent Assay (ELISA) technique. Out of 90 study subjects tested, WNV IgG antibodies were present in 27 (30.0%) study participantswhile 63 (70.0%)study participants were seronegative for WNV IgG antibody.In relation to age, higher prevalence of WNV occurred among 61-70 years (31.3%, n= 5) compared to 41-60 (30.8%, n= 12) and 20-40 (28.6%, n= 10). 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 in relation to age and sex. 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 patients 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, Antibodies, IgG, seroprevalence, ELISA.

UNDER PEER REVIEW

1. INTRODUCTION

The West Nile Virus (WNV) 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 (Shomaker et al., 2013). 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 (Hughes et al., 1940; Castro-Jorge et al., 2019). 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 (Barakat et al., 2004), and in 1951, the first known WNV human epidemic was reported in Israel, with young children accounting for the majority of cases (Qian et al., 2014). 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 appears to be sporadic with symptoms of lymphadenopathy, sore throat, and diarrhoea (Castro-Jorge et al., 2019).

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 (Opaleye *et al.,* 2014; Blázquez et al., 2015; Sule &Oluwayelu, 2016). The virus can infect and cause disease in horses and humans while also maintaining an enzootic cycle between ornithophilic mosquitoes and birds (Lim et al., 2011). Infection in humans is initiated following a bite from a female mosquito (e.g., *Culex* mosquito species) infected with the WNV virus (Qian et al., 2014; Sule &Oluwayelu, 2016).

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 (Hughes et al., 1956; Castro-Jorges et al., 2019).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 (Colpitts et al., 2012).

In Northern Nigeria, WNV has been linked to mosquitoes and specific antibodies have been isolated in patients with febrile illness (Baba et al., 2015). 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 (Olaleyeet al., 1990; Hubalek&Halouzka, 1999).

UNDER PEER REVIEW

In a recent study reported by Sule and Oluwayelu(2016), the study showed that, there is no correlation between WNV infection and febrile illness in people of Southwestern Nigeria, and there is no evidence of WNV exposure among humans in this area. In this study we report the prevalence of West Nile Virus (WNV) among patients with febrile illness attending the University of Port Harcourt Teaching Hospital and also show that there is no correlation between age and WNV infection among study subjects.

MATERIALS AND METHODS

2.1. Study Area

The study area is University of Port Harcourt Teaching Hospital, located in municipal area of Port Harcourt. 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° East of Greenwich meridians and latitude 4°75$^{'}$ North of the Equator. Port Harcourt 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 is relatively constant, showing little variation throughout the course of the year. Average temperature is typically between 25°C-28°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 of 90 malaria patients with apparent symptoms of fever in the University of Port Harcourt Teaching Hospital (UPTH) in September, 2019 to December 2019.

2.3 Ethical Consideration

UNDER PEER REVIEW

Clearance from the health research ethical committee of University of Port Teaching Hospital (UPTH) was obtained in accordance with 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.

2.4 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 appropriately labeled 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 labeled Eppendorf tubes and stored at -20°C until analyzed for WNV antibodies. Samples were clearly 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°C-8°C up to five days after collection. For longer storage periods, samples were stored frozen at -20°C.

2.5. Serological Analysis of West Nile virus Antibody

Microplate were~~are~~ 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, it presents 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 antibody labeled with peroxidase (HRP) and anti-hlgM antibody labeled 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

UNDER PEER REVIEW

(Co), mathematically S/Co ≤0.9 as Negative, 0.9-1.1 as Equivocal and >1.2 as Positive. The resultswere then used to evaluate the sociodemographic characters obtained from the questionnaires administered to participants that were enrolled in the study.

2.6. 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 age range of 20-70yrs. The age group (41-60yrs) constituted the largest populations making up 43.3%, followed by age group (20-40yrs) (38.9%) while 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- 70	16 (17.8)
Sex	
Males	35 (38.9)
Females	55 (61.1)

UNDER PEER REVIEW

Total	90 (100.0)

3.2.Overall Seroprevalence of West Nile Virus

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 Figure 1.

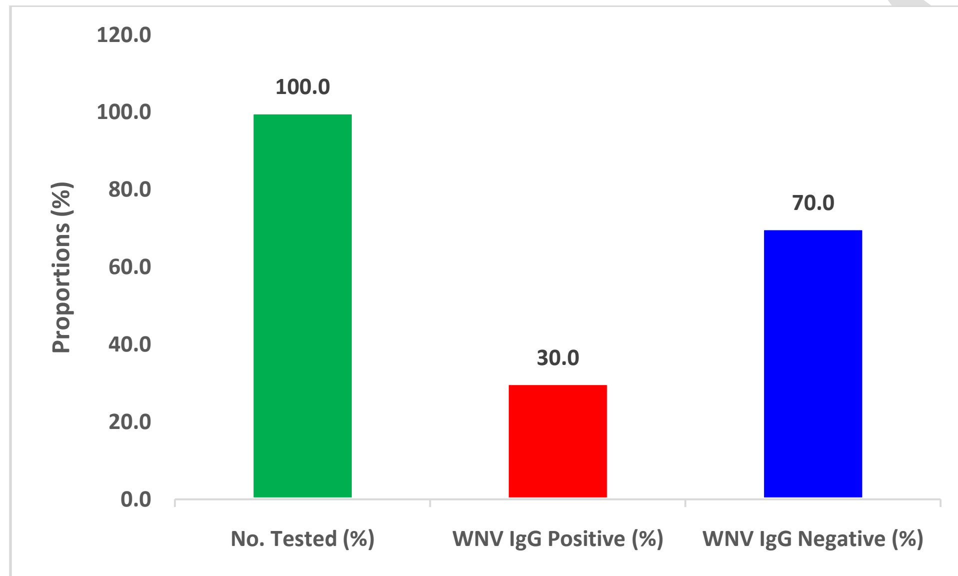

Figure 1: Overall Seroprevalence of West Nile Virus

3.3. Seroprevalence of West Nile Virus in Relation to Age

In relation to age, higher prevalence of WNV 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 Figure 2. This difference was not statistically associated (X^2 = 0.971947, df= 2, p>0.05).

UNDER PEER REVIEW

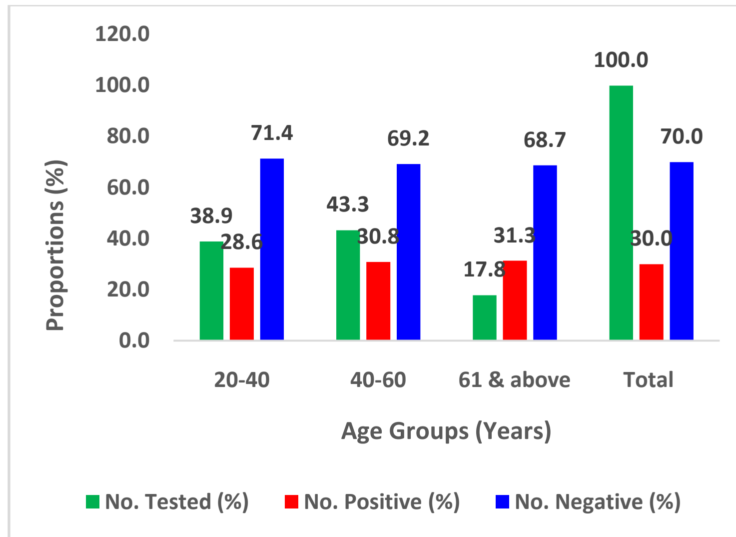

Figure 2: Seroprevalence of West Nile Virus in relation to age

3.4 Seroprevalence of West Nile Virus in Relation to Sex

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

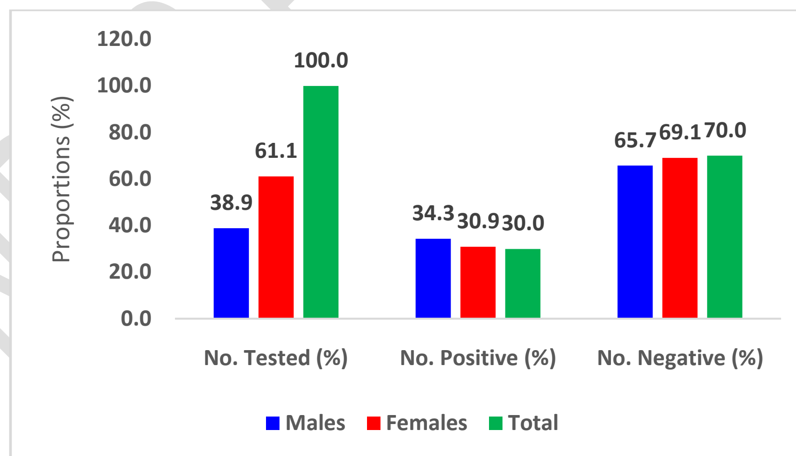

Figure 3: Seroprevalence of West Nile Virus in relation to sex

4. DISCUSSION

UNDER PEER REVIEW

This study provides scientific data on the seroprevalence of West Nile virus (WNV) among patients who were recruited in the study with febrile illness in Port Harcourt, Rivers State. The seroprevalence of West Nile Virus in certain parts of Nigeria is well documented (Baba et al., 2006; Sule &Oluwayelu, 2016). 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. Out of this number, 27(30.0%) were positive to anti-WNV IgG antibodies while 63(70.0%) were negative. In recent years, more seroprevalence studies have been reported, similar to the findings of this study (Baba et al., 2006). The 30.0% reported here is lower than the 80.2%reported by Baba et al. (2006) among patients with febrile illness suspected of malaria/typhoid fever. Baba et al. (2006) 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% (18/40) of febrile participants had WNV antibodies but were negative for malaria parasite and Widal tests, thereby accounting for undifferentiated febrile illness (Sule *et al*., 2016).Increasingly, surveys suggest a high seroprevalence of WNV antibodies in Nigerian populations. 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 (Sule *et al*., 2018).

In this present study, the elderly (61-70yrs)was more susceptible to WNV infection (suggesting underlining diseases or immunosuppressed state) than young adults(20-40yrs) and middle-aged (41-60yrs) but the difference was not statistically significant.

Also, this study indicated that higher prevalence of WNV IgG antibodies occurred in male subjects (34.3%) than in female subjects (30.9%) but the difference was not statistically

UNDER PEER REVIEW

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 previous report on gender and acquisition of WNV infection (Kolawole et al., 2018).

Factors which could influence the acquisition of WNV infection and its prevalence may include housing and roofing type, abundance of surrounding bushes and trees which can become a favorable 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.

4.2 Conclusion

In this study low prevalence of WNV was shown among febrile patients in Rivers State, Nigeria. This study indicated that there is no association between WNV infection in relation to age and sex.Although, estimating the global burden of this infection is important for appreciating the scale of an epidemic, 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 practice and the correct use of mosquito treated nets as this will significantly reduce exposure to mosquito bites and the risk for West Nile Virus (WNV) infection in people living in mosquito endemic regions.

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UNDER PEER REVIEW

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UNDER PEER REVIEW