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ANTI-WEST NILE VIRUS IMMUNOGLOBULIN G AND M PROFILES OF PATIENTS WITH PYREXIA OF UNKNOWN ORIGIN IN ILORIN, NIGERIA

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ABSTRACT

West Nile Virus (WNV) is a member of flaviviruses which contribute significantly to morbidity and mortality especially in countries where it is endemic. In resource limited countries like Nigeria, fever resulting from the infection is often classified as pyrexia of unknown origin (PUO) due to poor diagnostic infrastructure and low level of awareness of the infection. This study determined the prevalence of anti-WNV IgM and IgG antibodies among patients with PUO in Ilorin metropolis. A total of one hundred and eighty-two (182) blood samples were collected from consenting patients and the corresponding sera were screened for WNV IgM and IgG antibodies using indirect enzyme-linked immunosorbent assay (ELISA). Anti-WNV IgM and IgG antibodies were detected in 8.2% and 6.6% of the samples respectively. Prevalence of anti-WNV IgM was higher in females (10.8%) than males (3.2%) while anti-WNV IgG was similar in both genders (6.7 % vs 6.5%). The study did not establish any statistically significant association ($p>0.05$) between age, marital status, occupation, use of mosquito nets and level of education and occurrence of the antibodies. However, statistical significant relationship was established between occurrence of the antibodies and travel history. WNV remains a serious health concern and may be responsible for most cases of PUO in Ilorin-Nigeria. Screening for WNV is therefore advocated for all febrile patients.

KEYWORDS: Immunoglobulin G, Immunoglobulin M, Pyrexia of Unknown Origin, West Nile Virus

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INTRODUCTION

West Nile Virus (WNV) is one of the arboviruses of public health importance in both humans and animals (Petersen *et al.*, 2013). It belongs to the genus *Flavivirus* within the family of *Flaviviridae*. The first isolation of the virus occurred in 1937 from a febrile patient in the West Nile district of Uganda (Smithburn *et al.*, 1940) while the first reported human epidemic occurred in Israel in 1951 (Bernkopf *et al.*, 1953). Since then, WNV has witnessed significant geographical spread around the world aided by activities such as globalization, land use, and international travel.

Mosquitoes, belonging to *Culex spp* and *Aedes spp* are the principal vectors (Calistri *et al.*, 2010). In nature, the virus is maintained by mosquito – bird – mosquito transmission cycle. Humans and other vertebrates such as horses are considered “dead-end” hosts; being susceptible but unable to transmit to mosquitoes as a result of transient and low viremic titer (Sule *et al.*, 2018). Consequently, they do not play a role in the transmission cycle of the virus although they can manifest severe disease or death as a result of the infection (Rizzoli *et al.*, 2015). Following incubation, symptoms appear in humans between 3-14 days while in severe cases may be biphasic with symptoms prolonged up to 60 days (Hayes *et al.*, 2005). Human infection by WNV presents in different forms including an asymptomatic infection; a mild febrile syndrome termed West Nile Fever (WNF) and a neuroinvasive disease (West Nile Neuroinvasive Disease, WNND) with life threatening encephalitis, meningitis and acute flaccid paralysis (Chowers *et al.*, 2001; Fall *et al.*, 2017).

The often non-specific symptoms of West Nile Virus infections in human makes differential diagnosis complicated (Monath *et al.*, 2001). In resource-limited countries like Nigeria, this is even more complicated due to poor diagnostic infrastructure and low level of awareness of arboviruses leading to

underdiagnosis and/or misdiagnosis (Baba *et al.*, 2006). Consequently, cases of WNV infections could often times be classified as pyrexia of unknown origin (PUO) leading to wrong treatment with attendant high morbidity and mortality. Hence, this study was conducted among patients with PUO to ascertain the level of exposure to the virus and highlight the need for WNV test for febrile patients.

MATERIALS AND METHODS

Study design and population

This health institutions-based cross-sectional study was conducted within Ilorin metropolis, Kwara state Nigeria. The institutions included in this study are University of Ilorin Health Centre, Civil Service and Sobi Specialist Hospitals. Ilorin is located geographically at 80° 30¹ North, 4° 30¹ East, 300 km from Lagos, and 500 km from Abuja, the Federal Capital Territory. It is located in the guinea savannah vegetation zone, which breeds different species of mosquitoes (Okogun *et al.*, 2005) and characterized by a semi-arid climate with hot, dry, and wet- mild seasons which may differ from year to year.

The study participants included 182 (62 males and 120 females; age range: 6-70 years) consenting in- and out- febrile patients irrespective of age and gender. Inclusion criteria is presence of fever of >38.3°C. Patients with a confirmed diagnosis associated with the occurrence of fever were excluded from this study.

Sample Collection and Analysis

About 5 ml of venous blood sample was collected from each study participant into plain sample bottle, separated into serum and packed cells and stored at -20°C until assayed. Relevant socio-demographic information was obtained by administration of well-structured questionnaires.

Presence of anti-WNV IgM and IgG antibodies were detected using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Sunlong

Biotech Co., Ltd, China) for the qualitative detection of IgM and IgG antibodies to WNV in human serum/plasma. The assay was carried out according to the kit's manufacturer's instructions.

Ethical Consideration

The protocol for this study was approved by the Ethics and Health Research Board of the Ministry of Health, Ilorin, Kwara State (MOH/KS/EU/777/286). The protocol complied with the Helsinki Declaration for the use of human subjects. Informed consent was obtained from all the participants and from the parents/legal guardians (in case of participants who are less than 18 years old) before sampling. All results were delinked from patient identifiers and anonymized.

Statistical Analysis

Results were analyzed using the Statistical Package for the Social Science (SPSS) version 23 software package. The significance of the values obtained was determined at $P < 0.05$.

RESULTS

Out of the 182 samples tested, 15 of them were positive for anti-WNV IgM and only 12 for IgG. This represents 8.2% recent/ongoing infections and 6.6% past infections respectively among the study participants (Figures 1A and 1B).

Analysis showed no statistical difference ($p > 0.05$) in the occurrence of IgM and IgG antibodies with respect to all the demographic variables (gender, age range and marital status) analyzed (Table 1).

The results were also analyzed based on some risk factors of acquisition including; occupation, travel history, use of mosquito nets and level of education. Similarly, there is insufficient statistical evidence ($p > 0.05$) that occupation, use of mosquito nets and level of education play any role individually on the presence of anti-WNV IgM and IgG

antibodies. However, with p -values < 0.05 , there is a statistically significant association between travel history and observation of anti-WNV IgM and IgG in the subjects. While participants with travel history outside of the country had anti-WNV IgM and IgG prevalence of 40% and 20% respectively, their counterparts with no history of travel outside the country had anti-WNV IgM and IgG prevalence of 4.3% and 4.9% respectively (Table 2).

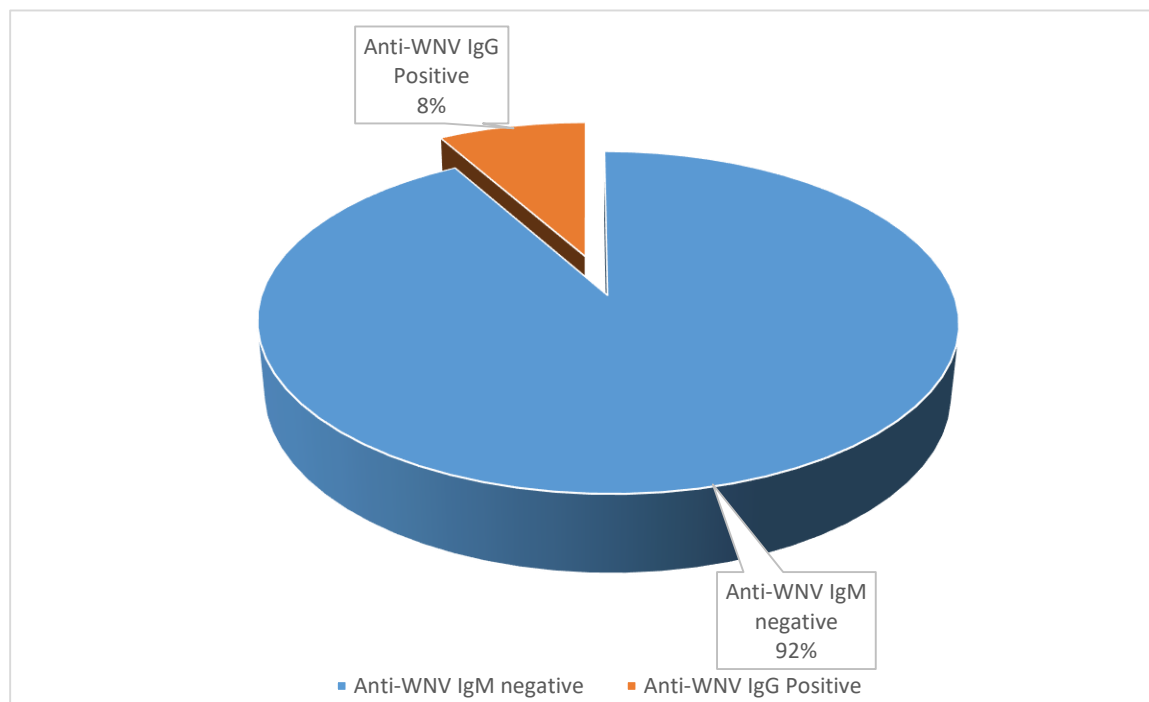


Figure 1A: Anti-WNV IgM antibodies prevalence among patients with PUO in Ilorin, Nigeria

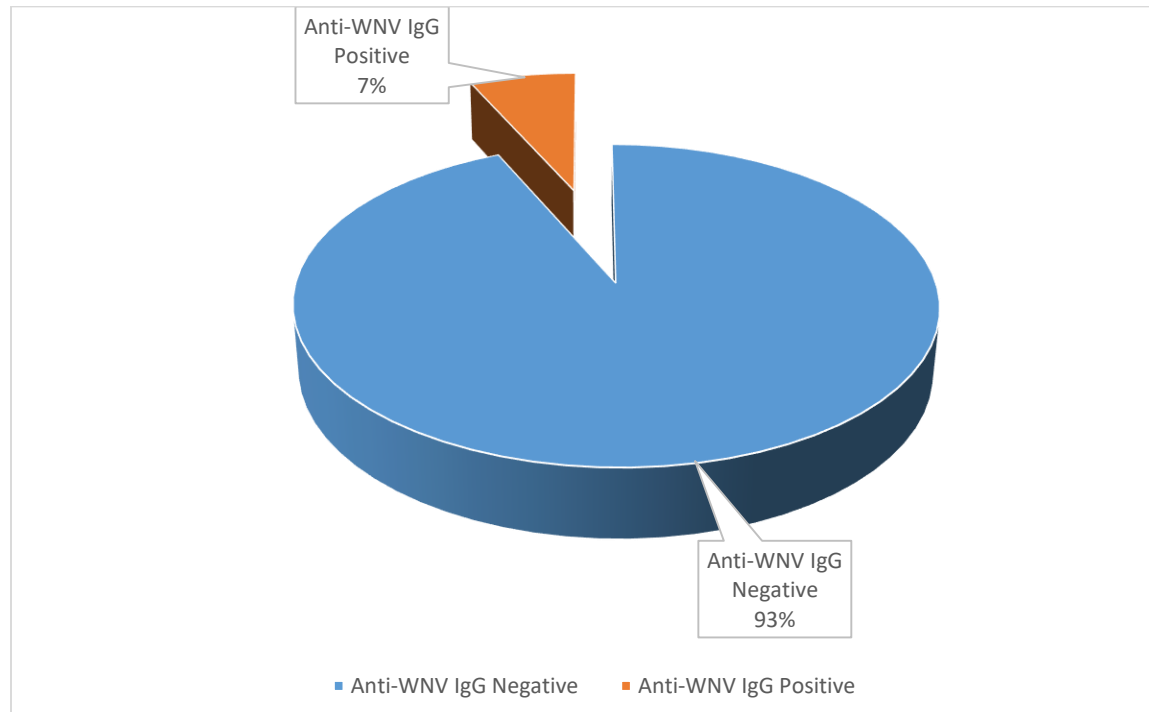


Figure 1B: Anti-WNV IgG antibodies prevalence among patients with PUO in Ilorin, Nigeria

Table 1: Relationship between demographic variables and distribution pattern of anti-WNV IgM and IgG antibodies among patients with PUO in Ilorin, Nigeria

Variables	Number tested	Number (%) positive			
		IgM	χ^2 (p-Value)	IgG	χ^2 (p-Value)
Gender					
Male	62	2 (3.2)	3.128(0.077)	4 (6.5)	0.003(0.956)
Female	120	13 (10.8)		8 (6.7)	
Total	182	15 (8.2)		12 (6.6)	
Age range (years)					
≤10	10	0 (0)	2.913(0.820)	0 (0)	1.494(0.960)
11-20	52	6 (11.5)		4 (7.7)	
21-30	70	6 (8.6)		4 (5.7)	
31-40	16	1 (6.3)		1 (6.3)	
41-50	16	1 (6.3)		1 (6.3)	
51-60	10	0 (0)		1 (10)	
61-70	8	1 (12.5)		1 (12.5)	
Total	182	15 (8.2)		12 (6.6)	
Marital status					
Married	60	4 (6.7)	0.294(0.588)	4 (6.7)	0.001(0.978)
Single	122	11 (9.0)		8 (6.6)	
Total	182	15 (8.2)		12 (6.6)	

p<0.05: Significant

p>0.05: Insignificant

Table 2: Relationship between some risk factors of transmission and distribution pattern of anti-WNV IgM and IgG antibodies among patients with PUO in Ilorin, Nigeria

Variables	Number tested	Number (%) positive			
		IgM	χ ² (p-Value)	IgG	χ ² (p-Value)
Occupation					
Unemployed	11	2 (18.2)	2.148(0.342)	2 (18.2)	2.600(0.272)
Civil servants	57	3 (5.3)		3 (5.3)	
Students	114	10 (8.8)		7 (6.1)	
Total	182	15 (8.2)		12 (6.6)	
Travel history					
Yes	20	8 (40)	29.966(<0.001)	4 (20)	6.557(0.010)
No	162	7 (4.3)		8 (4.9)	
Total	182	15 (8.2)		12 (6.6)	
Use of mosquito nets					
No	25	1 (4)	0.690(0.406)	1 (4)	0.316(0.574)
Yes	157	14 (8.9)		11 (7.0)	
Total	182	15 (8.2)		12 (6.6)	
Level of education					
Formal education	172	13 (7.6)	1.934(0.164)	10 (5.8)	3.088(0.079)
No formal education	10	2 (20)		2 (20)	
Total	182	15 (8.2)		12 (6.6)	

p<0.05: Significant

p>0.05: Insignificant

DISCUSSION

WNV infection remains a public health concern especially in countries where it is endemic including Nigeria (Abdullahi *et al.*, 2020). In this study, 8.2% and 6.6% of the study participants tested positive to anti-WNV IgM and IgG antibodies respectively. These represented recent/ongoing and past infections respectively. The rate of recent/ongoing infections in this study is appreciably higher than the previously reported rates in Kaduna (Ma'aji *et al.*, 2020), Osun (Opaleye *et al.*, 2014) and another semi-arid zone in Nigeria (Baba *et al.*, 2006). This rate is also higher than the reported 3.3% among blood donors in Qatar (Darghama *et al.*, 2021). However, higher prevalence of anti-WNV IgM antibodies have been reported from other places such as Ibadan and Ogbomoso (Omilabu *et al.*, 1990). The prevalence of 6.6% anti-WNV IgG observed in this study is similar to prevalence of 7.4% reported from Ethiopia (Eshetu *et al.*, 2020) but significantly lower than prevalence reported from Qatar and Iran (Darghama *et al.*, 2021; Kalantari *et al.*, 2019).

Higher prevalence of IgM than IgG in this study is a bit puzzling. Since IgG antibodies usually are detected shortly after IgM antibodies and usually persists for many years unlike IgM antibodies that disappears within a few months to years, IgG antibodies should expectedly cumulate in the population with higher prevalence. The observed high IgM prevalence in this study could reflect the level of recent/ongoing infections in this study area. Since flaviviruses (especially members within the Japanese encephalitis complex) are known to share epitopes, resulting in cross-reactive serological tests (Allwinn *et al.*, 2002), the observed high IgM prevalence could also reflect presence of other flaviviruses which are known to circulate in the region (Udeze and Odebisi-Omokanye, 2021), hence the need for result confirmation by neutralizing antibody testing (CDC, 2019). Furthermore, the higher IgM level than IgG

could also be explained partly by the nature of the study participants (PUO patients) who have no other common causes of fever reaffirming the suspicion of WNV infection. This could therefore suggest the possibility of viremia and acute infection of these patients by WNV and other cross-reacting flaviviruses especially those of the Japanese encephalitis serogroup.

Since WNV-specific IgG antibodies usually are detected shortly after IgM antibodies and usually persists for many years, higher IgG than IgM antibody prevalence is expected in the population and with advancing age group in the population. However, the lower IgG prevalence observed in this study can be attributed to a number of factors including wane in antibody titer (Murray *et al.*, 2013) and reversion to seronegativity over time (Pierro *et al.*, 2013). A study in Houston, showed that 42%, 34%, and 23% of the study participants had anti-WNV IgM antibodies approximately 1, 6, and 8 years post-infection respectively, while almost one-half of the participants (46%) had undetectable anti-WNV IgG antibodies by 8 years post-infection (Murray *et al.*, 2013). Reversion to IgG seronegativity after previous positive status has been associated with asymptomatic primary infection of WNV (Pierro *et al.*, 2013). On the other hand, long-term persistence of WNV IgG and sometimes even IgM antibodies is witnessed in symptomatic infections and could be explained by a strong initial stimulation of the immune system which results in long-term antibody memory (Cassese *et al.*, 2003). Since most WNV infection in Nigeria is asymptomatic/subclinical, the 6.6% IgG prevalence in this study may be a gross underreporting and may not reflect the true burden of the infection as many may have missed detection as a result of waned antibody titre to below detection limit. Detection method is another factor which can lead to false negative results (sensitivity < 100%).

The prevalence of anti-WNV IgM antibodies is similar in both male and female populations. This is consistent with the report of Ma'aji *et al.* (2020) in Kaduna and differs from the report of Olaleye *et al.* (1990) in Ibadan. Similarly, prevalence of anti-WNV IgG antibodies was similar in female (6.7%) and male populations (6.5%).

This study could not establish any statistical significant association between other variables including; age, marital status, occupation, use of mosquito nets and level of education and the occurrence of anti-WNV IgM and IgG antibodies signifying little or no contribution of these factors to the spread of the infection in this part of the world. However, a strong statistical significant relationship ($p < 0.05$) was established between occurrence of both anti-WNV IgM and IgG antibodies and travel history of the study participants. Participants with travel history outside of the country had higher prevalence of both anti-WNV IgM and IgG antibodies compared to their counterparts with no such history. Travel to endemic countries have been recognized as a potent factor for the spread of emerging infections (especially arboviral infections) (Freedman *et al.*, 2006; Chen and Wilson, 2010; Cleton *et al.*, 2012; Cleton *et al.*, 2015; Schlagenhauf *et al.*, 2015). Few studies have investigated the impact of this variable with occurrence of WNV making comparison difficult.

CONCLUSION

This study has reaffirmed the endemicity of WNV in most parts of Nigeria including Ilorin and may be responsible for most cases of PUO in the region. The study also showed a strong association between both recent/ongoing and past infections and travel history of the participants suggesting that this factor may be the driving force of this infection in the region. There is therefore the need for active surveillance of the infection and screening of febrile patients for WNV to

reduce cases of PUO and proper management of patients.

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