Content available at: iponlinejournal.com

International Journal of Clinical Biochemistry and Research

Journal homepage: www.innovativepublication.com

Original Research Article

Molecular characterization of dengue and chikungunya viruses and their association with the liver profile

Abhinav Manish[1], Tariq Masood[1], Amit Verma[1], Narotam Sharma[1], Rajeev Singh Kushwaha[1,*]

[1] *Dept. of Biochemistry, SGRR Medical College and SMI Hospital, Dehradun, Uttarakhand, India*

ARTICLE INFO

Article history:
Received 15-11-2019
Accepted 02-01-2020
Available online 13-03-2020

Keywords:
Febrile illness
Dengue
Chikungunya
Hepatotoxic
RTPCR

ABSTRACT

The episode of acute febrile illnesses increases day by day due to poor socioeconomic status in developing nations. Dengue and Chikungunya are emerged as important arboviral and alpha virus diseases respectively, with significant impact on disease burden residing these areas. Both viruses, seems to have hepato-toxic effect. Affiliation of liver in form of derangement in liver function tests is common. Present study focuses on the hepatic manifestations of the liver injury in dengue and chikungunya patients. A cross-sectional study was conducted on patients attending Out-patients and In-patients Department of Medicine at Shri Mahant Indiresh Hospital Dehradun. 161 cases were included in the study. 5 ml of blood sample from each of them is collected in a sterile test tube. The blood samples were analyzed via RT-PCR technique at Central Molecular Research Lab in Biochemistry Department of Shri Guru Ram Rai Institute Of Medical and Health Sciences Dehradun and Liver profile was analyzed at SMI Hospital. Liver enzymes were found to be raised in male as well as in females for both dengue and chikungunya. But dengue was found to be more hepato-toxic when compared to chikungunya with statistical significance value (<0.0001).

1. Introduction

Febrile illness is one of the most common reasons for seeking medical attention. Febrile illnesses which are caused by different etiological agents are the leading cause of morbidity and mortality, particularly in developing nations.[1] Apart from parasites and bacterial diseases; viral etiologies, namely dengue fever virus (DENV), Chikungunya virus (CHIKV), influenza virus, rota- and adenoviruses have also been reported.[2,3] DENV and CHIKV are RNA viruses that are transmitted to humans by Aedes mosquitoes and cause major disease burden in tropical and subtropical countries worldwide. Dengue fever is caused by a virus of the genus Flavivirus and there are four serotypes (DENV 1-4) while Chikungunya is caused by an alphavirus.[4,5]

Maternal-fetal transmission-Pregnant women infe cted with Chikungunya virus are at potential risk for atypical disease. Maternal-fetal transmission of Chikungunya virus has been reported , and miscarriage is also associated.[6,7]

The highest risk of maternal-fetal transmission in symptomatic cases is seen during the intrapartum period (two days before delivery to two days after delivery). During this period, vertical transmission occurs in approximately half of cases, Cesarean delivery was not protective against vertical transmission.[6]

Episodes of acute febrile illnesses increase day by day due to poor socioeconomic status in developing nations. Dengue and Chikungunya are emerged as important Arboviral and Alpha Viral diseases respectively, with a significant impact on the disease burden residing in these areas. Both the viruses seem to have hepatotoxic effects. CDC recommends that all suspected cases of Chikungunya be managed as Dengue fever initially; this is particularly true as dengue fever is a major cause of morbidity and

* *Corresponding author.*
E-mail address: abhinav5manish@gmail.com (R. S. Kushwaha).

https://doi.org/10.18231/j.ijcbr.2020.025

mortality. The use of Non Steroidal Anti Inflammatory Drugs in Chikungunya is helping but can lead to severe life-threatening hemorrhage in dengue fever. So it is important to differentiate it as early as possible. RT-PCR is a very rapid technique but available to research setups only, so we conducted this study to compare both viral diseases on the basis of liver transaminases.

2. Material and Methods

After taking the ethical clearance from the ethical committee, a hospital-based prospective, cross-sectional study was conducted from August 2018 to November 2018, on patients attending the Out-Patient and In-Patient departments, of Medicine Department at Shri Mahant Indiresh Hospital Dehradun. A total number of 161 cases of acute febrile illnesses were included in the study. 161 cases (64 of dengue and 77 of chikungunya) were selected for the study, 20 cases were febrile but negative for both dengue and chikungunya. Only ELISA (Enzyme linked immunosorbent assay) based, card test confirmed dengue and Chikungunya patients were included in the study from all age groups except <6 months of age. 5 ml of the blood sample from each of them was collected in a sterile test tube. The serum was separated from the blood and nucleic acid (RNA) was extracted from serum samples using silica column based technique described below and mixed with master mix containing random primers and probes, and were analyzed via Real Time-PCR technique of QIAGEN which gives qualitative detection of specific RNA in the form of CT value at the Central Molecular Research Lab[8] in Biochemistry Department of Shri Guru Ram Rai Institute Of Medical and Health Sciences, and Serum liver profile SGPT(Aspartate Transaminase)[9] SGOT(Alanine Transaminase)[10], ALP(alkaline phosphatase)[11], GGT(gamma glutamyl transpeptidase)[12] were analyzed at Central Clinical Laboratory of the Shri Mahant Indiresh Hospital using the vitros 5600 autoanalyzer of the Ortho Clinical Diagnostics.

2.1. Statistical analysis

The unpaired ' t ' test was used to establish the comparison using SPSS (Statistical Package for the Social Sciences) version 20.

Amplification and detection of RNA viral gen es in serum specimens.

Isolation of RNA by silica column method for Molecular detection of RNA virus by QIAGEN viral RNA mini kit (catalog no. R0115308)

2.2. RNA isolation

In 140μl of serum sample 560μl of lysis buffer, and 5.4μl carrier RNA was added, and incubated for 10 minutes with intermittent vortex at every 3 minutes for 2 to 3 seconds. Then 560μl of absolute ethanol was added and vortexed. Out of this MCT (micro centrifugation tube) 700 μl of sample was transferred to the silica column and centrifuged at 10000 rpm for 1 minute on 4 degree Celsius in cooled centrifuge and decant the supernatant. After it the column was again centrifuged at 10000 rpm for dry washing. Then the silica column was transferred to the new freshly labeled MCT. 60μl of elusion buffer was poured in the centre of the silica column, and was incubated at room temperature for 4 minutes. MCT with the column was centrifuged at 10000 rpm for 1 minute in cooling centrifuge. The silica column was discarded, and the extracted nucleic acid was used as a template for detection.

3. Result

Out of 161 febrile cases 39.75% were dengue, 47.82% were chikungunya and 12.43% patients were due to some other febrile illness. Out of 64 dengue patients, 39 (60.94%) were males and 25 (39.06%) were females. Out of 77 Chikungunya positive cases, 39 (50.64%) were males and 38 (49.36%) were females.

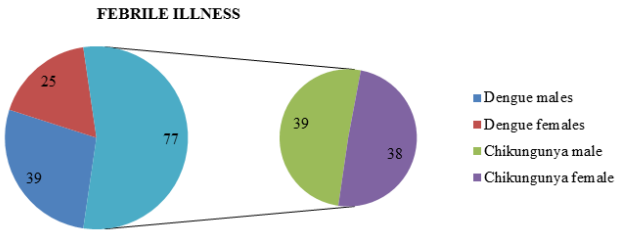


Fig. 1:

When we compare dengue and chikungunya, for both males and females, results showed that after having the lower Ct- value (which represents the no of cycles to generate exponential growth in thermocycler) with more viral load, chikungunya virus affects the liver enzymes to a lesser degree.

In Chikungunya males the liver enzymes were SGPT (122.53±64.67), SGOT (116.42±73.55), ALP (89.68±34.10), and GGT (81.76±66.39). On the other hand, in Dengue males liver enzymes were SGPT (219.18±141.58), SGOT (242.72±163.69), ALP (156.51±97.32), and GGT (116.26±56.57).

In Chikungunya females the liver enzymes were SGPT(123.23±82.59), SGOT(110.50±70.25), ALP(88.95±50.40), GGT(73.58±61.28), On the other hand, in Dengue females liver enzymes were SGPT(165.44±96.11), SGOT(170.52±84.64), ALP(94.16±41.84), and GGT(73.96±40.89).

Comparison between chikungunya male and Chikungunya female was not significant.

Dengue males were affected more SGPT (219.18±141.58), SGOT (242.72±163.69), ALP (156.51±97.32), and GGT (116.26±56.57) when compared with Dengue females SGPT (165.44±96.11), SGOT (170.52±84.64), ALP (94.16±41.84) and GGT (73.96±40.89).

Dengue virus is found to be more hepato-toxic than chikungunya virus.

In the Real-Time PCR thermocycler of QIAGEN, during the amplification of the targeted nucleic acid sequence their growth curves are also generated. The exponential growth curves indicate the positive Dengue and Chikungunya cases.

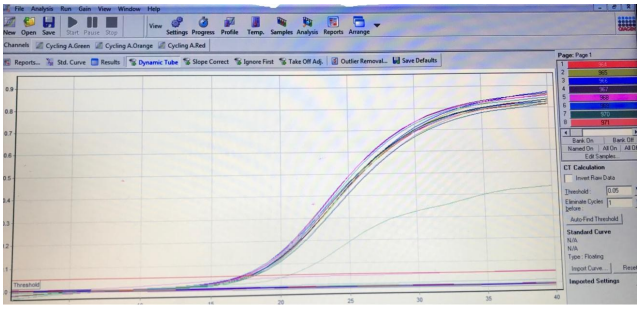
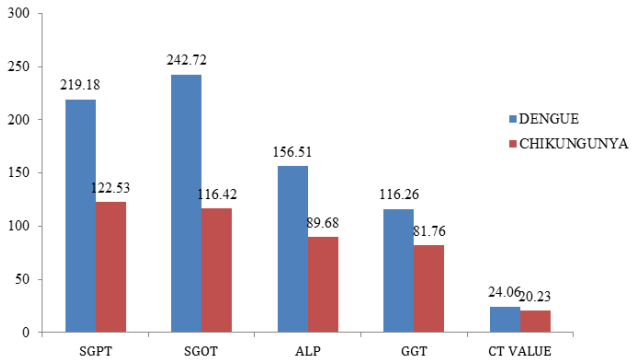


Fig. 2: Molecularly detected exponential curves of specific targets. Comparison between Dengue hikungunya male.



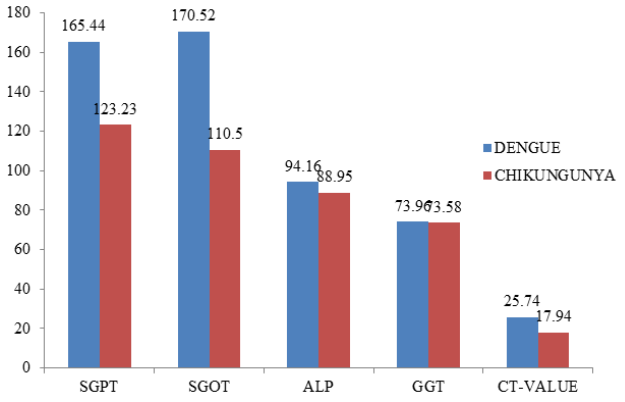
Graph 1: Comparison between dengue male and chikungunya male.

Graph 1 shows higher CT value of Dengue cases reflects less viral load, but derangements of liver enzymes to a higher degree.

Graph 2 shows higher CT value of Dengue cases reflects less viral load, but derangements of liver enzymes to a higher degree.

The lower limit of CT value in males reflects the high viral load hence liver derangements in the case of Dengue males is more than Dengue females.

When we compared Chikungunya males with Chikungunya females no statistically significant difference is seen.



Graph 2: Comparison between Dengue female and C hikungunya female.

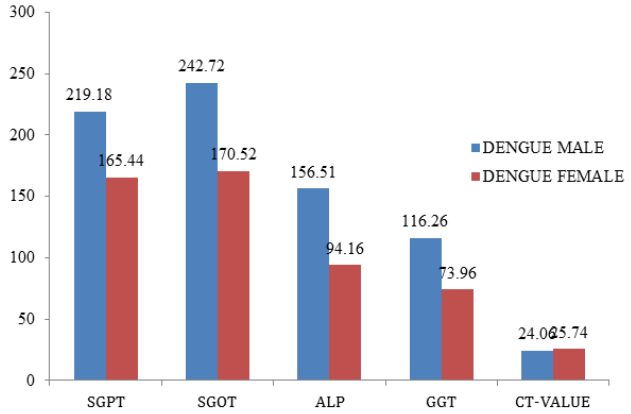


Fig. 3: Comparison between Dengue male and Dengue female.

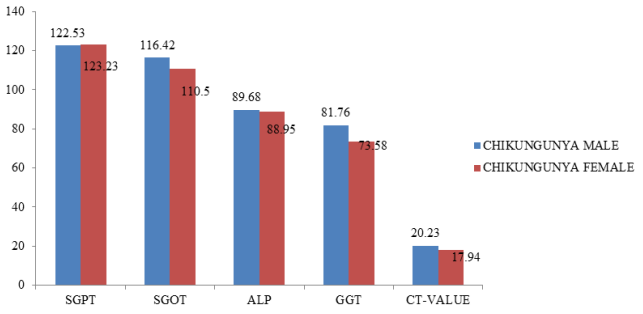


Fig. 4: Comparison between Chikungunya male and Chikungunya female.

Table 1:

Parameter	DENGUE MALE(39) Mean±SD±SE	CHIKUNGUNYAMALE(39) Mean±SD±SE	t- value	p-value	significance
SGPT(U/L)	219.18±141.58±22.69	122.53±64.67±10.36	-3.9	0.0002	ES
SGOT(U/L)	242.72±163.69±26.23	116.42±73.55±11.79	-4.3	<0.0001	ES
ALP(U/L)	156.51±97.32±15.60	89.68±34.10±5.47	-4.0	=0.0001	ES
GGT(U/L)	116.26±56.57±9.07	81.76±66.39±10.64	-2.4	=0.01	S
CT-value	24.06±7.44±1.19	20.23±6.56±1.05	-2.5	=0.01	S

ES= extremely significance, S= significant

Table 2: Comparison between dengue and chikungunya female

Parameter	DENGUE FEMALE(25) Mean±SD±SE	CHIKUNGUNYAFEMALE(38) Mean±SD±SE	t- value	p-value	Significance
SGPT(U/L)	165.44±96.11±18.16	123.23±82.59±13.4	-1.8	0.005	ES
SGOT(U/L)	170.52±84.64±16.0	110.50±70.25±11.40	-3.0	0.003	ES
ALP(U/L)	94.16±41.84±7.91	88.95±50.40±8.18	-0.4	0.66	NS
GGT(U/L)	73.96±40.89±7.73	73.58±61.28±9.94	-0.02	0.97	NS
CT-value	25.74±6.90±1.30	17.94±7.05±1.14	-4.33	<0.0001	ES

ES= extremely significance, NS= not significant

Table 3: Comparison between Dengue male and Dengue female.

Parameter	DENGUE MALE(39) Mean±SD±SE	DENGUE FEMALE(25) Mean±SD±SE	t- value	p-value	Significance
SGPT(U/L)	219.18±141.58±22.69	165.44±96.11±18.16	-1.6	0.10	NS
SGOT(U/L)	242.72±163.69±26.23	170.52±84.64±16.0	-2.0	0.04	S
ALP(U/L)	156.51±97.32±15.60	94.16±41.84±7.91	-3.02	0.003	ES
GGT(U/L)	116.26±56.57±9.07	73.96±40.89±7.73	-3.22	0.002	ES
CT-value	24.06±7.44±1.19	25.74±6.90±1.30	0.9	0.36	NS

ES= extremely significance, NS= not significant, S=significant

Table 4: Comparison between Chikungunya male and female

Parameter	CHIKUNGUNYAMALE(39) Mean±SD±SE	CHIKUNGUNYAFEMALE(38) Mean±SD±SE	t- value	p-value	significance
SGPT(U/L)	122.53±64.67±10.36	123.23±82.59±13.4	.04	0.96	NS
SGOT(U/L)	116.42±73.55±11.79	110.50±70.25±11.40	-0.31	0.71	NS
ALP(U/L)	89.68±34.10±5.47	88.95±50.40±8.18	-0.07	0.94	NS
GGT(U/L)	81.76±66.39±10.64	73.58±61.28±9.94	-0.5	0.57	NS
CT-value	20.23±6.56±1.05	17.94±7.05±1.14	-1.4	0.14	NS

NS= not significant

4. Discussion

Dengue and chikungunya fever have similar symptomatology. Both spreads by Aedes mosquitoes, and can present with a wide range of symptoms, ranging from those of a benign febrile illness to those with severe complications that can result in shock and death. Clinical differentiation of the chikungunya fever and dengue feveris difficult; generally arthralgia is more common in chikungunya fever whereas, myalgia and thrombocytopenia (platelets <118,000/mm3) are more common in dengue fever.[13,14] Both illnesses may have a rash, and saddleback fever profile. A tourniquet test is more likely to be positive in dengue fever; however, this is not consistently seen in the literature.[15]

To date, there are two hypotheses for explaining the liver damage in dengue patients. The first is the immune enhancement hypothesis. In 2004, a strong correlation was found between T-cell activation and hepatic cellular infiltration in immune-competent mice infected with dengue virus. It was noted that kinetics of liver enzymes elevation and T cell activation was correlated.[16] One of the studies detected the appearance of different helper cells and cytokines in human WBC's cultures, infected invitro with dengue virus and reported cytokines production during infection.[17] The second hypothesis relates the damage in the liver to the direct virulence of the virus[18]. According to these studies, we hypothesized the same mechanism responsible for the liver damage which occurred

in our patients. The aspartate aminotransferase (AST) levels in dengue infection tend to be greater than alanine aminotransferase (ALT) levels[19,20] these results are in accordance with our study . This differs from the pattern in viral hepatitis but is similar to that seen in alcoholic hepatitis. The exact cause of this is uncertain, but it has been suggested that it may be due to excess release of AST from damaged monocytes during dengue infection.[18] We also noted a preferential elevation of liver enzymes, with AST being significantly higher than ALT. This abnormality may act as an early indicator of dengue infection.

Chikungunya is an emerging viral infection spreads along with its Aedes mosquito vectors, through tropical and subtropical countries, causing explosive epidemics of both acute illness, and persistent disabling arthritis.[21] Following acute infection, typically lasting for 1–2 weeks, approximately 35% of patients develops a second phase of illness; disabling, potentially chronic arthritis. Chikungunya fever is often diagnosed clinically, especially in resource-limited settings. General laboratory workup often reveals lymphopenia, thrombocytopenia, elevated creatinine, and elevated hepatic Transaminases.[21,22] Specific laboratory tests do exist to confirm a diagnosis of Chikungunya virus, and are available through the CDC and several state health departments. Viral culture can detect the RNA in three days of illness. RT-PCR can detect viral RNA in the first eight days of illness, and serology can detect IgM and IgG antibodies towards the end of the first week of illness.[21]

A comparison of the laboratory parameters of patients with dengue and chikungunya fever was carried out to support the clinical diagnosis of physicians in diagnosing dengue fever and chikungunya fever based on liver enzymes; alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) , and Gamma Glutamyl Transferase (GGT). The blood samples from patients were tested for said laboratory parameters; results were then statistically analyzed and concluded the evident risk of bleeding and the risk of developing liver damage are significantly higher among dengue fever patients as compared to chikungunya fever patients.[23] Molecular assays are more sensitive for diagnosis in the early stages of illness (within 4 days) when antibodies are not detected. So molecular detection or early detection of viral RNA before the production of antibodies is an effective tool in preventing rheumatism related to chikungunya.[24] Sison RC, Nieto XA, Bautista JL, Ferrer JM, Parado MA, et al. also demonstrated the more toxic effect of dengue virus on liver transaminases than chikungunya virus. These results are in accordance with our study.

5. Conclusion

We concluded that the risk of developing liver damage is significantly higher among dengue fever patients as compared to chikungunya fever patients. Which clearly states that the Dengue virus is more hepato-toxic than the chikungunya virus. It can help in differentiating dengue from chikungunya in resource limited areas and so aids in planning early treatment lineup for better prognosis.

5.1. Limitations of this present study

Dengue and chikungunya is prevalent in most of the Asian region our study is only confined to the Uttaranchal region specifically. Dengue hemorrhagic fever and dengue shock syndrome patients are not separated with Dengue fever which could be a shortcoming of this study. The co-infection of Dengue and Chikungunya is also neglected in this study.

5.2. Acknowledgements

The authors are grateful to Hon. Chairman, Shri Guru Ram Rai Education Mission for his constant support andguidance in pursuing such studies in our laboratory. We are also indebted to the staff of the Department of Biochemistry specially Dr Nita Garg, SGRR Institute of Medical and Health Sciences for their technical assistance and guidance.

6. Conflict of interest

The authors declare that they have no conflict of interest.

References

1. WHO (2011) the top 10 causes of death: The 10 leading causes of death in the world, 2000 and 2011 ;. .
2. Hertz JT, Saganda W, Munishi OM, Crump JA, Howe S, et al. Chikungunya and Dengue Fever among Hospitalized Febrile Patients in Northern Tanzania. *Am J Tropl Med Hygiene*. 2012;86(1).
3. Hokororo A, Kidenya BR, Seni J, Mapaseka S, Mphahlele J, et al. Predominance of Rotavirus G1[P8] Genotype among Under-Five Children with Gastroenteritis in Mwanza, Tanzania. *J Trop Pediatr*. 2014;60(5):393–396.
4. Whitehorn J, Simmons CP. The pathogenesis of dengue. Elsevier BV ; 2011,. doi:10.1016/j.vaccine.2011.07.022.
5. O, Ml A. Biology and pathogenesis of chikungunya virus. *Nat Rev Microbiol*. 2010;8:491–500.
6. Gérardin P, Barau G, Michault A, Bintner M, Randrianaivo H, et al. Multidisciplinary Prospective Study of Mother-to-Child Chikungunya Virus Infections on the Island of La Réunion. *PLoS Med*. 2008;5(3):e60–e60.
7. Lenglet Y, Barau G, Robillard PY. Chikungunya infection in pregnancy: Evidence for intrauterine infection in pregnant women and vertical transmission in the parturient. Survey of the Reunion Island outbreak. *J Gynecol Obstet Biol Reprod*. 2006;35.
8. Lanciotti RS, Calisher CH, Gubler DJ, Chang GJ, Vorndam AV. Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction. *J Clin Microbiol*. 1992;30(3):545–551.
9. Bergmeyer HU, Horder M, Rej R. Approved Recommendation (1985) on IFCC Methods for the Measurement of Catalytic Concentration of Enzymes.Part3 .IFCC Method for Alanine Aminotransferase. *J Clin Chem Clin Biochem*. 1986;24.
10. Tietz NW. Textbook of Fundamentals of Clinical Chemistry. Philadelphia: WB Saunders ; 1987,. p. 369–371.
11. Tietz NW. Textbook of Fundamentals of Clinical Chemistry. Philadelphia: WB Saunders ; 1987,. p. 385–386.

12. Tietz NW. Textbook of Fundamentals of Clinical Chemistry . Philadelphia: WB Saunders ; 1987,. p. 391–392.
13. Control. Centre for Disease Control. Chikungunya information for healthcare providers ;. Available from: http://www.cdc.gov/chikungunya/pdfs/CHIKV_Clinicians.pdf.2014.Accessed.
14. Staples JE, Breiman RF, Powers AM. Chikungunya Fever: An Epidemiological Review of a Re-Emerging Infectious Disease. *Clin Infect Dis*. 2009;49(6):942–948. doi:10.1086/605496.
15. Nimmannitya S, Halstead SB, Cohen SN. Dengue and chikungunya virus infection in man in Thailand, 1962-1964. Observations on hospitalized patients with hemorrhagic fever. *Am J Trop Med Hyg*. 1969;18:954–971.
16. Chen HC, Sy L. lymphocyte activation and hepatic cellular infiltration in immunocompetent mice infected with dengue virus. *J Med Virol*. 2004;73(3):419–431.
17. Seneviratne SL, Malavige GN, de Silva HJ. Pathogenesis of liver involvement during dengue viral infections. *Trans Royal Soc Trop Med Hygiene*. 2006;100(7):608–614. doi:10.1016/j.trstmh.2005.10.007.
18. Kuo CH, Tai DI, Chang-Chein CS, Lan CK, Chiou SS, Liaw YF. Liver biochemical tests and dengue fever. *Am J Trop Med Hyg*. 1992;47(3):265–70.
19. Souza LJ, Gonçalvescarnerio H, Soutofilho JT, Souza TF, Cortes VA, et al. Hepatitis in dengue shock syndrome. *Braz J Infect Dis*. 2002;6(6):322–327.
20. Kalayanarooj S, Vaughn DW, Nimmannitya S, Green S, Suntayakorn S, et al. Early Clinical and Laboratory Indicators of Acute Dengue Illness. *J Infect Dis*. 1997;176(2):313–321. doi:10.1086/514047.
21. Staples JE, Breiman RF, Powers AM. Chikungunya Fever: An Epidemiological Review of a Re-Emerging Infectious Disease. *Clin Inf Dis*. 2009;49(6):942–948. doi:10.1086/605496.
22. Thiberville SD, Moyen N, Dupuis-Maguiraga L, Nougairede A, Gould EA, et al. Chikungunya fever: Epidemiology, clinical syndrome, pathogenesis and therapy. *Antiviral Research*. 2013;99(3):345–370. Available from: https://dx.doi.org/10.1016/j.antiviral.2013.06.009. doi:10.1016/j.antiviral.2013.06.009.
23. Sison RC, Nieto XA, Bautista JL, Ferrer JM, Paradoma. An Evaluation on the Hematologic Differentiation Between Dengue Fever and Chikungunya Fever Based On Complete Blood Count, Kidney Function Tests, and Liver Enzymes Test Results. *Am J Clinl Pathol*. 2014;142.
24. Rahman MM, Rahim R. Simultaneous Detection and Differentiation of Chikungunya Virus and Dengue Virus in Blood at Clinical Presentation by Real Time RT-PCR. *Bangladesh J Pulse*. 2017;7:6–11.

Author biography

Abhinav Manish Resident

Tariq Masood Professor and hEAD

Amit Verma Professor and Head

Narotam Sharma Scientist CMRL

Rajeev Singh Kushwaha Associate Professor

Cite this article: Manish A, Masood T, Verma A, Sharma N, Kushwaha RS. **Molecular characterization of dengue and chikungunya viruses and their association with the liver profile**. *Int J Clin Biochem Res* 2020;7(1):124-129.