

Prevalence and Molecular Detection of West Nile Virus (WNV) Among Renal Transplant Patients in Khartoum State, Sudan

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ABSTRACT

Background:-

West Nile virus (WNV) is an arbovirus from the Flaviviridae family .West Nile now represents one of the most common arboviral diseases worldwide that causes febrile illness. Also, significant number of patients develop severe neurological disease including meningitis , encephalitis ,and acute paralysis . This study was carried out to detect the frequency of West Nile virus IgM antibodies and virus nucleic acid among renal transplant patients in Khartoum state.

Methods:-

This was a descriptive study in which serum specimens were collected from 93 patient (68 male,25 female) and investigated for WNV specific immunoglobulin M(IgM) using enzyme- linked immunosorbant assay(ELISA) and for WNV RNA using real time PCR (RT-PCR) The study group age ranged from 20 to 80 years old.

Result:-

Out of the 93 patients tested, 7(7.5%) were positive for IgM and 86 (92.4%) were negative and no positive RT-PCR results were recorded.

Conclusion:-

The frequency of West Nile virus among renal transplant patients in Khartoum State ,Sudan was documented through detection of specific IgM antibodies

Keyword:-West Nile Viruses (WNV), IgM .ELISA and Real-Time PCR.

I. INTRODUCTION

West Nile virus (WNV) is an arthropod transmitted virus (arbovirus) from the *Flaviviridae* family ¹. It is closely related to a group of viruses that cause disease around the globe such as dengue fever, yellow fever, Japanese encephalitis and tick-borne encephalitis ². WNV infection now represents one of the most common arboviral diseases worldwide ³. Although

most individuals with WNV Infection are asymptomatic, a significant number of patients develop severe neurological disease, including meningitis, encephalitis, and acute flaccid paralysis. Smithburn et al published the first report of neurotropic WNV infection in 1940 and isolated the virus from the blood of a woman with fever residing in the West Nile district of Uganda ⁴. Subsequently, the virus became recognized as a cause of meningitis

and encephalitis in elderly patients in Israel in the 1950s. Epidemics of WNV infection have been reported in many countries, including South Africa, France, Romania, India and Indonesia. In endemic areas like Egypt, a 40% WNV seroprevalence rate has been described⁵. From 1937 until 1999, West Nile virus (WNV) garnered scant medical attention as a cause of febrile illness and sporadic encephalitis in parts of Africa, Asia, and Europe. After the surprising detection of WNV in New York City in 1999, the virus has spread dramatically westward across the United States, southward into Central America and the Caribbean, and northward into Canada, resulting in the largest epidemics of neuroinvasive WNV disease ever reported. From 1999 to 2004, >7,000 neuroinvasive WNV disease cases were reported in the United States. In 2002, WNV transmission through blood transfusion and organ transplantation was described for the first time, intrauterine transmission was first documented, and possible transmission through breast feeding was reported. This highlighted new information regarding the epidemiology and dynamics of WNV transmission, providing a new platform for further research into preventing and controlling WNV disease. The disease is transmitted to humans by the bite of infected mosquitoes. Birds act as amplifying hosts and infect mosquitoes, which then transmit disease to other birds. Humans, horses, and other non avian vertebrates are incidental hosts. *Culex* mosquitoes are the principal WNV vectors, but other mosquito species have been demonstrated as WNV carriers. Most non-avian species, including humans infected with WNV do not generally contribute to viral spread. This is because most develop transient and insufficient viremia to infect mosquitoes and contribute to the virus's cycle in nature⁶. A possible dialysis-related and blood transfusion transmission of WNV was also recorded in USA. The present study aimed to investigate the seroprevalence of West Nile Virus among hemodialysis patients in Khartoum State, Sudan.

II. MATERIAL AND METHODOLOGY

Study area

The study was conducted in Khartoum State during the period August to November 2016.

Patients criteria

Renal transplant recipients, who were not treated with antiviral therapy, were recruited into this study. These participants were recruited through the Ibin Sina Hospital and Dr. Salma Center for Transplantation and Haemodialysis, Khartoum State, between August and November 2016.

Blood Sample Collection:

Blood samples were collected from 93 renal recipient patients with age ranging from 20 years to 80 years old. Blood (2-5 ml) was collected in plain containers and transported on wet ice to the laboratory for immediate processing. Sera were separated from blood samples by centrifugation for 5 minutes at 3000 RPM. Obtained sera were used for ELISA and RNA extraction for real-time PCR.

ELISA IgM:

Serum samples were tested for the presence of WNV IgM antibodies using commercially available ELISA kits (EUROIMMUN, Germany), according to the manufacturer's instruction.

Real time PCR:

Real-time one step RT-PCR was done to detect viral RNA by using a commercial WNV kit following the manufacturer's instructions (Shanghai ZJ Bio-Tech Co, Ltd, China).

Ethical Approval

The study has been approved by the local ethics committee of Alneelain University. All participants in the study were given a written informed consent considering the aims of the study. Sample and clinical information were used anonymously.

RESULT:

A total of 93 renal transplant patients were enrolled in this study. Their ages ranged from 20 to 80 years. The results revealed that 7 patients (7.5%) were positive for WNV IgM while 86 (92.4%) were negative . Three (42.8%) of the 7 positive patients were females and 4 (57.1%) were males.

None of the 93 patients was proved to be positive for WNV RNA using RT-PCR.

ELISA WNV IGM

Table 1. results of IgM Elisa in 93 renal transplant patients

Group test	No	Positive result	Negative result
Renal transplant patient	93	7(7.5%) Males: 3(57.1%) Females: 4 (42.8%)	86(92.4%)

Table 2. Results of Rt-PCR in 93 renal transplant patients

Group test	No	Positive	Negative
Renal transplant patient	93	0(0%)	93 (100%)

III. DISCUSSION

Most human infections with WNV are asymptomatic and symptomatic infections may vary from flu-like malaise to serious neuroinvasive disease for which there is no specific treatment. Fewer than 1% of human infection progress to severe disease for which the most reported risk factors include advanced age, immune suppression, chronic medical conditions such as hyper tension, diabetes and chronic renal failure¹⁰. Earlier studies investigating prevalence of WNV antibodies in Sudan indicated seroprevalences that ranged between 54% - 63.7% for IgG and 12.1% -

15.4% for IgM among different populations (^{7,8}). The aim of this study was to determine prevalence of WNV using IgM ELISA and Real-time PCR among renal transplant recipients in Khartoum s State.

The results showed that WNV IgM antibodies were found in 7(7.5%) patients but none was positive for virus RNA (0%) in the tested samples.

Several incidences of WNV infection in solid organ transplant patients have been reported in the literature^{10,11,12,13} . In these cases, WNV infection was associated with a high incidence of neuroinvasiveness and severe morbidity and mortality (30%). The time to onset of symptomatic WNV infection was 13 days after

transplantation (range 5–37 days) with initial unexplained fever that did not respond to antibiotic therapy followed by rapid onset of neurologic deficits. Confirmation of WNV infection in these cases was made by testing serum and CSF for both WNV RNA by RT-PCR and WNV IgM by serological assays¹⁴.

Our present results showed that WNV IgM antibodies were found in 7(7.5%) and none was positive for virus RNA (0%) .In addition none of our patients showed fever or other severe symptoms of infection described in the cases mentioned above .Hence IgM sero-conversion in some of our test group most probably represent asymptomatic recent infection acquired through mosquito bites.

The detection of IgM in some of the patients in this study also indicates WNV activity in the area.

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