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ORIGINAL PAPER

Seroprevalence of Chikungunya Cases in a Tertiary Care Hospital of Assam

Raja Dina[1], Phukan Chimanjita[2], Killing Lunse[3]

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ABSTRACT

***Objectives**: To determine the prevalence of Chikungunya cases and to correlate the clinical symptoms of Chikungunya with serological findings in patients attending Gauhati Medical College and Hospital. **Material and Methods**:The study was carried out among 866 clinically suspected Chikungunya cases presenting with fever, headache, retro-orbital pain, back pain and arthralgia and the sample were tested for Chikungunya virus specific IgM antibodies, in the Department of Microbiology, Gauhati Medical College and Hospital. Detection of CHIK V IgM antibodies in serum of all subjects was carried out by ELISA kits procured from NIV, Pune. Age, sex wise distribution and the period of peak incidence of the positive cases was studied. **Result**: In the study, the seroprevalence of Chikungunya among the suspected cases was 9.93%. The prevalence of Chikungunya infection according to clinical symptoms were 97% fever, 67.44% headache, 30.23% retro-orbital pain, 30% back pain, 22% arthalgia. Gender wise distribution showed male and female ratio to be 2.7:1.The metro population were infected more than the rural population. The maximum number of seropositive was seen among Kamrup Metro followed by Barpeta. The peak season was in the month of September and in the 30-39 age group. **Conclusion**: Chikungunya is a newly emerging viral infection which had spread to new areas during this outbreak. Hence it is essential to have a proper diagnostic laboratory support, proper surveillance system and public awareness in order to prevent future epidemic in this region.*

***Keywords**: Chikungunya; Capture linked immunosorbant assay, seropositivity*

INTRODUCTION

Emerging viral infections have become a serious problem in recent years. Emergence or re-emergence of severe arboviralhemorrhagic fevers caused by mosquito borne viruses, such as dengue virus and Chikungunya (CHIK) virus, have been frequently reported in the Indian subcontinent in the past few years. Chikungunya is an emerging viral illnessin the majority of people presenting with fever, headache, myalgia, retro-orbital pain, backache, rashes and severe arthralgia. Since from clinical perspective the clinical manifestation are almost similar and it is very difficult to distinguish from one another. As the outcome of these infections vary on the basis of the infecting agent where the mortality rate of dengue is high. There is therefore a need for a means of definitive diagnosis and identification of the viral agent.[1]

The species chikungunya belongs to *Alpha virus* genus which consists of 28 viruses.[2,3] Chikungunya virus is serologically classified as a member of the Semliki Forest antigenic complex.[4] The disease is transmitted predominantly by *Aedesaegypti, Ae. albopictus*and*Ae. Polynesiensis*are commonly involved in the transmission although *Culex*mosquitoes has also been reported for the transmission in some cases, the same species involved in the transmission of dengue.[2,5]

The symptoms are most often clinically indistinguishable from those observed in dengue fever. Indeed, the simultaneous isolation of both dengue and Chikungunya from the sera of the same patients has previously been reported, indicating the presence of dual infections. In 2010, a hospital-based study revealed co-circulation of Chikungunya virus and Dengue virus in some areas of West Bengal, India with high morbidity.[6] It is, therefore, very important to clinically distinguish dengue from Chikungunya infection. A definitive diagnosis of Chikungunya infection can be made only with the aid of laboratory support

Address for Correspondence:
[1]Associate Professor **(Corresponding Author)**
Email: dinaraja2016@gmail.com
Mobile No: +919864039629
[2]Chimanjita Phukan, Associate Professor,
[3]Lunse Killing (PGT) Department of Microbiology, Gauhati Medical College, Guwahati), Guwahati, Assam

since clinically, symptoms resemble those of dengue fever.[7] As the outcomes of these infections vary on the basis of the infecting agent, they pose a diagnostic dilemma for the clinician. Laboratory diagnosis is therefore critical to establish the differential diagnosis.[7] Therefore our aim and objective is to determine the prevalence of Chikungunya cases and to correlate the clinical symptoms of Chikungunya with serological findings.

MATERIALS AND METHOD

The present study was undertaken to know the prevalence rate and correlation of clinical symptoms of Chikungunya with serological test IgM antibodies in patients attending Gauhati Medical College and Hospital during a period of one year from June 2013 to May 2014. During this period, a total of 866 samples were screened for IgMChikungunya antibodies from the clinically suspected cases of Dengue and Chikungunya. Special interest was given on clinical presentation, duration of illness, age, sex. Written inform consent were obtained from each patient.The study was a hospital based cross sectional study. IgMChikungunya ELISA antibody assay has been done on the serum samples of patients fulfilling the criteria of case definition. Permission was obtained to conduct the study from the Institutional Ethical Committee (IEC), Gauhati Medical College, Assam.

Inclusion criteria were the patients presenting with fever and arthralgia that are not explained by any other etiology, all the patients presenting with retro-orbital pain, rashes, severe headaches, myalgia, backache along with high or low grade fever typically lasting from several days up to a week,samples with clinically compatible illness from new geographical areas without active dengue circulation and Meningoencephalitis cases admitted in Gauhati Medical College and Hospital.Exclusion criteria were patients suffering from fever fever for less than 4 days , fever due to other etiological causes, and altered sensorium, seizure, swelling of legs, menorrhagia and pain abdomen which were not associated with Chikungunya infection.

Collection of samples: Under all aseptic and antiseptic condition, 5ml of venous blood was collected from the patient. Blood was allowed to clot and serum was separated by centrifuging at 3000 rpm in a centrifuge machine for 10 minutes. The samples were stored at -20 °C.

Serum samples of 866 of suspected Chikungunya cases were tested for Chikungunya specific IgM antibody by IgM Capture linked immunosorbant assay using IgMChikungunya ELISA kit procured from NIV Pune and all equivocal samples were tested with NOVALISA IgMμ-Capture ELISA, NOVATEC kit is produced by Novatech, Germany. The procedure that was followed was according to the kit insert.

Statistical analysis: Data was collected and entered in Microsoft office Excel and analyzed by using SPSS version 17 and graph pad. Description statistics were done for different study variables. Chi-square test and fisher's exact test was used for analysis of categorical vartiables. Criteria of significance was used in the study were $p<0.05$.

RESULTS AND DISCUSSION

Among all 866 suspected Chikungunya virus infected cases, most of the Chikungunya positive cases presented with symptoms of fever, headache, retro-orbital pain, back pain and arthralgia whereas fever, headache and arthralgia were the most common symptoms presented by Chikungunya negative cases. In our study, among all the 866 suspected Chikungunya cases 86 (9.93%) were found to be seropositive for Chikungunya by IgM ELISA.

Table 1 Demographic features of the IgM seropositive cases in GMCH in 2013

	VARIABLE	CHIK POSITIVE (%)	CHIK NEGATIVE (%)
AGE GROUP	Adult > 16 yrs	80 (93.02)	664 (85)
	Children 0-15 yrs	6 (6.97)	116 (14.87)
	P value	< 0.0492*	
GENDER	Male	63 (73.23)	528 (67.69)
	Female	23 (26.74)	252 (32.30)
	P value	0.329	
PLACE OF RESIDENCE	Metro	75 (87)	526 (67.4)
	Rural	11 (12.79)	340 (43.58)
	P value	< 0.0001*	
	Odd ratio	4.404 (CI 95% 2.307 to 8.421)	

*Fisher's exact test significant

The demographic table shows the prevalence of Chikungunya infection was more among the age group beyond 16 yrs and it was comparatively less among the children below 15 yrs of age and it was found to be statistically significant (p=0.0492). Gender distribution for Chikungunya infection was not found to be statistically significant and the male and female ratio was found to be 2.7:1. On comparing the occurrence of Chikungunya infection among metro and rural populations, it was found that the metro populations were infected more than rural population 87% vs 12.79%. Theodd ratio for metro occupants was 4.404 (95% CI: 2.307- 8.421) compared to rural and the p value was < 0.0001.

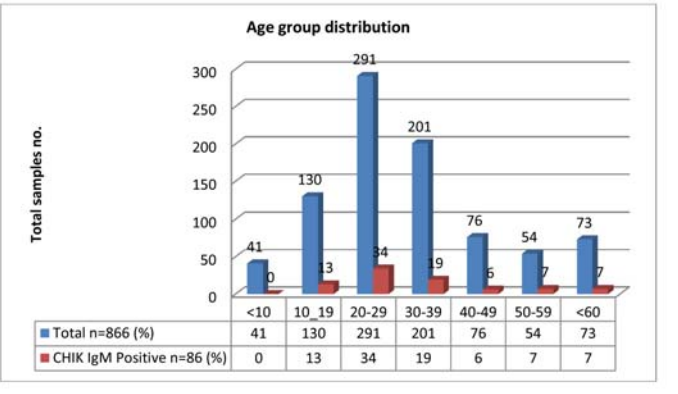

Figure 2 Shows the age distribution

The **Figure 1** shows the correlation of occurrence of Chikungunya infection with age wise distribution where the highest numbers of positive cases were seen in the age group of 20-29 (39.53%) followed by 30-39 (22.09%) and < 20yrs (15.11%) respectively. Chikungunya infected case was not seen below 10yrs of age.

Table 2 District wise distribution of Chikungunya virus infection in Assam

NAME OF THE DISTRICT	POSITIVE n=86	%	NEGATIVE n=780	%	TOTAL n=866	%
Kamrup Metro	75	87.2	526	67.43	601	69.39
Kamrup rural	0	0	94	12.05	94	10.85
Sivsagar	1	1.16	9	1.15	10	1.15
Golaghat	1	1.16	3	0.38	4	0.46
Nagaon	1	1.16	13	1.66	14	1.62
Bongaigaon	1	1.16	9	1.15	10	1.15
Barpeta	2	2.33	22	2.82	24	2.77
KarbiAnglong	1	1.16	3	0.38	4	0.46
Morigaon	0	0	11	1.41	11	1.27
Nalbari	1	1.16	27	3.46	28	3.23
Dhubri	0	0	15	1.92	15	1.73
Lakhimpur	0	0	9	1.15	9	1.04
Mangaldoi	0	0	7	0.89	7	0.81
Darrang	1	1.16	6	0.76	7	0.81
Dhemaji	0	0	6	0.76	6	0.69
Goalpara	1	1.16	8	1.02	9	1.04
Dibrugarh	0	0	5	0.64	5	0.57
Cachar	1	1.16	4	0.51	5	0.57
DimaHasao	0	0	3	0.38	3	0.35
Grand total	86	100	780	100	866	100

The maximum number of seropositive was seen among Kamrup Metro follower by Barpeta. Seropositive cases were not detected from the districts of DimaHasao, Dibrugarh, Dhemaji, Mangaldoi, Lakhimpur, Dhubri and Morigaon. Cachar, Goalpara, Darrang, Nalbari, Bongaigaon, Nagaon, Golaghat and Sivsagar districts detected only single seropositive cases each.

Table 3 Month wise distribution of Chikungunya cases in 2013

Month	No. Test done	Positive, n= 86 %	Negative, n= 780 %	X^2 P value
July	115	4 (4.65%)	111 (14.23)	0.0030**
August	142	8 (9.30)	134 (17.17)	
September	609	74 (86.04)	535 (68.58)	
Grand total	866	86	780	

** Significant

The **Table 3** shows that among a total of 866 clinically suspected Chikungunya cases, maximum numbers of seropositive cases were seen in the month of September followed by August and July which were found to be 86.04%, 9.30% and 4.65% respectively.

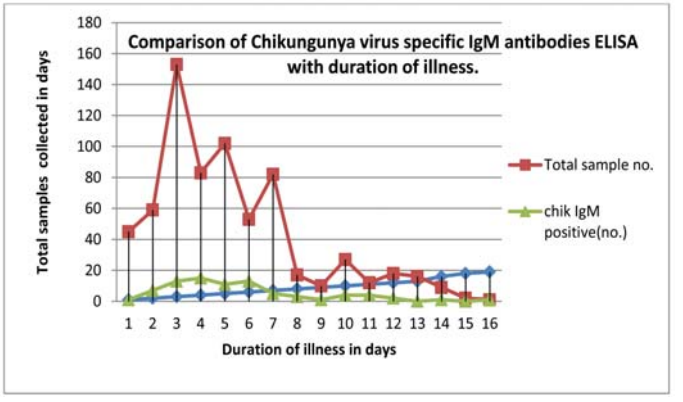

Figure 2 Comparison of Chikungunya virus specific IgM antibodies ELISA with duration of illness

Figure 2 depicts that on correlating the duration of illness with Chikungunya infection it was seen that maximum seropositivity was by 3 day of illness.

Table4 Seropositivity in relation to duration of illness

Duration of disease	IgM assay (86)
< 5 days	36 (41.86%)
5–10 days	33(38.37%)
10–20 days	12 (13%)

DISCUSSION

In our study, the seroprevalence of Chikungunya virus infection done by Chikungunya specific IgM antibody ELISA among the suspected cases were found to be 9.93% correlating to studies by Ravi at al[8] in contrast to the studies by Mahanty*etal*[9] Suryawanshi*et al*[10] and SaiGopal*et al*.[11] The low prevalence rate in our study may be due to the newly entry of the virus in this region although the presence of vector was reported from this region. More than 50% of the patients attending the hospital were before three days or after 6 days of illness where the sensitivity of IgM ELISA is comparatively low.[11] Another reason for low prevalence rate may be due to asymptomatic and mildness presentation of the disease in the young age group.

In our study,the prevalence of Chikungunya infection among the adult above 16 yrs of age showed the seropositivity rate to be 93.02% and the prevalence was comparatively less among the children below 15 yrs of age which was found to be statistically significant (p=0.0492) Vijayakumar*et al*[12] found Chikungunya infection more commonly in the adult age group.

Gender distribution for Chikungunya infection was not found to be statistically significant and the male and female ratio was found to be 2.7:1. The males were more affected than females because they go out for work at day time and get exposed to the vector Aedessp, which is domestic in nature and a day bite.

On comparing the occurrence of Chikungunya infection among metro and rural populations, it was found that the metro populations were infected more than rural population 87% vs 12.79%. The odd ratio for metro occupants was 4.404 (95% CI: 2.307- 8.421) compared to rural and the p value was < 0.0001. Debjani Taraphdar *et al*.[13] in their studyfound Urban populations (74.8%) were mostly infected than rural (25.2%) with a significant p value 0.001 whose findings are similar to our study.The reasons behind the increased infection rate of Chikungunya in Metro populations may be due to high density of Aedes mosquitoes with increasing urbanization which has lead to an abundance of mosquito breeding sites. Storage of drinking water and other urban water, containers including plant-pot bases, guttering, tarpaulins and tyres and discarded containers can all collect rain water and provide habitat for *Aedesaegypti*. Another point may be due to the highly populated areas along with existence of high density of Aedes mosquitoes in Metros which helps in easy transmission of Chikungunya virus from one viremic host to another.

In our study maximum numbers of positive cases were seen in the month of September, August and July which were found to be 86.04%, 9.30% and 4.65% respectively. Mahanty *et al* from Odisha, in their study reported more number of cases from the month of July to September and less during the month of January to March.[14]

The maximum number of seropositive was seen among Kamrup Metro followed by Barpeta. Ravi V in 2006 cited regarding the emergence of Chikungunya virus in India since its first isolation in Calcutta in 1963.[8] The last outbreak of chikungunya virus infection occurred in India in 1971. Reports of large scale outbreaks in Andhra Pradesh, Karnataka, Maharashtra, Orrisa. But no reports from the North east part of India.[15] P Dutta et al reported the first evidence of chikungunya virus infection in Assam, Northeast India during June-September 2008.[15] They also stated that the chikungunya positive patients did not travel to and from any endemic region confirming indigenous transmission. It was also the maiden report of chikungunya occurrence in Northeast India. M. Muniaraj reported that the entire North Eastern States such as Assam, Arunachal Pradesh, Manipur, Mizoram, Nagaland and Tripura excluding Meghalaya were not affected till this reporting. Although Jharkhand and Bihar were not affected till 2010, cases were seen 2011.[16] Resurgence of chikungunya is due to Urbanization, Increase in the mosquito population, Loss of herd immunity. As no active or passive surveillance carried out in the country and therefore, it 'seemed' that the virus had 'disappeared' from the Assam and the North east part of India.

CONCLUSION

Chikungunya is a newly emerging viral infection which had spread to new areas during this outbreak. Hence it is essential to have a proper diagnostic laboratory support, proper surveillance system and public awareness in order to prevent future epidemic in this region.

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