

Clinical Profiling Of Chikungunya Positive Patients By Rtpcr In Tertiary Care Center,Pune

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1 INTRODUCTION

Chikungunya is a public health problem in Asian, African and Latin American countries. Chikungunya caused by virus which is an enveloped positive strand RNA virus, this virus belongs to genus Alphavirus of family Togaviridae [1]. The first chikungunya outbreak was recorded in Calcutta [2], India in 1963 after which multiple epidemics were recorded in India's different parts upto 1973. [3,4] But after 1973 no such outbreaks of chikungunya were reported therefore it was assumed that Chikungunya virus had disappeared from South-east Asia and from Indian subcontinent [5]. But in 2005-06 many states of India have reported several Chikungunya outbreaks [6]. Malaysia, Philippines, Indonesia, Thailand also experience reemergence of Chikungunya [7,8].

The clinical differentiation of Chikungunya from dengue is very difficult as both diseases share similar clinical signs and symptoms leading to misdiagnosis of Chikungunya in areas where dengue is endemic. One more point need to be considered that most of the laboratory parameters were unremarkable at the time of admission. It clinically presents as fever, incapacitating arthralgia and skin rash [9]. Mortality rate in Chikungunya cases is low [10]. The typical features of it includes massive outbreak with high attack rate acutely after this there was a slow decline in cases as herd immunity develops [11,12].

Usually incubation period is 2-4 days. At the end of incubation period, patient presents with high grade fever, back pain, myalgia, headache, arthralgia [13]. Theoretically it was assumed that it can be transmitted through infected blood products and through organ donation but, it is yet not documented [14].

It's symptoms generally resolves within 7- 10 days, except for joint-stiffness and pain.

In some Indian studies, all patients of Chikungunya, Fever and arthralgia have been noted [15,16]. However it can be asymptomatic but usually almost all patients ranging from 72%-97% shows symptoms [17].

As Dengue fever has a high incidence and also high mortality rate and symptomatic patients are tested only for DENV and rarely it is tested for Chikungunya viral infection. That is why Chikungunya cases go undiagnosed in dengue -endemic regions, and the true burden of Chikungunya viral infection is unclear. Since the same mosquito vector can transmit dengue and Chikungunya, investigation

of both viruses should be done specifically in endemic regions. Also, accurate and early diagnosis of co-infections will help for appropriate management. Therefore, the present study is undertaken to find out chikungunya disease burden.

Introduction -Chikungunya is a public health problem in Asian, African and Latin American countries. The clinical differentiation of Chikungunya from dengue is very difficult as both diseases share similar clinical signs and symptoms leading to misdiagnosis of Chikungunya in areas where dengue is endemic.

Aims and objectives -1) To determine the clinical profiling of IgM ELISA/RTPCR positive patients. 2) To determine the distribution of patients diagnosed by RTPCR and ELISA

Method-Purposive sampling

Result-Out of 1405,320 showed presence of Chikungunya IgM antibodies. Among these 260/320 (81.25%) were Chikungunya positive alone while 60/320(14.6%) showed presence of both dengue and Chikungunya IgM antibodies indicating co-infection. 90 patients were having fever <5 days among this 16(17.7%) positive by IgM ELISA and among this 10(62.5%) show PCR positive and 6(37.5%) PCR negative.

Discussion -Total 90 patients were tested by RTPCR out of which 55(61.1%) were chikungunya positive. In contrast Philippe Renault et al in their study mentioned 6.17% case detection by RTPCR. In Jean Paul Carrera et al who reported that 44.7% Chikungunya cases detected by RTPCR.

Conclusion – The rate of positivity by RTPCR was highest in acute phase first 5 days indicates definitive diagnosis in very early stages.

Keywords -Chikungunya, Dengue, co-infection, RTPCR, ELISA

2.AIMS AND OBJECTIVES

- i) To determine the detail clinical profiling of IgM ELISA/RTPCR positive patient
- ii) To determine the distribution of patients diagnosed by RTPCR and ELISA

3.GENOME STRUCTURE AND REPLICATION

The Alphavirus genome is a single-stranded RNA molecule, positive polarity nearly 12 000 nucleotides in length, having a sedimentation coefficient of ca. 42–49S [21,22]. The genomic RNA mimics the structure of cellular messenger RNA: which contains a 30 polyadenylate sequence and a 50 methylguanylate cap structure.

The nonstructural viral proteins coded by the 50 two-thirds of the genome that are translated directly from this RNA immediately following its introduction to cytosol. Structural proteins coded by the subgenomic RNA which corresponds to the 30 one-third of the genome. The subgenomic 26S RNA, transcribed from the subgenomic promoter located on the minus-strand intermediate of the viral genome, is also polyadenylated and capped.

3.1 Morphology:

The CHIKV has four structural proteins, three envelope glycoproteins (GPs) (E1, E2 and E3) and one capsid (CP). These are synthesized as a polyprotein which is processed proteolytically.

Cryo-electron microscopy (cryoEM) structure of mature alphavirus particles and virus-like particles (VLPs) revealed that virions are made up of two icosahedral layers: the outer envelope layer and the inner nucleocapsid (NC) core, both have T = 4 quasi-icosahedral symmetry. The envelope comprises 80 membrane embedded spikes, and each spike is formed by a trimer of the E1–E2 heterodimer (Figure 1.A) [23].

Each CP interacts with the cytosolic domain of E2, and 240 copies of CP form an icosahedral NC core. This NC encloses the viral genomic RNA. (Figure 1.B, 1.C). E1 is a type II membrane of three domains: domain I links distal domain II and membrane proximal domain III. A fusion loop is located at the distal end of E1 domain II. Crystal structures of CHIKV E1E2 heterodimer revealed E2's three domains [24]. Domain A is located in the center of the spike surface, and also to the membrane proximal end of E2, while domain B and C are located at distal end (Figure 1.D).

The apex of domain A and a part of domain B have binding site for receptor which confirmed by recent identification of shared receptor, Mxra8, for a number of arthritogenic alphaviruses including CHIKV, Mayaro virus (MAYV), O'nyong nyong virus (ONNV), and Ross River virus (RRV), the recent identification [23]. E2 domain B protects the fusion loop at the distal end of E1 from activation, at neutral pH [25]. Cryo-EM structures of several alphaviruses have shown that E2 domain B is flexible as E2 domain B has a lower electron density compared to other domains. E2 domain B is disordered and the fusion loop at the tip of E1 domain II is exposed in the X-ray crystal structure of envelope proteins from the related alphavirus, at low pH.

3.2 Replication:

In acidified endosomes; low pH triggers conformational rearrangements in the envelope GPs to expose the fusion loop at the distal end of E1, where alphaviruses are transported after endocytosis into target cells. E1 forms a homotrimer, further exposing the fusion loops of each monomer at the end of the trimeric complex for insertion into host membrane when there is removal of E2 from the center of viral spike [26]. Membrane fusion between virus and host cell allows the penetration of nucleocapsids (NCs) into the cytosol and rapid disassembly. Viral nonstructural proteins translated from the incoming viral genomic RNA in the cytosol to form replication complexes which lead to synthesis of new viral RNA.

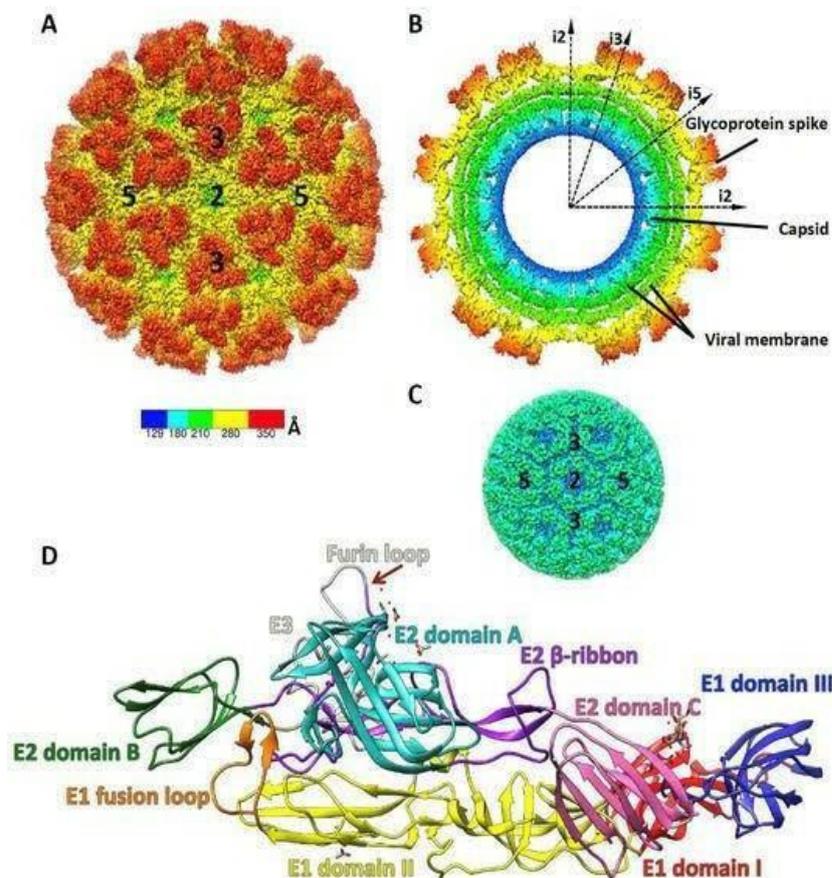


Figure 1. Structure of Chikungunya virus (CHIKV) and envelope glycoproteins. CryoEM density map of

- (A) CHIKV virus-like particles (VLP) [23].
- (B) CHIKV VLP viewed at the cross-section, and
- (C) CHIKV nucleocapsid core, colored according to the radial distance from center of virus
- (D) Structure of p62-E1 heterodimer[23].

Viral structural proteins are translated in the cytosol from the newly synthesized viral sub-genomic RNA. To nucleate the assembly of NCs the positively charged residues at the N-terminal domain of CP interact with viral genomic RNA. GPs are synthesized as a polyprotein (p62-E1) containing E1 and E3E2 (p62).

After co-translational proteolysis, the p62-E1 heterodimer assembles into trimeric spikes in the endoplasmic reticulum and through intracellular membrane trafficking it is delivered to the plasma membrane. Furin and furin-like host proteases priming the spikes for fusogenic activation by cleaving E3 during the transport through the trans-Golgi network to the plasma membrane.

The cytosolic domain of E2 binds to a hydrophobic pocket in CP on the surface of NC at the plasma membrane [27]. The icosahedral glycoprotein shell assembles in synchrony with the icosahedral symmetry of the NC, which finally resulting in budding of nascent virions. CPs can assemble independently of GPs in vitro and in situ icosahedral NCs. NCs it is prepared by in vitro assembly, and there is isolation from virions stripped of envelope, and or purification from infected cells. When visualized in vitro by cryoEM single particle analysis (SPA) only a small sub-population of these NCs were icosahedrally symmetric.

The organization of CPs in the isolated NCs differed from that inside mature virions. All these suggest that NCs are structurally heterogeneous and less stable compared to mature virions which undergoes GP-driven and/or lipid-driven structural changes upon envelopment.

CPs which are deficient in NC assembly were able to assemble and bud into wild-typelike Semliki Forest virus (SFV) virions which is driven by vertical spike–capsid interactions and horizontal spike–spike interactions [28]. This indicates a direct role for viral spikes in organizing the icosahedral conformation of alphavirus.

In situ electron tomography of chemically fixed SFV-infected cells suggested that trimeric GP spikes might already assemble into hexagonal lattices in cellular membrane compartments where they are required for virus budding prior to being delivered to the plasma membrane.

Mutations in the D- loop of the SFV E2 protein, which disrupt the contact between the viral E1 and E2 proteins also inhibit SFV budding which further suggests that the native organization of the GP lattice for virus budding is critical. Therefore, vertical interactions between GPs with CPs and horizontal interactions between GPs coordinately drive the formation of the icosahedral GP shell and induce membrane curvature formation which required for virus budding, which is similar to COPI and COPII complex-driven membrane vesicle budding for intracellular protein transport [24]. To sum up the replication cycle.

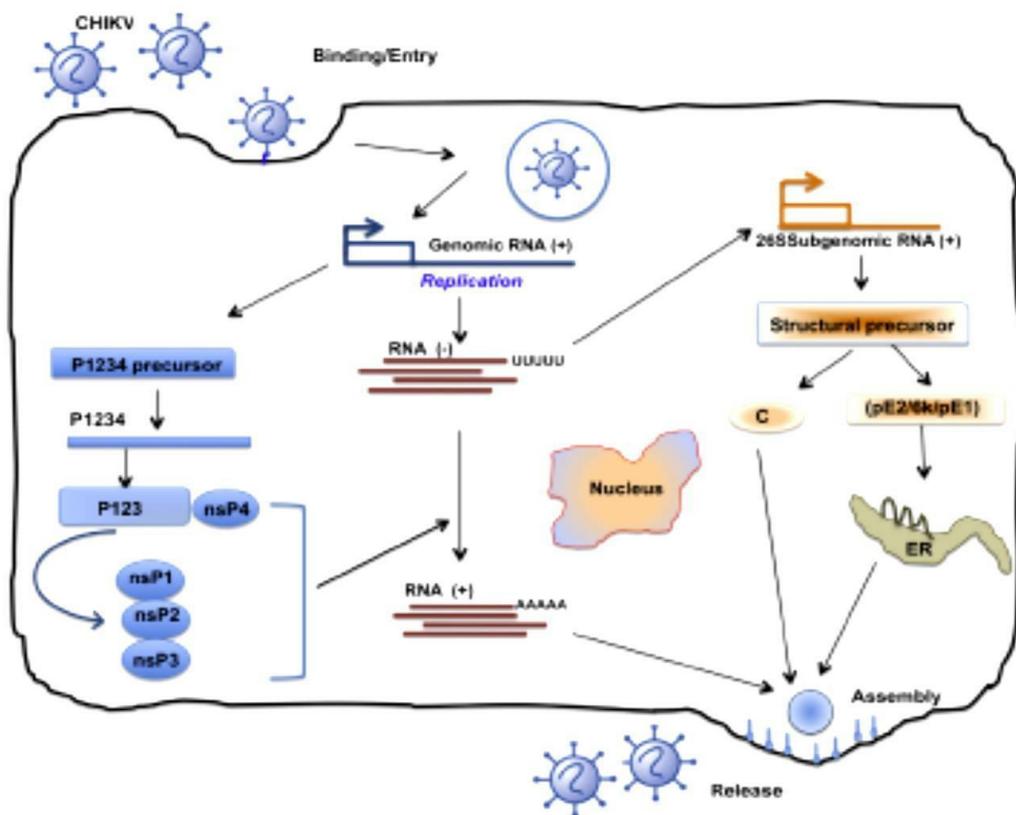


Figure 2. Life cycle of CHIKV in infected cells[29].

The replication cycle of CHIKV and other alphaviruses is similar. The nonstructural proteins (nsP1–4) and their cleavage intermediates are involved in RNA replication. The five structural proteins (C, E3, E2, 6k, E1) and cleavage intermediates, required

for budding and viral encapsidation [30]. Replication occurs in the cytoplasm, both in vertebrate and insect cells, which is in close association with the Golgi apparatus.

Virus enters cells at the plasma membrane, mostly by endocytosis, via a pH-dependent mechanism. It begins with attachment (E2 is primarily responsible for interactions with cellular receptors) and also the fusion of virus particles with the host membrane.

Positive-sense genomic RNA will act directly as mRNA and is partially translated (5' end) to produce nsP's which means non-structural proteins. These proteins are responsible for formation of a complementary negative strand and replication.

Sub-genomic mRNA (26S) replication occurs through the synthesis of full-length negative intermediate RNA, which is regulated by p123 and nsP4 precursor in early infection, and then by mature nsPs. Translation of the 26S sub-genomic RNA results in production of 5 structural proteins; which required for viral budding and encapsidation. Its release and maturation occur almost simultaneously while assembly occurs at the cell surface, and the envelope is acquired as the virus buds from the cell.

However, during presence of high concentrations of Arbidol (a molecule that typically blocks fusion) a CHIKV resistant mutant selected and adapted to growth it also included a substitution of single amino acid (G407R), localised in the envelope protein E2, which suggests that there is a close cooperation between proteins E1 and E2 which is necessary for the process of fusion [31].

3.3 Laboratory Diagnosis

Chikungunya virus infection should be suspected in patients with polyarthralgia and acute onset of fever and who meet the relevant epidemiologic exposure criteria (travel to an area or residency in where mosquito-borne transmission of chikungunya virus infection has been reported). The laboratory diagnosis of CHIKV infection can be achieved in the majority of cases by following two different strategies:

- i) the identification of the specific immune response (serological diagnosis)
- ii) Detection of viral RNA (virological diagnosis).

To this end, choosing the appropriate timing of specimen collection and of the use of the most suitable diagnostic methodologies is crucial for accurate diagnosis. The algorithm developed by the US Center for Disease Control and Prevention (CDC) to diagnose CHIKV infections is based on the timing of specimen collection and characteristics of CHIKV infection (Figure 3)

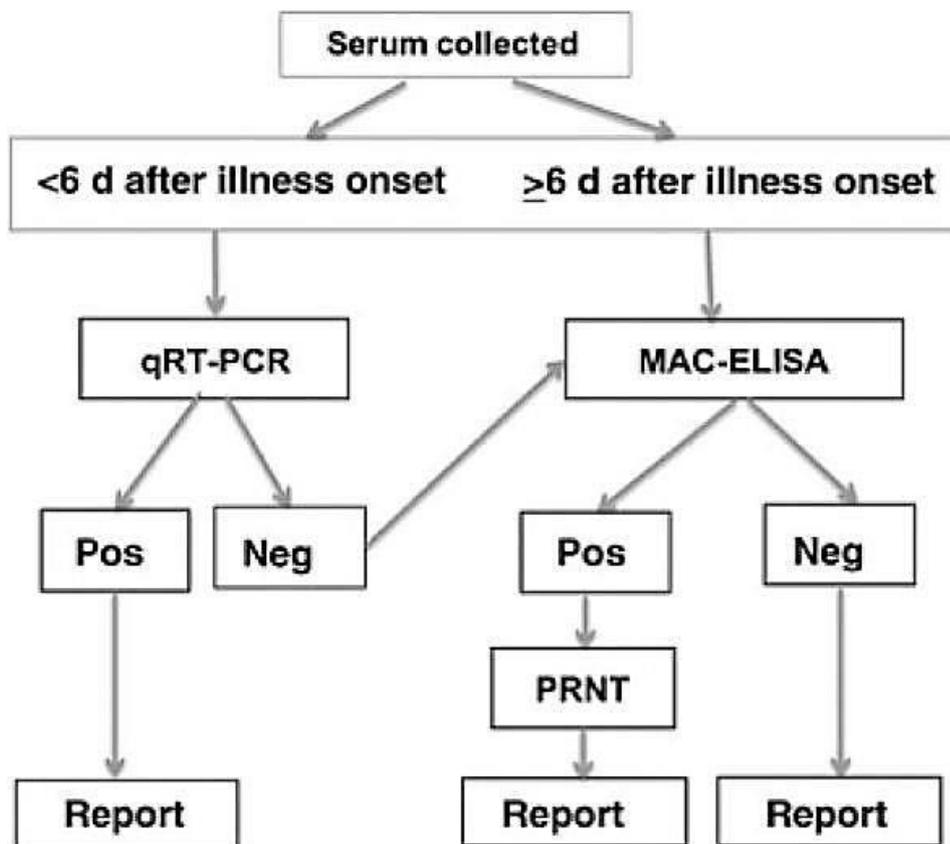


Figure 3. Algorithm by US for CHIKV infection, Source of CDC

CHIKV replicates rapidly to high titers in the host, immunoglobulin M (IgM) antibodies are normally detectable in serum by days 5–7 after onset of illness while viral RNA generally can be detected by real-time RT-PCR in the first week after onset of clinical illness [18].

Molecular assays (RT-LAMP assay, TaqMan real-time PCR and reverse transcription PCR) are more sensitive in the early stage of chikungunya fever (2–5 days p.o.i.) when CHIKV specific IgM is not yet detectable. In the later stages of chikungunya fever (>5 days p.o.i.), CHIKV specific IgM is a more reliable indicator.

Viral diagnosis depends on the characteristics of CHIKV infection and the time elapsed since the viral infection, clinical manifestation of infection and the type of patient (e.g., fetus, pregnant, etc.). Samples need to be sent to the laboratory quickly. The best type of tube for serological diagnosis is serum separator. EDTA and Heparin are unsuitable for antibodies CHIK testing [19]. Serum used for serological test can be used for virological diagnosis, but whole blood (leukocytes) or plasma is preferable.

3.4 Viral isolation

Viral isolation is generally a research tool [19,32-33]. For viral culture it is essential to preserve the complete virion. Culture sensitivity for chikungunya virus is high only in early infection (during the viremic phase) but it drops 5 days after onset of illness. Therefore, virus isolation is rarely used in the diagnosis of CHIKV infection because low sensitivity occurs after only a few days of post-infection, and it is laborious procedure and time consuming. Samples where chikungunya virus is suspected should be handled under Biosafety Level (BSL) 3 conditions.

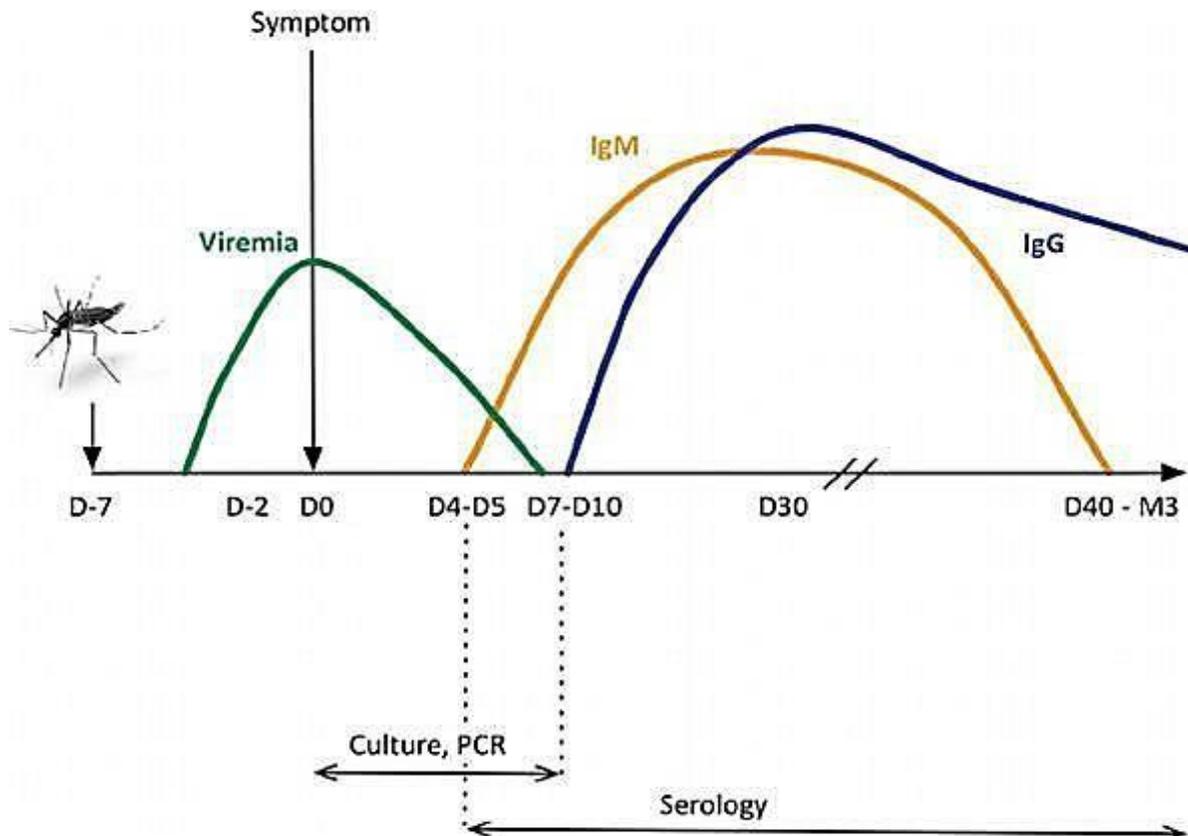


Figure 4. Duration of IgM and IgG detection in patient serum. Source of CDC

A wide variety of cells are used for in vitro CHIKV cultures in order to assess the full scope of the disease, including human blood monocytes, primary human skeletal muscle myoblasts [34-35], African green monkey kidney (Vero-E6) cells and C6/36 (*Aedes albopictus* clone cells) and also human osteoblasts [36]. Culture on HEL cells is sensitive more, and the cytopathic effect can be observed earlier than in Vero cells [37].

These cultures all need to be maintained under standard conditions.

Antigen detection There are commercial immunochromatography antigen detection kits which detect CHIKV in the early phase (up to 4–5 days after the onset of fever, when blood-enveloping proteins are still present) with high sensitivity. These tests also have high specificity as there is no cross-reactions with dengue virus are known to exist, and the method is simple and straightforward and does not require specially trained laboratory personnel [38].

3.5 Genomic detection (RT-PCR)

The diagnosis of CHIKV infection in the acute phase of infection is typically performed by the detection of viral RNA in serum (or other sample types) or plasma by RTPCR. The viral RNA can be detected by various molecular methods, such as real-time and nested PCRs [39]. Conventional and real-time PCRs have been used to amplify envelope protein genes (E3, E2, or E1) and nsP1, nsP2 [40-41]. Labeled probes or SYBR Green is used for real-time quantification for amplified PCR products [42-43].

Loop-mediated isothermal amplification (LAMP) under isothermal conditions without the use of a thermal cycler amplify nucleic acid which is a specific, fast, and cost-effective technique [44]. Monitoring by turbidity, also observation of color change after adding SYBR Green has also been described for the detection of CHIKV [44-45]. The CDC protocol for chikungunya infection diagnosis uses two RT-PCRs.

The nucleotide sequences of the two sets of probes and primers used are listed in Table 3 [46-47] The 3855 primer/probe set is specific to the ECSA genotypes although which is also capable of detecting both ECSA and Asian genotypes [47] The 856 primer/probe set targets the Asian genotype, slightly higher sensitivity than the 3855 set, prevalent in the Caribbean.

Genomic analysis is also used to characterize and classify viruses usually by Sanger sequencing method. The three principal strains of CHIKV (West African, ECSA, and Asian) have been typed using RT-PCR by nucleotide sequencing of a portion of the E1 region [48]

3.6 Serological diagnosis

A variety of serological methods (ELISA, complement fixation, hemagglutination inhibition and neutralization of viral infectivity using reference serum samples) are also used to characterize the alphavirus species [49-50] A fourfold increase in levels of CHIK virus IgG antibody in serum samples taken during the recovery and the acute phase is required to sero- diagnosis. Several serological assays have been developed, the large majority of which demonstrate high specificity and reliability.

The most common first-line serological techniques for CHIKV diagnosis are enzyme- linked immunosorbent assay (ELISA) and indirect immunofluorescence (IFA) assays, and the most suitable sample type is serum. Commercially available ELISA tests, for example, have been shown to demonstrate high specificity and sensitivity (ranging from 82 to 88%) in samples from recovering patients [51-52]

Table 1 - Primers and probes for Chikungunya infection [46-47]

Primer/genome 5' position	Nucleotide sequence
CHIKV3855F	GAGCATACGGTTACGCAGATAG
CHIKV3957C	TACTGGTGATACATGGTGGTTTC + TGCTGGTGACACATGGTGGTTTC
CHIKV3886FAM (probe)	ACGAGTAATCTGCGTACTGGGACGTA + ACGAGTCATCTGCGTATTGGGACGCA
CHIK856F	ACCATCGGTGTTCCATCTAAAG
CHIK962C	GCCTGGGCTCATCGTTATT
CHIK908FAM (probe)	ACAGTGGTTTCGTGTGAGGGCTAC

The presence of IgM antibodies indicates recent CHIKV infection^[53]. Detection of specific IgG antibodies, on the other hand, indicates previous CHIKV infection, which may be recent or as long ago as several months or years, given the anti-CHIKV IgG antibodies persistently^[54]

4. MATERIALS AND METHODS

Method- Purposive sampling

Study type- Descriptive/ observational cross sectional Sampling

Sample size-90

Duration of study: 1.5 year

5. DETAILED RESEARCH PLAN:

3-5ml blood collected in plain bulb along with data sheet detail history of patients collected. Their serum separated by centrifugation at 1500rpm for 10-15min. Test performed for presence of anti-chik IgM antibody MagRNA-II Viral RNA extraction kit , RT-PCR – HiGenoMB

a. SELECTION OF CASES:

Total 1405 samples tested for Chikungunya infection by ELISA. Out of which 260(18.5%) chikungunya positive and 60(4.2%) were coinfectd(Chik/Denv) were selected randomly, among 260 chikungunya patients 90(6.4%) were having fever less than 5 days. So as per the Algorithm given by US for CHIKV diagnosis(Figure 3) and NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME

guidelines this 90 samples were tested by RT-PCR .

Inclusion Criteria:

-As per the NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME guidelines will be followed for case definition

- **Suspected Case**-acute onset, high fever, 7 days duration, severe headache, myalgia, severe arthralgia, with or without rash

Probable Case-suspect case of Chikungunya, high vector density, presence of confirm case in area

Confirmed Case -Serological test positive for IgM antibody after 5th day of illness

Exclusion Criteria

- Patient who are already diagnosed as Chikungunya positive
- Those who are not willing to participate

5.1 Study Population:

Patients attending the Medicine department at our institute with Acute febrile illness were included as the study population. Written informed consent (translated in Marathi) (Appendix VII) was obtained from each patient at the beginning of the study. The study protocol was approved by the institutional ethics committee. Only those patients who fulfilled the eligible criteria were included in the study.

(a) MagRNA-II Viral RNA extraction kit

(b) RT-PCR – HiGenoMB®

RT-PCR – VIRAL RNA EXTRACTION KIT MagRNA-II

The entire procedure describe in Appendix V

Principle MDSTM Viral RNA Extraction Kit represent a well-established technology for general-use viral RNA preparation. The kit combines the selective binding properties of a silica-based membrane with the speed of microspin and is highly suited for simultaneous processing of multiple samples. The sample is first lysed under highly denaturing conditions to inactivate RNases and to ensure isolation of intact viral RNA. Buffering conditions are then adjusted to provide optimum binding of the RNA to the membrane, and the sample is loaded onto the spin column. The RNA binds to the membrane, and contaminants are efficiently washed away in 2 steps using 2 different wash buffers. High quality RNA is eluted in a special RNase-free buffer, ready for direct use or safe storage. The purified RNA is free of protein, nucleases and other contaminants and inhibitors. The special membrane guarantees extremely high recovery of pure, intact RNA in just 25 min without the use of phenol/chloroform extraction or alcohol precipitation.

5.2 RT-PCR HiGenoMB®

The entire procedure explained in Appendix IV

Principle

Real-time polymerase chain reaction, also called quantitative Polymerase Chain Reaction

(qPCR) or kinetic Polymerase Chain Reaction, is a laboratory technique based on the principle of PCR. This technique is used to amplify a targeted DNA sequence by use of hydrolysis probes that are short oligonucleotides that have a fluorescent reporter dye attached to the 5' end and a quencher dye to the 3' end. HiMedia's Chikungunya Detection Kit (Real-Time Probe Based PCR) is designed to detect the Non-structural gene of CHIKV in FAM channel and Internal Control in HEX channel in a single tube reaction. The kit allows sensitive and specific detection of CHIKV in a single tube reaction.

6 RESULTS

6.1 In the present cross sectional study during our study period

Total 1405 samples from clinical suspicion of Chikungunya infection were received, they were tested for presence of anti-chikungunya IgM antibodies by ELISA. Since clinically differentiation between dengue and chikungunya is difficult therefore this samples were also tested for dengue

IgM by ELISA. Out of 1405,320 showed presence of Chikungunya IgM antibodies. Among these 260/320(81.25%) were chikungunya positive alone while 60/320(14.6%) showed presence of both dengue and chikungunya IgM antibodies indicating co-infection(Chik/Denv). Apart from this

90 patients were having fever less than 5 days and satisfying inclusion criteria. So as per the

Algorithm given by US for CHIKV diagnosis (Figure 3) and NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME guidelines this 90 samples were tested by RT-PCR along with IgM ELISA.

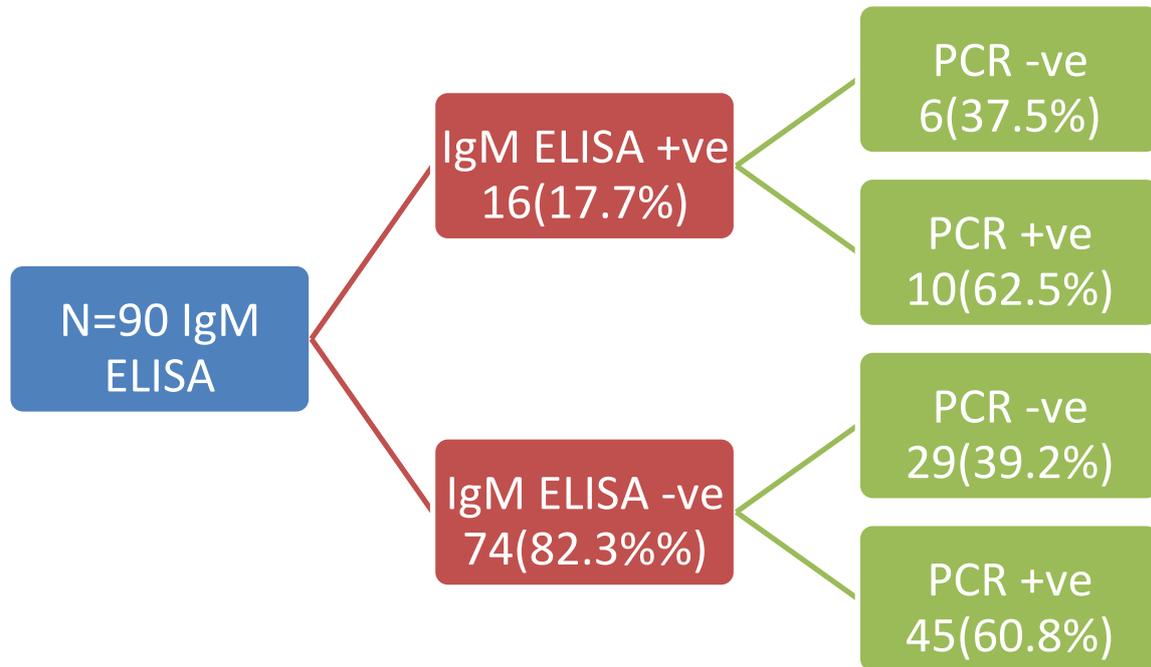


Figure 1. Showing Distribution of patients diagnosed by RTPCR and ELISA

In the present study, 90 patients who had fever <5 days and satisfying our inclusion criteria which shows 16(17.7%) positive by IgM ELISA and among this 10(62.5%) shows PCR positive and PCR negative 6(37.5%).

While remaining 74(82.3%) patients were negative by ELISA they were also tested by RTPCR among which 45(60.8%) were PCR positive and 29(39.2%) were PCR negative.

Table 1. Showing Distribution of clinical features of IgM ELISA/RTPCR positive patients

Clinical features	IgM ELISA positive (N-16)	PCR positive(N-10)
Fever	14(87.5%)	8(80%)
Headache	13(81.2%)	8(80%)
Bodyache	13(81.2%)	7(70%)
Weakness	12(75%)	6(60%)

In our study clinical features among IgM ELISA and RTPCR positive patients showing common viral infection symptoms which is not showing any significantly differentiating factor among both groups.

Table 2. Showing Distribution of IgM positive patients according to duration among clinically suspected chik. patients

Duration	Number (N-16)	Percentage (%)
0-1 day	4	25
2-3 days	3	18.8
4-5 days	9	56.2

Among this 16(17.7%) patients 9(56.2%) shows symptoms since 4-5 days which means it shows highest detection among 4-5 days by IgM ELISA.

Table 3. Showing Distribution of IgM ELISA/RTPCR positive patients according to duration

Duration	Number (N-10)	Percentage(%)
0-1 day	2	20
2-3 days	6	60
4-5 days	2	20

Among this IgM ELISA positive patients 16(17.7%) -PCR positive were 10(62.5%) among which patients showing symptoms highest among 2-3 days duration.

Table 4. Showing Distribution of clinical features of IgM ELISA negative,RTPCR positive(among this IgM ELISA) among clinically suspected chikungunya patients

Clinical features	IgM ELISA negative (N-74)	PCR positive(N-45)
Fever	72(97.3%)	43(95.5%)
Headache	67(90.5%)	10(22.2%)
Bodyache	64(86.5%)	39(86.6%)
Weakness	62(87.8%)	38(84.4%)

In our study clinical features among IgM ELISA negative and RTPCR positive patients showing common viral infection symptoms which is not showing any significantly differentiating factor among both groups.

Table 5. Showing Distribution of IgM ELISA negative and RTPCR positive patients according to duration among clinically suspected chik. patients

Duration	IgM ELISA negative Number (N-74)	RTPCR positive Number(N-45)
0-1 day	4(5.4%)	5(11.1%)
2-3 days	5(6.7%)	30(66.6%)
4-5 days	65(87.8%)	10(22.25)

Among the 74/90(82.2%) patients 45/90(60.8%) patients were RTPCR positive and most of positive patients detection occur among the duration of 2-3 days.

6. Discussion:

The clinical differentiation of Chikungunya from dengue is very difficult as both diseases share similar clinical signs and symptoms leading to misdiagnosis of Chikungunya in areas where dengue is endemic therefore the present study is undertaken focusing Chikungunya clinical profiling.

Total 1405 samples collected from patients with clinical suspicion of chikungunya were tested for Chikungunya infection by IgM ELISA. Out of which 320/1405(22.7%) sero-positive having 260/320(81.3%) chikungunya positive and 60/320(18.7%) were found to be

coinfected(Chik/Denv). There were 90 patients (6.4%) were having fever less than 5 days). So as per the Algorithm given by US for CHIKV diagnosis(Figure 3) and NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME guidelines this 90 samples were tested by RT-PCR for chikungunya also.

Urban area 40.2% and 11.5% in semi-urban areas^[55].

All the plasma samples were tested for the presence of CHIK virus -specific RNA by RTPCR. In this study, total 90 patients were tested by RTPCR out of which 55(61.1%) were chikungunya positive. In contrast Philippe Renault et al in their study mentioned meager 6.17% case detection by RTPCR. In Jean-Paul Carrera et al who reported that 44.7% Chikungunya cases detected by RTPCR[56-57].

In the present study, 90 patients who had fever <5 days and satisfying our inclusion criteria which shows 16(17.7%) positive by IgM ELISA and among this 10(62.5%) shows PCR positive and PCR negative 6(37.5%).

While remaining 74(82.3%) patients were negative by ELISA they were also tested by RTPCR among which 45(60.8%) were PCR positive and 29(39.2%) were PCR negative.

Distribution of clinical features of IgM ELISA/RTPCR positive patients

In our study clinical features among IgM ELISA and RTPCR positive patients showing common viral infection symptoms which is not showing any significantly differentiating factor among both groups. In IgM positive fever 14(87.5%), headache 13(81.2%) similarly in RTPCR positive patients fever and headache were seen in 8(80%). Therefore clinically it is difficult to diagnose Chikungunya infection in early stage because of generalised clinical manifestations.

In this study, IgM positive 16(17.7%) patients had 9(56.2%) showed symptoms since 4-5 days which means it shows highest detection among 4-5 days by IgM ELISA.

-The positivity rate of RTPCR is 61.1%(55/90) while for IgM ELISA it is 17.7%(16)

-The positivity rate of RTPCR is high compare to IgM ELISA in acute phase that is 2-3 days Thus it is difficult to diagnose case of chikungunya in early stage clinically as well as by IgM ELISA.

-Therefore it is important to diagnose suspected patients by RTPCR for Chikungunya in early stage

Kashyap et al. had similar findings in their study. They have reported PCR positivity (60%)

In a study carried out by **Vemu Lakshmi et al** 48.6% PCR positivity was reported ^[58]

Comparison of ELISA and RT-PCR positivity

Test	Vemu Lakshmi et al[145]	In our study	Kashyap et al[139]
IgM ELISA(Positive)	21.5%	17.7%	14%
PCR(Positive)	48.6%	61.1%	60%
PCR(Negative)	40%	38.8%	40%

The observation that the rate of positivity by RTPCR was highest in acute phase (first 5 days) and then decrease gradually .This indicates for definitive diagnosis in very early stages RT PCR plays important role.

The natural history of CHIK fever is not fully understood. Although it's mortality is rare ,vector control and early diagnosis will play an important role in preventing future epidemics. **Molecular diagnosis (RTPCR) is an important tool to identify viral illness such as CHIK**

Fever^[58]

].[58]Also Dengue fever has a high incidence and also high mortality rate and symptomatic patients are tested only for DENV and rarely it is tested for chikungunya viral infection. That is why chikungunya cases go undiagnosed in dengue -endemic regions, and the true burden of chikungunya viral infection is unclear. CHIKV causes both acute as well as chronic disabling trouble. Beginning prostrated fevers are predominantly followed by joint pain, frank arthritis, Guillain-Barre syndrome, vasculitis^[29] Since the same mosquito vector can transmit dengue and chikungunya, investigation of both viruses should be done specifically in endemic regions. Also, accurate and early diagnosis of co-infections will help for appropriate management especially those having chick rheumatism. Thus the present study indicates importance of laboratory diagnosis of chikungunya

7-8. SUMMARY AND CONCLUSION

IN OUR STUDY

- Total 90 having fever less than 5 days were tested for RTPCR out of which 58(64.4%) came positive
- **The observation that the rate of positivity by RTPCR was highest in acute phase (first 5 days) and then decrease gradually which is an important finding, this indicates definitive diagnosis in very early stages**
- **Molecular diagnosis RTPCR is an important tool to identify new vector-borne viral illnesses like CHIK fever, at an early stage**

9.CONFLICTS OF INTREST

NONE

Executive Summary

Introduction - Chikungunya is a public health problem in Asian, African and Latin American countries. As Dengue fever has a high incidence and also high mortality rate and symptomatic patients are tested only for DENV and rarely it is tested for Chikungunya viral infection.

Genomic Structure -The Alphavirus genome is a single -stranded RNA molecule, positive polarity nearly 12,000 nucleotides in length having sedimentation coefficient of ca.42-49S.

Laboratory diagnosis -CHIKV infection can be achieved in the majority of cases by following 2 different strategies

i)The identification of the specific immune response (Serological diagnosis)

ii) Detection of viral RNA (Virological diagnosis)

Conclusion -Rate of positivity by RTPCR was highest in acute phase, indicates definitive diagnosis in very early stages

Future aspects -RT-PCR will play a major role in future molecular diagnostic techniques. Commercialization efforts that have made PCR a gold standard in molecular diagnostics.

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