A clinico-epidemiological study of the first outbreak of Nipah virus in India – report from ground zero

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Introduction: The first Nipah Virus (NiV) outbreak occurred in India in the year 2001 at Siliguri. The second outbreak happened at Nadia in 2007. Nipah Virus exhibits neurological and pneumonic tropism with the predominant clinical presentation being encephalitis in humans.

Material and Methods: The present study was a record based prospective study on 67 cases admitted with pyrexia of unknown origin in North Bengal Medical College during the period from 18.02.2001 to 30.02.2001 and a parallel study on epidemiological record carried out by PSM department also taken into account. All necessary investigations including autopsy examination, pathological, and microbiological study were done.

Results: There was a clustering of cases around Bhaktinagar. There was a strong H/O Medinova Nursing Home Contact among the patients. 18 out of 20 cases were staff of that Nursing Home. Serum samples tested show NiV specific IgM and IgG in 9 out of 17 samples with one sample which was positive for IgG only suggesting past infection. The cases were admitted with predominant neurological symptoms (53.73% cases) but about 80% recovered with no residual neuro deficit. The natural reservoir of NiV is present in Bangladesh and in Northern India.

Conclusion: When NiV infection is suspected, infection control practices must be strengthened to avoid an outbreak in a hospital setting. Here the present study is presenting the experience in the first outbreak of the Nipah virus in India at Siliguri for awareness of clinical personnel to control further outbreak at the very beginning.

Keywords: Nipah virus (NiV), Zoonotic paramyxovirus
Introduction

There was an outbreak of febrile illness associated with altered sensorium during the month of February and March 2001 at Siliguri and adjacent areas. 67 cases were admitted to North Bengal Medical College and Hospital during this period and death occurred in 31 cases (CFR = 46.30%).

Japanese Encephalitis was initially suspected as it was endemic in that area, but epidemiological parameters suggested a different disease. Initially, laboratory investigations failed to identify the infectious agent [1]. Later Atypical measles from the National Institute of virology Pune, then Nipah virus, a zoonotic paramyxovirus was implicated as the cause identified from CDC Atlanta.

First Nipah outbreak occurred in Malaysia in 1998-99, then spread to Singapore and Bangladesh (2001, 2003 and 2004) and then to India (Siliguri in 2001 and Nadia in 2007) [2-9].

Malaysian outbreak was found to have occurred via exposure to infected pigs who consumed bat eaten fruits or exposed to bat urine or via human-to-human transmission. There was an outbreak in the Philippine via infected horses in 2014. The last outbreak was in Kozhikode in Kerala in May 2018 via bat (Pteropusgiganteus).

Nipah virus has neurological and pneumonic tropisms. With, Nipah virus, the predominant clinical syndrome in humans is encephalitis whereas, in pigs, it is characterized by acute fever and respiratory involvement with or without neurological signs. CFR is 40%-75%.

Methodology

Setting: The North Bengal Medical College and Hospital, Siliguri

Duration and type of study: The study was done during the period from 18.02.01 to 30.02.01, record based observational study.

Sampling methods: All patients admitted with pyrexia of unknown origin and those who fit inclusion criteria

Sample size: sixty-seven patients enrolled in the present study

Inclusion criteria: A suspected patient of NiV was one >15 years of age with acute onset of high-grade fever and headache.

A probable patient was one >15 years of age who had a high-grade fever and altered sensorium and encephalitis of unknown origin

Exclusion criteria: Fever of known cause

Data collection procedure: The standard laboratory investigations for Pyrexia of Unknown etiology and encephalitis were done. All cases were investigated for routine blood microscopy, MP, LFT, RFT, Sodium, Potassium, CSF microscopy and biochemistry, X-ray chest, and in some cases Ultrasonography. The clinical and investigation findings were documented in a semi-structured proforma.

An autopsy was done and material examined in Pathology, Microbiology departments of North Bengal Medical College. The material was also sent to the National Institute of Virology, Pune, and CDC, Atlanta for virological investigations maintaining the standard norm of transportation of samples. The outbreak was investigated by teams from AIIMS, Delhi, and the National Institute of Communicable Disease, Delhi.

Results

Table-1: Age and Sex wise distribution of cases.

<table>
<thead>
<tr>
<th>Age Group (in years)</th>
<th>Cases reported by Community Medicine</th>
<th>Cases in NBMC and H</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>&lt;15</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>15-20</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>21-30</td>
<td>19</td>
<td>15</td>
</tr>
<tr>
<td>31-45</td>
<td>14</td>
<td>9</td>
</tr>
<tr>
<td>&gt;45</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>43</td>
<td>30</td>
</tr>
</tbody>
</table>
Table-2: Variability of Clinical features with the outcome (N=67).

<table>
<thead>
<tr>
<th>Clinical feature</th>
<th>Death (n=31)</th>
<th>Survival (n=36)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number %</td>
<td>Number %</td>
</tr>
<tr>
<td>Fever</td>
<td>31 100</td>
<td>36 100</td>
</tr>
<tr>
<td>With chills and rigor</td>
<td>3 9.68</td>
<td>0 0</td>
</tr>
<tr>
<td>Headache</td>
<td>9 29</td>
<td>36 100</td>
</tr>
<tr>
<td>Convulsion</td>
<td>9 29</td>
<td>10 36</td>
</tr>
<tr>
<td>Respiratory distress</td>
<td>5 16.13</td>
<td>5 18</td>
</tr>
<tr>
<td>Unconsciousness</td>
<td>11 35.50</td>
<td>0 0</td>
</tr>
<tr>
<td>Altered sensorium</td>
<td>8 26.45</td>
<td>5 18</td>
</tr>
<tr>
<td>Vomiting</td>
<td>3 9.68</td>
<td>2 5.55</td>
</tr>
<tr>
<td>Watery stool</td>
<td>1 3.22</td>
<td>2 5.55</td>
</tr>
<tr>
<td>Haematuria</td>
<td>1 3.22</td>
<td>0 0</td>
</tr>
</tbody>
</table>

In all sixty-seven (67) cases were admitted to North Bengal Medical College and Hospital. Despite all standard efforts, death occurred in thirty-one (31) cases (CFR = 46.30%).

From Table-1, it was evident that cases admitted at NBMC and H and the report from the community medicine department in their field investigation were more or less similar. The majority (around 80%) of the cases were in the group of 21 to 45 years. This may be the fact that the particular age group is more exposed to the outside environment. It was also observed from the table that male was more affected than the female, the proportion being 60 and 40 respectively. The male preponderance might be due to more exposure to outdoor activity. A clustering of cases was observed around the Bhaktinagar area under the Siliguri Municipal Corporation, which might be due to the reason that majority of the affected staff of Medinova Nursing Home where the residents of the particular area.

From the data available from the Department of Community Medicine indicate that out of 30 Medinova Nursing Home contact, 22 were the staff of that particular nursing home and out of total 67 admitted cases in NBMC and H, twenty cases had the history of contact to Medinova Nursing Home. Out of the 20 cases, eighteen cases were the staff of that particular nursing home.

Blood samples were available for 18 hospitalized patients and for 13 family contacts of patients who died 2-3 weeks earlier. Six urine samples were collected, CSF, throat swabs and rectal swabs, necropsy material (brain, liver, lung tissue and blood clot from 5 fatal cases) were collected and processed at NIV, NICD, AIIMS, CDC, Atlanta, and Defense Research and Development Establishment (DRDE), Gwalior [10].

Discussion

In DRDE, Gwalior, 6 clotted blood samples were analyzed by RT-PCR for Flavivirus, Hantavirus, Nipah virus, and measles virus. In AIIMS, Delhi, agglutination tests for IgM and IgG antibodies to Mycoplasma pneumonia was done. Serum, CSF, throat and rectal swabs, brain biopsies, lung aspirates, and urine samples were processed of NICD, Delhi for Legionella pneumophilia, Measles, Plague, Malaria. Leptospirosis, Dengue, Hantavirus, Herpes Simplex Virus, and Enterovirus. NIV, Pune also carried out ELISA for IgM and IgG antibodies and virus culture to rule out Japanese Encephalitis, Dengue, Leptospirosis, West Nile, Measles, and Enterovirus infection.

Four serum specimens from cases that tested positive for measles IgM at NIV, Pune, four companions infected Vero cell culture lysates derived from the serum specimens, two case contact serum specimens (one reported as IgM positive and one as IgM negative for measles at NIV, Pune). Four urine specimens and one brain aspirate were sent to CDC, Atlanta.

The tests performed included CDC capture IgM and IgG assay on all 6 serum samples following gamma irradiation for measles and Nipah/Hendra virus. An IgM capture assay was performed within the BSL-3 laboratory using the non-irradiated specimens and appropriate controls and commercial IgM capture ELISA.

Serum samples were tested on 4-fold dilution from 1:100 to 1:6400. Samples were considered positive if the sum of adjusted optical densities (OD) from all dilutions (OD from infected antigen well – OD from mock-infected antigen) was >0.75 through the entire dilution series and titer was >1:400. For IgG to be positive adjusted OD from all dilution should be >0.9 and titer was >1:400. NIV specific IgM and IgG were detected on 9 of 17 serum samples. 1 sample was positive for IgG and negative for IgM suggesting a past infection [3-5,7-9].

Detection of NIV by RT-PCR and Virus Isolation: Two sets of primers were used for RT-PCR reactions. Primer set NVNBF-4 and NVNBR-4 amplified 159 nucleotides (nt) region of N gene of NIV and another primer set was NVBMFC-1 and NVBMFR-2, amplified 320 nucleotides (nt) region of M gene.

RT-PCR detected the N gene in 4 urine samples of NIV antibody patients and detected the M gene in...
3 of 5 samples. Isolated Siliguri N and M sequences were closely related to those of Bangladesh isolates than Malaysian isolates.

The Literature says: The main reservoir for NiV infection is thought to be fruit bats of the genus Pteropus and it was isolated from fruit bats in Malaysia and Cambodia and other parts of Southeast Asia [7,8,9,10,11]. In the Malaysian outbreak, commercially raised pigs were believed to be intermediate hosts. Presumably, the pigs were infected by virus shed from fruit bats and then transmitted the virus to humans. Although fruit bats with antibodies to NiV were captured in the outbreak areas of Bangladesh, no intermediate animal host was identified. Person-to-person spread was also seen during the NiV outbreak in Faridpur, Bangladesh, 2004 [4,5]. Therefore, the range of the proposed natural reservoir for NiV extends into Northeastern India, and since the geographic features of West Bengal are similar to those of Bangladesh, environmental circumstances that favor transmission of NiV to humans would likely also be found in West Bengal.

The Current episode: Epidemiological features for NiV outbreak in Siliguri were very much similar to Bangladesh and in both situations, there was no intermediate animal host found. In both outbreaks, transmission occurred in healthcare settings through contact with infected persons. In Siliguri, only adults were affected supported by the nosocomial transmission theory, as the number of children on the wards of hospitals was minimal. It was observed that suspected primary case was at Siliguri Subdivisional Hospital and he was transferred to Medinova Nursing Home and finally died. Contacts of this case later developed symptoms. During infection, NiV is present in respiratory secretions and in urine, and in both outbreaks, failure to use proper personal protective equipment probably contributed to the spread of the virus [12]. Analyzing the various cases, the incubation period was around 10 days with a range of 5-20 days.

The picture of the epidemic does not support the evidence of vector transmission rather it supports the possibility of droplet transmission. Regarding the time sequence of the epidemic, it was during the winter season and there were two prominent peaks of cases. One peak was observed around the 1st week of February 2001 and another in the 3rd week of February 2001. There were 11 cases in the 1st peak and 43 cases in the 2nd peak. A striking feature was that the death occurred in most cases of 1st peak (nearly cent percent) whereas the case fatality decreases in the 2nd peak of the epidemic. Death is both the above situation that occurred instead of all possible efforts of the district and state-level healthcare management. All the sixty-seven (67) cases admitted in NBMC and H were in the 3rd and 4th week of February 2001, i.e., along with the 2nd peak of outbreak reported by the Community medicine department. The difference in the picture may be due to a decrease of virulence of the microorganism in the second situation with the passage through the host.

Regarding the clinical manifestation there were two types of presentation:

1) Predominantly pneumonic type, (2) Predominantly encephalitic type.

The encephalitic type presented with abrupt onset of fever with vomiting, severe headache, and body ache. Patients were initially normally oriented and within a day or two few patients developed mental confusion and quickly progressed to unconsciousness. Some neurological features like ataxia, small unequal pupils were found in very few patients. Deep tendon reflexes were diminished with extensor plantar reflex and increased ICT. In a few cases, pupils were dilated with variable reactions to light with or without nystagmus. Papilledema was surprisingly absent. In some patients, there was proximal limb weakness. Initially, there was the preservation of sensory functions, although some developed myoclonic jerks or even convulsion. The above features indicate midbrain involvement.

The pneumonic type presented with fever and respiratory distress. They were conscious and mentally oriented throughout the course of illness. Initially, auscultation of chest was normal but later developed coarse crepitations with expectoration of pink frothy sputum; few patients showed frank hemoptysis. Features of pneumonitis (crepitations and rhonchi in the chest) were noted in both groups of patients. One or two patients had loose motion. Few patients apparently responded to treatment well with regaining consciousness, diminution of fever, and respiratory distress, only to be followed by deepening coma and gross features of ARDS and death. There was no hepatosplenomegaly. There were neither features of renal or other organ involvement nor any hemorrhagic diatheses observed.

The pneumonic patients showed complete recovery without any chest findings and follow up chest X-ray
Was normal. The encephalitic patients developed sequelae like limb weakness and paralysis; few ended with optic atrophy. Malarial parasites were not found in any of the cases. CSF pressure was found raised with total cell count = 0-4 cells, all lymphocytes, and normal protein and sugar. Blood film and haemogram showed a grossly elevated total count of WBC (15000-20000/cmm), with neutrophilia in most cases. The liver function and renal function tests were found within normal limits. Few cases showed albuminuria and microscopic haematuria. Mild to moderate hyponatremia was found in all cases (120-132 mEq/dL). The serological test excluded the possibility of Japanese Encephalitis, Herpes, and West Nile infections. X-ray of chest showed segmental pneumonitis in few cases and diffuse or patchy wooly opacities in others suggesting ARDS. Abdominal ultrasonography did not reveal any obvious abnormality.

An autopsy was done in some patients dying either from mainly respiratory or neurological illness. The findings are described below:

01. Brain – Trans nasal necropsy of the brain was done with Vim Silverman Parker needle. The findings were – (a) Puncture of meninges made a gushed-out flow of CSF, indicating raised intracranial tension. (b) On microscopy, there were non-specific neuronal damage, spongiform degeneration in some neurons (in sensory and motor cortex), and necrosis.

02. Lungs – showed interstitial pneumonitis with infiltration of mononuclear cells and hyaline eosinophilic material within the alveolar cavities indicating pulmonary edema.

03. Liver – There were fatty changes. Pseudolobules were found in one alcoholic patient.

04. Heart – There was evidence of nonspecific myocarditis with loss of striations in the myocardium. Mononuclear cell infiltration was noted in the interstitium.

05. Spleen – There was congestive splenomegaly, which is a nonspecific feature.

06. Testis – There were interstitial edema and degeneration of Sertoli cells.

07. Kidney – The glomeruli were hypercellular with degenerative changes in the tubular epithelium. Widening of interstitial spaces with lymphocytic infiltration was noted in a few cases.

The outbreak of the so-called unknown fever that occurred in February-March 2001, took away a good number of the liver (31 out of 67 cases admitted in North Bengal Medical College and Hospital), in spite of the best efforts made by this apical institute. The clinic-epidemiological, laboratory and autopsy findings suggested the probability of viral etiology with great suspicion Nipah or some other virus. The mode of transmission of the disease was not very clear, although it seems to be respiratory route transmission. The clustering of cases around a particular Nursing home also indicated the possibility of a focal ‘hot area’ of some vector that could not be supported by the entomological study.

Although a report from the CDC, Atlanta pointed toward the possibility of the Nipah virus, there was no history of animal (horse, pig, or bat) to man transmission. Again, a good number of patients in the present study had pneumonitic features with ARDS, which is an infrequent feature of Nipah virus infection [13].

The organism was probably highly virulent but the virulence decreased rapidly suggested by sharp tailing of propagation of the infective agent in the community altering the second peak of occurrence of cases. This is a common phenomenon with an epidemic situation, particularly if it is of a single/common source of origin.

Lastly, the outbreak of the disease has pointed out clearly the need for a constant epidemiological watch using a standard case definition and arrangement of an expert team with equipment to tackle such a situation whenever needed in this area.

Out of the total admitted 67 cases, 36 (53.73%) cases had predominant neurological manifestation. Of these 36 cases, 29 (80.56%) were recovered without any residual neurological deficit and the remaining 7 (19.44%) cases had the residual neurological deficit in the form of weakness and dizziness which persisted even during the follow-up period.

One developed paraplegia as sequelae. Patients predominantly of pneumonitic type showed complete recovery without any chest findings and follow up chest X-ray was found normal. The encephalitic type of patient developed sequence like limb weakness and paralysis, few ended with optic atrophy.

First Nipah virus outbreak occurred in Malaysia in 1998–1999, then spread to Singapore, and further epidemics have occurred later in Bangladesh.
(Sporadic between 2001 and 2008) and India (at Siliguri in 2001 and at Nadia in 2007, both in West Bengal). The Indian outbreaks occurred in the villages adjoining Bangladesh [14,15,16,17,18,19]. The Malaysian outbreak was found to have occurred via exposure to infected pigs which had consumed bat-eaten fruits or exposed to bats’ urine 15. Humans also get infected by consuming bat-eaten fruits or exposure to bats’ urine or via human-to-human transmission [20]. The Nipah outbreak was reported in the Philippines in the year 2014, probably via infected horses [21]. The incubation period in humans ranged from 4 days to 2 months, with more than 90% occurring at 2 weeks or less [15]. The last outbreak was at Kozhikode, Kerala in India in May 2018 [22].

**Treatment:** Currently there is no known treatment or vaccine available. Intensive supportive care with the treatment of symptoms is the main approach. Some observational data suggest that Ribavirin may be of use in reducing mortality among patients with encephalitis caused by Nipah.

**Conclusion**

In an infected person, NiV is present in respiratory secretions and urine. In both outbreaks, failure to use proper personal protective equipment probably contributed to the spread of the virus. Initiating adequate barriers and nursing techniques helped curtail further spread of infection. Bat is the natural reservoir of the Nipah virus.

**What does the study add to the existing knowledge**

The Indian flying fox Pteropus medius formerly known as P. giganteus is the apparent natural reservoir of NiV which is locally abundant in this area. So there is a chance of further outbreak to occur in Bangladesh and Northern India. On any suspicion of airborne disease, control measures should be taken to prevent an outbreak in a hospital setting like the one in Siliguri. Early suspicion and early detection are helpful to curtail the disease spread. Use personal protective equipment and isolation of the disease should be followed first.

**Author’s contribution**

All the authors; Dr. Rama Saha, Dr. Sudipan Mitra, Dr. Swapan Halder, Dr. Jaydip Deb, Dr. Anupam Patra, and Dr. Gautamnarayan Sarkar

Contributed for data collection, analysis and various steps in manuscript preparation.

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Dedicated to Dr Ajit and other doctors, Sister Barnali and other paramedical staffs who lost their life in this battle. Authors are thankful to Dr Prasenjit Maity and Dr Arunabha Aarkar for their relentless work at that time.

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