PREVALENCE OF WEST NILE VIRUS INFECTION IN INDIA

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Abstract. During the course of the virological investigation of cases of suspected viral fevers carried out at the National Institute of Virology (NIV), Pune, India, evidence of recent infection with West Nile (WN) virus was detected in 88 cases. Fever, general aches, headache, nausea and vomiting were the principal clinical features in 92% (81/88) of the cases; there were seven cases of encephalitis, in which WN virus-specific IgM class antibodies were detected in CSF samples. These cases of encephalitis were from Japanese encephalitis (JE) nonendemic areas, like Maharashtra and Rajasthan, as well as from JE endemic areas, like Goa and Orissa. Interestingly, neutralizing antibodies predominantly to WN virus were detected in CSF samples by the 50% cytopathic effect inhibition method; the titers ranged from 5 to 375.

Cases of WN virus infection associated with both encephalitis and classic features have been reported for the first time in recent years in India. Reports of unique urban West Nile virus encephalitis epidemics in New York, Romania, and Algeria in recent years have signaled the emergence of neurological infection due to West Nile virus as a novel public health threat. This study is important because it records evidence of WN virus infection in India.

INTRODUCTION

West Nile (WN) virus, a group B arbovirus, belongs to the family Flaviviridae. Infection generally causes a febrile illness with or without a rash; occasionally, infection can lead to hepatitis (George *et al*, 1987), acute pancreatitis (Perelman and Stern, 1974) or encephalitis (Flatau *et al*, 1981). Until recently it was not thought to be a serious public health problem. However, the epidemics of urban WN virus excephalitis in New York in 1999 and Romania in 1996 and 1997 indicated the emergence of a new public health problem.

After the first report of WN virus prevalence in India by Smithburn *et al* (1954) and other isolated reports (Dandawate *et al*, 1969; George *et al*, 1984), sufficient attention was not given to the virus. In this paper we report the results of virological studies performed on serum/CSF samples obtained from cases of suspected viral fever.

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MATERIALS AND METHODS

The study was conducted during 1992-2001 as an institutional activity.

Serum and CSF samples collected from patients with suspected flavivirus infection were referred from all over India to our institute for virological investigation. Samples are routinely tested by the indigenously standardized IgM antibody capture (MAC) ELISA against Japanese encephalitis (JE, P20778); West Nile (WN, G22886) and dengue viral antigens (DEN 23085).

Serological investigations

Ninety-three samples collected from 88 cases positive for IgM predominantly to WN virus in MAC ELISA were included in this study. These samples compriesed seventy-eight single sera, five single CSF, three paired sera and two paired sera/CSF samples.

In addition to the MAC ELISA positive samples (n=93), 60 samples collected from 52 cases, including 44 single sera and 8 paired sera negative in MAC ELISA, were further tested by the hemagglutination inhibition (HI) test (Clarke and Casals, 1958) and the *in vitro*

neutralization test (NT) using a Vero cell line and the cytopathic effect (CPE) inhibition method (Reed and Muench, 1938) against JE and WN viruses (depending upon the availability of samples). Hemagglutination inhibition (HI) titers of >20 and a dilution of sample that produced 50% CPE inhibition were considered positive.

RESULTS

MAC ELISA

Ninety-three samples collected from 88 cases gave positive reaction to WN virus in the MAC ELISA test. Eighty-one of the eighty-eight paatients presented with fever, headache, and nausea and vomiting. There were seven cases in which IgM was detected in CSF samples: these cases had been referred to our institute with a provisional diagnosis of viral encephalitis. There were three cases from Goa, two from Maharashtra, and one each from Rajasthan and Orissa.

Neutralization test

Single serum samples: Eighty-five samples were tested for both JE and WN viruses, while an additional 37 samples were tested only for WN virus. The results are summarized in Table 1.

Single CSF samples and paired serum/CSF: Six CSF samples were tested against WN virus, while three samples were tested against both WN and JE viruses. Neutralizing antibodies to WN virus were detected in all six samples, with the titers ranging from 5-375. Of the three CSF samples tested against JE virus, two gave a negative reaction, while the JE neutralizing antibody titer was 1:5 in one sample.

The neutralizing antibody titer to WN was 1:625; it was 1:125 to JE (a five-fold difference) in serum sample whose CSF was tested. Another pair of serum/CSF was not tested due to insufficiency of the samples. The results are summarized in Table 2.

Paired sera: The serum samples from all 11 pairs were tested against WN virus while the sera of six pairs were tested against both JE and WN viruses. WN neutralizing antibody titers ranged from 25-625. A four-fold or greater rise in the neutralizing antibody titer to WN virus was recorded for three pairs; a four-fold or greater drop in the titer was recorded of two pairs. For the remaining six pairs, the neutralizing titer was >625 in both of the samples of each pair.

Of the six pairs of samples tested against JE, a four-fold or greater drop in the titer was observed in four pairs, while there was a four-fold or greater rise in the titer in one pair. Interestingly, neutralizing titers to WN virus were very high (>625) in both the serum samples of each pair.

HI test

Single serum samples: Antibodies to both

Table 1
Results of the neutralization test (single serum samples).

Category	Results	Number
Category 1	Samples tested against both JE and WN viruses	85
	Samples +ve only to WN	31
	Samples +ve only to JE	8
	Samples +ve to JE and WN	45
	Samples -ve to JE and WN	1
Category 2	Additional samples tested against only WN	37
	Samples +ve	27
	Samples -ve	10

Table 2
WN encephalitis cases (N=7).

Case No.	Sample	Age	MAC WN	MAC JE	WN NT titer	JE NT titer
1	CSF and	58	+ve	-ve	25	ND
	serum		+ve	-ve	625	125
2	CSF and	12	+ve	-ve	ND	ND
	serum		+ve	-ve	ND	ND
3	CSF	35	+ve	-ve	25	5
4	CSF	22	+ve	-ve	375	<5
5	CSF	16	+ve	-ve	125	ND
6	CSF	30	+ve	-ve	5	<5
7	CSF	59	+ve	-ve	25	ND

ND: Not done.

WN and JE were detected in 89% (109/120) of the samples, while antibodies to only JE virus were recorded in 4% (5/120) of the samples. The results of the HI test are summarized in Table 3.

Paired sera: A four-fold or greater in antibody titers to WN were recorded in four pairs; similar reactivity to JE virus was recorded in 6 pairs. A WN virus antibody titer of ≥640 in both the samples of a pair was recorded in one case, while a similar titer (>640) to JE virus in both the samples of a pair was recorded in four pairs. A four-fold or greater drop in the titer to WV virus was recorded in two pairs, while a two-fold difference in titer was found in four pairs. A two-fold drop in the titer to JE virus was recorded in a pair. Due to cross-reactions among closely related flaviviruses it was difficult to arrive at a firm differential diagnosis.

Table 3
HI titers against JE and WN viral antigens.

HI titer	No. of samples positive to JE	No. of samples positive to WN
1,280	47	28
640	9	15
320	6	12
160	13	9
80	10	18
40	10	11
20	6	8
<20	19	19
Total	120	120

DISCUSSION

Mosquito-borne West Nile fever is an endemic febrile illness in Africa and the Middle East; in Australia and Europe fewer cases are seen. The disease is usually benign, although occasionally it can cause severe complications (George *et al*, 1987; Perelman and Stern, 1974; Flatau *et al*, 1981). In recent years, the disease has become more evident and has caused public health concern in Algeria (1994), Romania (1996), and New York (1999): places that suffered epidemics of WN virus encephalitis. High fatality rates were reported (~7%) from Romania and Algeria; in Romania, the older age group was involved, while in Algeria mainly children were affected (LeGuenno *et al*, 1996).

In India, the prevalence of WN virus was first established in 1952 by detection of neutralizing antibodies (Smithburn *et al*, 1954). In between the years, West Nile virus has been noticed as the result of hemagglutination inhibition (HI) antibody titers in serological investigations and through the finding of virus isolates in North Arcot Districts, Chennai and Chittore District, Andhra Pradesh (Dandawate

et al, 1969) and in cases of human febrile illness (Paul *et al*, 1970).

To date there has been no reported outbreak of illness caused by West Nile virus in India. In this study we have reported 88 sporadic cases of recent infection, as shown by the detection of WN virus specific IgM antibodies. Of these cases, 92% (81/88) presented as West Nile fever. Interestingly, there were seven cases of encephalitis that occurred sporadically; in these cases WN virus specific IgM antibodies and neutralizing antibodies predominantly to WN virus were detected in CSF; these cases are mainly among adults. Earlier, in 1984, isolation of WN virus was reported from brain of children who had died of encephalitis (George *et al*, 1984).

WN virus belongs to the JE antigenic subgroup, which was divided on the basis of neutralization using polyclonal hyperimmune serum (Calisher *et al*, 1989). WN virus usually coexists with Japanese encephalitis virus. However, anti-WN virus IgM antibodies in acute phase samples are mostly type-specific. (Tardei *et al*, 2000). In the present study, encephalitis cases were reported from JE non-endemic areas (Maharashtra and Rajasthan) as well as from JE endemic areas (Goa and Orissa).

In this study, virus specific IgM antibodies were detected in all the CSF samples. The first apprearence of IgM antibody is often reported as being in CSF (Thakare, 1992; Tardei *et al*, 2000); its presence does not merely reflect transudation from the systemic circulation (Thakare, 1992; Cerneseu *et al*, 1997) and often indicates intrathecal synthesis. The West Nile and Japanese encephalitis viral genomic sequences have been detected in cerebrospinal fluid from acute encephalitis cases in Karachi, Pakistan (Igarashi *et al*, 1994). WN virus has been isolated from CSF and brain tissue in WN encephalitis cases (George *et al*, 1984; Tsai *et al*, 1998): it is an indication of the neuroinvasive and neurovirulent properties of the virus. In Romania, sporadic cases of encephalitis were serologically diagnosed as being secondary to WN virus in 1955 and 1963 (Tsai *et al*, 1998). Almost three decades later, Romania experienced a life-threatening WN encephalitis epidemic (1996); transmission of the virus has been reported in certain parts of Romania (Cernescu *et al*, 2000). There are other examples of viruses whose virulence was underestimated: Rift Valley fever virus was considered a benign disease for 44 years until the first hemorrhagic cases were recognized in South Africa in 1975; the virus is now known to cause hundreds of deaths.

In the present study, we have reported on sporadic cases of recent virus infection that produced classic WN fever as well as, in a few instances, encephalitis. The results of HI and neutralization tests were inconclusive in spite of having been based an paired sera. Flaviviruses are known to have homology in HI domains while they are specific as far as neutralizing epitopes are concerned. However, cross-reactions, even in the neutralization test, have been reported (Yoshihiro *et al*, 1994.) As the result of detection by hemagglutination inhibition (HI) and neutralizing antibody assays and cases of recent infection, we conclude that WN virus is active and prevalent in India.

Different strains of WN virus vary in their behavior and biological properties; these variations may affect the viruses virulence in cultured and murine cells (Umrigar and Pavri, 1977). The potential of mosquitos to transmit WN virus is an important epidemiological consideration (Turell *et al*, 2000) and it would therefore be useful to conduct surveillance studies that consider the mosquito-bird maintenance cycle of WN virus. More research into the isolation of the pathogen and the application of RT-PCR may serve to complement CSF MAC ELISA testing.

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