2.1 Chikungunya

Chikungunya is an arboviral fever caused by Chikungunya virus, which is a member of the genus Alpha virus of the family Togaviridae (Porterfield 1980). CHIKV is closely related to the O'nyong'nyong virus, Semliki Forest Virus, Ross River and also the viruses that cause eastern equine encephalitis as well as western equine encephalitis (Enserink 2007; Vanlandingham et al., 2005). In the language of Swahili, the term “chikungunya” does mean “the bent walker” (Schuffenecker et al., 2006). Togaviridae consists of two genera, Alpha virus and Rubivirus. There are approximately 40 Alpha viruses, which infect various vertebrates such as humans, rodents, birds, and horses and in addition to other invertebrates (Calisher and Karabatsos 1988).

2.2 Structure and Genome organization

The electron micrograph of CHIKV virions, exhibits typical Alpha virus structure. Virus structure shows a roughly spherical shape with a diameter of 42 nm and the core sized 25–30 nm in diameter, which is surrounded by an envelope (Simizu et al., 1984). In general Alpha viruses are enveloped particles and their genome is a positive-sense single-stranded RNA molecule, which has around 12 kb nucleotides. CHIKV has 11.8 kb positive sense genomic RNA, could act as a cellular messenger RNA. The genomic RNA is capable of translating into non-structural proteins as well as structural proteins directly. The 5’ end is capped with a 7-methylguanosine, whereas the 3’ end is polyadenylated (Faragher et al., 1988; Strauss & Strauss, 1986).

The genome of other Alpha viruses as well as CHIKV is as follows: 5’ cap-nsP1-nsP2-nsP3-nsP4-(junction region) -Capsid-E3-E2-6K-E1-poly (A) 3’ (Calisher and Karabatsos 1988; Santhosh et al., 2009). Sub-genomic region (Last one third) codes for structural proteins, which is also mentioned as 26S RNA (Christine et al., 2007). The open reading frames has 7,422 nucleotides coding non-structural poly-protein, consisting of 2,474 amino acids and 3,744 nucleotides encoding structural poly-protein with 1,248 amino acids (Khan et al., 2002).

The non-structural proteins (nsP) are synthesised in the early course of infection and has role in transcription and replication of the viral RNA. Nsps are, directly from genomic RNA as a P1234 polypeptide further this polyprotein cleaves to form Nsp1
Figure 1: Electron micrograph of CHIKV virions (Solignat et al., 2009).

Figure 2: Organization of non-structural as well as structural genome and its products of CHIKV (Christine et al., 2007).
(535 amino acids), nsP2 (798 amino acids), nsP3 (530 amino acids), and nsP4 (611 amino acids). The Nsp1 is considered to play a role in the synthesis of negative sense-strand RNA for replication of viral RNA. The Nsp2 main functions are helicase and proteinase, which cleaves the non-structural poly-protein to form the individual proteins (Hardy and Strauss, 1989; Kim et al., 2004; Merits et al., 2001; Vasiljeva et al., 2001; Strauss et al., 1992). The exact role of Nsp3 in viral replication remains unknown; the Nsp4 is the viral RNA polymerase enzyme, which interacts with the N-terminal region of other non-structural proteins and unknown host cellular factors (Khan et al., 2002; Strauss and Strauss 1994).

The structural proteins viz., C (261 amino acids), E3 (amino acids), E2 (amino acids), 6K (61 amino acids) and E1 (439 amino acids) are synthesized as a single polypeptide, further both co-translationally and post translationally to form structural proteins (Strauss & Strauss, 1986, 1988; Faragher et al., 1988). Structural proteins has role in the replication of the virus as well as interaction with the host especially with antibodies (Griffin and Johnson 1977; Khan et al., 2002).

The junction region functions to promote transcription of an intracellular sub-genomic 26S RNA. The un-translated regions of the 5’-end is required for plus-strand RNA synthesis (Ou et al., 1983; Strauss & Strauss 1994) and the other one at the 3’ end between the stop codon of the E1 gene and the poly (A) tail is mainly involved in translation of viral proteins (Kuhn et al., 1991; Leathers et al., 1993).

Nucleotide Polymorphism: The analysis of whole genome sequence from the past three consecutive chikungunya outbreaks in Kerala, South India, were analysed for genetic differences by sequencing the 11798 bp whole genome of the virus. A total of 37 novel mutations were identified and they were predominant in the 2007 and 2008 isolates among the six isolates studied. E1 A226V mutation, which increases adaptability for multiplication in Aedes albopictus mosquito, was present in samples. Further observations have shown the presence of two coding region substitutions, leading to nsP2 L539S and E2 K252Q change in three isolates (2007 RGCB80 and RGCB120; 2008 RGCB355) by full-genome analysis (Sreekumar et al., 2010).
Studies have confirmed that in Alpha viruses, the infectivity and virulence-associated gene mutations are distributed both in the structural and non-structural protein coding regions of the viral genome (Fazakerley et al., 2002; Kielian et al., 1996; Mayuri et al., 2008). Several mutations in the structural and non-structural protein coding regions were detected in the CHIKV isolates from the Re’Union outbreak (Santhosh et al., 2009).

It has been hypothesized that explosiveness and high magnitude of the outbreak was attributed due to a point mutation in the E1 (A226V) protein during the 2005–2006 Indian Ocean epidemic (Santhosh et al., 2009). This mutation also believed to have altered the vector competence and epidemic potential of the CHIKV (Tsetsarkin et al., 2007; Vazeille et al., 2007). This mutation has also been documented from the Indian subcontinent, including those from Kerala (Kumar et al., 2008; Santhosh et al., 2008).

Whole genome analysis indicates that CHIKV has the tendency to get transported through travellers to anywhere and it can also adapt to the new vector populations (Xavier et al., 2008).

Genotypes: Emergence of various genotypes of CHIKV has been reported in both Africa and Asia during the past two decades between the outbreaks (Powers and Logue, 2007). During the period from 1950s to 1970s many African countries have experienced epidemics of CHIKV. The new Central/East African strains were isolated in 2000. These viruses are believed to have originated from the outbreak in the Democratic Republic of Congo (Pastorini et al., 2004).

Phylogenetic analysis indicates that CHIKV strains cluster into 3 distinct groups based on originating from West Africa, Central/East Africa or Asia (Bessaud et al., 2006; Khan et al., 2002; Parola et al., 2006; Pastorino et al., 2004; Powers et al., 2000; Schuffenecker et al., 2006; Yadav et al., 2003). Phylogenetic relationships were examined using the E1, E2, and nsP1 gene sequences of their isolates and all other available CHIKV sequences obtained from NCBI Genbank (Hasebe et al., 2002; Abubakar et al., 2007). All these genes equally shared the similarity with available CHIKV isolates in phylogenetic analysis (Abubakar et al., 2007).
Whole genome sequencing is a complex and cost prohibitive procedure (Sanger et al., 1977). Therefore, finding an alternative to this technique should be prioritized. Multi-locus sequence typing (MLST) is a nucleotide sequence based approach that could be applied to study the procaryotic and eucaryotic pathogen biology (Urwin and Maiden 2003). MLST could be successfully used as an ideal tool for long-term tracking in population structure studies, global epidemiology and long-term surveillance (Maiden et al., 1998).

Recently developed double locus sequence typing (DLST) based on the analysis of two partial sequences, the usefulness of DLST also been evaluated for epidemiological investigations with the results obtained by PFGE. In addition, the unambiguous definition of DLST types makes this method also more suitable for long-term epidemiological surveillance (Basset et al., 2010). DLST data should be considered similar to MLST (multi locus sequence typing) data (Kuhn et al., 2007). To the best our knowledge there has been no attempt using MLST or DLST analysis for genotyping viruses.

2.3 Diagnosis
Clinical diagnosis of chikungunya fever is based on the key signs in acute stage such as fever and arthralgia. It resembles closely to dengue and other fevers caused by arthropod-borne viruses of the genus Alpha virus. Therefore, laboratory evidence is essential for diagnosis of infection, which caused by either Chikungunya or Dengue virus (WHO, 2009).

There are some similarities of symptoms between dengue and chikungunya like leucopenia (3 to 4 days) and thrombocytopenia. These two biological parameters could be used to aid the diagnosis of chikungunya infection in the acute phase in a region free of dengue fever such as European countries (Staikowsky et al. 2009). However in dengue endemic region like Malaysia, these criteria might not be as helpful (Ali et al., 2011).

The laboratory diagnosis of CHIKV infection is made by the combination of clinical diagnosis of a patients presenting with typical symptoms and laboratory. For molecular
diagnosis Reverse Transcription – PCR (RT-PCR) is commonly performed to detect a part of nsP1 and E1 genes (Hasebe et al., 2002). Recently, one-step TaqMan Real-Time-PCR, a specific and sensitive assay, which can diagnose in both clinical and culture supernatant has also been reported by Pastorino et al (2005).

National Institute of Virology (NIV) Indian Council of Medical Research (ICMR), Pune, India was mainly involved in detecting and monitoring the CHIKV outbreaks. An ELISA test to detect IgM antibodies is available at NIV Pune, which has been found as very much useful tool for the screening of chikungunya infection (Yergolkar et al., 2006).

2.4 Epidemiology of Chikungunya

Chikungunya was first reported in 1952 in the Southern Province of Tanzania (Robinson, 1955; Lumsden 1955). CHIKV is geographically distributed in Africa, India, and South-East Asia. In Africa, the virus is maintained through a sylvatic transmission cycle between wild primates and mosquitoes such as *Aedes luteocephalus*, *A. furcifer*, or *A. taylori* (Jupp and McIntosh 1988). Since the 1952 Tanzania outbreak, CHIKV has caused outbreaks in East Africa (Tanzania and Uganda), in Austral Africa (Zimbabwe and South Africa), in West Africa (Senegal and Nigeria), and in Central Africa (Central African Republic and Democratic Republic of the Congo) (Jupp and McIntosh 1988).

The first documented Asian outbreak was from Bangkok in 1958, Thailand, and subsequent outbreaks have been reported from Cambodia, Vietnam, Laos, Myanmar, Malaysia, Philippines, and Indonesia (Johnston et al., 1996; Jupp and McIntosh 1988). There were numerous outbreaks in East and South Africa and in Southeast Asia during the last 50 years, (Schuffenecker et al., 2006). The most recent epidemic re-emergence was documented in 2001–2003 in Java, after 20 years (Laras et al., 2005).

In the recent past chikungunya fever has attracted more attention when CHIKV caused an outbreak in a temperate region such as Italy where approximately two hundred cases were reported (Lines 2007). Tourism is thought to be the reason for virus transmission to Europe (Chastel 2005; Depoortere and Coulombier 2006; Service 2007).
Following the first reported outbreak of Chikungunya fever during 1963-1964 in Kolkata, India (Shah et al., 1964), it was also noticed in the erstwhile Madras in 1965. However, the last epidemic in India was reported in 1973 from Barsi, Maharashtra (Padbidri et al., 1979). After a period of three decades, CHIKF in the recent past has been emerging and re-emerging public health problem world over (Enserink 2007; Ravi 2006). The magnitude of the outbreak was higher than observed in the past (Pavri 1986). The number of suspected cases estimated to be in excess of one million in the country (NVBDCP, 2007).

The first ever confirmed outbreak of chikungunya fever was reported during July and August 2006 in Port Blair, the capital town of the Union Territory of Andaman and Nicobar Islands. (www.andaman.nic.in). By July 2009, a total of 1,568,630 suspected cases were reported throughout India. (Krishnamoorthy et al., 2009; NVBDCP, 2009). More than one-half of these cases were reported from the southern Indian state of Karnataka (Krishnamoorthy et al., 2009; NVBDCP 2009). Large scale occurrences of fever caused by CHIKV infection in more than a few parts of Southern India have confirmed the re-emergence of this virus (Yadav and Murthy 2006). These areas where Chikungunya has been noticed are also endemic for Dengue and Leptospirosis (Baruah et al., 2006; Padbidri et al., 1995).

Transmission of CHIKV occurs by the bite of an infected Aedes spp., during feeding blood meal, the mosquito deposits virus-infected saliva extravascularly (Turell 1995). Primarily the day biting Aedes aegypti is the established vector quit and recently Aedes albopictus (Asian tiger mosquito) was also found to be an important vector (Calisher and Karabatsos; Enserink 2007; Hochedez et al., 2006; Reiter et al., 2006). These Aedes spp., mosquitoes are well established in majority of tropical countries, especially in Asian countries (Hawley 1988; Shriram et al., 2009). Travellers get infected with CHIKV during their visit to the places were the outbreak occurs and thereby became reservoirs of infection when they back (Bodenmann and Genton 2006; Hochedez et al., 2007; Krastinova et al., 2006).
2.5 Isolation of Chikungunya virus

C6/36 mosquito cell line is very sensitive to the Chikungunya virus. Human serum infected with C6/36 cell line with small number of virus in the sample, grows enormously. Cytopathic effect (CPE) may appear following prolonged incubation (Singh 1971; Igarashi 1978). The appreciable CPE development was observed in 48–96 h in Vero cell line monolayer (Oberste et al., 2002). The CPE was characterized by the rounding of cells, increased granularity and vacuolation, followed by cell death and disruption of the monolayer by detachment of the dead cells (Oberste et al., 2002).

2.6 Clinical manifestations

The classical clinical features in acute chikungunya infection are the triad of fever, arthralgia and irregular skin rash (Robinson 1955). However there are several observations on the frequency of occurrence of different symptoms of chikungunya infection (Borherini et al., 2007; Simon et al., 2007; Kennedy et al., 1980; Pialoux et al., 2007; Suryawanshi et al., 2009) In acute stage, mild to severe disease characterized by high fever (ranging from 38.5°C to 40°C) and associated symptoms such as headache, retro-orbital pain, photophobia, lumbar back pain, chills, weakness, malaise, rashes, nausea, vomiting and myalgia are seen. However, the presence or frequencies of symptoms are highly variable from individual to individual (Brighton 1981; McGill 1995; Munasinghe et al., 1966; Ozden et al., 2007).

Arthralgia is the key clinical presentation in the clinical diagnosis of chikungunya infection (Staikowsky et al., 2009; Win et al., 2010). Arthralgia is symmetrical with the involvement of more than one joint. The pain can be severe and involves fingers, wrist, elbows, toes, ankles and knees. This kind of arthralgia is described for most of the Alpha viruses infection (Tesh 1992). Swollen joints, inflammation, and restriction of movement are other associated features (Suryawanshi et al., 2009).

The manifestations such as rashes present on the face, trunk and limbs, which can occur when temperature declines and along with itching during acute illness (Win et al., 2010). Rashes in chikungunya are generalized, erythematous, nonpruritic and maculopapular in nature. Presence of aphthous ulcers also known as a canker sore also have been noticed in oral cavities (Suryawanshi et al., 2009).
The acute signs and symptoms were resolved after 2 weeks but arthralgia may persist for several months to years (Robinson 1955; Swaroop et al., 2007). Further the disease progresses into a second stage with chronic rheumatism as major feature, such as large joint effusions, hygromas, bursitis, and axial pain, especially in areas where arthritis or trauma earlier occurred. The patients who developed de novo bilateral vascular disorders (Raynaud phenomenon) were also reported and continued for several weeks during the second and third months after onset of disease and that were not associated with wrist tenosynovitis (Simon et al., 2007).

Persistent and disabling arthralgia in more than 60% of patients post 18 months infection have been reported. In a study of chikungunya infection it was observed that most working adults were disabled as a result of difficulties in movement of upper limb and lower limb and depressive reaction which persisted for weeks to months (Borgerini et al., 2007).

Clinical impacts are much more severe in older adults; increasingly leading to complete loss of self-support, deteriorating health status and sometimes death in debilitated or elderly people (Pialoux et al., 2007). There were no confirmed fatality following CHIKV infection but during outbreaks in Reunion archipelago and India, crude deaths (average number of deaths 1,000 per year) have been reported (Mavalankar et al., 2007). Mortality due to CHIK virus infection has also been reported (Chua et al., 2010).

The acute stage symptomatology across the age groups has been not documented earlier. On the other hand CHIKV infection resulting in rheumatoid arthritis syndrome has been documented (Bouquillard and Combe 2009; Fourie and Morrison 1979). Chronic arthritis following CHIKV infection has been well documented. Synovial fluid tested from chronic patients with persistent joint pain show high antibody titres against CHIK virus (Brighton et al., 1983). After the acute phase, polyarthritis may persist for several months to years (Power and Logue 2007). Quite a lot of individuals infected with CHIKV suffer persisting chronic and incapacitating arthralgia for several months (Schuffenecker et al., 2006).
As per the previous guidelines, there must be four of the seven criteria for classifying a patient as having rheumatoid arthritis and patients with two or more clinical diagnoses are not excluded. Criteria: (a) Morning stiffness, (b) Arthritis of three or more joint areas, (c) Arthritis of hand-joints and (d) Symmetric arthritis has to be present for at least 6 weeks. Criteria; (b) Arthritis of three or more joint areas, (c) Arthritis of hand joints (d) Symmetric arthritis and (e) Rheumatoid nodules must be observed by a physician. (Arnett et al., 1988; Cush and Lipsky 2005).

Specifically, in symptomatic acute infection is associated with neurological, renal, cardiac, respiratory, hepatic, and haematological complications, with a high risk for long term (>30 months) rheumatologic complications among patients older than 30 years (Borgherini et al., 2007). There are reports of cases with neurological complications such as encephalitis and transverse myelitis (Suryawanshi et al., 2009). Fluid in the synovial bursa is uncommon (Brighton 1983).

Neurological complications such as meningoencephalitis have been observed in the recent outbreaks in Re-Union Islands and India (Chatterjee et al., 1965; Quatresous 2006; Patrick and Blaise 2006). Mother to child transmission of CHIKV has also been reported (Robillard et al., 2006). Atypical presentations such as flaccid limb weakness have been observed in CHIKV infection (Singh et al., 2008). Similarly there are several undocumented atypical manifestations based on the systems affected such as neurological (encephalitis, seizures, neuropathy), ocular, cardiovascular (myocarditis, heart failure, arrhythmia), dermatological, renal (nephritis) and other miscellaneous manifestations.

However, until recently it has been considered that in chikungunya arthritis radiological findings are normal (Pialoux et al., 2007). The study of Penny et al., (2009) observes that CHIKV was responsible for 14% of suspected CNS infections. An unusually persistent level of viremia was confirmed on day 23 of illness from the sample in an 8-month-old girl for whom no acute-phase sample was available. The severity of her illness, with marked rash, hepatosplenomegaly, and digital gangrene could have been due to inability to clear the virus. Alternatively, the patient might have become infected with CHIKV during the stay in the hospital (Penny et al., 2009).
2.7 Pathogenesis in chikungunya infection

CHIKV infection in humans is thought to begin with the inoculation of virus by mosquitoes in the dermis of the host. Then the virus finds its way into the blood vessels before dissemination to the target tissues or organs (Kam et al., 2009). Although the exact route and mechanisms of early infection is poorly understood (Couderc et al., 2008), studies from other Alpha viruses indicates the involvement of different immune cell populations in the skin (Johnston et al., 2000; MacDonald et al., 2000; Ryman et al., 2000; Sourisseau et al., 2007) and migrating cells such as macrophages and/or dendritic cells (DCs) (Johnston et al., 2000).

But the events taking place during the acute blood phase of chikungunya infections have not been understood. Although the viraemic period in humans is relatively short (Borgerini et al., 2008; Parola et al., 2006), the levels of virus in plasma can go very high levels ($3.3 \times 10^9$ viral copies/ml) in CHIKV infected patients (Parola et al., 2006). This high viral load could suggest that blood leukocytes could also be infected with CHIKV and involved in viral production. Indirect evidence that blood leukocytes are not susceptible to CHIKV infection in-vitro, suggests that the blood virus are produced by cells from other tissues (Sourisseau et al., 2007). Unlike, other arboviruses, CHIKV has shown to target various blood cells in vivo and in vitro such as monocytes, dendritic cells or B lymphocytes (Kou et al., 2008; Lin et al., 2002).

On the other hand, if blood leukocytes as targets for CHIKV infection is important because many blood leukocyte subsets, and in particular monocytes, are involved in innate immune responses against viruses, and in the control of viral infections. These early responses play a role in shaping subsequent antiviral adaptive immune responses and may influence the development of immune-pathogenesis.

Natural killer cells are strongly activated within the first few days post infection and lead to a more sustainable CD4/CD8 response. Some, but not all, canonical cytokines and chemokines stimulate the immune response and the level of TNF-α is rather weak (almost undetectable) during the acute phase of CHIKV infection (Hoarau et al., 2010; Ng LFP et al., 2009).
Replication-cycle: CHIKV has the characteristic features for replication in the vertebrate host as well as in the invertebrate vector. The process of virus replication is principally the same, but the release of virions is altered. The process of exocytosis is in role for virus discharging from insect host cells through plasma membrane (Miller 1992; Stollar et al., 1975). Therefore, the lifelong infections of virus are supported by non cytopathic infection in insect cells, whereas virus is treated by the host immune system after infection among vertebrates (Ozden et al., 2007).

The entry of viruses generally occurs through receptor-mediated endocytosis and endosomal dependent fusion (Tuldeo and Kirchhausen 1998; Kolokoltsov et al., 2006; Marsh and Helenius 2006; Marsh et al., 1984). Alpha viruses used to attach with poorly characterized receptors on many different cell types in various species. The E1 structural protein spikes drive the fusion process followed by E2 protein which interacts with cellular receptors (Johnston and Peters 1996; Kielian and Rey 2006). Mainly domains I and II of the E1 protein get involved in E1 trimerization in the course of the viral fusion process at the time of infection. Then domain II mediates the E1–E2 interaction during the virus maturation and budding from infected cells (Mukhopadhyay et al., 2006; Zhang et al., 2002). Different viruses have diverse, but wide tropism, accounting slightly for different disease patterns (Johnson and Peters et al., 1996).

The cellular receptors for CHIKV are still unknown. However, the interaction between the envelope proteins of CHIKV and receptors of host cells is important to penetrate into vertebrate cells in viral uptake. The virus is transported into the cell by endocytosis of clathrin-coated vesicles. The pH reduction in the vesicle for the activation of the E1 protein from the E1-E2 complex promotes viral and endosomal membrane fuses, which results in the release of the nucleocapsid into the cytoplasm. The replication of CHIKV occurs in the cytoplasm, (Ozden et al., 2007).

The P1234 polyproteins are translated directly from the viral genome. Further the process of RNA replication is started by the synthesis of a full-length minus-strand RNA, which is used as a template for the synthesis of the viral genome. Subsequently gets transcribed into the 26S subgenomic plus-strand RNA from the internal promoter.
in the junction region. Both processes are interlinked. Subsequent cleavage from the P1234 polyprotein, Nsp4 associates with P123 and strange host partners to regulate the synthesis of minus-strand RNA. The switch from genome replication to transcription is regulated by non-structural proteins, which cleaves from the P123 polyproteins (Ozden et al., 2007).

The mature structural protein precursor (C-E3-E2-6k-E1) is co-translationally cleaved. The capsid protein is produced by autocatalytic cleavage from the N-terminal region of structural polyprotein precursor and encapsidates the viral genomic RNA, resulting in the rapid assembly of nucleocapsid cores in the cytoplasm. After being cleaved from the envelope polyprotein precursor, E2 and E1 are transferred to the plasma membrane. At last, the packaging of virus is performed in the cytoplasm by the process of assembly of nucleocapsid cores with glycoproteins and virus is released by budding through the cellular membrane to form an enveloped virion (Ozden et al., 2007).

The adults who accompanied CHIKV infected highschool children were also contracted the disease. But the adults suffered most severely from the chronic arthritic form of the disease than the children and some cases were with the episodic polyarthritis till 18 months after the onset of the disease. The low titre of Rheumatoid factors were also could be demonstrated in the circulation of patients with chronic symptoms.

In rheumatoid arthritis, the ESR tends to reflect clinical disease activity but usually mirrors other symptoms such as morning stiffness or fatigue ( Sox and Liang 1986; Wolfe and Michaud 1994). The decreased erythrocyte sedimentation rate (ESR) is positively correlated with a number of blood sicknesses in which red blood cells have an uneven or smaller shape that causes slower settling (Saadeh 1998; Sox and Liang 1986). Any form that elevates fibrinogen such as pregnancy, diabetes mellitus, end-stage renal failure, heart disease, collagen vascular diseases, malignancy may also elevate the ESR (Sox and Liang 1986). Reference ranges for the ESR in healthy adults are as follows; age < 50 years men 0 – 15 mm/hr and women 0 – 20 mm/hr, in case of age age > 50 years men 0 – 20 mm/hr and women 0 – 30 mm/hr (Bottiger and Svedberg 1967).
Postulation for possible role of destructive arthropathy following CHIKV infection is now available. The same can persist for years (Brighton et al., 1984; Krishnamoorthy et al., 2009). Arthritis following CHIKV infection mimics rheumatoid arthritis (Bouquillard and Combe 2009; Fourie and Morrison 1979). Chronic osteoarthritis can cause synovial thickening (Brandt et al., 2006; Fernandez et al., 1995) and tendinitis may have a different explanation (Gilliland 2005).

In case of long-lasting symptoms in patients could be explained by discovering CHIKV tropism for muscular satellite cells that are considered as reservoirs for virus or virus-encoded components for longer than expected periods (Ozden et al., 2007). A pathophysiological mechanism of CHIKV infection in particular to the cellular targets of CHIKV has not been understood.

The European health standards revised the strategy to identify previously unreported clinical presentations in clinical point of view during the Réunion outbreak. This outbreak was characterized by an atypical magnitude and virulence, with painful and overthrowing poly-arthritis, and with myalgia being reported as a major clinical symptom (97.7% of cases) (Paquet et al., 2006). Information on observations of rhabdomyolysis (damaged skeletal muscle tissue) with high creatine phosphokinase is available (Bachelet et al., 2006).

Further it was also studied in both by an ex-vivo approach in muscle biopsies from two infected patients with a myositis syndrome, and in vitro on cultures of human muscle satellite cells, that can be differentiated into myotubes (a developing skeletal muscle fibre with a tubular appearance) (Ozden et al., 2007). Magnetic resonance image (MRI) findings in chronic arthritis following CHIKV infection have not been documented earlier. CHIKV infection resulting in rheumatoid arthritic syndrome has been documented (Bouquillard and Combe 2009; Fourie and Morrison 1979).

2.8 Cytokines and their major functions
The term "cytokine" refers to immunomodulatory proteins, such as interleukins and interferons (Boyle 2005). They are a category of signalling molecules that are used extensively in cellular (Gilman et al., 2001). Cytokines are classified according to the
cells that produce them viz., lymphocytes produces lymphokines, monocytes produces monokines, chemokines which has chemotactic activities and interleukins secreted by one leukocyte to act on other leukocytes (Chang et al., 2003; Gene Mayer 2010).

2.8.1 Cytokine and chemokine response in viral infections
Type I interferons (IFN-I) organize numerous biological and cellular processes and are essential elements during host antiviral defences. Subsequently the immune system might identify the immune signatures which are highly conserved specific virus. Then a rapid complex network of signalling events might initiated which leads to IFN-I synthesis. These cytokines in a straight line induce a strong antiviral state and exert numerous immune-regulatory actions, which are aimed for preventing the virus spread. Alternatively, viruses may progress to escape or takeover the IFN-I system for their own benefit (Zuniga et al., 2007).

2.8.2 Cytokine and chemokine response in chikungunya infection
CHIKV induces cellular damage which could lead to secretion of certain cytokine and chemokine and other growth factors. However, information on these lines are limited (Lee N et al., 2006). Lisa F. P. Ng et al., 2009 showed increased levels of IL-1b, IL-6 and a decrease in RANTES were associated with disease severity of CHIKV infection by estimating a panel of cytokines.

Currently, the immunology behind the CHIKV is poorly understood. It has been documented that type I interferon (IFN) production from CHIKV infected chick embryo–like fibroblasts (Friedman 1964; Wagner 1964). Although IL-2, IL-10 and IFN-γ have been implicated in the pathogenesis of CHIKV, only limited studies have been conducted on the cellular damage and the pathway leading to the secretion of these factors (Krishnamoorthy et al., 2009).

Cytokines are inflammatory mediators and their balance is often associated with inflammatory disease (Barksby et al., 2007). The typical antiviral response in humans is characterized by the induction of cytokines such as IFN-α/β, IFN-γ, IL-12 and IL-18 and the activation of macrophages, NK cells, dendritic cells, neutrophils, and complement (Labadie et al., 2010). IFN-α/β up-regulation occurs very early in the
course of CHIKV infection (Labadie et al., 2010) and by day 5 post-infection the levels return to baseline. IL-10 has been shown to have a negative role in immunity against West Nile Virus infection (Bai et al., 2009).

IL-6 has been shown to play a critical role in both the initiation and perpetuation of immunologic dysfunction and inflammatory responses in various forms of autoimmune arthritis (Malemud 2009). Clinical trials have given compelling evidence that neutralizing IL-6-mediated signalling provided a significant clinical benefit in patients with RA (Malemud 2009). Clinical and radiological features in CHIKV arthritis are closely resembling those of RA (Manimunda et al., 2010). Therefore, IL-6 signalling blockade could potentially be an intervention strategy for CHIKV arthritis (Petersen and Pedersen 2005).

IL-6 has an anti-inflammatory effect also, which is mediated through its inhibitory effect on TNF-α and IL-1 and activation of IL-1RA and IL-10 (Petersen and Pedersen 2005). IL-1RA is mainly associated with T cell activation and is secreted by activated T cells (Rubin et al., 1985). IP-10 and MIG Both cytokines are chemo-attractants of monocytes, macrophages, T cells, NK cells and dendritic cells and promote adhesion of T cell to endothelium (Angiolillo et al., 1995; Booth et al., 2002).

Elevated levels of these chemokines that are associated with Th-1 type reaction along with elevated anti-inflammatory cytokine IL-10, suggesting a predominantly anti-inflammatory cytokine response in acute CHIKV infection, has been reported earlier (Ng LFP et al., 2009). Neurologic complications were reported occasionally in CHIKF (Chatterjee et al., 1965),

Raised levels of IL-8 in serum and synovial fluid of RA patients have been reported in several studies (Kaneko et al., 2000). IL-8 inhibition by monoclonal antibodies has been suggested as a potential treatment strategy in RA, though results from phase II trials promising results (Keystone 2003). Macrophages and fibroblasts infected with Ross River Virus (RRV) have been reported to up regulate MCP-1 and IL-8 mRNA (Mateo et al., 2000) indicating that IL-8 may be playing a similar role in EPA due to the Alpha virus, RRV as well.
MCP-1 is implicated in other viral arthritis including measles virus (Van Damme et al., 1994) and caprine arthritis encephalitis virus (Palacios et al., 1998). Elevated level MCP-1 in serum and synovial fluid of active RA patients has also been reported (Stankovic et al., 2009). MIP-1α and MCP-1 has been identified as the most prevalent chemokines detected using adjuvant or collagen-induced arthritis models in rodents (Lidbury and Mahalingam 2000).

Studies indicate that these cytokines (MCP-1 and MIP-1α) play an important role in bone resorption and inhibiting these chemokines using antibodies improves artificially induced arthritis in rats (Orhan Sezer et al., 2005; Toh et al., 2010). Recent studies on non-human primates suggest that there is an increased expression of MCP-1 during acute infection (Labadie et al., 2010) but joint pathology could not be observed (Stephen et al., 2010).

A study in transgenic mice over expressing IL-5 has indicated that IL-5 plays a role in bone homeostasis, although it is not clear whether this cytokine has a direct effect or if it is mediated through eosinophils or B cells (Macias et al., 2001). Platelets are a major reservoir of RANTES in the peripheral circulation (Ellis et al., 2005) Severe CHIKF has been characterized by thrombocytopenia. Thrombocytopenia can also reduce levels of circulating RANTES. Low levels of RANTES correlate with disease severity and mortality in individuals with severe malaria (John et al., 2006).

Various disease associations have been described with increased macrophage migration inhibitory factor (MIF) protein expression (Barton et al. 2003). The MIF role in inflammatory arthritis as well as in juvenile idiopathic arthritis has been documented (Barton et al., 2003; Donn et al., 2002).

2.9 Antibodies in chikungunya infection
The persistence of the specific IgM antibodies for months after the initial infection has already been observed in several alpha viral infections, probably due to viral persistence but the underlying mechanism is poorly understood (Kuno 2001). However reports suggesting that infected individuals become positive for IgM by 7 days of
illness or post infection and the antibody may last for 6 months (Edwards et al., 2007). Further studies on CHIKV infected subjects also reveal that IgM and neutralizing antibodies were detected in less than 65% of samples collected within the first week of illness; therefore, negative result of samples tested IgM or neutralizing antibodies in the first week of illness cannot be used to rule out CHIKV infection.

Preferably, samples negative for antibodies must be tested for CHIKV by culture or by RT-PCR or a convalescent sample should be collected after day 7 of illness to repeat the serological testing. The studies have revealed that anti-CHIKV IgM antibodies can persist for 18 months in one-third to one-half of CHIK patients; therefore, it also understood that a positive CHIKV IgM test result occasionally might also reflect the past CHIKV infection (Borherini et al., 2008; Grivard et al., 2007).