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Chikungunya infection: A potential re-emerging global threat

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ABSTRACT

Infectious diseases are indeed a lifelong threat to everyone irrespective of age, sex, lifestyle and socio-economic status. The infectious diseases have persisted among the prominent causes of death globally. Recently, re-emergence of Chikungunya viral infection harmed many in Asian and African countries. Chikungunya was considered as a major threat in developing and under-developed countries; the recent epidemiological outbreak of Chikungunya in La Reunion urges the global researchers to develop effective vaccine against this viral disease. In this review, Chikungunya, pathogenesis and epidemiology were briefly described.

1. Introduction

Since the evolution of human race, infectious diseases also have made headway. Even with the rapid development of modern health care systems like usage of synthetic or recombinant drugs, we are still a step backward to nature, as some new diseases occur time-to-time challenging the humanity. An infectious disease is defined as a clinically manifest infection, resulting due to the pathogenic microorganisms, normally illustrated as contagious (communicable) having ability to transform from one person/species to other through physical contact or it may be vector-borne or air-borne. Even though many medical advances have been made during the past two decades of the 20th century, there are still new and re-emerging diseases, adding up with the massive spread of antibiotic resistant pathogens and disease carrying insects that are resistant to insecticides, are still scary challenge to human health. Still it is a nightmare situation for public health system due to the emergence of new diseases and also the re-emergence of old ones.

Emerging and re-emerging infectious diseases may occur anywhere across the world and its effect will be severe and unpredictable. Advancement in the field of medicine during the past few decades made to believe that the infectious diseases are under the control of humankind. Recently, re-emergence of old infectious diseases debilitated many people globally. Re-emerging diseases are already identified diseases that have come back after a significant delay in incidence. Some epidemic diseases spread from one continent to another that affects the country's economy and livelihoods. Global food trade is one of the major factors that cause the infectious diseases to flourish across the world. Many emerging human diseases originate from animals and transmitted to humans *i.e.,* zoonosis. It has been reported that the 75% of emerging and re-emerging pathogens are zoonotic [1]. The zoonotic emerging infectious diseases threaten humans as well as animal health. Much attention is given to the zoonotic infectious diseases because of their potential to transmit from animals to humans and also from human to human transmission way. The areas rich in wildlife are always the hotspots for most of the zoonotic diseases [2].

Infectious diseases are potentially contagious caused by microorganisms. Virus that has RNA as genetic material causes most of the infectious diseases. The great impact of RNA viruses in causing diseases are due to the fact that they are easily adapt to the varying environmental conditions because of the high error rates of the virus enzymes (RNA polymerases) that replicate their genomes, which doesn't have proof reading activity [3]. However, a complex interplay of factors can influence disease emergence

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like genetic variation that occurs in the virus genome and also the environmental factors like social and health care [4].

2. Tropical countries and infection

Historically contagious diseases hit the tropical regions more as compared to the temperate regions. The most common disease carrying vectors are mosquitoes and arthropods [5]. Human movement is one of the key factors that influence the exposure to vectors and the transmission of pathogens. Normally, the outbreaks of many human diseases in the tropical region occur during the wet season than in the dry season. The major reason being the disease vectors, like mosquitoes and flies are more abundant during the wet season [6]. Mosquito is one of the major disease causing vectors that spreads worldwide. In tropics, it is impossible to avoid the omnipresence of mosquito and its bite where mosquitoes and humans co-exist. Even though there are more repellents exist commercially, none seems to be effective against these lean mean-biting machines. Frequently female mosquito is the one, transmitting the diseases since the blood meal is obligatory for the mosquitoes in order to lay eggs. For laying eggs, mosquitoes mostly prefer freshwater, some species prefer brackish water [7]. The larvae from the eggs release into the water, where they complete their larval life cycle and emerge as adult mosquitoes. The most dreaded infections caused by mosquitoes are mainly Malaria, Dengue and Chikungunya.

3. Chikungunya

The word Chikungunya (CHIK) is used to represent both the virus and disease. Chikungunya virus (CHIKV) is an arthropod borne virus, belongs to the family Togaviridae and genus Alphavirus, which is responsible for Chikungunya fever in humans [8]. CHIKV believed to be originated from Africa and subsequently transferred to Asia. CHIKV infection was first reported in Tanzania in 1952 and first isolated in 1953 [9]. Chikungunya is a word meaning 'The bent walker/stooped walk' referring to the posture of the affected patient acquired due to unbearable pain in the joints and other rheumatologic complications [10]. CHIKV is geographically distributed in tropical and sub-tropical regions of many Asian and African countries. The virus was commonly transmitted by arthropods like mosquitoes *Aedes aegypti* (*Ae. aegypti*) and *Aedes albopictus* (*Ae. albopictus*), where it is maintained in 'sylvatic cycle' but the common reservoirs for the virus are monkeys and other vertebrates [11]. Mainly humans are the reservoirs for the Chikungunya virus (Urban Cycle) during epidemic period. Thus, alphavirus are maintained in mosquito vectors and vertebrate hosts. Phylogenetic analysis revealed that there are 3 different CHIKV isolates *i.e.,* West African and East Central South African and Asian genotypes with distinct antigenic and genotypic characteristics [12]. Due to the serious risk imposed by Chikungunya on humans, it was categorized as a category C priority pathogen by US National Institute of Allergy and Infectious Diseases in 2008 [13].

4. CHIK virion structure

Alphaviruses are membrane enveloped viruses, which consists of a single-stranded RNA as a genetic material and an icosahedral like nucleocapsid of size (60–70) nm diameter [14]. The alphavirus genome consists of single-stranded, positive sense RNA of about 11.8 kb with a 5′ cap structure and 3′ polyadenosine tail [9].

CHIKV contains two open reading frames (ORF) that code for structural and non-structural proteins. The amino acid sequence identity between CHIK and other alphavirus range from 58% to 85% and 42%–85% in case of non-structural and structural proteins, respectively [9]. Non-structural polyproteins (nsP1-4) are synthesized directly from the sub-genomic RNA that is essential for viral replication and processing. The junction region between two ORF is untranslatable composed of 68 bp that contains an internal promoter and start site for the transcription of sub-genomic RNA. The nonstructural proteins are mainly involved in viral pathogenesis by interacting with viral and host cell components [8].

All the structural proteins such as capsid, E1, E2, E3 and 6K are produced from the 26S sub-genomic RNA as a single polypeptide, which undergoes cleavage and post-translational modifications to form major three proteins capsid, E2, E1 and two minor proteins E3 and 6K that are involved in viral encapsidation and budding [15]. The CHIKV envelope protein E1 consists of 435 amino acids (45 kDa) which are largely covered by E2 in the viral envelope. Similar to E1, pE2 protein (65 kDa) is the type I transmembrane glycoproteins, which is the precursor for E3 and E2 proteins that undergoes post-translational modification to form E2 and E3 proteins. pE2 and E1 glycoproteins form heterodimers in the endoplasmic reticulum (ER), then E3 is cleaved from pE2 by furin in the golgi complex that eventually forms the E1–E2 heterodimers and later develop as spike on the virus envelope [16]. E2 protein belongs to the immunoglobulin super gene family that has three regions; ectodomain (260 amino acids), stem region (100 amino acids) and transmembrane helix. About 33 amino acids present in carboxy-terminal domain of E2 interact with the nucleocapsid core, which drives the budding process. E3 protein is a small α/β protein consists of 64 amino acids (11 kDa) that have disulfide isomerase activity helps in proper folding and disulfide bond formation in the viral glycoproteins. CHIKV 6K is small, hydrophobic protein essential for the viral particle assembly, which acts as a signal sequence for the processing of E1 protein [15,17]. The role of 6K protein in viral replication is not fully resolved.

Capsid protein is 261 amino acids (30 kDa) long, expressed as a part of structural polyprotein, which has conserved autoprotease domain in the C-terminal end that helps to release itself from the polypeptide string after synthesis of structural proteins [18]. The capsid is surrounded by a lipid bilayer derived from the plasma membrane of host cell in which almost 240 copies of E1 and E2 heterodimers in turn assembled into trimers (80 Copies) are embedded and forms the rigid structure across the membrane [19]. These envelope glycoproteins help in the attachment of viruses to the host cell during infection.

5. Replication of alphavirus

The envelope glycoprotein present on the surface of the viral capsid interacts with the membrane bound receptors and enters into the host cell by receptor mediated endocytosis [15]. The cell surface receptors like ICAM-3 grabbing non-integrin and laminin like receptors have been reported for other alphavirus, but for CHIK, no cell surface receptors have been identified both in mosquito and humans till date [8]. However, it was also reported that some viruses utilize clathrin dependent and caveolin dependent pathways for cellular entry [20,21]. The

envelope protein E1 mediates the low pH triggered membrane fusion of virus into the host cell. Inside the endosomes, the conformational change of E1-E2 heterodimer occurs, which is mediated by low pH that exposes the furin peptide towards the target membrane. The E2 protein has been responsible for receptor binding during the course of viral infection and delivers the nucleocapsid along with viral RNA into the host cytoplasm [16].

After entering the host cell cytoplasm, the capsid protein binds to large ribosomal sub-units and releases the viral RNA. The viral genome is then translated from two open reading frames to produce non-structural and structural polyproteins [22]. The N-terminal ORF encodes a nonstructural polyprotein of 2474 amino acids, nsP123 and nsP4. This precursor nsP123 is proteolytically cleaved and forms nsP1, nsP2, nsP3 and then join with nsP4. A leaky termination codon was observed in most of the alphavirus genome after nsP3; hence the protein synthesis occurs at 10%–20% efficiency that eventually leads to an excess of nsP123 than nsP1234. Hence, nsP4 is relatively low when compared to other nsPs. Furthermore, nsP4 is stable in the form of replication complex and degraded when it is in excess [23,24].

After the synthesis of non-structural polyproteins, it forms the replication complex within virus induced cytopathic vacuoles (CPVl) and initiates positive strand genomic and subgenomic RNA synthesis [15,25]. The sub-genomic RNA (26S) translates and produces viral structural polyprotein (1244 amino acids). After synthesis, capsid protein is cleaved off from the rest of the structural polyproteins due to its auto-proteolytic cleavage activity. The capsid protein recognizes the specific packaging signals in the 5′ half of the genome and packed with the RNA genome to form nucleocapsid [26]. The N-terminal signal sequence of E3 protein directs the remaining polyprotein to the endoplasmic reticulum, where it is processed by host signal peptidase. Similarly small 6K peptide acts as a signal sequence for the E1 protein processing. After modification, E1 protein interacts with pE2 (E3 + E2), thereby forming the heterodimers and transported *via,* golgi complex [16,27]. At the late stage of transport, pE2 is cleaved by host furin like protease, which eventually weakens the E1 and E2 heterodimer priming the exposure and activation of fusion peptide at the time of low pH exposure [8]. On the virion surface, E1 and E2 are closely paired and together form trimers that appear as ‘spikes’. Then the fully assembled virion particles acquire the lipid bilayer from the host cell plasma membrane and finally released from the host cell by budding process [28].

6. Vectors for CHIKV

Mosquitoes are the common vectors for most of the arbovirus and it is responsible for the 5 major diseases in India *viz*., Malaria, Filaria, Dengue, Chikungunya and Japanese Encephalitis. *Ae. albopictus* and *Ae. aegypti* is the major culprit for Chikungunya disease. Though *Ae. aegypti* is the primary classical vector for CHIKV, both the species is known to be susceptible to CHIKV infection. *Ae. albopictus* was responsible for the 2005–2006 Chikungunya epidemic in Reunion island and identified as a major vector in Europe, whereas *Ae. aegypti* is the dominant CHIKV carrier in India [29].

Aedes mosquitoes are known to be important vector for dengue and other arbovirus [30]. *Ae. albopictus* easily adapts in both the rural and urban environments that makes it a ideal viral vector. Besides, the mosquito's eggs are highly resistant even in dry period, giving rise to larvae in rainy season. All of these characters make *Ae. albopictus,* an important vector for spreading this disease. *Ae. albopictus* is geographically distributed in Asia, Europe, Middle East and America [29]. Thus worldwide urbanization and expanding geographical range of *Aedes* mosquitoes have driven the rise in Chikungunya infections globally. During the epidemic period, human beings act as reservoirs for the Chikungunya virus, whereas the major reservoirs are mosquitoes, rodents and birds [31]. Till now, there is no existing vaccine or specific treatment available to treat Chikungunya infection.

7. Outbreaks of Chikungunya

Chikungunya become a disease of global distress following its recent revitalization. The possible factors that influence the re-emergence of CHIKV include, immunologically compromised human population, global trade, international travel and most importantly the adaptation of the virus to anthropophilic Asian tiger mosquito (*Ae. albopictus*) [32]. After the first report from Tanzania in 1952, Chikungunya epidemics have been reported in major parts of Africa and Asia, in particular Southeast Asia, India, Pakistan, Sri Lanka, Myanmar, Thailand and in many islands of the Pacific Ocean like Madagascar, Mauritius and the Reunion Island (Indian Ocean) [33]. In 1958, CHIKV was reported in Asia for the first time in Bangkok [34]. In 1963, Chikungunya first made its headway in India (Kolkata), followed by Pondicherry and Vellore (1964), Visakhapatnam, Rajmundry and Kakinada (1965), Nagpur (1965) and Barsi (1973) [35].

From 1973 to 2005, no cases were reported in India. In 2006, Chikungunya suddenly re-emerged as a major outbreak in India after 32 years. Over 1400000 cases were reported during the outbreak [31]. Since 2005, several states in India experienced massive outbreaks of epidemic that badly affects the many people. In 2005–2006, Chikungunya hit Andhra Pradesh and Tamil Nadu, India, which was the recent major outbreak. The major complaint during that outbreak was high fever and crippling joint pain. In recent past (2007), Chikungunya outbreak in French La Reunion island, where mortality was reported and almost one third of the population (35%) was affected by Chikungunya fever [36,37].

After which, Chikungunya has been identified in almost 40 countries including Indonesia, Thailand, Malaysia, Philippines the Indian Ocean islands of Mayotte, Seychelles, Mauritius and La Reunion. Before the Chikungunya outbreak in Italy, the major question in 2007 was ‘When and where the next Chikungunya epidemic’ will re-occur? At that time the probable answer was tropical and sub-tropical countries. But after the outbreak in Italy, the risk imposed by Chikungunya was severe threat for both temperate and tropical world.

8. Symptoms and signs

After the CHIKV infected *Ae. aegypti* or *Ae. albopictus* mosquitoes bite humans, viruses start its multiplication in the human body that eventually causes arthralgic syndrome/Chikungunya fever. During epidemics, the attack rate was >50% and almost two thirds of the infected patients had to be

hospitalized [38,39]. Chikungunya infection is not fatal always but mortality was also reported in recent outbreaks in India, Italy and La Reunion [40]. Symptoms of Chikungunya fever were similar like dengue and often it is clinically difficult to distinguish from the dengue or O'nyong–nyong infection, which accounts for misclassification [8]. The major symptoms of CHIKV infection include fever, headache and joint pains/polyarthralgia that last for several days to months and the fatality was rare in most cases; whereas tenosynovitis, myalgia, headache, nausea, vomiting, lymphadenopathy, asthenia and dysgeusia were also reported in few patients [41,42]. After the infected mosquito bites the human, the virus starts replication and distributes to the liver/joints and damages the collagen and disrupts the connective tissue metabolism leads to acute arthritis [43]. The latent period for CHIKV normally ranges from (2–12) days. Usually CHIKV infection appears to be mostly symptomatic but asymptomatic infection was also reported in 3%–25% of people [44].

9. Diagnosis, treatment and prevention

Chikungunya virus can be diagnosed by viral culture but the facilities needed for culturing the viruses are not widely available in many underdeveloped and developing countries. Molecular tools such as RT-PCR and serodiagnostic methods like Enzyme-linked immunosorbent assay, indirect immunofluorescent method, hemagglutination inhibition, or neutralization techniques for the detection of IgM and IgG antibodies against Chikungunya virus in sera can also be used [31,45].

Currently there is no anti-viral therapy available to inhibit viral replication during human infection; hence the infected patients are given analgesics, anti-inflammatory drugs and paracetamol [46] to reduce the viremia symptoms. Taking non-steroidal anti-inflammatory drugs may reduce the symptoms of fever and pain [31]. Apart from this, vector control measures are recommendable that would probably reduce the density of *Ae. aegypti*/*Ae. albopictus*.

10. Current status of CHIKV vaccines

Several groups around the world are working on to develop CHIK vaccines using various strategies including inactivated viral vaccines, live-attenuated viruses, chimeric viruses, recombinant viral vaccines, consensus-based DNA vaccines, recombinant subunit vaccines and more recently, a virus-like particle vaccine [47–51]. The first live attenuated vaccine was reactogenic in clinical trials and chimeric alphavirus by employing structural protein showed good promise for CHIKV prevention in pre-clinical trials [52]. A virus like particle vaccine expressing Chikungunya polyprotein induced high antibody titres and protects monkeys from CHIKV infection after challenge [47]. A DNA vaccine based on Chikungunya envelope proteins elicit high neutralizing antibody titres and protected the mice from intranasal CHIKV challenge [48].

11. Conclusions

Chikungunya is a re-emerging disease which affects many people at regular intervals. However the clinical features and number of affected people are keep on increasing compared to its previous outbreak. The proper control measures and preventive strategies are essential to prevent this epidemic. The recent outbreak of Chikungunya has affected many countries and hence it is essential that researchers develop vaccine to control this disease. Still there is no anti-viral treatment, however many vaccines are in development or clinical trials. The collaboration of several researchers might helpful to develop an effective treatment against this infection.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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