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***Research article***

# A STUDY ON CHIKUNGUNYA OUTBREAK IN ANDHRA PRADESH, SOUTH INDIA

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**ABSTRACT:** The present study investigated 161 individuals who developed signs and symptoms compatible with CHIKV infection during April - May 2007. CHIKV infection was confirmed by RT-PCR/IgM strip analysis. Studies on Severity and prevalence of various symptoms in CHIKV confirmed patients were further assessed. All age groups were affected. 71.09% of the affected patients were in the age group of 21-50 yrs. The percentage of infection was observed to be higher in females (87%) than that of their male counterparts (72.6%). The major symptoms observed in >70% of CHIKV patients were fever (99.2%), arthralgia (97.7%), myalgia (89.1%), difficulty in walking (78.1%), joint stiffness (77.3%), morning stiffness (76.6%), sleeping disturbances (75.8%) and headache (70.3%). Asymptomatic patients were encountered. Weight gain (3.9%) and hospitalized cases(2.3%) were observed. Significant association was observed between CHIKV symptoms with respect to age group and gender of the patient. A high morbidity rate with no mortality was observed. RT-PCR/ CHIKV IgM rapid strip analysis clearly suggested CHIKV as the aetiological agent responsible for the outbreak. Fever, arthralgia, myalgia, difficulty in walking, joint stiffness, morning stiffness, sleeping disturbances and headache were observed in majority of the patients. Strengthening of surveillance and IEC activities will further help in controlling the spread of CHIKV epidemic.

**Key words:** *Chikungunya virus*, Questionnaire, Symptomatology, Diagnosis, RT-PCR.

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## INTRODUCTION

Re-emergence of arboviral diseases, such as Chikungunya (CHIK), has been frequently reported in the Indian subcontinent for the past one decade (Naresh Kumar and Sai Gopal., 2010; Mohan et al., 2010). CHIK fever has an abrupt onset with fever, chills, headache and severe joint pain. The incubation period ranges from 1 to 12 days. Infected patients suffer from severe arthralgia, characteristically migrating and predominantly involving small joints of hands, wrists, ankles and feet with lesser involvement of larger joints (Ramana and Prakash., 2009). The disease is almost self-limiting and rarely fatal (Carey et al., 1969).

Other clinical features demonstrated by infected patients include chronic joint pains and generalized myalgias (Brighton., 1984). In some patients cutaneous manifestations may be found as a maculopapular rash on face and trunk; photophobia and conjunctival redness may be observed in others and a few patients develop pharyngitis (Carey et al., 1969). CHIKV infection was first reported to affect the nervous system during 1960s (Chatterjee et al., 1965). Various neurological complications were described in the recent epidemic: meningo-encephalitis, meningo-encephalo-myeloradiculitis, myeloradiculitis, myelitis, myeloneuropathy, Guillain-Barre' (GB) syndrome, external opthalmoplegia, facial palsy, sensorineural deafness, and optic neuritis (Murthy., 2009). Pathophysiology studies revealed that CHIKV targets human epithelial cells, endothelial cells, fibroblasts, macrophages, and human muscle satellite cells (Gerardin et al., 2011). Recent findings in a non-human primate model showed that CHIKV persistence in macrophages triggers a sustained low-noise T helper 1 cell (Th1) response leading to chronic inflammation and fibrosis (Labadie et al., 2010). The present study was organized to achieve a better understanding of the symptoms prevalent in CHIKV infected patients and to assess their severity with respect to age group and gender.

## MATERIALS AND METHODS

### Study area and Questionnaire preparation

Sri Venkateswara University Health Centre (SVUHC) is a 20 bedded hospital located in the heart of Sri Venkateswara University. It caters to the needs of nearly 20,000 people including students, non teaching staff, the faculties and their family members, technical staff. A CHIK questionnaire was prepared with the assistance of medical officer, SVUHC. The first half of the questionnaire enquired about the patient's age, gender, contact address, telephone number, about his/her family members and their CHIKV infection status. The second part of the questionnaire contained multiple choice questions to be answered by ticking the appropriate boxes and focused on the clinical signs and symptoms observed in the patients. The duration of each symptom was made a note of. Details of mosquito breeding sites, preventive measures adopted and medications used by the patients were also noted.

### Sample and data collection

In our earlier studies we have identified and reported the circulation of CHIKV in Andhra Pradesh (Naresh Kumar et al., 2007; Naresh Kumar et al., 2010; Mishra et al., 2011; Naresh Kumar and Sai Gopal., 2013). In the month of April 2007, SVUHC observed a huge influx of patients having fever associated with/without arthralgia. Blood samples were drawn from these patients (n=161) (by SVUHC staff) visiting SVUHC during $7^{th}$ – $19^{th}$ April 2007 and transported to Virology Department, S.V. University in wet ice. Serum was separated and stored at 4º C until use. The importance of the study was explained to the patients. Prior to sample collection 'Informed oral consent' were obtained from all the patients and their parents (in case of minors). The patients were also briefly informed about the various diagnostic tests to be used for confirming the CHIKV infection. Irrespective of their CHIKV status (which was later tested), questionnaire was filled from all the 161 patients. The medical officer, SVUHC examined the patients and assisted us in recording the symptoms and filling the questionnaire form. Specific attention was drawn to note any new found symptom also. Systemic examination was based on the CHIK symptoms as described by NICD, focusing on the musculo-skeletal system by enquiring about the presence or absence of arthralgia, myalgia, oedema, etc (Kannan et al., 2009). The study was approved by University ethics committee of S.V.University, Tirupati.

### Sample analysis

Sixty acute phase samples were screened for the presence of CHIKV specific RNA by RT-PCR assay using primer pair DVRChk-F/ DVRChk-R as described by us earlier (Naresh Kumar et al., 2007). Briefly total RNA was extracted from 250 µl of Patient serum samples using TRI Reagent® BD (Sigma-Aldrich, USA) and the pellets were dissolved in nuclease free water. cDNA was synthesized in 20 µL reaction volume using Oligo $(dT)_{18}$ primer. PCR amplification was carried out using DVRChk-F:5$^{!}$-ACCGGCGTCTACCCATTCATGT-3$^{!}$ (forward primer) and DVRChk-R 5$^{!}$-GGGCGGGTAGTCCATGTTGTAGA-3$^{!}$ (Reverse primer) in a 20 µL of reaction volume as described by us earlier (Naresh Kumar et al., 2007). The convalescent phase samples (n=101) were screened for the presence of CHIKV specific IgM antibodies using one step IgM antibodies to CHIKV test according to manufacturer's instructions (Standard diagnostics, Inc., South Korea).

### Telephonic interview and Data analysis

Only those patients who were positive for CHIKV were further interviewed after every 15 days up to a month and thereby after every 3 months up to 18 months of the study period (April 2007- November 2008). In case of minors, the assistance of their parents was taken in recording the symptoms. The duration and severity of various major and minor symptoms were noted. Departmental contact numbers was provided to the patients and were asked to intimate us regarding any relapses during the study period. At the end of the study period, the information gathered from the questionnaire was entered into an excel database (Microsoft) and data analysis was performed using PASW SPSS 18.0 version and MS-Excel. Mean and standard deviation values were calculated. Statistical analysis was performed using Chi-square test with Yates correction. The results were considered significant for a 95% confidence interval (95% CI) ($p<0.05$).

**Extension activities**

CHIK pamphlets containing information regarding CHIKV, its mode of spread, symptomatology, prevention and control measures were prepared in English and Telugu (local language) in collaboration with SVUHC. The CHIK pamphlets (n=3000) were distributed freely among the patients visiting SVUHC and the Primary health centers (PHC) in and around Tirupati region to educate the people about the CHIK disease and prevention strategies to be adopted.

## RESULTS AND DISCUSSION

Of the 161 patients, 77 (47.83%) were female and 84 (52.17) were males. The participants mean age was 34.20 ± 13.33 years (Range: 6-75) and mean age was similar for both genders (Female: 34.78 ± 11.73, Range: 12-61; Male: 33.68 ± 14.68, Range: 6-75). The male to female (M: F) ratio was 1.0:1.1. Out of sixty acute phase samples screened 51(85.0%) were positive for CHIKV by RT-PCR (Fig 1). Out of 101 convalescent phase samples 77(76.23%) were positive for CHIKV specific IgM antibodies. In total 128 patients (79.5%) were confirmed of CHIKV infection. Age and gender distribution of 128 CHIKV confirmed patients are shown in fig. 2.

All age groups were affected. 71.09% of the affected patients were in the age group of 21-50 yrs while 15.6% and 13.28% of the patients belonged to age groups >50 yrs and 5-20 yrs respectively. The percentage of infection was observed to be higher in females (67, 87%) than that of their male counterparts (61, 72.6%). Symptoms of clinically diagnosed Chikungunya fever cases (n=128) during the outbreak in Tirupati is shown in table 1.

We observed 2 male patients (1.56%) in the age group of 21-35 years who had fever of one day duration. The fever subsided on the second day and both of them resumed work as usual without showing any CHIKV disease symptoms. Blood drawn from these patients on the second day showed CHIK positive by RT-PCR**.** One of the sample (RT-PCR product) was further cloned into pGEM-T Easy vector and sequenced from Eurofins genomics India Pvt Ltd., Bangalore. The obtained sequence was deposited in GenBank database under the accession number EU383028. It is evident that depending upon the immune status, both of them might have acted as asymptomatic carriers.

An attempt was made to study the association of CHIKV symptoms with respect to age group (Table 2) and gender (Table 3). Duration of various symptoms (major and minor) in CHIKV infected patients with respect to gender factor was studied in detail (Table 4). The Medical officer, SVUHC prescribed the patients with the combination of Diclofenac (analgesic) + Cefixime (antibiotic) + Tantaprozole (antacid) for the initial 4 days and the patients were asked to visit the SVUHC on 5$^{th}$ day. During the subsequent follow up studies it was noted that only 24.2% patients (n=31) visited back SVUHC for follow up while 74.2% (n=95) of patients got treated from private physicians and unqualified health providers (Private medical practitioners). Of these 73.4% (n=94) of patients took injections (probably analgesics though patients were not able to tell the name of the medicine used in the injection), to get relieved from arthralgia. 8.6% of patients (n=11) were administered saline (Table 5). One patient (0.8%) went on for homeopathic treatment in addition to allopathy. Prevention and treatment measures of CHIKV infected patients is shown in table 5.

A high morbidity rate with no mortality was observed in the present study. CHIKV infection affected all age groups. Nearly 71.09% of the affected patients were in the age group of 21-50 yrs. CHIKV studies from India indicated 20-40 yrs and 31-40 yrs as the most affected age group (Lakshmipathy and Dhanasekaran., 2008; Lakshmi et al., 2008; Dumpala et al., 2014). In India and Reunion more cases were reported in the adult age groups even though all age groups were affected (Kannan et al., 2009; Quatresous., 2006; Jain et al., 2007). A serosurvey conducted at Calcutta a decade ago revealed that only 4.37 % of the serum samples were positive for CHIKV antibodies with highest seropositivity rate in age group of 51-55 yr and none detected in younger population. These findings probably suggest that there is indeed a lack of herd immunity to CHIKV (Ravi., 2006). Lack of herd immunity to CHIKV probably accounts for more infection rate in patients <50 yrs of age when compared to the elderly patients (>50 yrs) as suggested earlier (Suryawanshi et al., 2009). The proportion of CHIKV infections also varied with age, also possibly because different age groups are exposed to different environments and have different immune responses to infections (Staikowsky et al., 2008). The percentage of infection was observed to be higher in females (87%) when compared to males (72.6%). Similar results indicating higher disease incidence in females was reported earlier from India and Reunion Islands (Lakshmi et al., 2008; Staikowsky et al., 2008). The observed difference was possibly due to the differential exposure of men and women to the mosquito vector by their activities and their environments (Staikowsky et al., 2008).

**Table 1: Symptoms of clinically diagnosed Chikungunya fever cases (n=128) during the outbreak in Tirupati**

| S No | Symptoms | Number of patients (Percentage) | | |
|---|---|---|---|---|
| 1 | Fever | 127 (99.2) | | |
| 2 | Headache | 90 (70.3) | | |
| 3 | Pain intensity | 125 (97.7) | Mild | 15 (11.7) |
| | | | Moderate | 9 (7.0) |
| | | | Severe | 101 (78.9) |
| 4 | Arthralgia | 125 (97.7) | Neck | 68(53.1) |
| | | | Shoulder | 86 (67.2) |
| | | | Elbow | 62 (48.4) |
| | | | Wrist | 88 (68.8) |
| | | | SFH | 108 (84.4) |
| | | | Hip | 50 (39.1) |
| | | | Knee | 104 (81.3) |
| | | | Ankle | 82 (64.1) |
| | | | Feet | 68 (53.1) |
| | | | Toes | 69 (53.9) |
| | | | Rachis | 43 (33.6) |
| | | | SCJ | 44 (34.4) |
| 5 | Morning stiffness | 98 (76.6) | | |
| 6 | Joint stiffness | 99 (77.3) | | |
| 7 | Sleeping disturbance | 97 (75.8) | | |
| 8 | Walking difficulty | 100 (78.1) | | |
| 9 | Myalgia | 114 (89.1) | | |
| 10 | Lombalgia | 55 (43.0) | | |
| 11 | Oedema | 73 (57.0) | Face | 31 (24.2) |
| | | | Shoulder | 14 (10.9) |
| | | | Elbow | 16 (12.5) |
| | | | SFH | 41 (32.0) |
| | | | Knee | 39 (30.5) |
| | | | Ankle | 41 (32.0) |
| | | | Feet/Toes | 47 (36.7) |
| | | | Legs | 24 (18.8) |
| 12 | Rashes | 7 (5.5) | Face | 1 (0.8) |
| | | | Trunk | 3 (2.3) |
| | | | Hands | 5 (3.9) |
| | | | Legs | 2 (1.6) |
| 13 | Retro-orbital pain | 24 (18.8) | | |
| 14 | Eye congestion | 43 (33.6) | | |
| 15 | Asymptomatic carrier | 2 (1.56) | | |
| 16 | Chills | 65 (50.8) | | |
| 17 | Cough | 22 (17.2) | | |
| 18 | Running nose | 23 (18.0) | | |
| 19 | Abdominal pain | 37 (28.9) | | |
| 20 | Nausea | 54 (42.2) | | |
| 21 | Vomiting | 48 (37.5) | | |
| 22 | Oral ulcer | 8 (6.3) | | |
| 23 | Weight gain | 5 (3.9) | | |
| 24 | Weight loss | 52 (40.6) | | |
| 25 | Lethargic | 62 (48.4) | | |
| 26 | Anorexia | 68 (53.1) | | |
| 27 | Diarrhoea | 7 (5.5) | | |
| 28 | Giddiness | 61 (47.7) | | |
| 29 | Hospitalization | 3 (2.3) | | |

SFH: Small fingers of hand; SCJ: Sternocostal joints

**Table 2: Comparisons of symptoms observed among CHIKV patients in different age groups**

| S.No | Symptoms | % of symptoms among different age groups (yr) | | | |
|---|---|---|---|---|---|
| | | 5-20 (n = 17) | 21-35 (n = 46) | 36-50 (n = 45) | > 50 (n = 20) |
| 1 | Fever | 17 (100.0) | 46 (100.0) | 44 (97.7) | 20 (100.0) |
| 2 | Headache | 13 (76.5) | 29 (63.0) | 32 (71.1) | 16 (80.0) |
| 3 | Pain intensity* | 17 (100.0) | 43 (93.5) | 45 (100.0) | 20 (100.0) |
| | Mild | 7 (41.2) | 5 (11.4) | 3 (6.7) | 0 (0.00) |
| | Moderate | 1 (5.9) | 5 (11.4) | 1 (2.2) | 2 (10.0) |
| | Severe | 9 (52.9) | 33 (75.0) | 41 (91.1) | 18 (90.0) |
| 4 | Arthralgia | 17 (100.0) | 43 (93.5) | 45 (100.0) | 20 (100.0) |
| | Neck* | 8 (47.1) | 17 (37.0) | 27 (60.0) | 16 (80.0) |
| | Shoulder | 10 (58.8) | 29 (63.0) | 31 (68.9) | 16 (80.0) |
| | Elbow | 6 (35.3) | 19 (41.3) | 22 (48.9) | 15 (75.0) |
| | Wrist | 9 (52.9) | 28 (60.9) | 35 (77.8) | 16 (80.0) |
| | SFH | 14 (82.4) | 34 (73.9) | 41 (91.1) | 19 (95.0) |
| | Hip | 4 (23.5) | 15 (32.6) | 20 (44.4) | 11 (55.0) |
| | Knee | 12 (70.6) | 36 (78.3) | 39 (86.7) | 17 (85.0) |
| | Ankle* | 6 (35.3) | 25 (54.3) | 34 (75.6) | 17 (85.0) |
| | Feet* | 6 (35.3) | 17 (37.0) | 29 (64.4) | 16 (80.0) |
| | Toes* | 7 (41.2) | 17 (37.0) | 29 (64.4) | 16 (80.0) |
| | Rachis* | 3 (17.6) | 12 (26.1) | 16 (35.6) | 12 (60.0) |
| | SCJ | 5 (29.4) | 11 (23.9) | 17 (37.8) | 11 (55.0) |
| 5 | Morning stiffness | 12 (70.6) | 30 (65.2) | 39 (86.7) | 17 (85.0) |
| 6 | Joint stiffness | 15 (88.2) | 32 (69.6) | 35 (77.8) | 17 (85.0) |
| 7 | Sleeping disturbance | 12 (70.6) | 32 (69.6) | 35 (77.8) | 18 (90.0) |
| 8 | Walking difficulty | 14 (82.4) | 30 (65.2) | 39 (86.7) | 17 (85.0) |
| 9 | Myalgia | 15 (88.2) | 38 (82.6) | 43 (95.6) | 18 (90.0) |
| 10 | Lombalgia | 4 (23.5) | 20 (43.5) | 22 (48.9) | 9 (45.0) |
| 11 | Oedema* | 6 (35.3) | 21 (45.7) | 32 (71.1) | 14 (70.0) |
| | Face | 2 (11.8) | 9 (19.6) | 16 (35.6) | 4 (20.0) |
| | Shoulder | 0 (0.0) | 4 (8.7) | 6 (13.3) | 4 (20.0) |
| | Elbow | 1 (5.9) | 6 (13.0) | 5 (11.1) | 4 (20.0) |
| | SFH | 2 (11.8) | 14 (30.4) | 16 (35.6) | 9 (45.0) |
| | Knee* | 0 (0.0) | 13 (28.3) | 17 (37.8) | 9 (45.0) |
| | Ankle* | 1 (5.9) | 11 (23.9) | 21 (46.7) | 8 (40.0) |
| | Feet/Toes* | 2 (11.8) | 13 (28.3) | 21 (46.7) | 11 (55.0) |
| | Legs | 2 (11.8) | 7 (15.2) | 11 (24.4) | 4 (20.0) |
| 12 | Rashes | 0 (0.0) | 2 (4.4) | 3 (6.7) | 2 (10.0) |
| | Face | 0 (0.0) | 1 (2.2) | 0 (0.0) | 0 (0.0) |
| | Trunk | 0 (0.0) | 0 (0.0) | 1 (2.2) | 2 (10.0) |
| | Hands | 0 (0.0) | 2 (4.4) | 1 (2.2) | 2 (10.0) |
| | Legs | 0 (0.0) | 0 (0.0) | 1 (2.2) | 1 (5.0) |
| 13 | Retro-orbital pain | 3(17.6) | 4 (8.7) | 10 (22.2) | 7 (35.0) |
| 14 | Eye congestion | 5 (29.4) | 14 (30.4) | 12 (26.7) | 12 (60.0) |
| 15 | Asymptomatic carrier | 0 (0.0) | 2 (4.3) | 0 (0.0) | 0 (0.0) |
| 16 | Chills | 10 (58.8) | 21 (45.7) | 21 (46.7) | 13 (65.0) |
| 17 | Cough | 2 (11.8) | 9 (19.6) | 8 (17.8) | 3 (15.0) |
| 18 | Running nose | 4 (23.5) | 9 (19.6) | 6 (13.3) | 4 (20.0) |
| 19 | Abdominal pain | 6 (35.3) | 11 (23.9) | 14 (31.1) | 6 (30.0) |
| 20 | Nausea | 8 (47.1) | 17 (37.0) | 19 (42.2) | 10 (50.0) |
| 21 | Vomiting | 7 (41.2) | 15 (32.6) | 19 (42.2) | 7 (35.0) |
| 22 | Oral ulcer | 1 (5.9) | 2 (4.3) | 3 (6.7) | 2 (10.0) |
| 23 | Weight gain | 1 (5.9) | 2 (4.3) | 1 (2.2) | 1 (5.0) |
| 24 | Weight loss | 3 (17.6) | 17 (37.0) | 22 (48.9) | 10 (50.0) |
| 25 | Lethargic* | 6 (35.3) | 18 (39.1) | 23 (51.1) | 15 (75.0) |
| 26 | Anorexia | 7 (41.2) | 27 (58.7) | 20 (44.4) | 14 (70.0) |
| 27 | Diarrhea* | 0 (0.0) | 1 (2.2) | 2 (4.4) | 4 (20.0) |
| 28 | Giddiness | 6 (35.3) | 22 (47.8) | 24 (53.3) | 9 (45.0) |
| 29 | Hospitalization* | 0 (0.0) | 0 (0.0) | 0 (0.0) | 3 (15.0) |

*Symptoms are not associated with respect to age group (Chi square test) SFH: Small fingers of hand; SCJ: Sternocostal joints

The major symptoms observed in >70% of CHIKV patients were fever (99.2%), arthralgia (97.7%), myalgia (89.1%), difficulty in walking (78.1%), joint stiffness (77.3%), morning stiffness (76.6%), sleeping disturbances (75.8%) and headache (70.3%) (Table 1). Multiple joints in decreasing order of their affliction involved small fingers of hand (84.4%), knees (81.3%), wrist (68.8%), shoulders (67.2%), ankle (64.1%), toes (53.9%), neck (53.1%), feet (53.1%), elbow (48. 4%), hip (39.1%), sternocostal joints (34.4%) and rachis (33.6%) (Table 1). Of 128 CHIKV confirmed patients 101 (78.9%) patients suffered from severe pain, 9 (7.0%) had moderate pain and the rest 15 (11.7%) experienced mild pain. Rashes, retro-orbital pain, cough, running nose, oral ulcers, weight gain and diarrhoea were reported in <20 percent of the patients.

Rashes were observed in 7 patients (5.5%) 3-4 days after the onset of fever and were localized on face (0.8%), trunk (2.3%), hands (3.9%) and legs (1.6%) respectively. The frequency of rashes varied from 50 - 80 % during the Reunion island outbreaks (Staikowsky et al., 2008). Weight gain (3.9%), rashes (5.5%) and diarrhoea (5.5%) were the lowest recorded symptoms among CHIK patients. Oedema was observed in 57% of the patients and was majorly seen in feet/toes (36.7%), ankle (32.0%), small fingers of hand (32.0%), knee (30.5%), face (24.2%), legs (18.8%) and less frequently observed in elbow (12.5%) and shoulder (10.9%) respectively.

Five patients (3.9%) claimed to have gained weight as a result of CHIKV infection. Restricted body movements due to crippling arthralgia and prolonged rest during CHIKV infection might have helped in weight gain of the patients. Digestive disorders such as nausea (42.2%), vomiting (37.5%), anorexia (53.1%) and diarrhoea (5.5%) were observed in our study patients. Lombalgia, eye congestion, chills, abdominal pain, weight loss, lethargic nature and giddiness were observed in varying frequencies (Table 1). Three patients (2.3%) of >50 yrs of age were hospitalized in the present study. Duration of their hospital stay ranged from 3-5 days. The patients suffered from chronic joint pain which restricted their body movements and limited the patients in performing everyday tasks. During the current outbreak the percentage of hospitalized patients ranged from 2.7% to 33.3% (Suryawanshi et al., 2009; Staikowsky et al., 2008). CHIK infected children requiring intensive care were encountered during the current outbreak (Menon et al., 2010). A previous study from India reported rashes, headache, joint pains, swelling of joints, abdominal pain, cough and vomiting to be significantly associated with CHIKV confirmed patients (Ray et al., 2012).

Asymptomatic patients (n=2) were encountered during the present study. Blood donated by asymptomatic patients during the viremic stage will be infectious and the possibility of CHIKV transmission cannot be underestimated (Naresh Kumar CVM, Sai Gopal., 2008). Asymptomatic or paucisymptomatic forms of CHIKV were also revealed during 1996-97 CHIK outbreaks in Senegal (Staikowsky et al., 2008)**.** Approximately 3% - 28% of people infected with CHIKV remained asymptomatic (Staples et al., 2012). In a recent study it was observed that the estimated risk of viremic blood donation was high during CHIK outbreaks (Brouard et al., 2008). Estimated transfusion risks range as high as 150 per 10 000 donations during outbreaks (Petersen et al., 2010)**.** Our present observation in agreement with recent reports, supports the fact that improper blood screening may to some extent help in spread of CHIKV. Although probability of such a mode of transmission might be low, but we cannot take any chance against the CHIKV virus which has created havoc around the globe. During the outbreaks, as a precautionary measure CHIKV screening should be made mandatory in blood banks**.** Possible measures to prevent CHIKV transfusion transmission include deferral of symptomatic donors, discontinuing blood collections in affected areas, and CHIKV nucleic acid screening of donations (Petersen et al., 2010)**.** If proper mosquito prevention measures are not taken, these asymptomatic patients can spread the disease by mosquito bites to healthy humans (Staples et al., 2012).

## CHIKV symptoms with respect to age group

A significant difference was observed in pain intensity, arthralgia (neck, ankle, feet, toes, rachis), oedema (knee, ankles, feet/toes), diarrhoea, lethargic nature, and hospitalization cases and these symptoms were reported to be lower (11.8-100%) in younger age group than in older age group (55-100%) (Table 2). The prevalence of symptoms like arthralgia (shoulder, elbow, wrist, small fingers of hand, hip, knee and sternocostal joints), joint stiffness, sleeping disturbances, oedema (shoulders, small fingers of hand) and weight loss was observed to be higher with increase in age group (Table 2). Irrespective of the age group, the prevalence of the symptoms such as morning stiffness, walking difficulty, myalgia, lombalgia, eye congestion, chills, cough, abdominal pain, nausea, oral ulcers, weight gain and giddiness were observed to be almost same (Table 2).

## CHIKV symptoms with respect to gender

It is observed that the symptoms pain intensity, arthralgia (wrist, small fingers of hand, hip, ankle, feet, toes, rachis and sternocostal joints), walking difficulty, lombalgia, oedema (small fingers of hand, knee, ankle and toes), rashes, abdominal pain, lethargic nature and anorexia are associated with gender. It was also noted that the frequency of the CHIK associated symptoms were more in females when compared to their male counterparts (Table 3). In previous studies it was noted that, there were no significant symptom wise differences between two genders except for swellings which occurred more frequently in women than men (Kannan et al., 2009; Staikowsky et al., 2008).

**Persistence of symptoms**

The symptoms such as fever, headache, retro-orbital pain, eye congestion, chills, diarhhoea, giddiness, vomiting and nausea were observed in majority of the patients for less than 8 days duration (Table 4). Other symptoms such as morning stiffness, joint stiffness, sleeping disturbances, walking difficulty, lombalgia, rashes, cough, running nose, oral ulcers, anorexia, and lethargic nature were observed in majority of the patients for less than one month duration (Table 4). Arthralgia persisted for 79 (63.2%) patients for less than 1 month; 9 (7.2%) patients for 2 months; 15 (12%) patients for 3 months; 22 (17.6%) patients for > 3 months of which, 8 (6.4%) patients for 6-7 months; 11 (8.8%) patients for 13 months and 3 (2.4%) patients for 16 months respectively. Myalgia persisted for 102 (89.5%) patients for less than 1 month, 7 (6.1%) patients for up to 2 months and 5 (4.4%) patients for 3-4 months respectively. Oedema persisted for 48 (65.8%) patients up to 1 month; 12 (16.4%) patients for 2-3 months; 13 (17.8%) for more than 3 months, of which 10 (13.7%) patients for 5 months and 3 (4.1%) patients for up to 16 months respectively.

"CHIKV reheumatism" was observed earlier as well as during the current CHIKV outbreak (Fourie and Morrison., 1979; Brighton et al., 1983; Borgherini et al., 2007; Simon et al., 2007; Borgherini et al., 2008; Sissoko et al., 2009; Manimunda et al., 2010; Larrieu et al., 2010). Six months to 2 years after the acute CHIKV infection, nearly 48% rheumatic manifestations were observed in the CHIKV patients in France (Simon et al., 2007; Larrieu et al., 2010). In Reunion Islands 57% and 64% of CHIKV patients experienced permanent or recurrent polyarthralgia at 15 and 18 months post infection (Borgherini et al., 2008; Sissoko et al., 2009). Fourty four percent of patients from Reunion Islands suffering from prolonged CHIKV arthritis had a previous history of joint pain (Borgherini et al., 2008). Co-morbidity with osteoarthritis was identified as a significant risk factor for non-recovery of CHIKV patients (Sissoko et al., 2009). A recent study showed that among CHIKV infected individuals, 60% of polyarthralgia could be attributed to CHIKV (Gerardin et al., 2011). Early escape of CHIKV from blood monocytes and its relocation to synovial macrophages forms the basis of musculoskeletal pain and chronic arthropathy (Her et al., 2010). The recent development of a non-human primate model confirms the important role of the macrophages as the main target cell for CHIKV dissemination and persistence within the organism as well as being the cornerstone for CHIKV rheumatic pathophysiology (Labadie et al., 2010; Her et al., 2010; Jaffar-Bandjee et al., 2009; Hoarau et al., 2010). Thus, an inadequate host innate immune (Th1) response to CHIKV could damage cartilage and lead to osteoarthritis degenerative lesions as observed for RRV and Rheumatoid arthritis (Gerardin et al., 2011; Lidbury et al., 2008).

**Treatment**

In the present study it was not possible to assess the response of any particular drug. The patients were also educated about the self-limiting nature of CHIKV disease and were advised against indiscriminate usage of antibiotics and injections, thus avoiding unnecessary health complications.

As the joint pain lasts for a long period of time, medications for the treatment of CHIKV are likely to be overused (Murhekar et al., 2011). The median out-of-pocket expenses among CHIKV patients was 550 Indian rupees (Range: 0-15,000 Rs) for the course of the illness (Murhekar et al., 2011). A study from Maharashtra showed the use of Ibuprofen, Paracetamol, Diclofenac, Chloroquine, Hydroxychloroquin and steroids for treating CHIKV infected patients (Suryawanshi et al., 2009). In yet another study from Maharashtra it was observed that the combination of Nimesulide, Paracetamol and Cefixime was the most prescribed one for treating CHIKV infected patients (Mukadam et al., 2009). In addition to this, about 80% of patients were treated with intravenous fluids (Mukadam et al., 2009). Paracetamol and Corticoids were prescribed to CHIKV patients in Reunion Islands (Staikowsky et al., 2008). Because no specific antiviral therapy exists for CHIKV infection, treatment consists of supportive care, including administration of analgesics and anti-inflamatory medications for joint symptoms (Lakshmi et al., 2008). Indiscriminate use of antibiotics and NSAIDs should be avoided, as it leads to gastrointestinal bleeding and vomiting. This may in turn lead to renal failure and indirectly contribute to mortality due to CHIK fever (Mohan, 2006).

**Prevention**

Quite interestingly, it was found that the environmental factors of the patients favoured mosquito breeding a lot. Possible indoor mosquito breeding sites included refrigerators (30.5%), coolers (12.5%) and indoor plants (21.1%), while sewage (48.4%) and outdoor fields (10.9%) accounted for outdoor breeding sites for mosquitoes (Table 5). Surprisingly 41.4% patients (n=53) were unaware of the role of mosquitoes in spreading CHIKV. The CHIK pamphlet in English and Telugu was distributed among the patients and the general public. The public were educated about the disease and the use of personal protective measures in stopping the spread of the disease.

**Table 3: Comparison of CHIKV symptoms with respect to gender factor**

| S.No | Symptoms | | Gender | |
|---|---|---|---|---|
| | | | Male | Female |
| | | | Count (%) | Count (%) |
| 1 | Fever* | | 61(48.8) | 66(51.9) |
| 2 | Headache* | | 45(50.0) | 45(50.0) |
| 3 | Pain intensity | Mild | 9(60.0) | 6(40.0) |
| | | Moderate | 6(66.7) | 3(33.3) |
| | | Severe | 43(42.6) | 58(57.4) |
| 4 | Arthralgia | Neck* | 58(46.4) | 67(53.6) |
| | | Shoulder* | 34(50.0) | 34(50.0) |
| | | Elbow* | 45(52.3) | 41(47.7) |
| | | Wrist | 28(45.2) | 34(54.8) |
| | | SFH | 36(40.9) | 52(59.1) |
| | | Knee* | 48(44.4) | 60(55.6) |
| | | Ankle | 49(47.1) | 55(52.9) |
| | | feet | 34(41.5) | 48(58.5) |
| | | Toes | 23(33.8) | 45(66.2) |
| | | Rachis | 24(34.8) | 45(65.2) |
| | | SCJ | 16(37.2) | 27(62.8) |
| | | | 16(36.4) | 28(63.6) |
| 5 | Morning stiffness* | | 47(48.0) | 51(52.0) |
| 6 | Joint stiffness* | | 51(51.5) | 48(48.5) |
| 7 | Sleeping disturbances* | | 51(52.6) | 46(47.4) |
| 8 | Walking difficulty | | 42(42.0) | 58(58.0) |
| 9 | Myalgia* | | 54(47.4) | 60(52.6) |
| 10 | Lombalgia | | 22(40.0) | 33(60.0) |
| 11 | Oedema | Face* | 29(39.7) | 44(60.3) |
| | | Shoulder* | 12(38.7) | 19(61.3) |
| | | Elbow* | 6(42.9) | 8(57.1) |
| | | SFH | 7(43.8) | 9(56.3) |
| | | Knee | 12(29.3) | 29(70.7) |
| | | Ankle | 13(33.3) | 26(66.7) |
| | | Feet/Toes | 11(26.8) | 30(73.2) |
| | | Legs* | 15(31.9) | 32(68.1) |
| | | | 10(41.7) | 14(58.3) |
| 12 | Rashes | Face* | 1(14.3) | 6(85.7) |
| | | Trunk* | 0(0.0) | 1(100.0) |
| | | Hands | 1(33.3) | 2(66.7) |
| | | Legs* | 0(0.0) | 5(100.0) |
| | | | 0(0.0) | 2(100.0) |
| 13 | Retro-orbital pain* | | 9(37.5) | 15(62.5) |
| 14 | Eye congestion* | | 23(53.5) | 20(46.5) |
| 15 | Asymptomatic carrier* | | 2(100.0) | 0(0.0) |
| 16 | Chills* | | 31(47.7) | 34(52.3) |
| 17 | Cough* | | 12(54.5) | 10(45.5) |
| 18 | Running nose* | | 11(47.8) | 12(52.2) |
| 19 | Abdominal pain | | 12(32.4) | 25(67.6) |
| 20 | Nausea* | | 29(53.7) | 25(46.3) |
| 21 | Vomiting* | | 22(45.8) | 26(54.2) |
| 22 | Oral ulcer* | | 5(62.5) | 3(37.5) |
| 23 | Weight gain* | | 2(40.0) | 3(60.0) |
| 24 | Weight loss* | | 27(51.9) | 25(48.1) |
| 25 | Lethargic | | 26(41.9) | 36(58.1) |
| 26 | Anorexia | | 28(41.2) | 40(58.8) |
| 27 | Diarrhoea* | | 5(71.4) | 2(28.6) |
| 28 | Giddiness* | | 26(42.6) | 35(57.4) |
| 29 | Hospitalization* | | 1(33.3) | 2(66.7) |

*Symptoms are not associated with respect to gender (Chi square test) SFH: Small fingers of hand; SCJ: Sternocostal joints

**Table 4: Duration of symptoms**

| S.No | Symptoms | Duration | Male | Female | Total |
|---|---|---|---|---|---|
| | | | Count (%) | Count (%) | Count (%) |
| 1 | Headache (n = 90) | 1 - 5 | 22 (48.9) | 30 (66.7) | 52 (57.8) |
| | | 5 - 10 | 13 (28.9) | 12 (26.7) | 25 (27.8) |
| | | > 10 | 10 (22.2) | 3 (6.7) | 13 (14.4) |
| 2 | Arthralgia (n= 125) | 0 - 10 | 14 (24.1) | 9 (13.4) | 23 (18.4) |
| | | 10 - 30 | 30 (51.7) | 26 (38.8) | 56 (44.8) |
| | | 30 - 60 | 3 (5.2) | 6 (9.0) | 9 (7.2) |
| | | 60 - 90 | 4 (6.9) | 11 (16.4) | 15 (12.0) |
| | | > 90 | 7 (12.1) | 15 (22.4) | 22 (17.6) |
| 3 | Myalgia (n= 114) | 0 - 15 | 36 (66.7) | 29 (48.3) | 65 (57.0) |
| | | 15 - 30 | 12 (22.2) | 25 (41.7) | 37 (32.5) |
| | | 30 - 60 | 4 (7.4) | 3 (5.0) | 7 (6.1) |
| | | > 60 | 2 (3.7) | 3 (5.0) | 5 (4.4) |
| 4 | Oedema (n= 73) | 1-10 | 11 (37.9) | 9 (20.5) | 20 (27.4) |
| | | 10-30 | 13 (44.8) | 15 (34.1) | 28 (38.4) |
| | | 30-60 | 2 (6.9) | 10 (22.7) | 12 (16.4) |
| | | > 60 | 3 (10.3) | 10 (22.7) | 13 (17.8) |
| 5 | Fever (n = 127) | < 7 | 44 (72.1) | 51 (77.3) | 95 (74.8) |
| | | > 7 | 17 (27.9) | 15 (22.7) | 32 (25.2) |
| 6 | Retro-orbital pain (n = 24) | 1-8 | 8 (88.9) | 14 (93.3) | 22 (91.7) |
| | | 8-15 | 1 (11.1) | 1 (6.7) | 2 (8.3) |
| 7 | Eye congestion (n = 43) | 1-5 | 21 (91.3) | 19 (95.0) | 40 (93.0) |
| | | 5-10 | 2 (8.7) | 1 (5.0) | 3 (7.0) |
| 8 | Chills (n = 65) | 1-4 | 28 (90.3) | 25 (73.5) | 53 (81.5) |
| | | 4-8 | 3 (9.7) | 9 (26.5) | 12 (18.5) |
| 9 | Giddiness (n = 61) | 1-3 | 21 (80.8) | 32 (91.4) | 53 (86.9) |
| | | 3-7 | 5 (19.2) | 3 (8.6) | 8 (13.1) |
| 10 | Diarrhoea (n = 7) | < 5 | 5 (100) | 2 (100) | 7 (100) |
| 11 | Morning stiffness (n = 98) | <30 | 39 (83) | 40 (78.4) | 79 (80.6) |
| | | >30 | 8 (17) | 11 (21.6) | 19 (19.4) |
| 12 | Joint stiffness (n = 99) | <30 | 43 (84.3) | 39 (81.3) | 82 (82.8) |
| | | >30 | 8 (15.7) | 9 (18.7) | 17 (17.2) |
| 13 | Sleeping disturbances (n = 97) | 15-20 | 42 (82.4) | 39 (84.8) | 81 (83.5) |
| | | >20 | 9 (17.6) | 7 (15.2) | 16 (16.5) |
| 14 | Walking difficulty (n = 100) | >30 | 29 (69) | 41 (70.7) | 70 (70) |
| | | <30 | 13 (31) | 17 (29.3) | 30 (30) |
| 15 | Lombalgia (n = 55) | <15 | 17 (77.3) | 26 (78.8) | 43 (78.2) |
| | | >15 | 5 (22.7) | 7 (21.2) | 12 (21.8) |
| 16 | Rashes (n = 7) | <10 | 1 (100) | 6 (100) | 7 (100) |
| 17 | Cough (n = 22) | <10 | 12 (100) | 10 (100) | 22 (100) |
| 18 | Running nose (n = 23) | <10 | 11 (100) | 12 (100) | 23 (100) |
| 19 | Abdominal pain (n = 37) | Infrequent | 12 (32.4) | 25 (67.6) | 37 (100) |
| 20 | Nausea (n = 54) | <7 | 29 (100) | 25 (100) | 54 (100) |
| 21 | Vomiting (n = 48) | 1-2 | 22 (100) | 26 (100) | 48 (100) |
| 22 | Oral ulcers (n = 8) | <15 | 5 (100) | 3 (100) | 8 (100) |
| 23 | Lethargic (n = 62) | <30 | 26 (100) | 36 (100) | 62 (100) |
| 24 | Anorexia (n = 68) | <15 | 28 (100) | 40 (100) | 68 (100) |

**Table 5: Prevention/Treatment measures of CHIKV**

| Parameter | Mode | Description | Count (Percentage) |
|---|---|---|---|
| **Mosquito breeding sites** | Indoor | Refrigerator | 39 (30.5) |
| | | Cooler | 16 (12.5) |
| | | Indoor plants | 27 (21.1) |
| | Outdoor | Sewage | 62 (48.4) |
| | | Fields | 14 (10.9) |
| **Preventive measures** | | Coils | 59 (46.0) |
| | | Bednets | 28 (21.8) |
| | | Coils + Bednets | 37(28.9) |
| | | Repellents | 4 (3.12) |
| **Treatment** | Allopathy | Tablets | 128 (100.0) |
| | | Injection | 94 (73.4) |
| | | Saline | 11 (8.6) |
| | Homeopathy | | 1 (0.8) |
| | Physiotheraphy | | - |

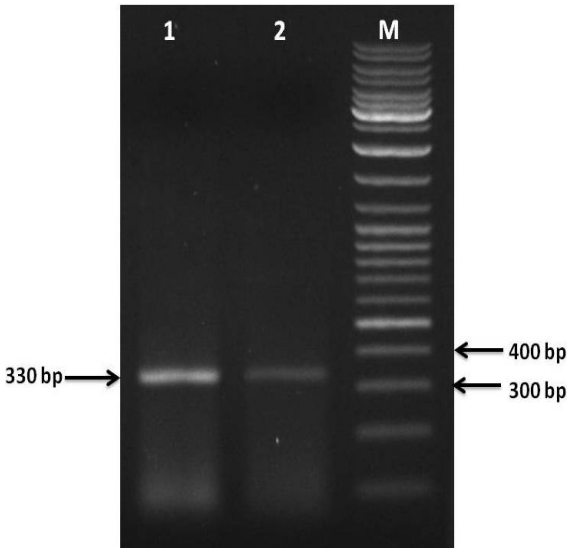


**Fig. 1: Gel photograph showing 330bp fragment of E1 gene of *Chikungunya virus* by Reverse Transcriptase - Polymerase Chain Reaction (RT-PCR)**
**Lane M: GeneRuler™ DNA Ladder (Fermentas)**
**Lane 1,2: CHIKV positive clinical samples**

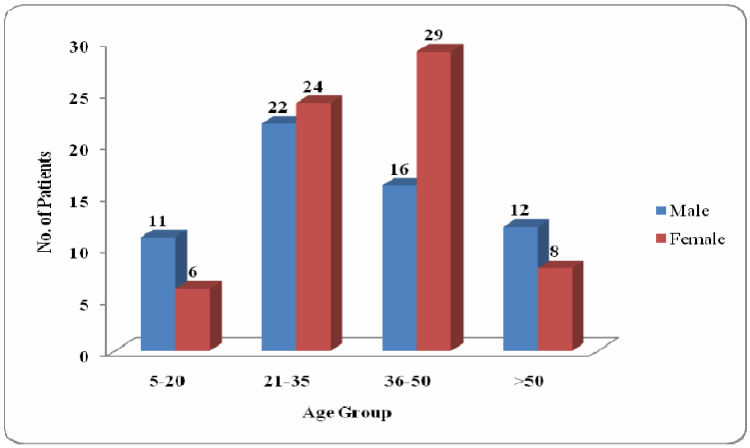


**Fig. 2: Age and gender distribution of 128 Chikungunya confirmed patients.**

Coils (46%), bednets (21.8%), coils + bednets (28.9%) and repellants (3.12%) were used by the patients (Table 5). But it didnot appear to stop the spread of the CHIKV disease as, 26 (20.3%) patients reported 100% infection in their family members while 57 and 45 patients reported 50-80% and <50% infection in their family members.

Patients with febrile illness that is suspected to be due to CHIKV were advised to avoid mosquito bites for at least 7 days after the onset of illness, to reduce the likelihood of transmitting the virus to local mosquitoes, which might then transmit the virus to other humans (AbuBakar et al., 2007).

Our study has some limitations. During the follow up studies the patients were not physically examined by physicians either to rule out differential diagnoses or to assess and confirm the complaints. We had to solely depend upon the details/descriptions provided by the patients to assess the disease severity. These reasons may have skewed the estimation of some of the subjective symptoms assessed in this study. Secondly we did not look into the neurological complications, as SVUHC lacked the required facilities to carry out such work.

## CONCLUSION

In conclusion, the CHIKV outbreak in Tirupati region of Andhra Pradesh was severe and the impact on human health generally long lasting particularly with reference to prolonged arthralgia. The clinical manifestations in the present study matched the known description of the disease. Asymptomatic cases were encountered during the present study. The most susceptible age group was 21-50 yrs and percentage of infection was observed to be higher in females. In the absence of a vaccine against CHIKV, a drastic change in the outlook of the community and public health authorities with regard to hygiene and mosquito control measures is essential to stand a chance in the war against the mosquitoes.

### Conflict of interest statement

We declare that we have no conflict of interest

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