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RESEARCH ARTICLE

PREVALENCE OF WEST NILE VIRUS (WNV) AMONG MULTI BLOOD TRANSFUSED PATIENTS IN KHARTOUM STATE, SUDAN

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ABSTRACT

Background: This study was carried out to determine the prevalence of WNV IgG and IgM among multi blood transfused patients in Khartoum, State Sudan.
Material and Methods: Ninety one sera samples were collected from multi blood transfused patients from Khartoum teaching Hospital during the period from August to November 2013 and subjected to Enzyme Linked Immunosorbent Assay (ELISA) to detect WNV IgG and IgM antibodies.
Results: Out of ninety one 50(54%) were positive for WNV IgG antibodies, while 11(12.1)% were positive for WNV IgM antibodies.
Conclusions: In conclusion, the incidence of WNV among multi blood transfused patients in Khartoum State, Sudan was documented through detection of WNV-specific IgG and IgM antibodies. Further study using various diagnostic methods should be considered to determine the prevalence of WNV disease at the national level.

INTRODUCTION

The West Nile virus (WNV) was first isolated in 1937 from the blood of an infected woman in Uganda. (Smithburn *et al.,* 1940) WNV a 50 nm icosahedral, enveloped, ssRNA virus that is a member of Flaviviridae family from the genus *Flavivirus* that belongs to the Japanese encephalitis virus (JEV) serogroup1. (Mackenzie *et al.,* 2002) Other closely related flaviviruses include yellow fever (YF) virus and dengue virus types 1 to 4. (Centre for Diseases Prevention and Control 2012) West Nile virus (WNV) is most widely spread flavivirus in temperate areas, it has been isolated in parts of Europe, Middle East, Africa, Asia, America and Australia. Migratory birds are responsible for dispersal of the virus. (Hayes *et al.,* 2005; Reiter 2010; Kilpatrick 2011) The most common route of transmission of WNV to human is through the bite of an infected culicine mosquito. (Turell *et al.,* 2001) Transmission of WNV has been documented to occur via red blood cell, plasma and platelet transfusions. (Centers for Disease Control and Prevention 2002) Organ Transplantation, Breast-feeding, laboratory acquisition and transplacental transmission also documented. (Centers for Disease Control and Prevention 2002) Serological studies have shown that the majority of infections (approximately 80%) remain asymptomatic. (Campbell *et al.,* 2002; Hayes and Gubler 2006) Only about 1% of infected persons become seriously ill with neurological symptoms (meningitis, encephalitis, paresis or paralysis with poliomyelitis-like symptoms (Kramer *et al.,* 2007) Immunocompromised patients have a higher risk of more severe course of disease. Approximately 40–60% of immunosuppressed patients developed severe neurological disease as a result of WNV infection.

West Nile virus–specific IgM can be detected in serum or CSF by IgM capture ELISA in 90% of patients either, or after the eighth day of illness. Once IgM antibodies appear it may persist for more than 6 months after illness and as long as 500 days in most of the patients. West Nile virus–specific IgG antibodies appear by the seventh day of illness, which can be detected by 3 weeks after infection in most patients. In acute infection, IgG titers should increase between day 7 and 21. (Campbell *et al.,* 2002) Detection of WNV IgM in serum represents probable WNV infection, whereas the detection WNV IgM in cerebrospinal fluid is considered diagnostic of central nervous system involvement by WNV. (Marfin and Gubler 2001; Tardei *et al.,* 2000) There are several types of serological tests routinely used for WNV diagnosis; The gold standard WNV serological test is still the plaque reduction neutralization test, and The micro-virus neutralization test (micro-VNT) which is a modification of the PRNT. (Lindsey *et al.,* 1976; OIE West Nile Fever 2008) ELISAs are preferred screening tools because of their rapidity, sensitivity, reproducibility and affordability. Three different assays are commonly used; The competitive ELISA, the indirect IgG

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ELISA, and the IgM antibody-capture (MAC) ELISA. (Tardei *et al.,* 2000)

Due to very low viraemia at the time of clinical onset, nucleic acid detection methods and WNV culture are not useful diagnostic tools. (Lanciotti *et al.,* 2000; Marfin and Gubler 2001) An outbreak of acute febrile illness occurred during August and September 1989 in the Northern Province of Sudan. The prevalence of IgG antibody was 59% for West Nile IgG AND 5%FOR IgM (Watts *et al.,* 1994) An atypical outbreak of West Nile virus (WNV) occurred in Nuba Mountains, Sudan, from May to August 2002 during it Blood samples of 3 children were examined: eight were cases with neurological sequelae, five were convalescent and 17 were controls. Seven of the eight children (87.5%) with neurological sequelae were positive for blood IgM and IgG of West Nile, one blood sample showed signs of recent infection, and all others were negative for WNV. (Evelyn Depoortere *et al.,* 2004)

Blood transfusion--associated transmission (TAT) of West Nile virus (WNV) in the United States was first identified in 2002 (Pealer *et al.,* 2003) In 2003, blood collection agencies (BCAs) responded by screening donations for WNV by using nucleic acid--amplification tests (NATs) (CDC. West Nile virus activity 2003) In 2003, blood-donation screening for WNV resulted in the impounding of approximately 800 blood components potentially containing WNV. However, six reported cases of transfusion-associated WNV disease were associated with units of blood components with viral concentrations too small to be detected by minipool NAT (CDC. Epidemic/Epizootic West Nile virus in the United States 2003).

MATERIALS AND METHODS

Data collection

The collected data through a questionnaire included, gender, age, and number of blood transfusions, clinical symptoms and place of samples collection.

Inclusion criteria and sample collection

A total number of 91 blood samples were collected from groups of patients included sicklier, leukemic, A plastic anemia and platelets disorders who received more than two bags of blood from Khartoum Hospital between August to November 2013, Blood samples (5ml) in EDTA, were collected from the cubital vein and then centrifuged at 4000 rpm for 5 minutes to obtain the serum. The sera was taken immediately and stored at -20 ˚C until used.

Serology

Capture ELISA for IgM The enzyme-linked immunosorbent assay was used to detect the specific WNV IgM antibodies. Commercial ELISA Kits (Panbio, Australia) were used as described by the manufactures. In brief, 100 µl of the diluted plasma was incubated in microplate wells coated with goat anti-human IgM incubate for 1h. Subsequently, the wells were washed (six times), and WNV antigen –MAb tracer that mixed and incubate for 1h before using was added. To minimize unspecific reactivity, control antigen consisting of uninfected cellular components is added to the conjugate. After another washing step to eliminate unbound material, a solution of enzyme substrate and chromogen is added. The blue colour changed to yellow after adding of stop solution (100µl). Results were read at 450 nm as indicated by the manufacturer. Index values for the patient samples and controls were obtained by dividing the absorbance of the patient or control well by the absorbance of the calibrator.

Indirect ELISA for Ig G

The enzyme-linked immunosorbent assay was used to detect the specific WNV IgG antibodies. Commercial ELISA Kits (panbio, Australia) were used as described by the manufactures. In brief, 100 µl of the diluted plasma (1:101) was incubated in microplate well coated with WNV antigen at 37 ºC for 1 hr. The well was then washed three times (350 µl washing solution) to remove residual plasma, and enzyme-labelled antibodies to human IgG conjugate were added (300 µl + 15 ml conjugate diluents) then incubated at 37 ºC for 30 minutes. After another washing step to eliminate unbound material; an enzyme substrate solution (280 µl of chromogen TMB + 14 ml substrate buffer) was added (100 µl /well). The blue colour changed to yellow after adding of the stop solution (100 µl), the final reaction product was measured in a spectrophotometer at a wavelength of 450 nm. An index value was obtained for both control and patient samples by dividing the absorbance value of the patients and controls by the absorbance value of the calibrator (cutoff control).

RESULTS

Out of Ninety one multi blood transfused patients 50 (54%) were found to be ELISA positive for WNV IgG antibodies, and 11(12.1) % were positive for WNV IgM antibodies. The age of the study populations between 5 to55 years old demonstrated in (Table 1).WNV IgG antibodies were highest in age group from 46-55 years old (90%) ,high prevelant of WNV IgM show in age from 5-15 years old (43.7%) Table (1). The majority of populations were male (55%). WNV infection was insignificantly associated with disease groups (P>0.05), The frequency and results of WNV seroprevelance among

Table 1. Comparison between ELISA IgG and IgM, for the detection of WNV in sera samples collected among multi blood transfused patients (age groups) in Khartoum State, Sudan (2013)

Age Group (years)	ELISA IgG		ELISA IgM		Total
	Positive	Negative	Positive	Negative	
5-15 year	26(55.3%)	21(43.7%)	7(14.9%)	40(85.1%)	47
16-25	6(40%)	9(60%)	2(13.3%)	13(86.7%)	15
26-35	4(50%)	4(50%)	1(12.5%)	7(87.5%)	8
36-45	5(45.5%)	6(54.5%)	1(9.1%)	10(90.9%)	11
46-55	9(90%)	1(10%)	0(0%)	10(100%)	10
Total	50(54%)	41(45%)	11(12.1%)	80(87.9%)	91

Table 2. Comparison between ELISA IgG and IgM, for the detection of WNV in sera samples collected from multi blood transfused patients (disease groups) in Khartoum State, Sudan

Disease Groups	Frequency	ELISA IgG		ELISA IgM	
		Positive	Negative	Positive	Negative
sicke cell anaemia	48	26(54.2%)	22(45.8%)	10(20,8%)	38(79.1%)
A plastic anaemia	13	4(30.7%)	9(70.3%)	0(0%)	13(100%)
CRF	15	11(73.3%)	4(26.7%)	1(6.7%)	14(93.3%)
Leukaemia	10	5(50%)	5(50%)	0(0%)	10(100%)
pH disorder	5	4(80%)	1(20%)	0(0%)	5(100%)
Total	91	50(54.9%)	41(45%)	11(12%)	80(87.9%)

Table 3. Comparison of ELISA IgG and IgM, for the detection of WNV in sera samples collected from multi blood transfused patients (Male & Female) in Khartoum State, Sudan

Gender	ELISA IgG		ELISA IgM	
	Positive	Negative	Positive	Negative
Male	31(62%)	24(58.5%)	8(72.7%)	47(58.8%)
Female	19(38%)	17(41.5%)	3(27.3)	33(41.2)
Total	50	41	11	80

disease groups was summarized in Table (2). WNV IgG and WNV IgM was significantly higher in male (62%, 72% respectively) than in females (38%, 27% respectively) as show in Table (3).

DISCUSSION

West Nile virus (WNV) is a widespread re-emerging pathogen That con cause severe neurological symptoms specially in immunosuppressed patients. The present study was the first survey of WNV seroprevalence in multi blood transfuse patient in Khartoum State. This study revealed that the prevalence of WNV in multi blood transfuse patient is very high, anti- WNV IgG antibodies was 54% of the cases, while 12% of the subjects tested positive for anti- WNV IgM. There are no published studies in the post transfused setting in sudan and thus, we can only compare our results to past-outbreak seroprevalence studies. Previously seroprevalence rates for WNV IgG antibody was 59% and5% for IgM were reported for 185 febrile individuals from Northern Province of Sudan by

Watts *et al*. (1989)

In this study seroprevalenc was not significantly associated with disease group, and time of donation of blood components**.** No vaccine exists to prevent WNV infection in humans. Vector control and health education on how to preventmosquito bites and screening blood bag are the only preventive methods available. In June 2003, blood-collection agencies (BCAs) implemented investigational WNV nucleic acid--amplification tests (NATs) to screen all blood donations and identify potentially infectious donations for quarantine and retrieval (CDC. West Nile virus activity 2003)

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