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Contemporary Issue

Discovery of fifth serotype of dengue virus (DENV-5): A new public health dilemma in dengue control

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ABSTRACT

Dengue fever is a re-emerging public health problem with two-fifths of the world population being at risk of infection. Till now, dengue fever was believed to be caused by four different serotypes. The fifth variant DENV-5 has been isolated in October 2013. This serotype follows the sylvatic cycle unlike the other four serotypes which follow the human cycle. The likely cause of emergence of the new serotype could be genetic recombination, natural selection and genetic bottlenecks. There is no indication of the presence of DENV-5 in India. Recent clinical trials with the promising Chimerivax tetravalent vaccine suffered a setback. Discovery of DENV-5 and more such sylvatic strains in future may further impede the Dengue Vaccine Initiative. Integrated Vector Management holds the key to sustainable dengue control. Further epidemiological and ecological studies are needed to detect additional sylvatic dengue strains.

Introduction

Dengue fever has re-emerged as a major public health challenge worldwide; with 2.5 billion people at risk of infection, more than 100 million cases and 25,000 deaths being reported annually.[1] As there is no licensed vaccine or specific treatment against dengue, preventive measures are the best strategy, which consist mainly of environmental management, spraying insecticides, and personal protective measures.

Till now, dengue infections were believed to be caused by four antigenically distinct serotypes, Dengue Virus (DENV)-1, DENV-2, DENV-3, and DENV-4; each generating a unique host immune response to the infection. These four serotypes are genetically similar and share approximately 65% of their genomes.[2] Dengue virus is transmitted to non-human primates (sylvatic form) and humans (human form) via a mosquito vector; primarily of the genus *Aedes*.

The new virus

The fifth and latest addition to the existing serotypes of dengue viruses is DENV-5 which has been announced in

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October 2013. DENV-5 has been detected during screening of viral samples taken from a 37 year old farmer admitted in hospital in Sarawak state of Malaysia in the year 2007. The infection in the farmer was initially thought to be an ordinary case of sylvatic dengue caused by DENV-4 which circulates among primates and *Aedes nivalis* mosquitoes in the forests of South East Asia.[3]

However, when the virus was isolated and a full genetic sequence was carried out, it was observed that the virus was phylogenetically distinct from the three previous forms of sylvatic DENV-4 and bore some similarity with DENV-2.[3] In the Sarawak outbreak, only one case was admitted and the other confirmed cases were treated on an outpatient basis, thereby indicating that the disease caused by DENV-5 is mild.

Since no new serotype of the virus had been reported for the last 50 years, it was initially believed that the new virus could be a variant of the dengue 4 serotype. However, when rhesus macaque monkeys who were pre-infected with the other four serotypes and had already recovered from the infection were infected with DENV-5, they produced a significantly different set of antibodies. This proved beyond doubt that the new virus was indeed a new serotype and not a variant of DENV-4.

Secondly, the viral titre of the secondary infections was four times higher than other serotypes, which follows the classification of a flaviviruses into serotypes based on the degree of viremia.[4]

Possible reasons for emergence of new virus

Co-circulation of multiple dengue serotypes coupled with increased human activity increases the likelihood of genetic changes, leading to diversity in virus populations. Genetic recombination, natural selection and genetic bottlenecks have been implicated as factors which may lead to the emergence of new serotypes.

Dengue viruses, being RNA viruses, have high mutation frequencies with mutation rates being more than 100 times greater than the mutation rates of DNA genomes. The accumulation of mutations is a continuing process, which, together with the possibility of intramolecular recombination due to simultaneous infections with different dengue virus serotypes, could lead to the emergence of a novel dengue virus serotype differing at one or more critical neutralising epitopes.[5]

Following extensive phylogenetic analysis, it has been hypothesised that the earlier four lineages of dengue viruses evolved in non-human primate reservoirs thousands of years ago and then jumped over from these ancestral sylvatic progenitors to humans due to clustering of sylvatic strains with human strains as a result of increased human activity. This ancestral sylvatic-DENV transmission cycle still exists and is maintained in non-human primates and Aedes mosquitoes in the forests of South East Asia and West Africa.[6]

The exact reason for emergence of DENV-5 is not clear. The new serotype has only been found in forests of Sarawak. This serotype circulates primarily amongst non-human primates and follows the sylvatic cycle unlike the other four serotypes of dengue which are transmitted between humans. Though sylvatic dengue virus strains have infected humans before, isolation of these sylvatic strains has revealed that they were closely related to one of the four current serotypes.

As was the case with the other four dengue virus serotypes, it has been suggested that DENV-5 has been circulating among non-human primates in the forests of South East Asia for centuries before jumping the human barrier. The virus has been maintained in macaques with a spillover into humans, resulting in the Sarawak outbreak. Phylogenetic evaluation revealed that DENV-5 is genetically similar to the other four serotypes, thereby hinting to a common ancestral origin.

The present threat of the surfacing of sylvatic DENV by spillover in human populations has been elicited in recent studies using laboratory models for the replication of sylvatic DENV in humans.[6] The replication profiles of low-passage DENV-2 strains representing all major genotypes were evaluated in two surrogate models – human monocyte-derived dendritic cells (moDCs) and severe combined immunodeficient (SCID) mice xenografted with human hepatoma (Huh-7) cells. It was observed that though the strains differed in their replication profiles, there was no marked difference between sylvatic and human strains. However, the replication of sylvatic DENV-2 in moDCs was remarkably similar to that of human DENV-2 from the American genotype.

Besides, the replication of human and sylvatic strains was measured in cultured cells from human (Huh-7 cells), monkey (Vero cells) and mosquito (C6/36 cells from *Aedes albopictus*) hosts; wherein human DENV strains produced considerably more progeny only in the human cells when compared to their sylvatic counterparts.

The results of the experiments involving surrogate human models and the cultured cells indicate that little or no adaptive barrier exists to prevent the emergence of sylvatic DENV in a wide range of primate hosts including humans, as has been demonstrated by the genomic analysis of the Sarawak outbreak isolate.

Jumping the human barrier by DENV-5 may be attributed to deforestation. As sylvatic dengue is native to the natural forests, deforestation activities such as uncontrolled population movement, unplanned and substandard housing, poor water storage facilities and improper waste disposal management systems provide ideal conditions for the emergence of DENV-5 by disturbing the ecological niche.[7]

Impact on dengue control

Occurrence of new cases DENV-5 may lead to new challenges in dengue control. DENV-5 has so far been linked to only one outbreak in 2007, thereby indicating that the new serotype probably has a low transmission rate. However, fresh outbreaks cannot be ruled out. Moreover, the serotype may spread to virgin areas to further complicate the situation.

As dengue has re-emerged with vengeance as a major public health problem and has spread from urban to rural areas and to countries where it was non-existent, immediate surveillance and control measures need to be put in place before DENV-5 also assumes epidemic proportions just like its predecessors. Presently, the new serotype is believed to be limited to the forest canopies of South East Asia, but in the

present age of air travel, transmission to other countries cannot be ruled out.

There is no indication of the presence of DENV-5 in India. It may be assumed that as human DENV circulates in abundance in India, it may be providing cross-immunity against sylvatic dengue by competitive exclusion.[8] However, complacence should not set in as the vector mosquito *Aedes niveus s l*, and the ideal sylvatic hosts in the form of NHPs are available in our country. It may very well be possible that a hitherto undetected sylvatic transmission cycle may be present in the forests of India; which may not be amenable to easy detection owing to the lack of public health infrastructure including diagnostic modalities in the country.

The detection of DENV-5 has also raised speculation that there might be more serotypes which have not been identified till date. Further research is underway to address the unanswered questions on the evolution of DENV-5.

Vaccine development prospects

For a dengue vaccine to be effective, it must give protection against all homotypic and heterotypic infecting serotypes. Development of safe and cost-effective tetravalent dengue vaccine has been on the top agenda of the public health stakeholders since the last decade.

Primary infection with a single serotype confers long-lasting homotypic immunity for that particular serotype. However; immunity to other serotypes is short lasting. In fact, secondary heterotypic infection is associated with an increased risk of potentially fatal DHF and dengue shock syndrome through antibody dependent enhancement (ADE) of infection.[9] Because of the above pitfalls, a safe and effective dengue vaccine is yet to be licensed. To neutralise the ADE phenomenon, research has focussed on the development of a tetravalent vaccine which is capable of providing long-term immunity against all virus serotypes.

There are several candidate vaccines being developed and evaluated in clinical trials after initial steps were taken by Mahidol University, Thailand and Walter Reed Army Institute of Research (USA). These include several live-attenuated virus vaccines, live chimeric virus vaccines, inactivated virus vaccines as well as live, recombinant, DNA and subunit vaccines.[9] Recent technological advances are focussing on virus-vectored and virus-like particle-based vaccines which are being evaluated in pre-clinical studies.

Live viral vaccines have demonstrated varied immunogenicity to the four serotypes and viral interference phenomenon. Subunit vaccines focussing on the E-protein have failed to elicit a balanced antibody response to the four serotypes.

The most advanced vaccine at present is a live-attenuated Chimerivax Dengue tetravalent vaccine (CVD1-4) developed by the US Centers for Disease Control and Prevention (CDC) which utilises the licensed yellow fever 17D vaccine as backbone. It is based on Chimerivax™ system, which was initially used to develop a candidate live-attenuated Japanese Encephalitis vaccine. This approach replaces the E-gene of the 17D yellow fever (YF) vaccine with the analogous gene of the vaccine-targeted flavivirus. Chimaeric YF/DEN viruses have been constructed for all four serotypes, utilising the donor genes from DENV-1, PUO-359; DENV-2, POU-218; DENV-3, PaH881; and DENV-4, 1228 strains.

Though pre-clinical and Phase I studies demonstrated that the Chimerivax dengue tetravalent vaccine is stable, immunogenic and safe, Phase II b studies suffered a setback with only 30 percent efficacy; the vaccine being found to be efficacious against only DENV-1, DENV-3 and DENV-4 serotypes,[10] thereby pointing to the fact that the risk of ADE phenomenon following vaccination still looms large and further modifications are needed before safety can be guaranteed. The vaccine has moved on to Phase III efficacy trials and has been licensed for manufacture to Sanofi Pasteur.

Even after more than six decades of research, a licensed vaccine against dengue is still elusive. Even if a tetravalent DENV vaccine is found to be safe and immunogenic and gets licensed in the near future, it may not be able to offer protection against additional DENV serotypes which may be existing in the sylvatic cycle. Future discovery of such sylvatic strains would severely hamper the dengue vaccine initiative.

The present spillover of sylvatic DENV-5 suggests that the adaptive barrier for the emergence of sylvatic DENV in humans is either non-existent or too low to be of significance. Hence, even if vaccination programmes using the present tetravalent vaccine are able to control dengue for a short while; the long-term prospect of dengue eradication may not be feasible due to the existence of sylvatic DENV reservoirs in jungle canopies.

Therefore the development of dengue vaccine should be seen only as an adjunct to other public health measures such as vector control, community participation and political will.

Dengue control measures

Dengue prevention and control will depend upon effective, long-term management of the Aedes vector. To be cost-effective and sustainable, dengue control needs to be achieved through integrated community-based action.

Integrated Vector Management (IVM) is a multi-pronged, rational strategic framework for regulation of dengue control activities.[11] It focuses mainly on integration of social mobilisation, environmental management, epidemiological and entomological surveillance, use of insecticides targeting the adult mosquitoes and their larval stages, and biological control using natural predators. The objective is to achieve dengue control in a cost-effective and environment friendly manner, utilising the efforts of the local community in collaboration with the public and private sectors. IVM ensures proper insecticide management and also empowers the local community, thereby bringing about a behaviour change at the grass root level.

As the Aedes vector dwells in domestic and peri-domestic settings and lays eggs in artificial water containers that have been created because of daily living activities undertaken by the communities themselves, the communities need to be educated about this specific behaviour of the vector and the corrective environmental modification (permanent and long-lasting) and environmental manipulation (temporary and short lived) measures that they need to take to reverse Aedes breeding. School-based dengue control programmes are also needed to educate generations for the future and bring about a sense of social responsibility.

The role of information, education and communication material in dengue control cannot be over-emphasised. As India has a diverse culture, the attitude and practices of all the major ethnic, social, linguistic and cultural groups should be studied and analysed, and health education material should be developed targeting all such groups.

As no vaccine is likely to be available against dengue in the near future, integrated community-based interventions hold the key toward achieving sustainable dengue control.[12]

Conclusion

The discovery of DENV-5 epitomises an autonomous cross-species transmission of sylvatic form of dengue to humans. As little or no adaptive barrier exists to the emergence of sylvatic DENV in the human population, re-emergence of sylvatic DENV in the human transmission cycle is a realistic prospect.

However, as the interaction between sylvatic strains and human populations is poorly understood, investigation of the epidemiology of sylvatic DENV becomes essential. Besides; ecological studies are urgently needed to detect the existence of additional sylvatic dengue strains amongst non-human primates and their modes of transmission to humans, including the role of the implicated vectors in dengue transmission.

However, the same is an uphill task as such a surveillance will require dedicated health staff to work in the jungles and state-of-the-art health and laboratory infrastructure to distinguish sylvatic dengue from the human form.

Conflicts of interest

All authors have none to declare.

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