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ORIGINAL RESEARCH

Outbreak of Chikungunya in Ahmedabad: A Report

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ABSTRACT

Chikungunya (CHIK) fever is caused by a CHIK virus and was analyzed in 757 referral cases in post-monsoon of 2016 at our Supratech-Micropath Research Laboratory. The temperature during this season ranges from 20 – 40˚C and is favorable for mosquito breed. The patients were asked to fill consent forms after which blood samples were taken to detect seropositivity of the virus by Real Time – PCR. It was first confirmed by preliminary symptoms like sudden onset of fever, headache, severe muscle pain, nausea, fatigue and rashes as per clinician reports. The percentile detected cases were 36.7% in six month referral cases (278/757). As per our RT-PCR reports, maximum percent patients (35.2%) were detected in October, November and December, 2016. Males were affected maximum (55%) in this post monsoon season (148/267). Additionally, these cases were correlated with low lymphocyte counts and altered aspirate aminotransferase (AST) in the serum affecting immune function and fast cardiac rate in our study. This fever also increased with age in this academic year. Hence, it is suggestive that this season is a period for such diseases and hygienic conditions like mosquito eradication, clean environment and other conditions are to be controlled by Health Departments in Ahmedabad city.

KEY WORDS: *Chikungunya fever, Virus, Real Time-PCR, Lymphocytes, Transaminases, Clinical Report*

Introduction

Chikungunya (CHIK) fever is caused by an alpha virus, native of Africa and Asian continent. It is transmitted to human by *Aedes* mosquitoes, *A.aegypti and A.albopictius* .It is first reported in Tanzania in 1952 – 53 and India in 1963 (Jadhav *et al*., 1965). It shares some clinical signs with dengue and can be diagnosed in areas where dengue is common. It is characterized by an abrupt onset of fever and joint pain followed by other common signs of muscle pain, headache, nausea and rash (WHO, 2016). India reported a massive CHIK epidemic in 2006 where its attack increased to 45% in some places (Mavalankar *et al*., 2008). In the state of Gujarat, the episode of chikungunya occurred in more than 72589 cases. Municipal Health Centres and Hospitals in Ahmedabad accounted 60,777 cases in 2006 (Mavalankar *et al*., 2010). Though control measures are being applied the patients suffering from chikungunya re-emerge with dengue and are compared with clinical manifestations and laboratory tests (Lee *et al*., 2012; Mittal *et al*., 2008). Most chikungunya cases do not result in severe complications and treatment is symptomatic unlike dengue haemorrhagic fever (DHF) or dengue shock syndrome (DSS) cases. But sometimes organ failures can happen are fatal especially in elderly with co-morbidities (Econompoulou *et al*., 2009).Based on these parameters, this study was undertaken, to investigate the epidemiology of Chikungunya cases at our centre in last six months, i.e. July – December, 2016, where temperature ranges from 20˚C – 40˚C during this time in Ahmedabad.

Materials and Methods

Patient Selection

Patients (757) suspected for this fever based on clinician suggestion were referred to our centre from July to December, 2016. After, the consent form was filled by the patient; blood was collected and used for blood profile assessment and RNA extraction. This project was approved by Human Ethical Committee (HEC) of Gujarat University (GUHEC-001-2015) for investigation.

Haematological profile

Complete Blood Count (C.B.C.) including Hb levels were done using cell counter (Siemen's, Advair2010i, Germany. Serum transaminases were estimated using kit (Dimension EXL, Germany) and expressed as U/L and compared.

Viral RNA Isolation and RT-PCR

Viral RNA was extracted from EDTA plasma samples using the Automated Perkin Elmer Viral RNA/DNA 200 extraction kit or QIAamp® Viral RNA mini kit (Qiagen, Germany) as per manufacturers' instruction. The RT-PCR assay used in this study for the detection of the chikungunya virus was RealStar® Chikungunya RT-PCR Kit 2.0 (Altona Diagnostics, Germany). The assay allowed for a qualitative detection of chikungunya virus specific RNA, with reverse-transcription of the RNA into complementary DNA (cDNA) followed by PCR for the amplification of specific target sequences. The probes utilized were specific for CHIKV RNA labelled with the fluorophore FAM™ and specific for the Internal Control (IC) is labelled with the fluorophore JOE™. The data were analyzed concentrating on post-monsoon season using ANOVA and Student's 't' test. A value of P<0.05 is considered significant.

Results

The CHIK cases referred in our centre were 757 from July to December 2016. The percent of seropositive patients ranged from 0 to 17.7% within these six months (278/757; 36.7%).In post monsoon season i.e. October, November and December 2016 maximum cases were detected positive (35.2%) being maximum in November followed by October and December. (Fig.1, Table-1).Amongst four age groups viz. 0 – 20, 21 – 40, 41 – 50 and 51 & above year age groups, the positivity increased with age and males were maximally affected 55% in October to Dec 2016 (Table -2).

Blood Cell Count

In all age groups, cell counts were within normal range including haemoglobin levels. Lymphocyte counts were significantly ($p< 0.05$; $p<0.01$) decreased in all age groups (Table-3).

Table 1: Total Prevalence distribution of CHIKV in six months

Month	Total	Affected	Percentage Affected
July	13	1	0.13%
August	29	0	0%
September	289	10	1.32%
October	101	71	9.40%
November	199	134	17.70%
December	126	62	8.19%
Total	757	278	36.70%

Transaminase level

The SGPT levels were within normal range (14 – 59 U/L). However, SGOT /ALT levels were significantly (P<0.01) increased in all age groups being maximum in the 41 – 50 age group (183%). The normal range is 0 – 37 U/L (Table-4).

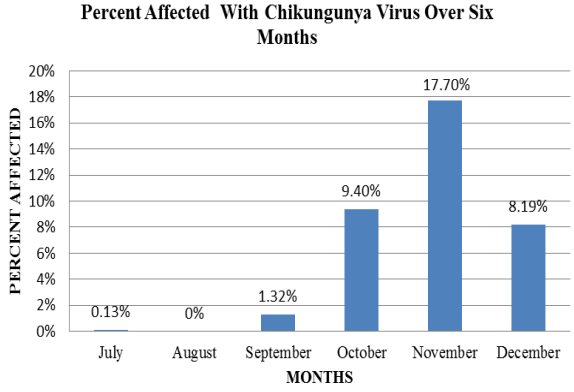

Figure 1: Percent Affected with Chikungunya Virus over Six Months

Table 2: Group-wise CHIK Cases in our study (Oct – Dec, 2016)

Age Group	Male	Female	Total Positive
0-20 (50)	17 (59%)	12 (41%)	29 (11%)
21-40 (106)	29 (47%)	33 (53%)	62 (23%)
41-50 (65)	26 (53%)	23 (47%)	49 (18%)
51 Above(205)	76 (60%)	51 (40%)	127 (48%)
Total	148 (55%)	119 (44%)	267 (100%)

Table 3: Blood cell count in Chikungunya cases (Oct – Dec, 2016)

Age Group (Yrs)	Platelet Count 150000 - 410000 /ul	RBC 3.8 - 4.8 Million/Cumm	WBC 4000-10000 /ul	Lymphocytes Count 1000-3000 /ul	Hb 12.0 -15.0 G%
0-20 (4)	222191±65796^{NS}	4.39 ±0.05^{NS}	7046 ±2028^{NS}	844±243*	12.5 ±1.64^{NS}
21-40 (17)	198615±66841^{NS}	4.40 ±0.60^{NS}	7300±1295^{NS}	670 ±363**	12.5 ±1.77^{NS}
41-50 (18)	198611 ±63416^{NS}	4.41 ±0.60^{NS}	7569 ±1586^{NS}	765±257*	12.5±1.67^{NS}
51&Above (36)	197698 ±66188^{NS}	4.40 ±0.59^{NS}	7281 ±1753^{NS}	761 ±286*	12.5 ±1.76^{NS}

Seropositivity by RT-PCR

The amplification of FAM™ and JOE™ dyes was interpreted as seropositive with amplification of CHIKV specific RNA (Fig-1.) whereas an absence of amplification of FAM™ and an amplification of only JOE™ was interpreted as negative where the sample does not contain detectable amounts of CHIKV specific RNA (Fig.-2). Out of 757 referral cases 278 were seropositive (36.7%). The month of July had 0.13%, August 0%, September 1.3%, October 9.3%, November 17.7% and December 8.19% positivity respectively (Table-1; Fig.-2).

Table 4: Serum transaminase levels in our cases of three months

Age Group (Yrs)	SGPT 14-59 U/L (100%)	SGOT 0-37 U/L (100%)
0-20	83%	155%
21-40	93%	138%
41-50	87%	183%
51 & Above	94%	137%

SGPT: Serum glutamic pyruvic transaminase; SGOT: serum glutamic-oxaloacetic transaminase

Discussion

Chikungunya (CHIK) fever is caused by two vectors mainly, *Ae. albopectus* and *Ae. aegypti*. In India *Ae. aegypti* was dominant. In 2005 – 2006,it's out-break occurred in Tanzania followed by India in 2006. In Ahmedabad, CHIK epidemic was also occurred in same time (Mavalankar *et al*., 2008, 2010). It still emerges in Ahmedabad, though the preventive measures are taken. Hence, we studied the outbreak of CHIK in 2016 during July to December 2016 ranging temperature from 20ºC to 40ºC. The data revealed that detected cases by RT-PCR were maximum in October, November and December 2016, (35.2%) in all age groups constituting 36.7% of the total patients during six months (278/757). However, the degree of infection increased with age as observed in Kerala where CHIK was higher in age groups studied (Kumar *et al*., 2011).

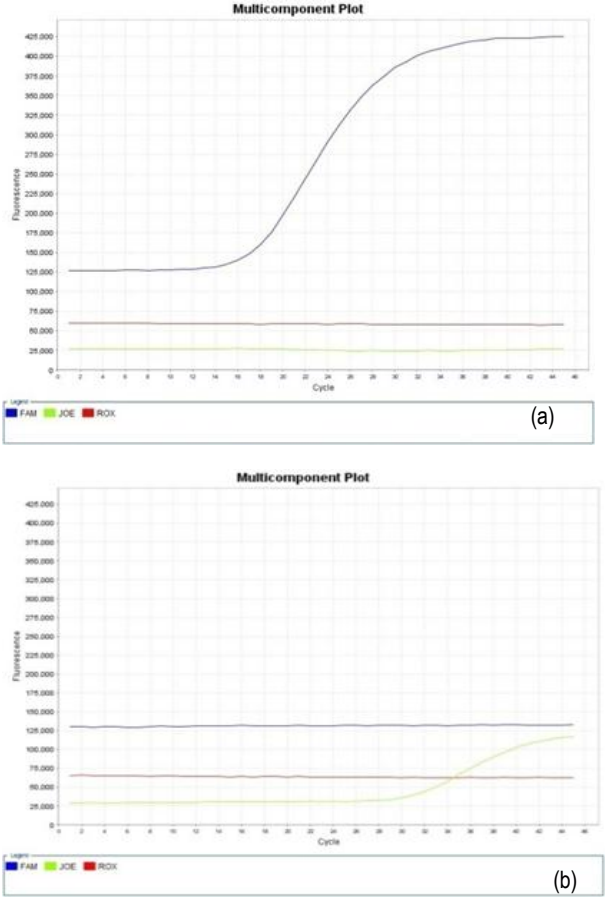

Figure 2: a. RT-PCR result for a positive sample for Chikungunya. b. RT-PCR result for a negative sample for Chikungunya

Same data was also reported by Mavalankar *et al*., (2008,

2010), where this Disease was maximum in these months of 2006. Gender differences were found where males were infected than females who are probably exposed to high risk factors in the environment (Kumar *et al*., 2011).This could also be attributed to unhygienic condition during this post-monsoon season where temperature is suitable for mosquito breeding. These results were also supported by clinical variables like fever, headache, and muscle pain as per our clinical reports. Lee *et al*. (2012) used several predictable laboratory tests for detecting CHIK like drop in lymphocyte count, higher platelet, leucocyte and neutrophil counts to separate Dengue types .But, in our report, lymphocyte count was remarkably below normal range. It indicated that these patients had increased tendency of antigen infection. Detection of infection with serological methods in addition to RT-PCR (http://infection.thelancet.com, 2001) was also used by measuring IgG and IgM levels (Kumar *et al*., 2011). We also assayed serum transaminases i.e. ALT/SGPT and AST/SGOT for detecting liver and heart functions (Karmen *et al*., 1995; Paul and Gibony., 2005). In our data, a significant elevation in AST/SGOT level (137% – 183%) was observed leading to heart and liver dysfunction. Heart-dysfunction was also supported by fast heart-beat in our infected patients. Gaze (2007) mentioned the measurements of troponins are better indicators for cardiac anomaly than AST/SGOT levels. Other variables like altered bilirubin, creatinine and proteins are also laboratory predictors for Chikungunya fever versus dengue (Lee *et al*., 2009). The changed variables done in our study, hence clearly pointed out that in post-monsoon season (October – December, 2016) majority of cases were suffered with CHIK viral infection in all age groups. These laboratory test variables like a drop in lymphocyte count, increased SGOT, fever, heartbeat, myalgia etc. are good indices for this viral detection as suggested by Lee *et al*. (2012) recently.

It is, hence suggested that in post-monsoon, measures are considered by Health departments in regard to eradication of mosquito breed areas, provision of vaccines, immunity boosting, poverty removal measures and other factors to prevent outbreak of such viral diseases including dengue in Ahmedabad city.

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