REVIEW ARTICLE

Chikungunya virus; an emerging arbovirus in Pakistan

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Abstract

Chikungunya virus (CHIKV), an arbovirus belongs to the family Togaviridae and was discovered in Tanzania in year 953-54. In November 2016, an outbreak occurred in Karachi and approximately 30,000 individuals were infected with CHIKV. More than 4,000 cases were confirmed by qualitative reverse transcriptase polymerase chain reaction. However, actual numbers of cases are expected to rise. For the diagnosis of chikungunya virus, several methods including viral culture, detection of viral antigen, anti-CHIKV immunoglobulin M, immunoglobulin G antibodies and viral nucleic acid can be used. The recommended therapies include use of analgesics, antipyretic, anti-inflammatory medications like paracetamol and non-steroidal anti-inflammatory drugs (NSAIDs). In severe cases, where NSAIDs are not effective, disease modifying anti-rheumatic drugs (DMARDs) can also be used. For prevention, mosquito nets and mosquito repellents are vital and must therefore be used effectively.

Keywords: Chikungunya virus, Arbovirus, Togaviridae, Pakistan.

Introduction

The term "chikungunya" refers to a viral disease transmitted to the humans by mosquito bite and is characterised by an abrupt onset of fever, with debilitating joint pain that may last up to a few days to weeks. The chikungunya patients also report other symptoms, such as skin rash, muscle pain, fatigue, headache and nausea. However, gastrointestinal, cardiac and neurological complications are reported less frequently. Because of the milder nature of symptoms, chikungunya infection can easily be misdiagnosed, particularly in dengue-prevalent areas.

The virus was isolated in the year 1952-53 from a patient with febrile illness while an outbreak on the Makonde plateau in the Southern province of Tanzania was going on.[1] The name chikungunya was derived from Swahili or Makonde language word Kan-qunwala means "to become contorted" or "twisted". The chikungunya virus (CHIKV) is an arbovirus, which is transmitted by mosquito species such as *Aedes albopictus* and *Aedes aegypti*.[1] The virus belongs to the family Togaviridae and genus alpha-virus. Based on antigenic properties, CHIKV is grouped into different sero-complexes.[2]

It is widely believed that CHIKV was originated in Africa and at least two genetically distinct lineages known as "West African and the East Central and Southern African (ECSA) lineage have been identified. Moreover, the ECSA also includes some Asian genotypes.[3] After the initial discovery of CHIKV, various smaller outbreaks were recorded in Africa. The earliest confirmation of the CHIKV in Asia was done during an outbreak in Philippines in the year 1954, which was followed by subsequent outbreaks in the years 1956 and 1968. Later on in the year 1970s, frequent outbreaks occurred in South and Southeast Asian territories. However, incident rate gradually decreased and only localized outbreaks were reported during the year 1982-1985. Previously, sporadic cases of CHIKV have been reported from Sri Lanka, Thailand, Cambodia, Vietnam, Pakistan, Laos, Philippines, Burma and India.[4] With the first report of the outbreak, in the year 1998 the virus was further disseminated to Malaysian territories.[5] Recently, CHIKV has re-emerged in African and Asian countries and became a substantial public health threat, particularly because of its increasing association with larger outbreaks. The frequent occurrence of CHIKV outbreaks have been attributed to the evolutionary adaptation of the virus in the mosquito vector.[6] In a viral collection of year 2006, mutation in enveloped protein (E1; A226V) was identified, that presumably contributes in the viral fitness inside the *Aedes albopictus* mosquito.[7] Both virological and serological confirmations of the virus have been reported from wider areas of the world, including countries of the West, East, South and Central Africa. Most recent epidemiological data suggests CHIKV has re-emerged in Kinshasa, Democratic Republic of Congo, after 39 years of the last official report. Similarly, in 2001-2003 the virus was once again reported in Indonesia after 20 years.[8] In the year 2004, reports from the coastal towns of Kenya, Lama and Mombasa confirmed CHIKV that was further spread to the Comoros Islands (Grande Comore, Moheli, Anjouan and Mayotte). During this time, in Grande

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Comoro Islands total population was 341,000 and approximately 215,000 were infected with CHIKV.[9] In May 2005, CHIKV infection was reported in Reunion Island and until early 2007, total 244,000 cases were reported with 203 infections associated deaths.[10] In the same island, sporadic cases were confirmed in the year 2009 and another outbreak occurred in year 2010 in which 100 cases of CHIKV infections were confirmed. Active transmission of the virus in this territory has been linked to *Aedes albopictus* and its reintroduction from Madagascar.[11] Similarly, a massive outbreak occurred in an Indian Ocean island in the year 2005 and that was due to a new lineage of the virus originated from Africa called Indian Oceans lineages (IOLs). It arrived in India, Southeast Asia and Southern Europe.[12] Recently, in year 2013, a CHIKV strain originated from Asia has re-emerged and was reported in South, Central and North American territories.[13]

In 2014, autochthonous transmission of ECSA genotype of the virus was reported in Brazil.[14] The virus was disseminated to different areas of Brazil. However, the index case contained no E1: A226V mutations.[15] According to the Pan American Health Organisation (PAHO) the virus dispersed to 26 Islands and 14 mainland countries within a year, leading to an estimated more than one million cases of infection.[15]

Most recently, a CHIKV outbreak occurred in Karachi. In Pakistan as early as 1983, the CHIKV was found in rodents.[16] A few cases of CHIKV were reported during the dengue fever outbreak in Lahore in year 2011.[17] The major outbreak in Karachi started in the middle of November 2016. According to the different healthcare authorities in Karachi, approximately 30,000 individuals were infected. By qualitative reverse transcriptase polymerase chain reaction (RT-PCR) more than 4,000 cases were confirmed by the National Institute of Health (NIH) and Armed Force Institute of Pathology (AFIP), Pakistan.[18] Outbreak continued from January to early May 2017, and the World Health Organization (WHO) was informed on April 13 by the Ministry of National Health Services, Regulations and coordination of Pakistan. From December 19, 2016, to March 30, 2017, a total of 1,018 suspected cases were reported.[19] According to the NIH, out of 157 suspected samples collected during this period, 121 were confirmed positive for the CHIKV infection.[19] In Karachi, the highest numbers of the

***Marked are the Areas of Karachi from where CHIKV cases were frequently reported during the outbreak of year 2017**

Figure-1: The suspected cases of Chikungunya virus was reported from the different areas in Karachi.

suspected cases were reported from the areas of Ibrahim Hyderi, Keamari, Malir and Lyari (Figure-1).[19] In fact the actual numbers of the CHIKV cases during the recent outbreak in Karachi might be much higher, yet no evidence based epidemiological data has been reported so far. The prolonged duration of warm weather, poor sanitation system, stagnant water in different parts of the city and less effective mosquito eradication programmes may further complicate this outbreak.

Transmission, lifecycle and pathogenesis of CHIKV

In the Asian-Pacific region, female mosquitoes such as *Aedes aegypti* or *Aedes albopictus* are the predominant vectors. In Africa, however, *Aedes furcifer*, *Aedes taylori* and *Aedes vittatus* have also been involved in the transmission of CHIKV.[20] The virus has two discrete transmission cycles; sylvatic cycle and urban cycle (Figure-2). The sylvatic cycle involves viral transmission between forest mosquitoes and non-human primates such as monkeys and up to some extent rodents. While in the urban cycle, transmission mainly occurs between mosquitoes and humans living in the urban environments.[21] The vertical transmission of the virus from mother to foetus and via blood transfusions has also been reported.[22,23] Following the mosquito bite, limited viral replication has been witnessed inside the endothelial cells. Moreover, virus can invade fibroblasts, pertaining to the involvement of muscles, connective tissues and joints. The virus replicates in lymph nodes, rising up to the titre 10^8 virions per millilitre of the blood, this may further disseminate the virus into brain, liver, muscles and joints. Overall, circulatory blood cells seem to be the most resilient cells against viral invasion.[21,23] In humans, it takes at least one week to mount appropriate adaptive immune response. Innate immune response against CHIKV is initiated by pattern recognition receptors, toll-like receptors 3 (TLR3), TLR7 and TLR8. These receptors initiate signalling cascades, which leads to the stimulation of type I interferons and cytokines. Interferon-stimulated genes encode more than 300 proteins and play a pivotal role in the host defence. Mouse model experiments showed that TLR adaptor protein MYD88 (myeloid differentiation primary response gene 88) dependent immune

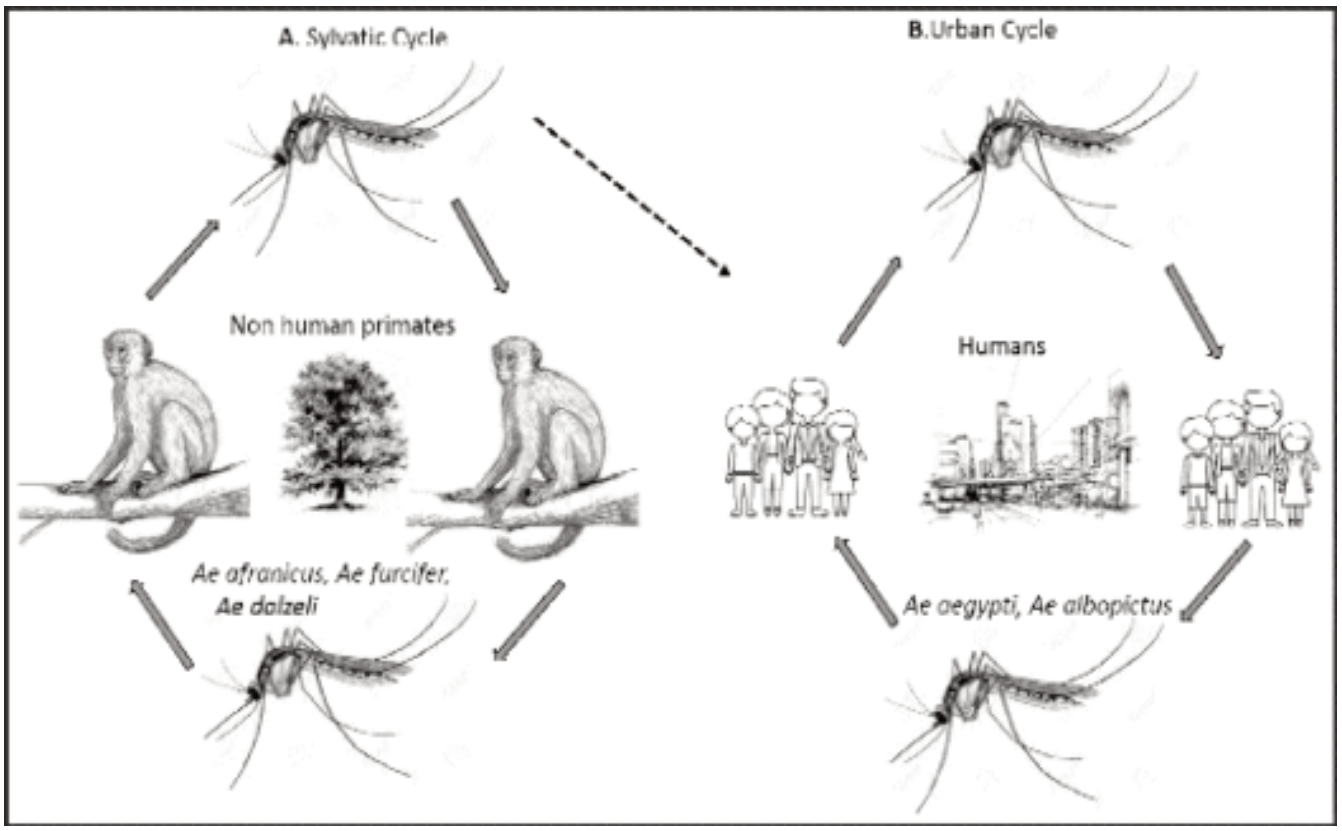

Figure-2: Lifecycle of the Chikungunya virus. Part A represents sylvatic cycle, where transmission is between mosquitoes-monkeys mosquitoes. Part B represents urban cycle where transmission is between mosquitoes-human-mosquitoes. Dashed arrow represents occasional interconnection between sylvatic and urban cycle.

response also plays a role in protection against the CHIKV virus.[24] Though not much is known about the exact role of the adaptive immune response against the CHIKV infection, mouse models lacking T and B cells (recombination activating gene 2 [RAG2]) show higher level of viraemia endorsing the vitality of the adaptive immune response in CHIKV elimination. Recently, the involvement of cluster of differentiation 8+ (CD8+) T lymphocyte and CD4+ T lymphocyte during early and late stages of infections was confirmed.[25]

Signs and Symptoms

The typical signs and symptoms include severe joint pain and high-grade fever associated with the acute phase of infection.[26] The arthralgia may affect entire body, mainly the distal joints.[27] Other symptoms such as skin rash, muscle pain, fatigue, headache and nausea are reported by chikungunya patients. However, gastrointestinal, cardiac and neurological complications are reported less frequently. CHIKV can cause acute, sub-acute and chronic infections. The long-term consequences include arthralgia, arthritis, depression, alopecia, fatigue, and mood disturbance, and sleep disorder, headache, hearing difficulties, impaired memory, blurred visions, skin lesion/rashes and digestive complications.[26]

Diagnosis of CHIKV

Epidemiological, clinical and laboratory correlation are vital for the diagnosis of CHIKV infection. The severe arthralgia or arthritis with acute onset of fever, not explained by any other medical conditions may indicate CHIKV infection, particularly if the individual visited or lived in the epidemic areas. However, due to its epidemiological and clinical similarities with dengue virus precise diagnostic of CHIKV remains challenging, particularly in the areas vulnerable to the dengue infection. Therefore, clinicians may misdiagnose it as dengue fever, which supports the notion that its actual incidence rate might be much higher than currently reported. The appropriate and rapid diagnosis is therefore vital to counter any epidemic.[21]

Clinical and Laboratory Diagnosis

In symptomatic patients, the infection is characterised by backache, headache, fatigue, poly-arthralgia followed by high fever. The symptoms usually appear within 4-7 days. Joint pain is reported in more than 85% of the patients while cutaneous manifestations such as maculo-popular rash on face and trunk are reported among 40-50% of the cases.[20,23] Laboratory and differential diagnosis is crucial to aid physicians in differentiating CHIKV from other etiological agents. For this purpose acute phase serum sample obtained within seven days of the infection offers best diagnostic option as it contains higher titer of the virus, suitable for the nucleic acid detection and viral isolation. PCR seems to be the rapid and sensitive technique for the detection of viral nucleic acid. CHIKV nucleic acid detection is used for the early diagnosis, particularly by performing RT-PCR and real-time loop mediated isothermal amplification (TR-LAMP) methods.[27] In low-resource countries such as Pakistan, the TR-LAMP may also be used as an alternative assay as it does not require PCR instruments.[28] In addition, Vero cells, mosquito cells (C6/36) or mouse models can be exploited for culturing CHIKV. However, culturing may take at least one week, requires expertise and specialised laboratory set-up with biosafety level-3 facilities.[3] Yet, the isolation and culture of the virus from the blood of the infected individual is the most reliable method.[28] Anti-CHIKV immunoglobulin M (IgM) is detected after 3-5 days of infections and remains elevated for 3-6 months.[28,29] After two weeks of the infection, anti-CHIKV IgG antibodies are detected and may remain elevated for years. CHIKV titer in the blood can also be detected by enzyme-linked immunosorbent assay (ELISA) and chromatographic techniques.[28] Serological assays such as ELISA, immunofluorescence, complement binding and haemagglutination are important alternative diagnostic tools but offer limited sensitivity and specificity.[30]

Treatment

Till to date, no approved antiviral or vaccines are available for the CHIKV infection. Recommended therapies include the use of analgesics, antipyretic and anti-inflammatory drugs like paracetamol and non-steroidal anti-inflammatory drugs (NSAIDs).[20] The use of aspirin should be avoided due the potential risk of bleeding or development of Reyes syndrome. In severe cases, where NSAIDs are not effective the disease modifying anti-rheumatic drugs (DMARDs) like methotrexate and sulfasalazine could be used.[31] For the treatment of chronic CHIKV arthritis chloroquine phosphate has long been reported to be effective.[28]

Prevention and Control

For the effective prevention of CHIKV infection, top priority should be public awareness, especially information regarding mosquito breeding and density of the vector is crucial. Stagnant water ponds, old automobiles tires, and risk factor associated with the

use of water for plants and gardening should be given higher attention. In endemic areas various protective measures such as mosquito repellent spray, long-sleeved clothes and mosquito net are highly recommended.[15] For the control of mosquito, larvicides and insecticides should be used. Recently, anendosymbiotic bacterium Walbachiapipientis is used for the control of arboviral transmission by infecting *Aedes aegyptia*. The use of this bacterium may reduce replication and transmission of different arboviruses, including CHIKV.[32] Moreover, various strategies have been and are being used for the development of vaccine against CHIKV which include live attenuated, inactivated, chimeric, sub-unit protein, virus like particle and deoxyribonucleic acid (DNA) vaccine.[33] Recently, a few vaccines are being evaluated in preclinical and early phase of clinical trials.[33]

Conclusion

Various factors are contributing in the recent emergence of CHIKV, including urbanisation, viral adaptation, human travel, absence of effective control measures and spread of new vectors. The vaccines are possibly the best preventive measures, however, none of the vaccine were commercially approved till to date. The increase in the incidence of the arabovirus infections such as CHIKV, dengue and Zika virus in recent years highlights the urgent need to recognise cost-effective strategies for the control of vectors (*Aedes aegypti* and *Aedes albopictus*). The entomological surveys are extremely important in highlighting vector density and can help in devising timely strategies for the effective vector control. In short, to minimise the spread of vector-borne diseases like CHIKV at global level, a collaborative approach should be given high priority. In developing countries like Pakistan, there is an urgent need for the national surveillance programme, particularly for the infectious diseases. Institutional vigilance and preparedness should be given high priority at national and international levels.

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