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ORIGINAL ARTICLE

An approach towards peptide-based antibody detection for diagnosis of Chikungunya infection

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Abstract

Chikungunya infection, although rarely fatal, is associated with significant morbidity which necessitates its diagnosis in the initial stages. Currently for diagnosis, together with clinical symptoms, immunological methods such as IgG/IgM detection, molecular methods such as real-time reverse transcriptase–polymerase chain reaction and viral isolation methods are available but they are either not very specific or they require high-level sophisticated infrastructures. In the present study, an enzyme-linked immunosorbent assay to evaluate antibody responses to peptides designed from the CHIKV E2 envelope glycoprotein was performed. Synthesized peptides were evaluated with confirmed Chikungunya and non-Chikungunya serum samples for antibody detection. The results demonstrate that the synthetic peptide-based diagnosis of Chikungunya can be an efficient and a more accessible approach in immunodiagnostics.

Keywords: *Chikungunya diagnosis; peptides; ELISA*

Introduction

Chikungunya fever is an acute, debilitating, arthropod-borne disease caused by a virus (CHIKV) transmitted to humans by mosquitoes, mainly *Aedes aegypti* or *Aedes albopictus*. CHIKV, indigenous to tropical Africa and Asia is an arbovirus belonging to the genus *Alphavirus* and family *Togaviridae*. After remaining dormant for nearly 32 years, there was an explosive re-emergence of Chikungunya in December 2005 affecting South East Asian countries and Africa, with more than a million cases in the Indian Ocean since the beginning of the outbreak in 2005 (Lahariya & Pradhan 2006). In India, Andhra Pradesh, Karnataka and Maharashtra were the most badly affected states with large numbers of patients (Naresh Kumar et al. 2007). The hospitals were flooded with patients with a clinical presentation of fever, maculopapular rashes and incapacitating arthralgia primarily affecting the peripheral small joints associated with excruciating pain.

Due to the sudden re-emergence and similarity of symptoms of Chikungunya fever with other viral infections such as Dengue, Sindbis, Ross river, West Nile virus, etc. (Saxena et al. 2009), its diagnosis was the main problem in controlling the spread of the disease. Presently IgG and IgM enzyme-linked immunosorbent assay (ELISA) detection methods are used for diagnosis but they have variable specificity and sensitivity, varying with different kits and protocols. Virus isolation and molecular tools such as reverse transcriptase–polymerase chain reaction (RT-PCR) and the more advanced real-time loop-mediated isothermal PCR methods (Parida et al. 2007) are continuously being used for obtaining confirmatory results. However, these methods can be carried out only in sophisticated referral laboratories with hi-tech equipment and skilled and trained technicians. Moreover, they are time-consuming, making these methods inaccessible to small laboratory set-ups, especially in developing countries. Because of these problems, emphasis should

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be laid on the development of rapid, specific and easily accessible diagnostic methods.

To overcome the deficiencies in Chikungunya diagnosis, we set out to identify specific protein markers unique to serum samples collected from patients with Chikungunya. By analysing the total sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) protein profile of such serum, we observed a 40-kDa protein band specific to these patients. These early investigations led us to hypothesize that molecular identification of the CHIKV-specific 40-kDa protein would allow the development of specific reagents and protocols for the rapid and accurate diagnosis of Chikungunya. In the present report, the identified protein was subjected to liquid chromatography–tandem mass spectrometry (LC-MS/MS) for characterization, and after characterization immunogenic peptides were designed and synthesized. The synthesized CHIKV peptides were evaluated in serum samples of Chikungunya patients for development of an antibody detection-based ELISA system.

Materials and methods

Selection of patients

A total of 28 samples were selected for the study, out of which 19 samples were confirmed positive by both clinical diagnosis and RT-PCR and real-time PCR assays. The remaining nine samples were used as control samples, confirmed negative by RT-PCR and real-time PCR assays or virus isolation. The RT-PCR, real-time PCR and virus isolation assays were performed at the Virology Department, DRDE, Gwalior. All the blood samples which were collected for the study were from subjects who either came to the Central India Institute of Medical Sciences (CIIMS), Nagpur, India or from the Bhilgaon village (Nagpur district) from July 2006 to September 2006. Patients within the age group of 5–85 years were included in the study on the basis of clinical symptoms including fever, headache, myalgia, joint pain with or without swelling, and the presence or absence of a rash on the body. The samples were categorized as confirmed Chikungunya or non-Chikungunya. A case was considered as confirmed when the clinical picture was consistent with Chikungunya and the sample was analysed as positive by either RT-PCR and/or real-time PCR or virus isolation. RT-PCR, real-time PCR and viral isolation were carried out at the Department of Virology, Defence Research and Development Establishment, Gwalior. Patients' consent was obtained for all the samples collected from all study groups for use in this study The Ethical Committee of the Central India Institute of Medical Sciences, Nagpur, approved the study. All analyses were performed double blinded.

One-dimensional electrophoresis by SDS-PAGE

The serum samples collected from the patients were subjected to one-dimensional SDS-PAGE. SDS-PAGE was performed on a vertical slab gel system (Broviga, Chennai, India) using the standard Laemmli method with 10% running and 5% stacking gels at 200 V power. Along with the samples, molecular weight markers were also run so as to study the molecular weight profile of the separated protein bands. The gel was developed by Coomassie Brilliant Blue GR-250 and the 40-kDa band was excised from the gel for LC-MS/MS analysis.

LC-MS/MS analysis

To partially purify the 40-kDa protein, serum was resolved by one-dimensional SDS-PAGE and the 40-kDa protein band was sliced and pre-equilibrated in the elution buffer (0.15 M phosphate-buffered saline (PBS), pH 7.4). The gel was electroeluted in a whole gel eluter system (Biotech India, New Delhi, India) for 1 h at 30 V and harvested from the unit and dialysed against PBS, and the protein content was measured by a Bio Lab kit. This partially purified material was again resolved by SDS, the gel was stained with Coomassie Blue and the 40-kDa band was excised from the gel. The excised 40-kDa protein band was sent to Kendrick Labs, Madison, WI, USA for LC-MS/MS analysis. Kendrick Labs characterized this protein using the following protocol: each gel piece was destained (50% methanol + 10% acetic acid) and washed (50% CH_3CN/0.1 M Tris–HCl, pH 8.0) prior to in-gel digestion, then protein bands were excised, digested and treated with trypsin after reduction and alkylation agents (100 mM iodoacetamide (IAA)) were added prior to the analysis on the LC-MS/MS. In-gel digestion was carried out in 50 mM NH_4HCO_4 buffer, pH 8.5 at 37°C for approximately 4 h. An equal volume of the digestion buffer was added, depending on the volume of the gel piece, and usually ranged from 20 to 50 µl. The amount of proteolytic enzyme (Promega trypsin, modified, sequencing grade; Promega, Madison WI, USA) that was used depended on both the size of the gel piece and the estimated amount of protein within the gel band. Typically, 200 ng to 1 µg trypsin was used per gel band. Acetonitrile (CH_3CN), in a volume equal to 3–5 times the volume of the digestion buffer, was then added to the digestion mix to extract the peptides. The samples were then centrifuged at high speed for 5 min. The supernatant was transferred to a clean microfuge tube with a gel-loading pipette tip and dried in a SpeedVac on medium heat. For the reduction step, alkylation agents (100 mM IAA) were added prior to analysis with LC-MS/MS. The dried sample was dissolved in 0.5% acetic acid (HOA_c) for LC-MS/MS analysis. A Finnigan (ThermoFinnigan, San Jose, CA, USA) LCQ ion trap MS in-line coupled with a high-performance liquid

chromatography (HPLC) system was used for LC-MS/MS. A 75 µm (ID) × 10 cm length, 3 µm packing C18 capillary column, which was packed in-house, was connected to a specially designed nanoSpray device, which is capable of delivering a stable electrospray at flow rates of 100–1500 nl min^{-1}. The mobile phases included Solvent A (2% CH_3CN, 97.9% H_2O, 0.1% formic acid) and Solvent B (90% CH_3CN, 9.9% H_2O, 0.1% formic acid). For this analysis, the ion trap MS was set to operate in a data-dependent mode with the automatic gain control (AGC) on. The MS/MS data were first evaluated against several internal quality control (QC) standards. After passing the QC standards, the MS/MS data were loaded into the proprietary ProtQuest search engine to search the most recent non-redundant protein database. The results from the ProtQuest search were then manually analysed. The endoproteinase trypsin (sequencing grade) was obtained from Promega. The ammonia bicarbonate (analytical grade) and HOA_C (>99.8% purity) were obtained from Sigma (St Louis, MO, USA). CH_3CN, methanol (MeOH) and water were each HPLC grade and obtained from Sigma.

Selection and designing of identified peptides

We have identified the E2 Chikungunya envelope glycoprotein (structural protein) by using EXPASY proteomic server – UniProtKB/Swiss-Prot Q5WQY5 (POLS_CHIKN) after the characterization of the 40-kDa protein. The antigenic peptides were determined on the basis of the Kolaskar and Tongaonkar (1990) method by using online software–Molecular Immunology Foundation–Bioinformatics software (http://www.immunax.dfci.harverd.edu/tools/antigenic.html). Seventeen peptides were designed with varying antigenicity. These designed peptides were scrutinized with online available software (Innovagen, http://www.innovagen.se/) for suitability of antibody production.

Peptide synthesis

The peptides were prepared by Caslo Laboratories (Lyngby, Denmark) as per the following procedure by using solid-phase methods employing N-Fmoc/t-Bu protection strategy and chemistry. The following side-chain protection strategy was employed for standard amino acid residues: Asp(OtBu), Glu(OtBu), Arg(Pbf), Lys(Boc), Trp(Boc), Ser(tBu), Thr(tBu), Tyr(tBu), Asn(Trt), Cys(Trt), Gln(Trt) and His(Trt). Solid-phase assembly was carried out in a stepwise manner on an AAPPTEC Apex396 multiple peptide synthesizer using HBTU/DIEA coupling chemistry at 0.11–0.15 mmol resin scale (Fmoc Rink resin). For each coupling cycle, 3 equiv. of N-Fmoc-amino-acid, 6 equiv. of DIEA and 3 equiv. of HBTU were used. The coupling time was 1 h. Fmoc deprotections were carried out with two treatments of a 20% piperdine in DMF solution, each for 8 min.

Peptide cleavage and global deprotections

The target peptides were cleaved/deprotected from their respective peptidyl resins by treatment with a trifluoroacetic acid (TFA) cleavage mixture as follows: a solution of TFA/thioanisole/1,2-ethanedithiol/triisopropylsilane/water (70:10:10:1:3:5) (4 ml) was added to each well in the reaction block, which was then mixed for 3 h. The TFA solutions from the wells were collected by positive pressure into vials located in a matching block on bottom of the reactor. The resins in the wells were rinsed twice with an additional 0.5 ml of TFA mixture, and the rinses were combined with the solutions in the vials. Then the cleavage solution was added to 45 ml of cold ether and the peptides were precipitated out. These were washed with ether three times and then dried to yield the crude peptides.

Peptide purification

Preparative HPLC was carried out on the Varian Pro-Star system. A linear 0.1 TFA/CH3CN/water gradient with 1% CH_3CN increase per min was used with UV detection at 220 nm. The desired product eluted was usually collected in a single 15–20 ml fraction and the final peptides were obtained as white powders by lyophilization.

ELISA for antibody detection

The peptides were in lyophilized form and were reconstituted in PBS. Some of the peptides were highly hydrophobic in nature. Such peptides were first dissolved in acetonitrile and then in PBS.

Standardizing peptide concentration for ELISA

Prior to development of the ELISA-based antibody detection, the peptide concentration for sensitization was standardized using different concentrations of peptides (12.5, 25 and 100 ng 100 μl^{-1}). One hundred microlitres of different of peptides were coated in microwells and incubated for 3 h at 37°C. The wells were then blocked with 0.5% bovine serum albumin (BSA)/PBS after washing once and kept for 2 h at 37°C. Then the wells were washed and stored at 4°C overnight. Next day, serum samples were added to the wells in four different concentrations (1:100, 1:200, 1:400 and 1:800) and incubated for 45 min at 37°C. After washing the wells with PBS, 100 µl of secondary antibody (goat antihuman horse radish peroxidase (HRP)-conjugated antibody, 1:10000) was added and incubated for 30 min at 37°C. One hundred microlitres of tetramethyl benzidine (TMB)/H_2O_2 substrate solution was then added and incubated for 10 min. This colour development reaction was stopped by adding 100 µl of 2.5 N H_2SO_4. Absorbance was read at 450 nm.

Antibody detection by ELISA

Peptides were coated in microwells at a concentration of 25 ng 100 μl^{-1} in each well and incubated for 3 h at 37°C. After washing with PBS, wells were blocked with 0.5% BSA/PBS for 2 h at 37°C. The wells were washed once and stored at 4°C overnight. Next day 100 µl of serum samples in different concentrations (1:100, 1:200, 1:400 and 1:800) were added to the wells and incubated for 45 min at 37°C. The wells were then washed three times with PBS and 100 µl of secondary antibody (goat antihuman HRP-conjugated antibody, 1:10000) was added and incubated for 30 min at 37°C. After washing the wells with PBS, 100 µl of TMB/H_2O_2 substrate solution was added and incubated for 10 min. This reaction was stopped by adding 100 µl of 2.5 N H_2SO_4 in each well. The absorbance of each well was read at 450 nm.

Statistical analysis

The sensitivity and specificity of the developed peptide-based antibody detection ELISA test for diagnosis of Chikungunya infection were calculated. The positive and negative samples were determined on the basis of RT-PCR and real-time PCR assays. A receiver-operating characteristic (ROC) curve was used to calculate the cut-off value, and comparison between the Chikungunya and non-Chikungunya groups was done by the χ^2 test.

Results

The serum samples of patients admitted to the CIIMS hospital were grouped into two different categories on the basis of clinical observation and RT-PCR and/or real-time PCR and/or virus isolation. These serum samples were then subjected to one-dimensional SDS-PAGE analysis to observe the total protein profile and to demonstrate and assign the specificity of the bands to CHIKV. The band pattern obtained, showed the presence of a specific band in the region of 40–45 kDa in all the samples of patients with CHIKV, otherwise absent in all the control samples (Figure 1). As the SDS-PAGE analysis strongly suggested the 40-kDa protein as a marker in the diagnosis of CHIKV, a full characterization of the 40-kDa protein was initiated. The serum sample of CHIKV patients was resolved by SDS-PAGE. A more highly purified 40-kDa protein band of the serum of CHIKV patients was recovered by electroelution. Analysis by LC-MS/MS of peptides derived through a trypsin digestion of the 40-kDa band revealed the presence of E2 envelope glycoprotein (strain Nagpur) (Figure 2).

After confirming the presence of the E2 envelope glycoprotein in the 40-kDa region, to design a more specific, sensitive and simple diagnostic assay, we targeted peptides from the sequence of the E2 immunogenic protein. The complete sequence of the protein was obtained from Expasy proteomic server – Swiss-Prot database. The antigenic peptides were identified from the complete sequence of the E2 glycoprotein using

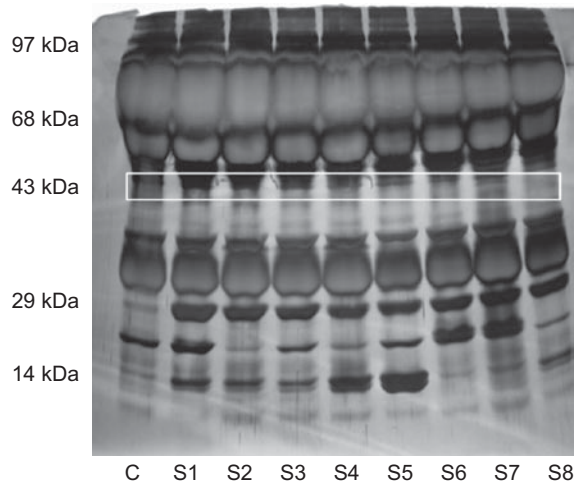

Figure 1. One-dimensional gel electrophoresis of serum samples of Chikungunya (lanes S2–S8) and non-Chikungunya patients (lane C).

326 SIKDN	FNVYKATRPY	LAHCPDCGEG	HSCHSPVALE	RIRNEATDGT
LKIQVSLQIG	IKTDDSHDWT	KLRYMDNHMP	ADAERAGLLV	RTSAPCTITG
TMGHFILARC	PKGETLTVGF	TDGRKISHSC	THPFHHDPPV	IGREKFHSRP
QHGKELPCST	YVQSNAATAE	EVEVHMPPDT	PDRTLMSQQS	GNVKITVNSQ
TVRYKCNCGD	SNEGLTTTDK	VINNCKVDQC	HAAVTNHKKW	QYNSPLVPRN
VELGDRKGKI	HIPFPLANVT	CRGPKARNPT	VTYGKNQVIM	LLYPDHPTLL
SYRNMGEEPN	YQEEWVTHKK	EVRLTVPTEG	LEVTWGNNEP	YKYWPQLSTN
GTAHGHPHEI	ILYYYELYPT	MTVVVVSVAS	FVLLSMVGVA	VGMCMCARRR
CITPYELTPG	ATVPFLLSLI	CCIRTAKA	748	

Figure 2. Sequence of the E2 glycoprotein of the Chikungunya envelope viral protein (strain Nagpur). Total amino acids 423.

Molecular Immunology Foundation software (based on the Kolaskar and Tongaonkar method) and Innovagen software for suitability of antibody response. Seventeen peptides were synthesized and selected to evaluate in selected samples of CHIKV and non-CHIKV patients (Table 1).

Evaluation of serum samples with the identified peptides was done according the above-mentioned ELISA protocol. The sensitivity and specificity of each peptide were calculated using ROC curve analysis. Finally, four peptides 3, 4, 8 and 13 showing sensitivity and specificity above 60% were selected as potential peptides for development of an immunodiagnostic assay for CHIKV (Table 2). The reactivity of serum samples of Chikungunya and non-Chikungunya patients with the selected four peptides is shown in the respective scatter plots (Figure 3).

Table 1. List of peptides designed from the E2 glycoprotein of Chikungunya.

S. no.	Peptides	Peptide sequence
1	Chikungunya peptide 1	EGHSCHSPVAL
2	Chikungunya peptide 2	LKIQVSLQI
3	Chikungunya peptide 3	RAGLLVRTSAPCT
4	Chikungunya peptide 4	GHFILARC
5	Chikungunya peptide 5	HGKELPCSTYVQS
6	Chikungunya peptide 6	YNSPLVPRN
7	Chikungunya peptide 7	KEVRLTVPTEGLE
8	Chikungunya peptide 8	HGHPHEIILYYYEL
9	Chikungunya peptide 9	PGATVPFLLSLICCI
10	Chikungunya peptide 10	EGHSSHSPVALC
11	Chikungunya peptide 11	LKIQVSLQIC
12	Chikungunya peptide 12	RAGLLVRTSAPSTC
13	Chikungunya peptide 13	HGKELPSSTYVQSC
14	Chikungunya peptide 14	YNSPLVPRNC
15	Chikungunya peptide 15	KEVRLTVPTEGLEC
16	Chikungunya peptide 16	HGHPHEIILYYYELC
17	Chikungunya peptide 17	PGATVPFLLSLISSIC

Discussion

The results obtained in the present study demonstrate that a peptide-based antibody detection assay for Chikungunya can be an effective method for the diagnosis of the infection. The lack of a differential diagnosis of Chikungunya from other similar viral infections such as Dengue, O'nyong-Nyong, etc. was realized during its widespread outbreak in 2007. The available standard molecular methods RT-PCR, real-time PCR and viral isolation are quite useful to diagnose the infection but are cumbersome, requiring trained professionals and still not available in many centres in less developed and developing countries.

IgM detection assays are also being used for screening and diagnosis of Chikungunya (Gerardin et al. 2008). The immunolateral flow assay which is commercially available has also been used for diagnosis of Chikungunya IgG (Rampal et al. 2007). However these commercially available antibody, i.e. IgM/IgG, detection kits have variable sensitivity and specificity. Therefore to surmount the problems of variation it was necessary to develop an alternative immunoassay that will be able to overcome this limitation.

In an attempt to develop a rapid, reliable, simple and cost-effective test for diagnosis of Chikungunya, we analysed serum samples of CHIKV patients along with non-CHIKV patients. The SDS-PAGE protein profile of CHIKV patients' serum samples demonstrated the presence of a 40-kDa protein. Thus the 40-kDa protein band was partially purified from the serum and the excised band was digested with trypsin and analysed by LC-MS/MS. These studies cumulatively identified the CHIKV antigen E2 envelope glycoprotein (Chikungunya Nagpur strain).

The E2 envelope glycoprotein, together with E1, form the spikes composed of triplets of a heterodimer of these two glycoproteins. They cover the viral surface in the form of a membrane anchored type. The E2 envelope protein is a type I transmembrane glycoprotein responsible for

Table 2. Sensitivity and specificity of the 13 selected peptides.

	Cut-off	Sensitivity(%)	Specificity(%)	AUC
CHIKV peptide 3	1.095	67	78	0.641
CHIKV peptide 4	1.304	89	78	0.765
CHIKV peptide 5	1.764	89	44	0.556
CHIKV peptide 8	1.23	78	78	0.765
CHIKV peptide 9	1.327	33	89	0.556
CHIKV peptide 10	1.139	56	78	0.691
CHIKV peptide 11	2.134	56	89	0.642
CHIKV peptide 12	0.733	33	100	0.654
CHIKV peptide 13	1.931	89	78	0.691
CHIKV peptide 14	1.323	44	89	0.593
CHIKV peptide 15	1.241	33	89	0.556
CHIKV peptide 16	1.573	56	89	0.679
CHIKV peptide 17	1.295	56	67	0.556

AUC, area under the curve.

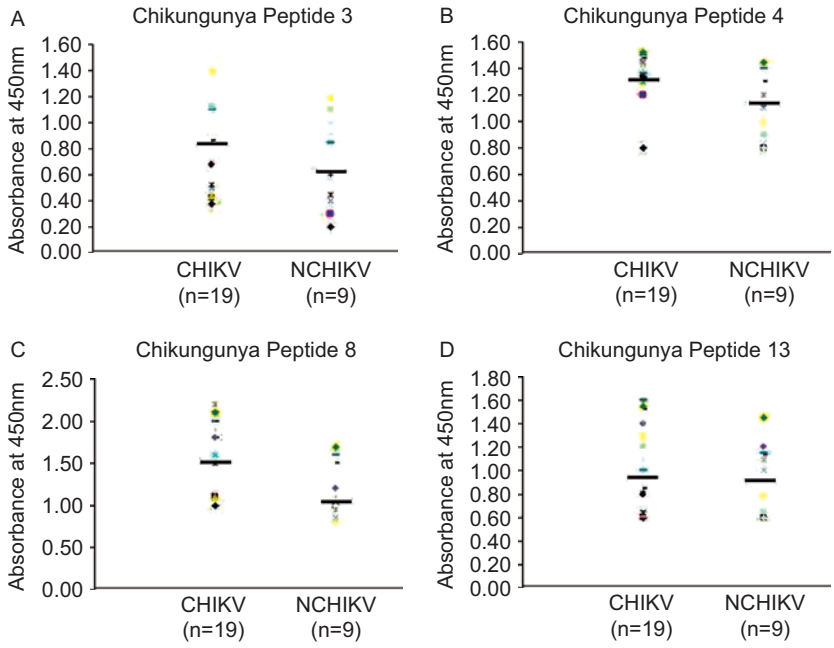

Figure 3. Scatter plots showing the reactivity of serum samples of Chikungunya and non-Chikungunya patients. (A) Chikungunya peptide 3; (B) Chikungunya peptide 4; (C) Chikungunya peptide 8; (D) Chikungunya peptide 13.

receptor binding during the course of the *Alphavirus* cycle, while E1 plays a role in membrane fusion during virus infection. The E1 protein is imbedded in the virus structure giving the CHIKV E1 envelope protein less chance of exposure to the host immune system. The E2 protein is considered as an important determinant of virulence as it interacts with cellular receptors.

Earlier studies have reported the use of envelope proteins of different viruses for diagnosis of their respective infections. Edwards et al. (2007) designed a RT-PCR-based diagnostic assay by using a part of the nucleotide sequence of the E1 glycoprotein of Chikungunya. Cho et al. (2008) reported recombinant Chikungunya virus E1 and E2 envelope proteins as a useful diagnostic reagent for CHIKV infection. E2 protein plays a role in attachment of the virus to the host cell. A viral envelope protein of Dengue virus has already been used for developing diagnostic assays.

In the present study, instead of using whole antigen, a peptide-based approach is being used to develop an immunoassay for diagnosis. Earlier various investigators have identified potential peptides to be used as a diagnostic marker for vector-borne infections. In a study by Dos Santos et al. (2004), they designed an IgG ELISA for diagnosis of Dengue using a polypeptide from the envelope protein region of DENV-2 giving a sensitivity and specificity of 100%. In another study, Pattanaik et al. (2006) reported the role of fusogenic peptides designed from the envelope glycoprotein of Dengue for diagnosis. Smith et al. (1986) used this peptide-based approach for Epstein–Barr virus diagnosis by using a peptide designed specifically against the Epstein–Barr virus nuclear antigen (EBNA). Similarly, Zheng et al. (2002) developed an immunodiagnostic assay using peptides for Hantavirus infection. For designing such peptides a specific region of the viral protein is being targeted which is responsible for its virulence or host–virus attachment and against which antibodies are produced in the host cells.

In a similar manner the present study was intended to design, synthesize and evaluate such peptides for Chikungunya diagnosis. After confirming the presence of the E2 envelope glycoprotein of CHIKV in the serum sample of CHIKV patients, we designed and synthesized 17 peptides for further evaluation in selected serum samples of CHIKV and non-CHIKV patients. Four peptides (Chikungunya peptide 3 (RAGLLVRTSAPCT), 4 (GHFILARC), 8 (HGHPHEIILYYYEL) and 13 (HGKELPSSTYVQSC)) showing sensitivity and specificity above 60% were finally considered as potential biomarkers for CHIKV diagnosis.

An issue of concern in the present study is the viremia of these CHIKV samples. The concept is that the antibody titre must be low in samples that have been detected positive by RT-PCR or real-time PCR as the antibody appears in the body or comes into the picture 8–10 days after the onset of the symptoms of infection. However in our study the collected samples included a few old cases, i.e. of 10–15-day-old infections, where the patients' samples showed positive results for RT-PCR due to its high viral load. Similarly in a study by Devi Goorah et al. the acute phase of the Chikungunya infection persisted even up to 15 days after the onset of symptoms where viremic load

was high in patients' samples (Devi Goorah et al. 2009). There is one more study which reports that the viremic load is high in Chikungunya samples compared with other viral infections (Pialoux et al. 2007). Our unpublished data of RT-PCR and real-time PCR analysis done on our samples also support that the viral load can be high in samples even after 10–12 days of the infection enabling our assay to detect the antibody in RT-PCR- and real-time PCR-confirmed samples.

This peptide-based immunoassay is being developed keeping in view the need of laboratories with small infrastructure. This immunoassay requires simple to handle technology which gives specific and reliable results. But the important part of this study is the choice and design of the peptides for a successful peptide ELISA. Specificity is enhanced in peptide-based assays by averting the selection of cross-reactive sequences from the antigenic proteins. A major advantage of the use of peptides as antigens in serological diagnosis is that the peptides are comparatively easy to produce in a reproducible manner compared with the whole antigen or virus (Greijer et al. 1998). Another advantage that can be associated with this immunoassay is that peptides can be produced in large quantities at a time, so the problem of batch to batch variation (Shen et al. 2009) can be avoided and more tests can be performed in similar conditions.

The results obtained from the present study demonstrate that the E2 glycoprotein of Chikungunya virus can be considered as a biomarker of Chikungunya infection and the peptide antigen derived from its sequence can be used to develop an ELISA-based immunoassay that will be more specific, rapid and less cumbersome compared with other conventional methods of Chikungunya diagnosis.

Declaration of interest

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