

Commentary

Chandipura virus – what we know & do not know

The Chandipura virus was discovered in 1966 by Bhatt and Rodrigues, scientists of the Virus Research Centre (VRC) established by the Rockefeller Foundation in 1952 in Poona (now Pune)¹. They were investigating persons with fever in Chandipura in northern Maharashtra, near Nagpur, for dengue or chikungunya virus aetiology¹. The virus thus detected was hitherto unknown, and Bhatt and Rodrigues named it Chandipura after the geographic location of its discovery¹.

In 1967 VRC was handed over to the Indian Council of Medical Research (ICMR) which renamed it as the National Institute of Virology (NIV). The main focus of VRC was to identify and document all Indian ‘arboviruses’ (arthropod-borne viruses -- viruses of different families and genera that are transmitted by blood-sucking arthropod vectors – insects and ticks).

Not much importance was given to Chandipura virus by virologists for a number of years. Scientists of VRC/NIV had isolated it on various occasions from humans with short fever, hedgehog, sandflies (*Phlebotomus* spp.) and *Aedes aegypti* mosquitoes. The virus is presumed to have wide geographic presence in India, as evidenced by the detection of virus-specific antibodies in humans and various animals in different parts of India. Much of these data had remained unpublished since the virus had no particular importance for human disease. In 1983, Rodrigues *et al*² reported the isolation of Chandipura virus from the blood of an 11 yr old child who died with acute encephalopathy syndrome. In 1988, a 35 yr old man had uneventful mediastinal surgery in our hospital in Vellore for a benign lesion; a week later he developed fever and intense headache and neck-stiffness; cerebrospinal fluid (CSF) examination showed lymphocytosis. The diagnosis was aseptic meningitis. In CSF we found a rapidly growing cytopathogenic virus in Vero cell culture (unpublished). The virus was enveloped –

as shown by loss of infectivity when treated with a lipid solvent; thus it was not an enterovirus, the most common cause of viral meningitis. Unable to identify it, we sent it to Centers for Disease Control (CDC) in the USA. Scientists in CDC were baffled at first as electron microscopy showed what looked like rabies virus. The patient had fully recovered in a week; his second CSF sample had no virus but had virus-neutralizing antibody. With this information, CDC scientists explored further and classified it as a member of *Rhabdoviridae* family and finally identified it as Chandipura virus (Pallansch M, personal communication). We surmised that the patient got infected while in the hospital, most probably by mosquito-bite, most likely *Aedes aegypti*. NIV scientists had earlier proved the vectorial capacity of mosquitoes to transmit Chandipura virus³.

Chandipura virus came to the limelight in 2003, with the publication of a report by NIV scientists on a large outbreak of an acute neurological illness of young children with high case-fatality, diagnosed as encephalitis and putatively associated with infection with the virus⁴. There were 329 cases with 183 fatalities⁴. A subset of hospitalized children was investigated and plausible evidence for Chandipura virus aetiology was reasoned out⁴. A retrospective analysis of another set of 104 children hospitalized in Hyderabad showed that the clinical features were those of encephalopathy (without leucocytosis in CSF) rather than encephalitis⁵. The illness was characterized by acute onset fever, altered sensorium, seizures, diarrhoea and vomiting. Death or recovery occurred rapidly, within 2-3 days; there was no sequel in survivors. Most of those who died did so within 24 h of onset of illness⁵. These findings led to the clinical diagnosis of ‘brainstem encephalitis’ partly to explain the lack of increase of cells in the CSF; the aetiology was described as Chandipura virus⁵.

Another outbreak of ‘acute encephalitis’, again investigated by NIV scientists and diagnosed with

Chandipura virus infection, was reported in eastern districts of Gujarat in 2003, mostly among tribal children⁶. There were 26 cases and 18 fatalities⁶. These reports have re-kindled scientific interest in Chandipura virus, believed to cause large scale outbreaks and death of hundreds of children. Several new studies have been reported⁷⁻¹², including one by Jodi and colleagues in this issue of the journal¹³. The latter paper describes the growth kinetics of Chandipura virus in several cell lines and embryonated hen's eggs¹³. Cells of mammalian origin (Vero and RD cells) and of insect origin (*Ae. aegypti* and *Phlebotomus papatasi* cell lines) were susceptible to virus replication¹³. The original virus discovery was made in infant mice used for specimen inoculation¹. NIV scientists have shown that Chandipura virus inoculated in the brain causes fatal encephalitis in mice⁴. Chick embryo also is susceptible to infection^{9,13}. These observations confirm the presence of virus receptors in a very wide range of host species, invertebrate and vertebrate. From evolutionary viewpoint it is a very ancient virus.

Chandipura virus is classified under *Rhabdoviridae* family, genus *Vesiculovirus*. Members of this genus include Lyssa (rabies and bat Lyssa viruses), vesicular stomatitis virus (VSV, the cause of vesicular stomatitis of cattle) and many 'minor' viruses such as Isfahan and Chandipura. They tend to have distinct geographic prevalence – for example, VSV belongs to the American continents. Bat Lyssa viruses are predominantly American and Australian, while Isfahan and Chandipura are Asian viruses. Isfahan virus is found in sand flies in Iran; Chandipura virus infects sand flies, mosquitoes and mammals including humans, in India and Sri Lanka¹⁴. Earlier believed to be exclusively Asian, Chandipura virus has been reported in western Africa (in sand flies)^{15,16}. The importance of transcutaneously transmitted Lyssa viruses to human and canine disease and death and that of VSV to epizootics in cattle are well known. VSV and Chandipura are transmitted by arthropods. Until the NIV reports on Chandipura virus causing 'encephalitis' epidemics with high case fatality, it was believed to be an inconsequential agent causing at best no illness or at worst short-lived febrile illness, with very rare instances of viral meningitis and possibly acute encephalopathy.

Some experts have raised doubts about the validity of evidences for Chandipura virus aetiology of epidemic encephalopathy¹⁷⁻²¹. An arbovirus expert in CDC has stated: "Chandipura virus, an infrequently recognized rhabdovirus, was attributed to large

outbreaks of viral encephalitis; however, compelling evidence suggests that the relationship of illness and the virus are questionable"¹⁷. The 2003 acute brain disease epidemic of Andhra Pradesh⁴ was investigated independently by pediatric neurologists¹⁸. The detailed neurological findings were interpreted to show that the outbreak was not one of 'encephalitis', but of an acute catastrophic event in the brain¹⁸. The site of lesion was pinpointed to the brain supply territory of the middle cerebral artery. The nature of the arterial pathology was not investigated, but it was suggested to be spasm or transient obstruction due to vasculitis, rather than thrombo-embolism¹⁸. That 'acute brain attack' could occur in an outbreak was new information^{18,19}. There was no clinical evidence of invasion by any pathogen, or of inflammatory lesions in pathology^{4,18}. If Chandipura virus aetiology is correct, the disease is mediated by vasculitis, not encephalitis^{18,19}.

Yet another study of acute encephalitis in Andhra Pradesh has provided intriguing results²⁰. In a hospital in Hyderabad all cases of encephalitis in children from May 2005 to April 2006 were prospectively investigated²⁰. Of the 90 cases, 25 yielded evidence of Chandipura virus infection by PCR, IgM antibody or seroconversion; however, no virus could be isolated²⁰. Among contacts of cases who were <15 yr of age, 71-73 per cent and among those who were >15 yr, 94-97 per cent had antibody evidence of past Chandipura virus infection²⁰. Thus Chandipura virus infection seems to nearly saturate the population by about 15 yr of age. Yet, reported outbreaks of acute brain disease supposedly caused by it have been inexplicably very few. However, basic studies on Chandipura virus must continue and as much details of its biology learned since it is a common agent of infection in a variety of species of hosts in India. Moreover, it is a *vesiculovirus* and much can be learned about the host-virus interactions of the genus, to which belongs rabies virus also.

Warangal district in Andhra Pradesh had, 2 years earlier, a very similar outbreak of acute encephalopathy which was investigated by NIV scientists who reported the isolation of measles virus directly from CSF of a few children, confirming measles virus aetiology²¹. Earlier an epidemic acute encephalopathy syndrome was reported in Haryana, with measles virus directly isolated from the CSF of several children²². Could very similar, if not identical, clinical disease affecting the brain and causing high case-fatality be caused by two very common viruses, measles and Chandipura? Genetic studies had not revealed major mutations in Chandipura virus to account for recent acquisition of virulence¹⁰.

In short, it is possible that the true aetiology was neither measles nor Chandipura virus in these outbreaks. Investigations to discover the true aetiology in future outbreaks should not be confined to confirming or excluding infection with Chandipura and measles virus. The temptation to quickly seek to identify the causative agent in outbreaks of unknown diagnosis/aetiology has misled many in the past. Instead every such outbreak should be thoroughly investigated using tools of epidemiology first, or at least in parallel with, investigating for aetiology. Often detailed epidemiology will lead to the correct aetiology.

The final word on the question whether Chandipura virus can cause outbreaks of encephalitis, encephalopathy or acute brain attack, will have to wait until they are confirmed in fresh epidemics by independent investigators. In science, truth is what is verifiable and verified for consistency and reliability.

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