

Research Article

Prevalence of West Nile Virus among Sudanese Participants in Gezira State

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Abstract

West Nile virus (WNV), a mosquito-borne pathogen belonging to the *Flavivirus* genus (family *Flaviviridae*), is primarily responsible for West Nile fever. In rare cases (< 1% of infections), it can lead to West Nile neuroinvasive disease (WNND), which carries an approximate fatality rate of 10%. This cross-sectional study aimed to assess WNV exposure among residents of Gezira State, Sudan, from 2019 to 2022. A total of 400 participants were included, with 5 mL of blood collected from each—2 mL in an EDTA tube and 3 mL in a plain container. Plasma and serum were separated via centrifugation, and WNV-specific IgG and IgM antibodies were detected using ELISA. The results revealed that 62.25% (249 cases) tested positive for WNV-IgG antibodies, while 37.75% (151 cases) were negative. None of the samples showed detectable WNV-IgM antibodies. Statistical analysis indicated no significant association between WNV-IgG seropositivity and age or gender ($p = 0.060$ and $p = 0.117$, respectively). However, significant correlations were observed with marital status and education level ($p < 0.05$). The study highlights the need for ongoing surveillance of WNV and other vector-borne diseases in the region to identify active West Nile Virus infections. Public health efforts should prioritize mosquito control measures, community education, and awareness campaigns about WNV symptoms and prevention.

Introduction

West Nile virus (WNV) is a single-stranded RNA mosquito-borne flavivirus that typically causes mild or subclinical infections in humans. However, in fewer than 1% of cases, it can lead to West Nile neuroinvasive disease (WNND), which has a fatality rate of around 10% [1].

First identified in Uganda in 1937, WNV was later detected in North America in 1999 [2,3]. Since then, the virus has been reported in Europe, Africa, Asia, Australia, and North America [2]. In the U.S., thousands of cases occur annually, peaking in August and September, with occasional outbreaks [2,3]. An unusual outbreak was documented in Ngorban County, South Kordofan, Sudan, between May and August 2002.

WNV is primarily transmitted through the bite of infected mosquitoes, which acquire the virus by feeding on infected birds. In rare cases, transmission can occur through blood transfusions, organ transplants, or from mother to child

during pregnancy, delivery, or breastfeeding [2]. However, the virus does not spread directly from person to person [3]. Individuals over 60 years old and those with underlying health conditions are at higher risk of severe disease. Diagnosis relies on symptoms and blood tests [2].

Factors such as viral virulence, population immunity, and age structure may influence disease severity. While all age groups are susceptible to infection, older adults (>60 years) face a greater risk of neurological complications and death [4,5].

In Sudan, a WNV outbreak affected 31 children (≤ 12 years old) in Ngorban County during a national polio immunization campaign [6]. Additionally, a cross-sectional study among blood donors in Khartoum, 2016 found that 44.4% (40/90) tested positive for WNV IgG antibodies, while 2.2% (2/90) had IgM antibodies, indicating recent exposure [7].

This research aims to investigate the epidemiology of WNV among participants in Gezira State, Sudan, and

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examine the relationship between age, gender, marital status, education level, and WNV infection. The findings may help prevent misdiagnosis (e.g., confusion with malaria or other febrile illnesses) and improve patient outcomes through accurate detection and treatment.

Materials and methods

This was a cross-sectional, laboratory-based study conducted across eight localities in Gezira State, Sudan, from February to December 2022. Participants included both symptomatic and asymptomatic individuals, selected based on predefined inclusion and exclusion criteria.

Sample collection and processing

From each participant, 5 mL of venous blood was aseptically collected: 2 mL in an EDTA tube (for plasma). 3 mL in a plain container (for serum). Plasma was separated immediately by centrifugation (3000 rpm for 5 minutes), while serum was obtained after allowing the blood to clot for 10 minutes, followed by centrifugation. Both plasma and serum samples were transferred into labelled Eppendorf tubes and stored at -20 °C until analysis.

Sample size determination

To ensure statistical reliability, the sample size was calculated to reflect the population proportion with a high confidence interval and minimal error margin. Using standard epidemiological formulas, 384 participants per locality were included to achieve representative results.

Data Collection and Analysis: Structured questionnaires recorded demographics, clinical symptoms, and risk factors. Data were analysed using SPSS (Version 22), with results interpreted at a 95% confidence level. Ethical approval was obtained from the Faculty of Medical Laboratory Sciences, University of Gezira, and the Gezira State Ministry of Health. Written informed consent was secured from all participants, ensuring confidentiality and restricted data usage.

Serological Testing (ELISA)

The study employed a semi-quantitative ELISA kit (EUROIMMUN) to detect WNV-specific IgM/IgG antibodies in serum/plasma: Antigen Binding: Samples were incubated in wells coated with WNV antigens; positive samples formed antigen-antibody complexes.

Detection: An enzyme-labeled anti-human IgM/IgG conjugate was added, producing a colorimetric reaction.

Interpretation: Optical density (OD) was measured, and results were expressed as a ratio (sample OD/calibrator OD). Cut-off values (EUROIMMUN criteria): Negative: Ratio < 0.8, borderline: Ratio 0.8–1.1, and positive: Ratio ≥ 1.1.

Results

Was performed to examine the WNV-IgG and IgM antibodies among blood samples collected from 400 Sudanese participants in Gezira state. 62.3% of the samples were positive, while 37.7% of the samples were negative for WNV-IgG antibodies, and 97% of the samples were negative, while 3.0% of the samples were borderline for WNV-IgM (Table 1 and Figure 1).

Based on the age distribution, the sample predominantly consisted of young to middle-aged adults, with nearly half (47.3%) falling within the 20-39 years age group, followed by those in the 1-19 years age group (34.0%). Middle-aged participants aged 40-59 years represented 16.0% of the sample, and elderly participants aged 60-80 years constituted the smallest group at 2.8% (Figure 2).

Table 1: The characteristics and patterns of WNV IgG and IgM antibodies in the study population.

WNV antibodies (n = 400)		Frequency	Percent
IgG antibodies	Negative	151	37.7
	Positive	249	62.3
	Borderline	0	0.0
IgM antibodies	Negative	388	97.0
	Positive	0	0.0
	Borderline	12	3.0

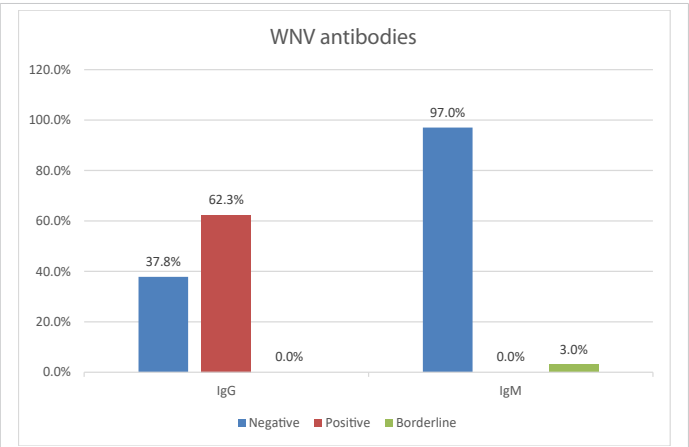


Figure 1: The characteristics and patterns of WNV IgG and IgM antibodies in the study population.

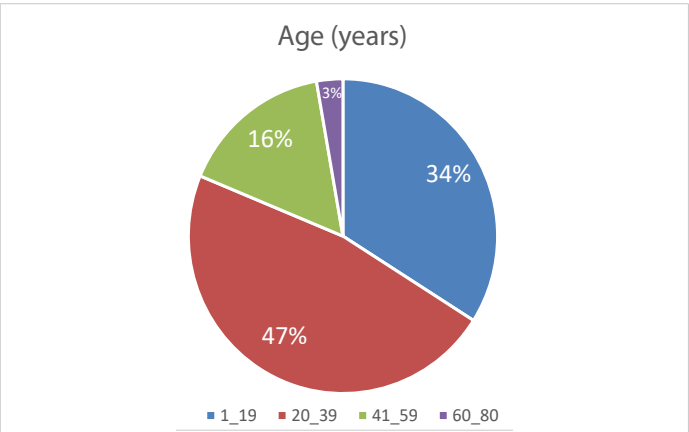
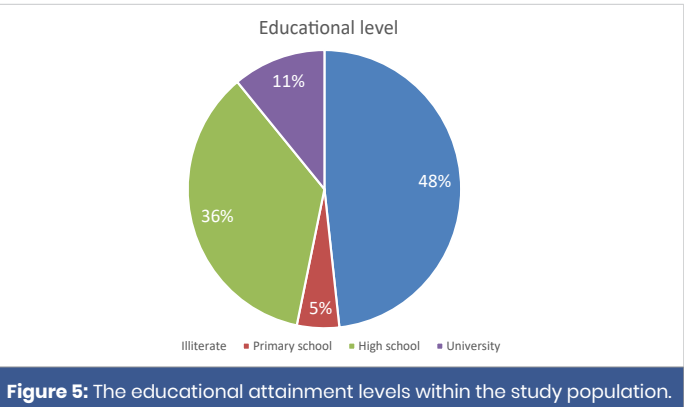
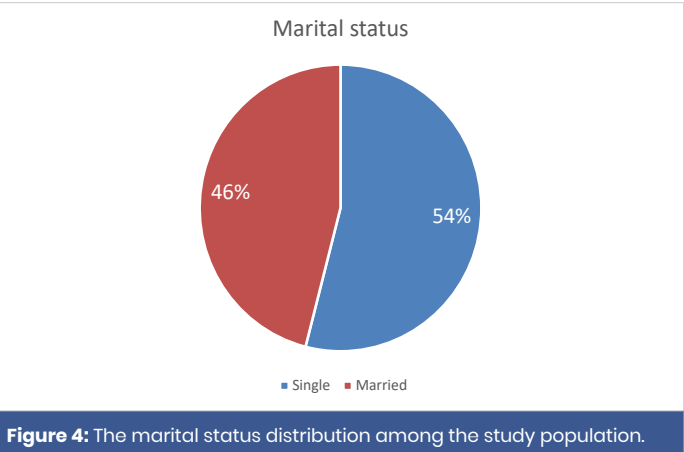
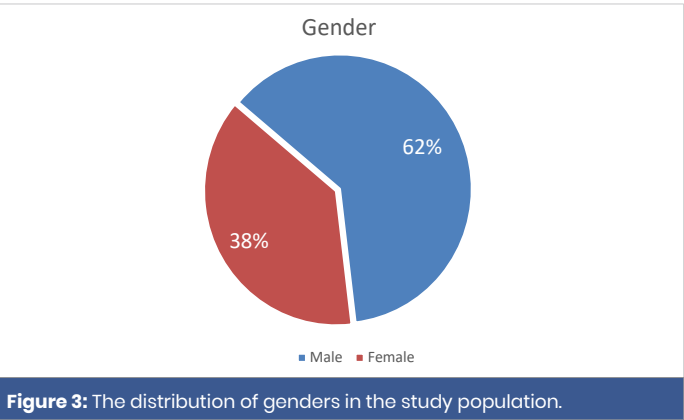


Figure 2: The age distribution within the study population.



The participant’s gender was demonstrated in Figure 3, which showed that 62.0 % of subjects were male and 38.0% were female. The population had their marital status as demonstrated in Figure 4, which showed that 46% of the participants were married, and 54% of the participants were single. The Education level of the study population is presented in Figure 5. Most of the participants are Illiterate (48.0%), followed by High school graduates (36.0%), followed by Primary school (5%), and University graduates (11%).

Table 2 presented the association between demographic variables and the presence of WNV-IgG antibodies. It was found that no significant association between age and gender with WNV-IgG positivity ($p = 0.060$ and $p = 0.117$,



respectively). The educational level variable showed a strong association with WNV-IgG antibodies, with a significant p - value of <0.001 . Those with no formal education (illiterate) had a higher proportion of negative WNV-IgG antibodies (63.6%), while higher education levels, such as university, had higher positive antibody proportions, marital status has significantly associated with WNV-IgG antibodies, with 62.2% of singles showing positive results compared to 37.8% of married individuals, with a highly significant p - value of <0.001 .

Table 3 analyzes the relationship between WNV-IgM antibodies and demographic variables, including age, gender, educational level, and marital status. No significant association was found between WNV-IgM serostatus and age ($p = 0.773$), gender ($p = 0.385$), educational level ($p = 0.679$), or marital status ($p = 0.384$).

Discussion

The research conducted in Gezira State, Sudan, from February to December 2022, monitored the prevalence of West Nile Virus among Sudanese patients. As it is known, the diagnosis of WNV depends on serological testing by

Table 2: The correlation between WNV-IgG antibodies and population characteristics.

Demographic Variables		WNV-IgG antibodies				Chi-Square	p value
		Negative (n = 151)		Positive (n = 249)			
		n	%	N	%		
Age	1-19 (n = 136)	39	25.8%	97	39.0%	7.408	0.060
	20-39 (n = 189)	81	53.6%	108	43.4%		
	40-59 (n = 64)	27	17.9%	37	14.9%		
	60-80 (n = 11)	4	2.6%	7	2.8%		
Gender	Male (n = 248)	101	66.9%	147	59.0%	2.459	0.117
	Female (n = 152)	50	33.1%	102	41.0%		
Educational level	Illiterate (n = 193)	96	63.6%	97	39.0%	23.352	<.001**
	Primary school (n = 20)	4	2.6%	16	6.4%		
	High school (n = 143)	40	26.5%	103	41.4%		
	University (n = 44)	11	7.3%	33	13.3%		
Marital status	Single (n = 216)	61	40.4%	155	62.2%	18.69 ^a	<.001**
	Married (n = 184)	90	59.6%	94	37.8%		

**..significant at the 0.01 level

**..significant at the 0.01 level

Table 3: The correlation between WNV-IgM antibodies and population characteristics.

Demographic Variables		WNV-IgM antibodies				Chi-Square	p value
		Negative (n = 388)		Borderline (n = 12)			
		n	%	N	%		
Age	1-19 (n = 136)	132	34.0%	4	33.3%	1.118	0.773
	20-39 (n = 189)	182	46.9%	7	58.3%		
	40-59 (n = 64)	63	16.2%	1	8.3%		
	60-80 (n = 11)	11	2.8%	0	0.0%		
Gender	Male (n = 248)	242	62.4%	6	50.0%	0.756	0.385
	Female (n = 152)	146	37.6%	6	50.0%		
Educational level	Illiterate (n = 193)	188	48.5%	5	41.7%	1.512	0.679
	Primary school (n = 20)	20	5.2%	0	0.0%		
	High school (n = 143)	137	35.3%	6	50.0%		
	University (n = 44)	43	11.1%	1	8.3%		
Marital status	Single (n = 216)	211	54.4%	5	41.7%	0.758	0.384
	Married (n = 184)	177	45.6%	7	58.3%		



using the ELISA technique to detect WNV-IgG or WNV-IgM antibodies. The sample population included 400 samples to study the relationship between age, gender, marital status, and education level, and the occurrence of WNV-IgG or WNV-IgM antibodies among the participants.

The serological detection by using WNV-IgG antibodies showed that 249 cases (62.25%) were positive, and 151 cases (37.75%) were negative. While all samples are negative for WNV-IgM antibodies, 12 of them were borderline. The pooled IgG seroprevalence of West Nile Virus was high (62.3%) in the samples. The high rate of IgG positivity suggests a significant portion of the sampled population has been exposed to the West Nile Virus at some point in their lives. IgG antibodies indicate a past infection or vaccination response.

IgM is typically used to identify recent infections, and its presence would indicate ongoing infection. This could mean that there was no recent transmission of the virus in the region at the time of the study or that ongoing cases were not detected among the 400 individuals sampled.

According to age group, the most positive cases (43.4%) age group 20-39, followed by the age group 1-19 years (39%). This result is consistent with the report of the WHO [3], which found that the incidence of WNV is high in children. The finding in this study also matches what that found by Yasir and Eltayib [7], who found the high incidence of WNV at the age of 28-37 years, while the incidence at the age groups 40-59 and 60-80 is 14.9% and 2.8 % respectively. Generally, in endemic countries, children usually present mild symptoms when infected with WNV, whereas a large proportion of the adult population has neutralizing antibodies [8-10].

There was no significant correlation between gender and the occurrence of WNV-IgG antibodies, $p > 0.05$. However, there was a significant correlation between the marital status and level of education and occurrence of WNV-IgG antibodies, $p < 0.05$. Necessarily, there is no correlation between the age groups, gender, marital status, and level of education and occurrence of WNV-IgM antibodies, IgM $p > 0.05$.

In the diagnosis of WNV using RT-PCR, the WNV was not detected among all study populations; 400 samples were negative (100%). This is due to the status of the patients, as most of them were in the chronic stage of infection (no active virus). Estimates differ from those reported in a previous study conducted in Africa, which indicated pooled seroprevalences of 70.3% for West Nile virus IgG in Sudan, and like another study in blood donors in Sudan, 2022, which showed 67(74.4%) of participants had positive WNV IgG. This study showed that the illiterate were more likely to have and transfer the infection, in which they showed (48.25%) positive WNV IgG. Also, the infection appears in males (147) from (248) than females (102) from (152) may

be due to the total number of males being more than females. Although the West Nile Virus is increasingly widespread in SUDAN, particularly in Gezira state, there is still a lack of clarity regarding its extent and distribution due to limited surveillance and inadequate diagnostic tests.

Conclusion

This study aims to monitor the prevalence of West Nile virus among Sudanese participants in Gezira State by serological and molecular methods. The specific objectives were to study the relationship between age, gender, and WNV infection. To estimate the prevalence of WNV among Sudanese.

Recommendations

These findings underline the necessity for continuous surveillance of WNV and other vector-borne diseases in the region to find active WNV infections. Public health initiatives could focus on preventive measures, education about mosquito control, and awareness campaigns regarding the symptoms of WNV.

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