Serofrequency of West Nile Virus among Hemodialysis Patients in Khartoum State

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Abstract:
Background: West Nile virus (WNV) is an arbo virus from Flaviviridae family. West Nile virus infection now represent one of the most common arboviral disease worldwide, significant number of patients develop sever neurological disease including meningitis, encephalitis, and acute paralysis. The hemodialysis patients is consider immunocompromised patients and dialysis can transmitted West Nile virus and causes severe illness. This study was carried out to detect the frequency of West Nile virus IgG antibodies among hemodialysis patients in Khartoum state and healthy individuals as control.

Method: this was descriptive study, in which serum specimens were collected from 90 participants 45 hemodialysis patients and 45 healthy individuals and analyzed by ELISA for WNV IgG antibodies and the result were correlated with age, gender, blood transfusion, number of dialysis per week, duration of dialysis per years, the study group age ranged from 25 to 76 years with 46 years mean for both group. out of them 29 were males and 14 females for both group. And generated data were analyzed by use SPSS.

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**Result:** Out of the total 90 participants from hemodialysis patients 10 (22.2%) were positive for IgG and 35 (77.8%) were negative while among healthy control group 6 (13.3) were positive and 39 (86.7) negative, statistical analysis showed insignificant association between seropositivity of West Nile virus and history of blood transfusion, age, gender, number of dialysis per week, and duration of dialysis per years.

**Conclusion:** The frequency of West Nile virus among hemodialysis patients in Khartoum state – Sudan was documented through detection of specific IgG antibodies.

**Key words:** West Nile Viruses (WNV), IgG .ELISA .hemodialysis patients, Khartoum, serofrequency

**Introduction:**

West Nile virus (WNV) is an arbovirus (arthropod transmitted virus) 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. Smith burn 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 described \(^5\). From 1937 until 1999, West Nile virus (WNV) garnered scant medical attention as the 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 breastfeeding was reported. This review highlights new information regarding the epidemiology and dynamics of WNV transmission, providing a new platform for further research into preventing and controlling WNV disease. West Nile virus (WNV) was first detected in the Western Hemisphere in 1999 during an outbreak of encephalitis in New York City. Over the next 5 years, the virus spread across the continental United States as well as north into Canada, and southward into the Caribbean Islands and Latin America. 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, are referred to as dead-end hosts and 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 nature. Other rare modalities of transmission that have been reported include transplacental transmission, breast-feeding, blood transfusion, organ transplantation, and percutaneous inoculation. A possible dialysis-related transmission of WNV was considered in Georgia division of public health was notified of two patients from the same country with confirmed West Nile virus disease who had received hemodialysis on the same day and on
the same dialysis machine. The two dialysis pt (pations A AND B) had the only confirmed cases of human WNV disease reported in their country in 2003. Review of dialysis center records indicated that another pt (pt B) had received dialysis on the same machine between this two pt on the same day. This report summarizes result of the epidemiologic investigation, which suggested That WNV might have been transmitted at the dialysis center9. this study aimed to seroprevalence of West Nile Virus among hemodialysis patients in Khartoum state – Sudan.

Material and method:

Design:
The present study was descriptive cross sectional study in which consenting hemodialysis patients and healthy control individuals at Khartoum state were enrolled in this study

Subject selection:
Patients receiving hemodialysis for test group and Healthy individuals' age of 18 – 50 for control group. During April to June 2015, were included in this study, ethical clearance was obtained from AL Neelain University Ethical board

Experimental work:

Collection of specimens:
The study was carried out in AL Neelain University Faculty of Medical Laboratory Sciences Research lab. Blood specimens were collected from a total of 90 individuals, 45 patients receiving hemodialysis and 45 healthy control. Specimens were collected and drawn in container contain no anticoagulant, centrifuged. Serum samples were then separated and kept on the ice compartment of the refrigerator, and immediately were stored at -20°C.
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Processing of specimens:
Serum samples were analyzed using Enzyme-linked immune sorbent assay kits (for anti-West Nile Virus (IgG)). Positive and -negative control was used, and the ELISA kits tested (Euroimmun, Germany) within analyzing the serum samples. The reagent and samples were allowed to reach the room temperature for at least 15-30 minutes. The washer buffer concentrate was checked for the presence of salt crystals. The washer buffer diluted 1 to 9 with distilled water. The strips needed were set in strip-holder and numbered sufficient number of wells including one negative control one positive control and three calibrators. The specimens were diluted with sample buffer well mixed by vortexing and incubated for 10 minutes at room temperature. The controls and calibrators are ready to use as supplied. 100μl of samples were added in to each well and 100μl from the three calibrators, positive and negative controls were added in to their appropriate wells. A separated disposal pipette tip was used for each specimen, Negative and positive controls as to avoid cross-contamination. The plate was covered with the plate cover and incubated for 60 minutes at 37°C. At the ends of the incubation the plate cover was removed and discard, then washed each well automatically 3 times with 450μl working strength wash buffer. Each time the micro wells were allowed to soaked for 30-60 seconds. After the final washing cycle, the plate was blotted on to a clean towel, to remove any remaining buffer. 100μl of peroxidase – labelled anti- human IgG were added in to each well. The plate was covered with the plate cover and incubated for 30 minutes at 25°C. Then the plate cover was removed and washed each well 3 times with 450μl working strength wash buffer. After the final washing cycle, the plate was blotted on to a clean towel, to remove any remaining buffer. 100μl of chromogen/substrate solution were added in to each well including the Blank, then was Incubated at 25°C for 15 minutes. 100μl of Stop solution was added in to each wells in the same order and the same
speed as the chromogen/substrate solution was introduced. The plate reader was calibrated with the calibrator well and read the absorbance at 450nm.

**Measurement:**
Photometric measurement of color intensity was be made at 450 wavelength within 30 minutes of added stop solution and the samples were shook before measuring.

**Calculation of results and interpretation:**
Results were calculated quantitatively from standard curve obtained by point to point plotting of the extinction value measured for the 3 calibration sera against the corresponding units (liner/liner).

- **Ratio<16 RU/ml:** negative
- **Ratio≥ 16 to <22 RU/ml:** borderline
- **Ratio ≥ 22 RU/ml:** positive

**Data analysis:**
The data retrieved from the questionnaires were analyzed using the Statistical Package for Social Sciences (SPSS) version 16 software program. The serofrequency of anti-West Nile Virus (IgG), gender, age, history of blood transfusion. Duration of dialysis history and times of dialysis session per week were analyzed among symptomatic patients. The degree of association of serofrequency (IgG) with gender, age, duration of dialysis history and times of dialysis session per week were determined using Statistical significance of p-value of less than or equal to 0.05 (p-value ≤ 0.05).
Result:

A total of 90 participants, 45 healthy control and 45 renal hemodialysis patients were enrolled in this study. Their ages ranged from 25 to 76 years, with 46.64 years mean for both group. Most of them were group O+ve, 29 were males, and 14 females for both group. The results revealed that the serofrequecy of WNV is 10 (22.2%) positive and 35 (77.8%) negative in patients under dialysis, while in healthy control group it was 6 (13.3) (table1), statistical analysis showed insignificant association between West Nile Virus and gender, age, duration of dialysis per years, number of dialysis per week and history of blood transfusion, however it was significant between serofrequency among hemodialysis patients Compared with control group.

<table>
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<th>Test</th>
<th>No</th>
<th>Positive result</th>
<th>Negative result</th>
<th>Total</th>
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<td>Hemodialysis group</td>
<td>45</td>
<td>10</td>
<td>(22.2%)</td>
<td>35 (77.8%)</td>
<td>45</td>
</tr>
<tr>
<td>Control group</td>
<td>45</td>
<td>6</td>
<td>(13.3%)</td>
<td>39 (86.7%)</td>
<td>45</td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
<td>16</td>
<td></td>
<td>74</td>
<td>90</td>
</tr>
</tbody>
</table>

Discussion:

Most human infection with West Nile Virus are A symptoms infection 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 sever disease for which the most reported risk factors include advance age, immunosuppression, and chronic medical condition but are not limited to hyper tension diabetes and chronic renal failure. West Nile Virus is wide spread re-emerging pathogen that can cause severe neurological symptoms especially in immune suppressed patient. The present study was the first survey of...
WNV seroprevalence in hemodialysis patients in Khartoum state. This study revealed that the prevalence of WNV in hemodialysis patient is 22.2 in case study, while it was 13.2 in healthy control. There are no published data in this study in Sudan, we can only compare our results to past outbreak seroprevalence studies, but previously seroprevalence rate for WNV IgG antibodies was (59%) and (5) for IgM were recorded for 185 febrile individual from northern province of Sudan by Watts's et al 1989, in thus study seroprevalence was not significant associate disease group and time of donation of blood component, and atypical outbreak of West Nile Virus occurred in Nuba Mountains –Sudan from May to August 2002 during it blood sample of 3 children were examined, eight were cases with neurological sequelae, five were convalescent and 17 were control, seven of the eight children (87.5%) with neurological sequelae were positive for blood IgM and IgM of West Nile Virus\textsuperscript{12}. This cluster of hemodialysis patients with WNV infection suggest possible transmission of WNV in the dialysis center, patients on dialysis are highly susceptible to infection because they often are immunocompromised and exposed routinely to invasive technics and devices. The possibility that WNV might be transmitted during dialysis, underscores the necessity for dialysis facilitators to strictly adhere to proper infection control at all time and must be all blood line attachment to the dialysis machine were disposable and discard after each dialysis session.

**Conclusion:**

In conclusion, frequency of West Nile Virus among hemodialysis patients in Khartoum state –Sudan was documented through detection of WNV specific IgG antibodies. Further study using various diagnostic method should be considered to determine the prevalence of WNV disease at national level.
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REFERENCE:

2. CDC (2012 west Nile Virus encephalomaylaitis intransplant recipients clinical laborities, diagnostic & neuropathological feature.
8. Kadiyala V. Ravindra, Alison G. Freifeld,2,a Andre C. Kalil, David F. Mercer, Wendy J. Grant, Jean F. Botha, Lucile E. Wrenshall, and R. Brian Stevens, West Nile


12. Evelyn Depoortere, Justine Kavle, Kees Keus, Hervé Zeller, Séverine Murri and Dominique Legros, Outbreak of West Nile virus causing severe neurological involvement in children, Nuba Mountains, Sudan, 2002 Tropical Medicine & International Health, June 2004Volume 9, Issue 6, pages 730–736,