Case Report

Encephalomyelitis Resulting from Chronic West Nile Virus Infection: A Case Report

Rafael Rodriguez[1], Liliana Rincon[2], Ahmed Yassin[3], Gerald A. Campbell[2*], Bi-Hung Peng[4], Corey Fulton[5], Juan C. Sarria[6], David H. Walker[2] and Xiang Fang[1*]

[1]Departments of Neurology, University of Texas Medical Branch, Galveston, TX 77555, USA

[2]Pathology, University of Texas Medical Branch, Galveston, TX 77555, USA

[3]Department of Neurology, Jordan University of Science and Technology, Irbid 22110, Jordan

[4]Neuroscience, Cell Biology and Anatomy, University of Texas Medical Branch, Galveston, TX 77555, USA

[5]Microbiology and Immunology, University of Texas Medical Branch, Galveston, TX 77555, USA

[6]Medicine, University of Texas Medical Branch, Galveston, TX 77555, USA

*Correspondence to:

Dr. Xiang Fang, MD, PhD, FAAN, FANA
Department of Neurology
University of Texas Medical Branch
301 University Boulevard Galveston
Texas 77555-0743, USA
Tel: 1-409-772-2870
Fax: 1-409-747-2400
E-mail: xiang.fang01@gmail.com

Dr. Gerald A. Campbell, PhD, MD
Department of Pathology
University of Texas Medical Branch
Galveston, Texas 77555, USA
E-mail: gcampbel@utmb.edu

Abstract

West Nile virus (WNV) is a mosquito-borne RNA flavivirus and human neuropathogen. The overall mortality rate for WNV disease is < 0.1% for all patients infected with WNV. Most WNV-related morbidity and mortality results from neurologic and respiratory involvement. For patients with WNV encephalitis, the mortality rate ranges from 12% to 15% and can range from 10-30% for patients with West Nile neuroinvasive disease. Mortality rates can reach up to 50% for patients presenting with acute flaccid paralysis and respiratory failure.

We report a fatal case of meningoencephalomyelitis due to chronic WNV infection in a 21-year-old male who presented with muscle weakness progressing to ascending paralysis and respiratory distress requiring mechanical ventilatory support. He was diagnosed with acute motor axonal neuropathy, a GBS variant, and had a prolonged hospital course until he died 8 months after the onset of symptoms. Microscopic examination of the central nervous system samples collected during autopsy revealed meningoencephalomyelitis. Subsequent serologic testing revealed antibodies to WNV, and immunohistochemistry detected presumed WNV antigens in neurons in multiple foci in the cerebrum, cerebellum, brainstem and spinal cord, later confirmed by an *in situ* hybridization technique. Myelitis and motor neuron degeneration in the spinal cord with subsequent atrophy in skeletal muscle were also observed.

Our case exemplifies chronic WNV infection causing meningoencephalomyelitis that manifested clinically as acute motor axonal peripheral neuropathy. WNV-associated encephalomyelitis as well as peripheral neuropathy should be considered in patients presenting with muscle weakness and paralysis, especially when occurring in known endemic regions.

Keywords

West Nile virus, Human neuropathogen, Guillain-Barre syndrome, Central nervous system, Paralysis

Case Report

A 21-year-old Hispanic male presented to a hospital in east Texas with a three-month history of progressive ascending muscle weakness requiring intubation, tracheostomy and PEG tube placement in 2013. The weakness

initially involved bilateral lower extremities, and progressively moved upward to involve the bulbar muscles, causing dysphagia, dysarthria and respiratory failure. There was no detectable sensory involvement, urinary incontinence or alteration of consciousness. The patient denied recent travel history, flu-like symptoms, fever, diarrhea, acute illness, vaccinations, or medication intoxication prior to the onset of acute weakness. Neurologic exam revealed weakness of grade 3/5 in upper extremities, 0/5 in lower extremities and global hyporeflexia. Sensory examination was intact. Laboratory analysis revealed neutrophilic leukocytosis (WBC 25,000 per mcL, 80% neutrophils), hyperCKemia (16419 U/L), elevated aldolase (72 U/L) and AST (489 U/L), and thrombocytopenia (platelets 66,000 per mcL). Cerebrospinal fluid (CSF) analysis was unremarkable (WBC 0, protein 37 mg/dL, and glucose 77 mg/dL). CSF infectious work up, including VDRL, fungal and viral cultures, and Lyme western blot, was negative. Initial EMG/NCS revealed acute motor axonal neuropathy, and then repeated EMG revealed generalized acquired sensorimotor polyneuropathy. Serum ganglioside panel, including GM1, GM2, GD1a, GD1b, and GQ1b, was negative, Smooth muscle actin antibodies, c-ANCA, p-ANCA, atypical p-ANCA, Anti-Ro, La, and urine porphobilinogen were negative. Heavy metal toxin screen, including lead, mercury, arsenic, and cadmium, was also negative. Additional infectious work-up including Rocky Mountain spotted fever, human monocytic ehrlichiosis (caused by *Ehrlichia chafeensis*), HIV, syphilis, hepatitis B and C, Lyme disease, leptospirosis, and human granulocyte anaplasmosis, was also negative. The patient had a muscle biopsy which showed muscle atrophy due to denervation, with evidence of reinnervation and mild myopathic changes. Sural nerve biopsy did not show evidence of demyelination. The patient was initially treated with intravenous solumedrol, 1000 mg/day for 3 days, with improvement of muscle strength. His strength continued to improve during his hospitalization, and he was then transferred to a regional medical facility. However, several months later, he developed respiratory distress and died after unsuccessful cardiopulmonary resuscitation. An autopsy was performed. In this case report, we follow HIPAA regulations and no protected health information (PHI) is disclosed.

Pathologic Findings

During the autopsy, aseptically collected lung tissue was sent for culture, sural nerve was dissected, and skeletal muscle sections were obtained for further examination. Blood samples were also collected for serologic testing for WNV. Serological enzyme immunoassay for West Nile IgG and IgM antibodies was performed on post-mortem blood by the Texas Department of State Health Services (DSHS) laboratory. Sections from CNS were formalin fixed and paraffin-embedded. Routine hematoxylin-eosin and myelin (Luxol fast blue) staining was done as well as additional special staining of slow myosin and fast myosin for muscle fiber typing, myelin and toluidine blue staining of nerve sections and immunohistochemical staining (IHC) with anti-WNV antibodies of CNS sections. IHC for WNV was performed using polyclonal immune serum

(gift of Dr. Robert Tesh, University of Texas Medical Branch), and titrated using sections of infected mouse brain (gift of Dr. Tian Wang, University of Texas Medical Branch). Immunostaining employed the standard avidin-biotin peroxidase technique (Vectastain, Vector Labs). Due to concerns about the specificity of the West Nile virus (WNV) antibody used for immunohistochemistry, the findings were later confirmed by an *in situ* hybridization technique. In this procedure, RNA *in situ* hybridization was performed using RNAscope 2.5 (Advanced Cell Diagnostics) using the manufacturer's instructions. Tissue sections were deparaffinized by xylene followed by 100% ethanol. Slides were then boiled in RNAscope Target Retrieval reagents and incubated with RNAscope Protease plus. Endogenous peroxidase activity was quenched using hydrogen peroxide. The probe targeting WNV was created by Advanced Cell Diagnostics (catalog #475091). Positive control tissue (mouse brain acutely infected with WNV) and negative control tissue (human spinal cord from confirmed non-infected) were stained simultaneously, similar to the IHC procedure. Sections were counterstained with Gill's hematoxylin.

Autopsy findings relevant to this presentation include a thick yellow exudate found in the tracheostomy, diffuse consolidations of both lungs, which had increased weights (right lung: 810 gm, left lung: 612 gm) and multiple hemorrhages on the pleural surfaces. Bacterial culture from lung tissue grew *Pseudomonas aeruginosa*, revealing that the immediate cause of death was bronchopneumonia caused by this microorganism. Gross examination of the brain and spinal cord did not reveal any abnormalities. Microscopic examination of the central nervous system samples collected during autopsy revealed meningoencephalomyelitis, see figure 1. Myelitis and motor neuron degeneration in the spinal cord (Figure 2) with subsequent atrophy in skeletal muscle were also observed. Subsequent serologic testing revealed antibodies to WNV, and
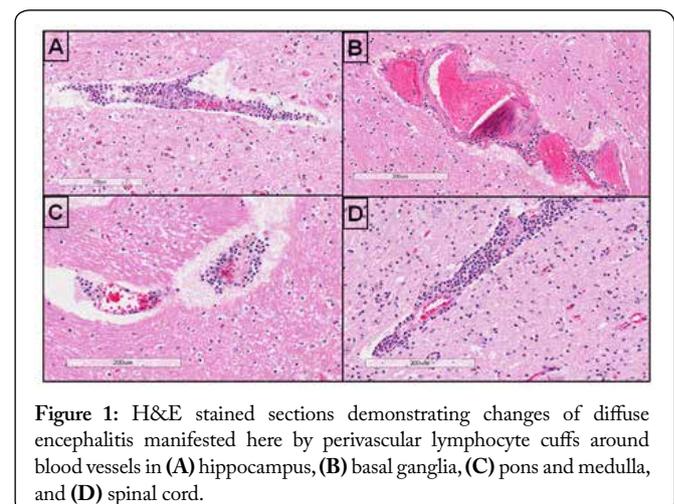

Figure 1: H&E stained sections demonstrating changes of diffuse encephalitis manifested here by perivascular lymphocyte cuffs around blood vessels in **(A)** hippocampus, **(B)** basal ganglia, **(C)** pons and medulla, and **(D)** spinal cord.

immunohistochemistry detected presumed WNV antigens in neurons in multiple foci in the cerebrum, cerebellum, brainstem and spinal cord (Figure 3). More recently, we were able to confirm the IHC findings using RNAscope *in situ* hybridization to demonstrate WNV RNA in the same CNS
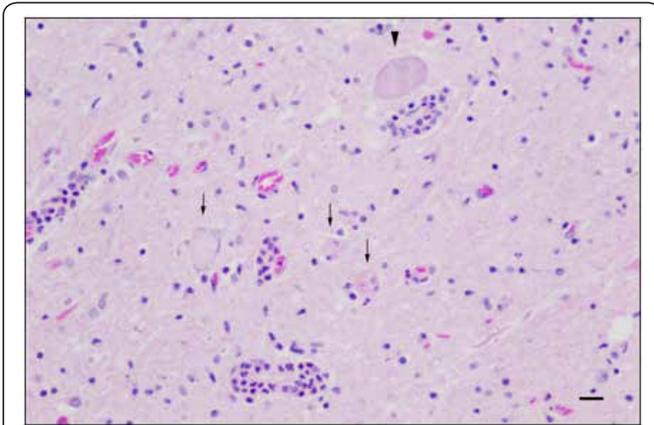
Figure 2: H&E stained section showing active neuronal injury in spinal cord anterior horn, including chromatolytic neuron with eccentric nucleus and distended cell body (arrowhead), and neuronophagocytosis (arrows). Scale bar represents 50 microns.
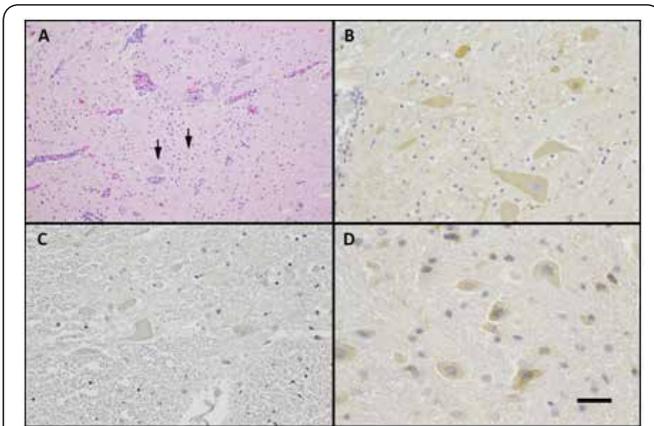

Figure 3: (A) H&E stain of the anterior horn region of the spinal cord showing perivascular inflammation and degenerating neurons (arrows). Several neurons with more normal morphology are seen above and right of center. **(B)** Higher magnification of same region in adjacent section showing neurons immunoreactive for WNV antigens (brown signal). Control sections using the same immunohistochemical technique include **(C)** uninfected human spinal cord (negative control), and **(D)** infected mouse cerebellum (positive control). Scale bar, lower right, represents 100 microns in **A**, 50 microns in **B** and **C**, and 25 microns in **D**.
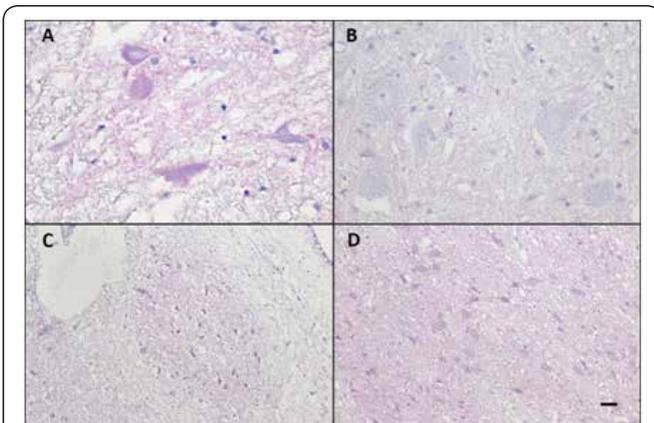

Figure 4: RNA scope in situ hybridization results, red signal is positive for WNV RNA. **(A)** Motor neurons in spinal cord anterior horn; **(B)** Negative control tissue: spinal cord anterior horn from uninfected human autopsy; **(C)** Hypoglossal nucleus; and **(D)** Pontine nuclei. Other positive brainstem nuclei in reacted sections included abducens nucleus and vestibular nuclei (not shown). Scale bar, lower right, represents 25 microns in **A** and **B**, 50 microns in **C** and **D**.

regions (Figure 4). From these findings, we can deduce that the patient's muscle weakness and atrophy were secondary to myelitis and motor neuron degeneration as part of diffuse involvement of the CNS.

Discussion

WNV is a mosquito-borne, single-stranded RNA flavivirus and human neuropathogen [1-7]. Approximately 80% of patients with WNV infection are asymptomatic and approximately 20% of patients present with a clinical illness manifested by a flu-like syndrome and maculopapular rash, termed West Nile fever (WNF) [1, 6-8]. West Nile neuroinvasive disease (WNND) occurs in up to 1% of patients infected with WNV, and is more frequently seen in patients of age 55 and above or patients who are immunosuppressed [9]. WNND has been reported to manifest in several ways, including West Nile encephalitis, West Nile meningitis, and acute flaccid paralysis, which can appear as a poliomyelitis-like syndrome or Guillain-Barré-like syndrome [1-4, 7-9]. These can present separately or with a considerable amount of overlap. West Nile meningoencephalitis or encephalomyelitis are terms that have also been used to describe patients with WNND [1-4, 7-9]. The overall mortality rate for patients with WNV disease in general is less than 0.1%, whereas the mortality rate for WNND can range between 10-30% [10]. Mortality rates range between 12-15% in patients presenting with West Nile encephalitis. However, mortality can reach up to 50% in patients presenting with acute flaccid paralysis and respiratory failure [9]. Acute WNV infection is usually cleared by an effective immune response after several days of viremia [4]. Several animal studies, however, suggest that long-term persistence of WNV occurs [1, 4, 10-12]. Persistence of WNV infection in humans has been detected in urine [12]. Since the original presentation of this patient was somewhat atypical for WNV polio-like disease, West Nile testing was not performed at the time of admission. Because he was confined to hospital or a rehabilitation center from the time of admission until his death, it is unlikely that he was infected during this period. Patients who survive WNND go on to exhibit a significant amount of morbidity due to sequelae from the disease [4, 6, 7, 9-12]. Chronic neurologic sequelae (which can persist for months to years) include fatigue, memory problems, cognitive impairment, muscle weakness, and impairment of daily activities as well as movement disorders (tremor, myoclonus) [4, 6, 7, 9-11]. However, rates of the chronic neurologic sequelae vary considerably between studies [7, 9-11].

Our case illustrates chronic WNV infection causing meningoencephalomyelitis that manifested clinically as acute motor axonal neuropathy. Autopsy demonstrated the presence of presumed WNV antigens in the cerebrum, cerebellum, brainstem, and spinal cord as well as evidence of motor neuron involvement. Note that the diagnostic IHC on brain tissue employed polyclonal antiserum, which has been suggested to possibly cross-react with other flaviviruses (not tested). Therefore infections by other flaviviruses, in particular both Zika virus and St. Louis encephalitis viruses that are both known to have infected humans in Texas, may not be

completely excluded by this technique. Epidemiologically, WNV activity occurs regularly in Texas, and there were no outbreaks of either Zika or St Louis encephalitis viruses in 2013 when this case occurred. The newer RNAscope *in situ* hybridization technique provided an independent confirmatory test for WNV. RNAScope includes multiple probes of 20-25 base pairs in length. Two probes must bind to the same RNA strand, adjacent to each other, for signal to be produced. Because two probes are unlikely to hybridize to non-target RNA, non-target binding is rare. One study found that RNAScope could differentiate between viruses with as much as 85% overall sequence identity [13]. Although reports of cross reactivity of this probe with other closely related flaviviruses are not available, a study using the same technology but targeting Zika virus reported no cross reactivity with WNV and dengue virus [14].

Although most cases of WNND are seen in patients with age > 50 and who are immunosuppressed [15], our patient was in his early 20's and did not have any history of immunosuppression. We postulate that the patient's symptoms of neuropathy (weakness and paralysis) and corresponding atrophy in skeletal muscle were secondary to myelitis and motor neuron degeneration in the spinal cord and brainstem due to chronic WNV infection. WNND should be considered in all patients presenting with not otherwise explained muscle weakness and paralysis in endemic areas.

References

1. Saxena V, Xie G, Li B, Farris T, Welte T, et al. 2013. A hamster-derived West Nile virus isolate induces persistent renal infection in mice. *PLoS Negl Trop Dis* 7(6): e2275. https://doi.org/10.1371/journal.pntd.0002275

2. Shrestha B, Gottlieb D, Diamond MS. 2003. Infection and injury of neurons by West Nile encephalitis virus. *J Virol* 77(24): 13203-13213. https://doi.org/10.1128/JVI.77.24.13203-13213.2003

3. Guarner J, Shieh WJ, Hunter S, Paddock CD, Morken T, et al. 2004. Clinicopathologic study and laboratory diagnosis of 23 cases with West Nile virus encephalomyelitis. *Hum Pathol* 35(8): 983-990. https://doi.org/10.1016/j.humpath.2004.04.008

4. Leis AA, Stokic DS. 2012. Neuromuscular manifestations of West Nile virus infection. *Front Neurol* 3: 37. https://doi.org/10.3389/fneur.2012.00037

5. Cushing MM, Brat DJ, Mosunjac MI, Hennigar RA, Jernigan DB, et al. 2004. Fatal West Nile virus encephalitis in a renal transplant recipient. *Am J Clin Pathol* 121(1): 26-31. https://doi.org/10.1309/G23CP54DAR1BCY8L

6. Watts DM, Granwehr BP, Shope RE, Solomon T, Tesh RB. 2006. Japanese encephalitis and West Nile and other flavivirus infections. In: Guerrant RL, Walker DH, Weller PF (eds) Tropical Infectious Diseases (Second Edition) Principles, Pathogens, & Practice. Churchill Livingstone, UK, pp 823-828.

7. Hart J Jr, Tillman G, Kraut MA, Chiang HS, Strain JF, et al. 2014. West Nile virus neuroinvasive disease: neurological manifestations and prospective longitudinal outcomes. *BMC Infect Dis* 14: 248. https://doi.org/10.1186/1471-2334-14-248

8. Sejvar JJ, Bode AV, Marfin AA, Campbell GL, Ewing D, et al. 2005. West Nile virus-associated flaccid paralysis. *Emerg Infect Dis* 11(7):1021-1027. https://doi.org/10.3201/eid1107.040991

9. Davis LE, DeBiasi R, Goade DE, Haaland KY, Harrington JA, et al. 2006. West Nile virus neuroinvasive disease. *Ann Neurol* 60(3): 286-300. https://doi.org/10.1002/ana.20959

10. Hughes JM, Wilson ME, Sejvar JJ. 2007. The long-term outcomes of human West Nile virus infection. *Clin Infect Dis* 44(12): 1617-1624. https://doi.org/10.1086/518281

11. Sejvar JJ, Kohl KS, Gidudu J, Amato A, Bakshi N, et al. 2011. Guillain-Barré syndrome and Fisher syndrome: case definitions and guidelines for collection, analysis, and presentation of immunization safety data. *Vaccine* 29(3): 599-612. https://doi.org/10.1016/j.vaccine.2010.06.003

12. Murray K, Walker C, Herrington E, Lewis JA, McCormick J, et al. 2010. Persistent infection with West Nile virus years after initial infection. *J Infect Dis* 201(1): 2-4. https://doi.org/10.1086/648731

13. Wang F, Flanagan J, Su N, Wang LC, Bui S, et al. 2012. RNAscope: a novel *in situ* RNA analysis platform for formalin-fixed, paraffin-embedded tissues. *J Mol Diagn* 14(1): 22-29. https://doi.org/10.1016/j.jmoldx.2011.08.002

14. Bhatnagar J, Rabeneck DB, Martines RB, Reagan-Steiner S, Ermias Y, et al. 2017. Zika virus RNA replication and persistence in brain and placental tissue. *Emerg Infect Dis* 23(3): 405-414. https://doi.org/10.3201/eid2303.161499

15. Murray K, Baraniuk S, Resnick M, Arafat R, Kilborn C, et al. 2006. Risk factors for encephalitis and death from West Nile virus infection. *Epidemiol Infect* 134(6): 1325-1332. https://doi.org/10.1017/S0950268806006339