

## **Evaluation of Hepatic Enzyme levels and Haematological Profiles in Chikungunya patients- An institutional study**

### **Abstract**

**Background:** Chikungunya is a viral infection caused by the Chikungunya virus (CHIKV) belonging to the *Togaviridae* family. The transmission of the virus is taking place through the bite of infected daytime biting female mosquitoes – primarily *Aedes aegypti* and *Aedes albopictus*. There were many reports which highlighted the number of reported cases of Chikungunya has been increasing in India in recent years, especially since 2016-2017.

The aim and objective of the present study was to understand the relationship between the severity of Chikungunya infection and its impact on levels hepatic enzymes and haematological profiles of individuals.

**Materials and Methods:** Blood samples were collected from patients diagnosed with having Chikungunya. Routine laboratory investigations were carried out for analysing haematological assays like platelets, hematocrit count, haemoglobin count, total leukocyte count. and serum enzyme profiles includes Liver Function Test.

**Result:** Our results highlighted that the alterations of haematological and hepatic dysfunction are prevalent in Chikungunya infection, where haematological indices were significantly different between the control and patient group. RBC count, Hb, and WBC count were significantly lower in the patients than in the healthy control group. Liver enzymes like SGPT rising significantly more than SGOT. Hepatic Enzymes levels appear to have a directly proportional correlation with severity of infections.

**Conclusion:** Our study demonstrated that haematological and hepatic enzymes levels should be explored as routine laboratory markers for assessing the severity of Chikungunya infection, as they will help in employing an appropriate patient therapy, and thus optimise the use of available resources.

**Keywords:** Chikungunya, liver enzymes, SGOT, SGPT, Thrombocytopenia

UNDER PEER REVIEW

## **Introduction**

Chikungunya is a viral illness transmitted by mosquitoes affecting all age groups, but severe complications are more often observed in children. Over the past two decades, there has been global increase in the frequency of Chikungunya and its epidemics. Symptoms generally start 4-7 days after the mosquito bite. The acute phase is characterised by joint and muscle pain, high fever, extreme weakness, headache, vomiting, and rashes. In the chronic phase, various neurological syndromes and non-neurological manifestations can occur [1]. In 2006, the CHIK virus (CHIKV) re-emerged in India after 32 years, causing the epidemic affecting more than 1.4 million people across the 13 States, and post epidemic, a declining trend was seen till 2011 [1-3].

The WHO South-East Asia Regional Office has reported a significant number of Chikungunya cases in almost 151 districts in 8 states of India suffered from many cases of chikungunya fever. The most affected states are Andhra Pradesh, Andaman & Nicobar Islands, Tamil Nadu, Karnataka, Maharashtra, Gujarat, Madhya Pradesh, Kerala and Delhi [1,2]. As usual, the outbreaks of Chikungunya infections are most likely to occur in the post-monsoon period when the vector density significantly increases. There are 3 epidemiological factors: the host (man and mosquito), agent (virus), environment (abiotic and biotic factors) agent for Chikungunya viruses. Human beings are considered as the reservoir for the chikungunya virus during the epidemic time.

The rapid spread of the disease in Southern part of India from 2004 has afflicted millions of people and left many of them with crippling disabilities. Chikungunya disease continues to cause epidemics in many countries in the region. The patient in this context acts as the reservoir of infection for others in the household and the community. Therefore, public health measures to minimise the transmission of infection become imperative to prevent and control the outbreak from spreading [3-5].

Fever with or without arthralgia is a prevalent manifestation of several other diseases. CHIK fever may not have typical manifestations, or it may co-exist with other infectious diseases like dengue fever or non-infectious diseases like rheumatoid arthritis [6].

The analysis and diagnosis of the case in the community need to be communicated immediately to the nearest public health official for identification of clusters by person, place and time and expansion of the control measures in the community and district levels.

The most significant features seen in CHIKV-positive patients are lymphopenia, leukopenia, hypocalcemia, and elevated transaminases; but these features were not consistently reported in all studies and vary depending the clinical phase of infection.

The general laboratory diagnosis of Chikungunya often reveals lymphopenia, neutrophils and thrombocytopenia, an elevated creatinine, and elevated hepatic transaminases. In this study we tried to establish the relationship between the severity of Chikungunya infection and its impact on levels hepatic enzymes and haematological profiles of individuals.

## **Materials and Methods**

**Study population:** This study was carried out in the Department of Biochemistry and General medicine at mamata Medical College and general Hospital, Khammam, Telangana from the period of July 2021 to November 2021.

### **Enrolment of patients:**

**Chikungunya:** Patients with acute febrile illness and high index of suspicion of Chikungunya infection were enrolled in the study.

**Patient Selection:** Patients of different age group with acute febrile illness fulfilling the following criteria were recruited in the study.

**Inclusion criteria:** Patients visited hospital or admitted to the medical wards with the history of acute undifferentiated febrile illness.

**Exclusion criteria:** Patients with immunosuppressive conditions including HIV infection, haematological malignancy, autoimmune disorders were excluded from the study.

**Study Design:** The first set of population was 50 healthy individuals who did not have a past history of Chikungunya infection or any other related viral infection, bacterial infection and hereditary disorders.

The second set of population was 50 patients admitted to the hospital with a history of fever of more than 38.5<sup>0</sup> and Chikungunya positive were selected using purposive sampling techniques. Clinical signs and symptoms, severity, and outcomes with relevant laboratory parameters were compared in detail. They are followed from the onset of fever to time of recovery or discharge according to WHO discharge criteria whichever is earlier. Same numbers of normal healthy individuals were also recruited for this study. Duly signed Informed consent forms were collected from all the participants during the study. Our study was in a prospective observational category study. This study was approved by the institutional scientific and ethical committee review board.

## **Routine Laboratory Investigations:**

### **Sample collection:**

Blood samples were collected aseptically from the study population by routine vein puncture method. Two ml of blood was collected in a sterile tube containing 3 mgs of anticoagulant (EDTA) for haematological experiments and 5 ml blood without anticoagulant was collected for enzyme assay. Immediately after the collection, blood samples were transported to the laboratory. Serum was separated by centrifugation and store at  $-20^{\circ}\text{C}$  for further use. All the serum samples were subjected to enzyme assay.

**Platelet count:** The diluent prevents coagulation, fixes the platelets and prevents them from clumping. No attempt is made to lyse the red cells. Platelets are identified by their size, shape and dark colour. The dye provides the back ground during cell counting. This dye does not stain the platelets, and it is not essential for the counting procedure.

**Estimation of Haemoglobin:** Haemoglobin is converted to acid hematin by the reaction of hydrochloric acid. The hematin solution is further diluted with distilled water until the colour method with the permanent standard of the comparative blank.

**WBC count** Blood is diluted exactly to 1:20 using WBC diluting fluid which contains weak acid to lyses RBC and to stain for staining the nucleus of WBC. WBC count was done as per the method described by Drabkin and Austin (1932).

**Liver Function Test:** SGPT, SGOT were estimated by using protocol prescribed by the International Federation of Clinical Chemistry. ALP, Total Protein, Albumin, Total Bilirubin were estimated by colourimetric assay.

**Statistical analysis:** The collected data analysis was done using SPSS software. The results were presented as mean  $\pm$  standard deviation (SD) and percentages. The data was analyzed using one way analysis of variance (ANOVA) and Student's t-test on SPSS

(statistical package for social sciences) Ver. 12 and the group means were compared by Duncan's Multiple Range Test (DMRT). The results were considered statistically significant if the  $p$  value was less than 0.05.

UNDER PEER REVIEW

## Results

The total numbers of 50 cases of acute febrile illness were recruited based on their clinical report as seropositive for CHIKV infection, an 50 number of healthy individuals also participated in this study. The patient characteristics and salient clinical features of acute chikungunya infection, and biochemical parameters are compared in Tables 1 and 2. The symptomology of chikungunya included headaches, joint pain, weakness, and restricted joint movement. No complications were reported. Deranged liver function was seen in all patients (100%).

**Table 1: Comparison of characteristics and clinical features in between normal healthy individuals and patients groups.**

Characteristics		Control Group (%)	Patient Group (%)	P value
Age Group (Years)	<20	2	8	Chi-square
	20-30	42	54	Dif - 18.14, 5
	31-40	24	22	
	41-50	16	6	P< 0.0028
	51-60	10	4	
	>60	0	6	
Gender	Male	80	74	Chi-square
	Female	20	46	Dif - 8.730, 1 P< 0.0031
Residence	Urban	48	44	P<0.5704
	Rural	52	56	
Symptoms	Fever	4	100	P<0.6890
	Joint Pain	2	62	
	Backache	0	30	
	Vomiting	0	8	

Out of 50 patients, 37 were male and 23 were female and in control group of 50 healthy individuals 40 were male and 10 were female. Most of the participants were in the age group

of 20 – 40 and none of them were above 70 years of age. All the patients had fever as a presenting complaint. Most of them had joint pain at the time of presentation.

**Table 2: Comparison of Haematological parameters in between normal healthy individuals and patients groups.**

Parameters	Control Group	Patient Group	p Value
Hb	13.15±1.82	11.55±2.36	0.5012
TLC	8560±2634	4781±2219	0.0082
PC	155,700±23,523	67,622±44,254	0.0015
HCT	36.67±4.52	38.66±6.07	0.523

Hb: Hemoglobin, TLC: Total leukocyte count, PC: Platelets count, HCT: Hematocrit,

Haematological parameters such as platelet count, Hb, TLC, HCT were studied in all the study groups and the comparison of these parameters between the study groups is depicted in Table 2. All of the measured haematological indices were significantly lower in the patients than in the healthy control group.

**Table 3: Comparison of Liver enzyme assay parameters in between normal healthy individuals and patients groups.**

Parameters	Control Group	Patient Group	p Value
SGPT	28.87±4.23	239.68±589.25	0.5215
SGOT	25.92±6.22	345.52±1356.52	0.633
ALP	34.21±14.21	144.21±110.26	0.2231
Total Bilirubin	0.98±0.29	1.84±0.28	0.001
Direct Bilirubin	0.51±0.18	0.98±0.33	0.001
Total Protein	7.4±0.59	6.29±0.89	0.0747
Albumin	4.11±0.47	3.15±0.69	0.0130

SGPT: Serum glutamate pyruvate transaminase, SGOT: Serum glutamic-oxaloacetic transaminase, ALP: Serum alkaline phosphatase,

The levels of liver enzymes such as ALP, SGOT, SGPT and proteins were measured and the mean values of all these enzymes are presented in Table 3. The mean values of ALP in chikungunya confirmed indicating higher values than the control group. However, the increase was significant only in chikungunya confirmed patients. Moreover, a significant

difference was observed between SGPT and SGOT values in chikungunya patients when compared to healthy group. Similar observation was also seen in Bilurubin and Total protein parameters between the study groups.

**Table 3: Comparison of deranged liver enzymes in between normal healthy individuals and patients groups.**

Parameters	Control Group	Patient Group	P value
<b>SGPT (U/L)</b>	60-180 (%)	2	<b>P&lt; 0.0069</b>
	181-500 (%)	0	
	501-1000 (%)	0	
	>1000 (%)	0	
<b>SGOT (U/L)</b>	38-120 (%)	0	<b>P&lt;0.001</b>
	121-500 (%)	2	
	500-1000 (%)	0	
	>1000 (%)	0	

Elevated liver enzymes were found in almost all patients. Of the liver enzymes, SGPT levels were significantly higher than SGOT levels.

**Table 4: Comparison of thrombocytopenia in between normal healthy individuals and patients groups.**

Platelets Count (/ $\mu$ L)	Control Group	Patient Group	P value
>150,000	52	4	<b>P&lt;0.002</b>
150,000-100,000	46	10	
100,000-50,000	2	40	
50,000-20,000	0	26	
<20,000	0	12	
<10,000	0	6	
Platelets transfusion	0	2	

Thrombocytopenia was noted in 43 patients, of whom 12 had bleeding, and 31 had no bleeding. Statistically, the Chi-square test was used to test the significance of thrombocytopenia and bleeding tendencies. There was no significant difference ( $p = 0.945$ )

found between the variations in the thrombocytopenia and the bleeding tendencies. This implies that bleeding tendencies does not depend upon thrombocytopenia.

### **Discussion**

Currently, Chikungunya is causing significant public health concern throughout the world, particularly in South-East Asian countries. Recently dengue outbreaks caused significant morbidity and mortality in certain parts of India mainly in Andhra Pradesh, Uttar Pradesh and Tamil Nadu [7]. Hepatic dysfunctions in dengue are common. It is due to either direct effect of the virus on hepatocytes or due to reactive hepatitis. Hepatic involvement in dengue fever is in the form of elevated serum aminotransferase [8]. Those patients with elevated liver enzymes are more likely to have increased risk of bleeding tendencies, shock, ARDS, renal failure. In addition to decreased platelet count, hepatic dysfunction plays a significant role in bleeding. Hence, it is mandatory to evaluate serum aminotransferases in all patients with dengue fever [9]. It is observed that, out of 50 patients, most of the patients had elevated liver enzymes, SGPT levels were significantly higher than SGOT levels, and these patients had severe complications.

Chikungunya viral infection is not generally fatal but can cause neurological and optical manifestations. Severe joint pain is the prominent clinical manifestation and can persist for months to a year [10]. As Dengue Fever (DF) has a high incidence and mortality rate, symptomatic patients are tested only for DENV and only in rare cases for chikungunya viral infection. This scenario is an important reason why chikungunya cases go undiagnosed in dengue-endemic regions, and the actual burden of the chikungunya viral infection has been missed. Thus, investigation for both viruses should be done, especially in endemic regions. Accurate and early diagnosis of coinfections would help inappropriate management [11, 12]. The number of cases increases during and after the monsoon months because higher humidity lengthens the life span of mosquitoes and increased temperatures shorten the extrinsic

incubation period [13]. Rising of the cases during the monsoon period with highest cases in different months had been reported in previous studies from India [11-14]. November was observed as a peak among all the months of the year in this study.

Earlier studies have reported increased incidences of atypical hemorrhagic manifestations [12-14] and hepatic dysfunction [15]. Hepatic dysfunction was the most common complication in our dengue and coinfection groups. SGPT levels were significantly higher than SGOT levels.

### **Conclusion**

The present study conducted haematological profile and hepatic involvement in Chikungunya fever patients. The haematological parameter such as thrombocytopenia was found in serologically confirmed Chikungunya patients which differed significantly from other study groups. Significant increase in the level of liver enzymes are associated with complications like bleeding, shock and organ impairment in Chikungunya confirmed patients as compared to other groups could also be considered as a potential predictor of Chikungunya infection and alternative diagnostic procedure for the Chikungunya investigation. A detailed study of these biomarkers would be very much useful for the clinicians to diagnosis the cases of Chikungunya during the early phase of illness and it will also help them to distinguish Chikungunya fever from other febrile illness.

## References

1. World Health Organisation. Strengthening implementation of the global strategy for dengue fever and dengue haemorrhagic fever, prevention and control. Report of the informal consultation. 18 – 20 October 1999. Geneva: WHO; 1999.
2. World Health Organisation. Scientific working group on dengue. Meeting report, Geneva, Switzerland, 3-5 April 2000. Geneva:WHO;2000.
3. Singhi S, Kisson N, Bansal A. Dengue and dengue hemorrhagic fever: Management issues in an intensive care unit. *J Peiatr (Rio J)*. 2007;83(2 suppl): S22-35.
4. Satarsinghe RL, Arulnithy K, Amerasena NL, Bulugahapitiya U, Sahayam DV. Asymptomatic myocardial involvement in acute dengue virus infection in a cohort of adult Sri Lankans admitted to a tertiary referral centre. *Br J Cardiol* 2007; 14:171-3.
5. Kamath SR, Ranjit S. Clinical features, complications and atypical manifestations of children with severe forms of dengue hemorrhagic fever in South India. *Indian J Pediatr* 2006; 73:889-5.
6. Singh M. Medical emergencies in children. 4th Ed. New Delhi: Sagar Publications; 2004.
7. Deeba F, Afreen N, Islam A, Naqvi IH, Broor S, Ahmed A, Parveen S. Co-infection with Dengue and Chikungunya Viruses. In: RodriguezMorales A, editor. *Current Topics in Chikungunya*. InTech; 2016 (London-United Kingdom) DOI:10.5772/64308. Available from: [http:// www.intechopen.com/books/current-topics-in-chikungunya/co-infectionwith-dengue-and-chikungunya-viruses](http://www.intechopen.com/books/current-topics-in-chikungunya/co-infectionwith-dengue-and-chikungunya-viruses). [Last accessed on 2018 Jan 30].
8. Taraphdar D, Sarkar A, Mukhopadhyay BB, Chatterjee S. A comparative study of clinical features between monotypic and dual infection cases with chikungunya virus and dengue virus in West Bengal, India. *Am J Trop Med Hyg* 2012;86:720-3.

9. Kalawat U, Sharma KK, Reddy SG. Prevalence of dengue and chikungunya fever and their co-infection. *Indian J Pathol Microbiol* 2011;54:844-6.
10. Carey DE, Myers RM, DeRanitz CM, Jadhav M, Reuben R. The 1964 chikungunya epidemic at Vellore, South India, including observations on concurrent dengue. *Trans R Soc Trop Med Hyg* 1969;63:434-45.
11. Afreen N, Deebea F, Khan WH, Haider SH, Kazim SN, Ishrat R, et al. Molecular characterization of dengue and chikungunya virus strains circulating in New Delhi, India. *Microbiol Immunol* 2014;58:688-96.
12. Mohanty I, Dash M, Sahu S, Narasimham MV, Panda P, Padhi S, et al. Seroprevalence of chikungunya in Southern Odisha. *J Family Med Prim Care* 2013;2:33-6.
13. Saswat T, Kumar A, Kumar S, Mamidi P, Muduli S, Debata NK, et al. High rates of co-infection of dengue and chikungunya virus in Odisha and Maharashtra, India during 2013. *Infect Genet Evol* 2015;35:134-41.
14. Kularatne SA, Gihan MC, Weerasinghe SC, Gunasena S. Concurrent outbreaks of chikungunya and dengue fever in Kandy, Sri Lanka, 2006-07: A comparative analysis of clinical and laboratory features. *Postgrad Med J* 2009;85:342-6
15. Krishna A.P, Chandrika, Suchetha, Manasa A and Shrikant L. Patil. Variation in Common lipid parameters in malaria infected Patients. *Indian Journal of Physiology and Pharmacology*, 2009; 53 (3): 271-274.