Original Article

Studying The Viral Hemorrhagic Fever In Indian Subjects Due To Chikungunya And Dengue Hemorrhagic Fever

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Abstract

Background: Viral hemorrhagic fever is caused by various viruses having single-stranded RNA namely Flaviviridae, Bunyaviridae, Filoviridae, and Arenaviridae prevalent globally. In India, the most common viral hemorrhagic fevers (VHF) are Crimean Congo hemorrhagic fever (CCHF), dengue fever, and Kyasanur forest disease (KFD). The data in India for VHF are scarce.

Aim: The present study aimed to assess the common etiology of VHF (viral hemorrhagic fever) in Indian subjects having Chikungunya, and dengue hemorrhagic fever.

Methods: From 102 subjects with suspicion of viral hemorrhagic fever, serum samples were collected. For detecting IgM antibodies specific to chikungunya and dengue, MAC-ELISA was done. This was followed by RT-PCR for the detection of chikungunya, and dengue-specific nucleic acid. For all subjects, clinico-demographic and laboratory profiles were assessed.

Results: Among 102 subjects, 32 subjects had confirmed diagnoses of viral hemorrhagic fever. Six subjects were diagnosed with co-infection of dengue-chikungunya and two subjects had confirmed diagnosis of chikungunya. The diagnosis in the study subjects was Grade I with chikungunya in 18.75% (n=6) study subjects, Grade I in 6.25% (n=2) study subjects, grade II in 37.5% (n=12) study subjects, grade III in 18.75% (n=60 study subjects, Grade IV in 12.5% (n=4) study subjects, and chikungunya in 6.25% (n=2) study subjects respectively. statistically, a significant difference was seen for retro-orbital pain seen in 81.25% and 37.14% of subjects with VHF and dengue and chikungunya respectively, and p=0.02. Hematocrit was significantly higher in confirmed VHF subjects with 42.3±10.8% compared to 35.3±10.3% in dengue and chikungunya-negative study subjects with p=0.03.

Conclusion**:** The present study, within its limitations, concludes that in Indian subjects, Crimean Congo hemorrhagic fever (CCHF) is not seen in human subjects residing in India and the hemorrhagic symptoms reported in Indian subjects can be of chikungunya infection.

Keywords: Chikungunya, Crimean Congo hemorrhagic fever, dengue, viral hemorrhagic fever, viral fever

INTRODUCTION

VHF or viral hemorrhagic fever is caused by various virus families taxonomically belonging to viruses having single-stranded RNA such as Flaviviridae, Bunyaviridae, Filoviridae, and Arenaviridae. These viruses are prevalent globally. In the Indian scenario, the viral hemorrhagic diseases include dengue infection, CCHHF (Crimean Congo hemorrhagic fever), and Kyasanur forest disease (KFD). The literature data is scarce concerning Viral hemorrhagic fever in the Indian context. In animal studies assessing the goat, sheep, and bovine animals in different geographic areas of India, anti-CCHV IgG antibodies have been detected.[1]

Owing to the involvement of the majority of the population in animal husbandry and agriculture, a possibility has been considered for transmission of the CCHF virus from animals to humans having a cycle of the enzootic tick to vertebrate and tick. Other than CCHF and dengue infection, chikungunya virus is not considered in the list of viral hemorrhagic fever that can present the manifestations of hemorrhagic fever as their atypical presentation. This can be attributed to overlapping symptoms and signs along with a commonly involved vector namely Aedes aegypti.[2]

CCHF (Crimean-Congo hemorrhagic fever) is caused by a virus born in the tick and leads to moderate to severe disease in human subjects. CCHF has a high mortality rate going up to 40%. CCHF has its name from the Congo and Crimea regions in 1956 and 1945 respectively where it was first reported. Concerning its antigenicity, the virus from the two

regions cannot be distinguished. The transmission of the CCHF virus in humans is caused by bites from infected ticks belonging to the genus Hyalomma mainly.[3] However, other modes of transmission to humans are through contact with tissues or blood of infected animals including nosocomial transmission from human to human. The vector species and associated viruses are globally widespread with the absence of endemic foci from Australia, South America, and North America. The distribution and transmission activities are dynamic. Recently, both vector species and viruses have been identified in newer regions, India reported occasional cases.[4]

Chikungunya fever is another viral disease that is arthropod-borne and is concerned a global healthcare burden since the year 2006. Earlier reports are suggestive of the presence of fever similar to chikungunya fever as epidemics along with the arthralgia which was prevalent in the year 1824. Chikungunya was first described in the year 1952 following an outbreak in South-eastern Tanzania. The Chikungunya term is derived from the kungunyala verb in the language Kimakonde meaning become contorted or dry up. A stooped posture is seen in the disease owing to the rheumatologic manifestations describing the Chikungunya. Buka-Buka is the term used in the Congo region which is suggestive of incapacitating joint pain.[5]

Dengue infection is caused by the virus from the Flaviviridae family and consists of four serotypes numbered from 1 to 4. Dengue infection is transmitted by mosquitoes Aedes albopictus and Aedes aegypti. Dengue virus, biologically, is maintained by vertical transmission and is highly adapted to the mosquito. Dengue virus is capable of causing a subclinical infection to a self-limiting disease of mild severity. In severe cases, dengue fever can be fatal advancing to dengue shock syndrome or dengue hemorrhagic fever. Dengue fever has a seasonal prevalence in India where the prevalence is determined by mosquito vectors in the subtropical and tropical region.[6]

Considering the scenario of these viral hemorrhagic fever diseases, the present study aimed to assess the viral hemorrhagic fever including CCHF, Chikungunya, and dengue virus in Indian subjects attending the tertiary health care center.

MATERIALS AND METHODS

The present cross-sectional analytical study was done to assess the viral hemorrhagic fever including CCHF, Chikungunya, and dengue virus in Indian subjects attending the health care center. The study subjects were recruited from the subjects visiting the Outpatient Department of the Institute. After explaining the detailed study design, informed consent was taken in both verbal and written format.

The study initially assessed 194 subjects who were fitting the criteria of viral hemorrhagic fever where 102 subjects were included finally in the study. Concerning the inclusion and exclusion criteria, the remaining 92 subjects were not included in the study that was screened initially. CDC (center for disease control) 2011 case definition criteria was followed was utilized in the study that was based on the epidemiological and clinical criteria.[7] Th inclusion criteria were subjects with a temperature of $>40^0$C and bleeding manifestations along with one or more clinical feature as tick splashing exposure, tick bite, contact with body fluid or blood, travel to a dengue-endemic area, thrombocytopenia, abdominal pain, diarrhea, vomiting, and trunk erythematous maculopapular rash three weeks before symptom onset. The exclusion criteria for the study were subjects having bleeding tendencies secondary to systemic diseases or any drug, classic dengue fever subjects with hemorrhagic tendencies with negative tourniquet test and positive thrombocytopenia (<1,00,000/cu mm), and with rickettsial disease diagnosis.

The blood samples from all the subjects were collected in sterile and aseptic condition from the antecubital vein in the quantity of 3ml to 5ml immediately after the inclusion of study participants following the inclusion criteria. The serum from the collected blood sample was separated and aliquoted to avoid repeated thawing and freezing. The serum was then divided into 3 serum vials for performing the RT-PCR (real-time Reverse Transcriptase-Polymerase Chain Reaction), and ELISA (enzyme-linked immunosorbent assay).[8] Biosafety procedures were followed while performing all the tests.[9]

Serological assays were done for detecting the anti-chikungunya IgM, dengue NS1 antigen, and anti-dengue IgM. In all 104 study subjects, MAC-ELISA (IgM capture enzyme immunoassay) was employed. The kits for chikungunya and dengue were taken from NIV (national institute of Virology), Puna. In 38 subjects from 104 participants, serum was collected within 7 days of illness marking the acute phase, and the Direct Sandwich ELISA was utilized for detecting the NS1 antigen (dengue nonstructural antigen). Strict instructions from the manufacturers were used to perform the test and do the subsequent calculations.

Concerning the RT-PCR, viral RNA was extracted from all 102 subjects in duplicates where one was used for internal control of chikungunya/dengue and the other for internal control of CCHF. Also, the extracted RNA was treated to multiplex real-time RT-PCR for detecting chikungunya and dengue RNA. Also, multiplex real-time RT-PCR was done for detecting the CCHF RNA. 70 subjects that were detected as negative for chikungunya or dengue on RT-PCR and/or MAC-ELISA were tested with real-time RT-PCR for detecting the CCHF viral RNA. Interpretation, assay validation, program setting, and reaction mixture preparation were identical in CCHF RT-PCR as was for the dengue.

The data gathered were assessed statistically using SPSS software version 26.0 (IBM, Chicago, IL) and fisher's extract test and Chi-square test. The level of significance was kept at the p-value of <0.005.

RESULTS

The present cross-sectional analytical study was done to assess the viral hemorrhagic fever including CCHF, Chikungunya, and dengue virus in Indian subjects attending the health care center. The study initially assessed 194 subjects who were fitting the criteria of viral hemorrhagic fever where 102 subjects were included finally in the study. In 102 study subjects with suspected viral hemorrhagic fever, 31% (n=32) subjects had confirmed diagnosis of VHF, 75% (n=24) subjects were diagnosed with dengue hemorrhagic fever, 6% (n=2) subjects were diagnosed to have chikungunya,

and 19% (n=6) subjects were diagnosed with the co-infection of dengue-chikungunya. No subject was diagnosed to have CCHFV infection in the present study. The diagnosis was made depending on positive MAC ELISA for dengue and dengue amplification with RT-PCR.[10] As no study sample was positive on RT-PCR for chikungunya MAC ELISA results were used to assessed chikungunya.[11] Hence, the present study considered chikungunya and dengue subjects as viral hemorrhagic fever. The mean age of the study participants was 33.2±3.46 years and the age range of 7 years to 70 years. In suspected subjects with VHF, 70% of subjects were male.

On assessing the symptoms in the study subjects, in subjects with a confirmed diagnosis of VHF, 2 subjects having chikungunya, all 100% (n=2) subjects had a fever, headache, breathlessness, arthralgia, sore throat, petechial rashes, and breathlessness each. In 6 study subjects having chikungunya and dengue, fever, headache, retro-orbital pain, myalgia, and petechial rashes were reported by all 100% (n=6) study subjects, arthralgia in 66.6% (n=4) study subjects with dengue and chikungunya, and sore throat, breathlessness, conjunctival suffusion, ascites, and pleural effusion in 33.3% (n=2) study subjects each with dengue and chikungunya. In 24 subjects with dengue, the headache was reported in 100% (n=24) study subjects, headache in 91.6% (n=22) study subjects, retro-orbital pain and myalgia in 83.3% (n=20) study subjects each with dengue, arthralgia, sore throat, and Malena in 25% (n=6) subjects each, diarrhea, ascites, and pleural effusion in 41.6% (n=10) subjects each, petechial rashes in 58.3% (n=14) study subjects, and conjunctival suffusion and bleeding gums in 33.3% (n=8) subjects each with dengue. In 70 subjects negative for dengue and chikungunya, fever was the most common symptom seen in 100% (n=70) subjects followed by headache and myalgia in 77.14% (n=54) subjects, petechial rashes in 68.57% (n=48) subjects, sore throat in 48.57% (n=34) study subjects, breathlessness in 28.57% (n=20) study subjects, arthralgia in 22.85% (n=16) study subjects, conjunctival suffusion in 20% (n=14) study subjects, pleural effusion, bleeding gums, and diarrhea in 17.14% (n=12) study subjects, and ascites in 2.85% (n=2) study subjects. All the parameters differed non-significantly among subjects with respective p-values of 0.46. 0.54, 0.68, 0.89, 0.79, 0.96, 0.67, 0.57, 0.52, 0.58, 0.77, and 0.19 for pleural effusion, ascites, conjunctival suffusion, Malena, bleeding gums, petechial rashes, breathlessness, diarrhea, sore throat, arthralgia, myalgia, headache, and fever. However, a statistically significant difference was seen for retro-orbital pain seen in 81.25% and 37.14% of subjects with VHF and dengue and chikungunya respectively, and p=0.02 as shown in Table 1.

Concerning the biochemical variables, ALP (alkaline phosphate) levels were 136.6±96.5 IU/L and 84.6±37.7 IU/L for dengue and chikungunya negative and confirmed VHF cases respectively which was a statistically significant difference with p=0.07. ALT (alanine transaminase) levels were 235.4±488.4 and 183.4±233.3 IU/L for dengue and chikungunya negative and VHF confirmed study cases with p=0.5 showing non-significance. AST (aspartate transaminase) levels were 132.2±130.02 and 125.5±140.3 IU/L in dengue and chikungunya negative and VHF confirmed study cases respectively with non-significant difference and p=0.7. ApTT (activated partial thromboplastin clotting time) was 40.6±10.2 seconds and 37.2±8.4 seconds for dengue and chikungunya negative and VHF confirmed study cases respectively with non-significant difference and p=0.3. PT (prothrombin time) was 16.1±14.1 and 15.7±3.3 seconds respectively with p=0.6 for dengue and chikungunya negative and VHF confirmed study subjects respectively. Platelet counts were 73932±47966 cells/mm3 and 96123±43867 in dengue and chikungunya negative and VHF-confirmed study subjects respectively with p=0.1. TLC levels were 10175±12354.1 cells/mm3 and 6956.4±6800.5 cells/mm3 for dengue and chikungunya negative and confirmed VHF subjects respectively with p=0.2. Hematocrit was significantly higher in confirmed VHF subjects with 42.3±10.8% compared to 35.3±10.3% in dengue and chikungunya-negative study subjects with p=0.03. Hemoglobin was higher in confirmed VHF cases with 11.7±1.7 gm% compared to 35.3±10.3gm% for dengue and chikungunya-negative study subjects. However, the difference was statistically non-significant with p=0.06 (Table 2).

For the DHF severity, in 30 subjects, the tourniquet test was positive in 73.33% (n=22) of the study subjects. Spontaneous bleeding was seen in 73.33% (n=22) of the study subjects. Narrow pulse pressure was seen in 20% (n=6) study subjects, not seen in 66.6% (n=20) study subjects, and was not detected in 13.3% (n=4) study subjects respectively. The profound shock was seen in 13.3% (n=4) of study subjects with dengue. Dengue hemorrhagic fever in grades I, II, III, and IV was seen in 26.66% (n=8), 40% (n=12), 20% (n=6), and 13.3% (n=4) study subjects respectively. For assessment in subjects with DHF, 20% (n=6) subjects were discharged and 13.3% (n=4) subjects died as depicted in Table 3.

On assessing the laboratory tests, Dengue NS1 antigen on ELISA was positive for 37.5% (n=12) of study subjects. Dengue IgM antibodies were positive in 75% (n=240 study subjects with ELISA. Chikungunya IgM antibodies were positive in 25% (n=8) of study subjects when assessed using the ELISA. On RT-PCR, dengue was positive in 43.75% (n=14) of study subjects. Chikungunya was positive in no subject on Rt-PCR assessment. The diagnosis in the study subjects was Grade I with chikungunya in 18.75% (n=6) study subjects, Grade I in 6.25% (n=2) study subjects, grade II in 37.5% (n=12) study subjects, grade III in 18.75% (n=60 study subjects, Grade IV in 12.5% (n=4) study subjects, and chikungunya in 6.25% (n=2) study subjects respectively as shown in Table 4.

DISCUSSION

The present study initially assessed 194 subjects who were fitting the criteria of viral hemorrhagic fever where 102 subjects were included finally in the study. In 102 study subjects with suspected viral hemorrhagic fever, 31% (n=32) subjects had confirmed diagnosis of VHF, 75% (n=24) subjects were diagnosed with dengue hemorrhagic fever, 6% (n=2) subjects were diagnosed to have chikungunya, and 19% (n=6) subjects were diagnosed with the co-infection of dengue-chikungunya. No subject was diagnosed to have CCHFV infection in the present study. The diagnosis was made depending on positive MAC ELISA for dengue and dengue amplification with RT-PCR. As no study sample was positive on RT-PCR for chikungunya MAC ELISA results were used to assessed chikungunya. Hence, the present study considered chikungunya and dengue subjects as viral hemorrhagic fever. The mean age of the study participants was 33.2±3.46 years

and the age range of 7 years to 70 years. In suspected subjects with VHF, 70% of subjects were male. These demographics were comparable to the previous studies of Kaul P[12] in 2015 and Mourya DT et al[13] in 2015 where authors assessed subjects with demographics comparable to the present study.

In the study subjects with a confirmed diagnosis of VHF, 2 subjects having chikungunya, all 100% (n=2) subjects had a fever, headache, breathlessness, arthralgia, sore throat, petechial rashes, and breathlessness each. In 6 study subjects having chikungunya and dengue, fever, headache, retro-orbital pain, myalgia, and petechial rashes were reported by all 100% (n=6) study subjects, arthralgia in 66.6% (n=4) study subjects with dengue and chikungunya, and sore throat, breathlessness, conjunctival suffusion, ascites, and pleural effusion in 33.3% (n=2) study subjects each with dengue and chikungunya. In 24 subjects with dengue, the headache was reported in 100% (n=24) study subjects, headache in 91.6% (n=22) study subjects, retro-orbital pain and myalgia in 83.3% (n=20) study subjects each with dengue, arthralgia, sore throat, and Malena in 25% (n=6) subjects each, diarrhea, ascites, and pleural effusion in 41.6% (n=10) subjects each, petechial rashes in 58.3% (n=14) study subjects, and conjunctival suffusion and bleeding gums in 33.3% (n=8) subjects each with dengue. In 70 subjects negative for dengue and chikungunya, fever was the most common symptom seen in 100% (n=70) subjects followed by headache and myalgia in 77.14% (n=54) subjects. All the parameters differed non-significantly among subjects with respective p-values of 0.46. 0.54, 0.68, 0.89, 0.79, 0.96, 0.67, 0.57, 0.52, 0.58, 0.77, and 0.19 for pleural effusion, ascites, conjunctival suffusion, Malena, bleeding gums, petechial rashes, breathlessness, diarrhea, sore throat, arthralgia, myalgia, headache, and fever. However, a statistically significant difference was seen for retro-orbital pain seen in 81.25% and 37.14% of subjects with VHF and dengue and chikungunya respectively, and p=0.02. These results were consistent with the previous studies of Mourya DT et al[14] in 2021 and Durrani M et al[15] in 2017 where authors reported fever and headache followed by petechial rashes in the subjects having viral hemorrhagic fever.

The study results showed that ALP (alkaline phosphate) levels were 136.6±96.5 IU/L and 84.6±37.7 IU/L for dengue and chikungunya negative and confirmed VHF cases respectively which was a statistically significant difference with p=0.07. ALT (alanine transaminase) levels were 235.4±488.4 and 183.4±233.3 IU/L for dengue and chikungunya negative and VHF confirmed study cases with p=0.5 showing non-significance. AST (aspartate transaminase) levels were 132.2±130.02 and 125.5±140.3 IU/L in dengue and chikungunya negative and VHF confirmed study cases respectively with non-significant difference and p=0.7. ApTT (activated partial thromboplastin clotting time) was 40.6±10.2 seconds and 37.2±8.4 seconds for dengue and chikungunya negative and VHF confirmed study cases respectively with non-significant difference and p=0.3. PT (prothrombin time) was 16.1±14.1 and 15.7±3.3 seconds respectively with p=0.6 for dengue and chikungunya negative and VHF confirmed study subjects respectively. Platelet counts were 73932±47966 cells/mm3 and 96123±43867 in dengue and chikungunya negative and VHF-confirmed study subjects respectively with p=0.1. TLC levels were 10175±12354.1 cells/mm3 and 6956.4±6800.5 cells/mm3 for dengue and chikungunya negative and confirmed VHF subjects respectively with p=0.2. Hematocrit was significantly higher in confirmed VHF subjects with 42.3±10.8% compared to 35.3±10.3% in dengue and chikungunya-negative study subjects with p=0.03. Hemoglobin was higher in confirmed VHF cases with 11.7±1.7 gm% compared to 35.3±10.3gm% for dengue and chikungunya-negative study subjects. However, the difference was statistically non-significant with p=0.06. These findings were in agreement with the previous studies of Sharma J[16] in 2017 and Ahmed NH[17] in 2015 where the reported laboratory parameters by the authors were comparable to that of the present study.

On assessing the DHF severity, in 30 subjects, the tourniquet test was positive in 73.33% (n=22) of the study subjects. Spontaneous bleeding was seen in 73.33% (n=22) of the study subjects. Narrow pulse pressure was seen in 20% (n=6) study subjects, not seen in 66.6% (n=20) study subjects, and was not detected in 13.3% (n=4) study subjects respectively. The profound shock was seen in 13.3% (n=4) of study subjects with dengue. Dengue hemorrhagic fever in grades I, II, III, and IV was seen in 26.66% (n=8), 40% (n=12), 20% (n=6), and 13.3% (n=4) study subjects respectively. For assessment in subjects with DHF, 20% (n=6) subjects were discharged and 13.3% (n=4) subjects died. These results were in line with the results of Khan SA et al[18] in 2016 and Abhishek K[19] in 2019 where the variables of DHF severity were similar to the findings of the present study.

Concerning the laboratory tests, Dengue NS1 antigen on ELISA was positive for 37.5% (n=12) of study subjects. Dengue IgM antibodies were positive in 75% (n=240 study subjects with ELISA. Chikungunya IgM antibodies were positive in 25% (n=8) of study subjects when assessed using the ELISA. On RT-PCR, dengue was positive in 43.75% (n=14) of study subjects. Chikungunya was positive in no subject on Rt-PCR assessment. The diagnosis in the study subjects was Grade I with chikungunya in 18.75% (n=6) study subjects, Grade I in 6.25% (n=2) study subjects, grade II in 37.5% (n=12) study subjects, grade III in 18.75% (n=60 study subjects, Grade IV in 12.5% (n=4) study subjects, and chikungunya in 6.25% (n=2) study subjects respectively. These laboratory tests were comparable to the findings of Dutta P et al[20] in 2011 and Sharma A et al[21] in 2021 where the laboratory parameters recorded in their study subjects were similar to the present study.

CONCLUSION

The present study, considering its limitation, concludes that in Indian subjects, Crimean Congo hemorrhagic fever (CCHF) is not seen in human subjects residing in India and the hemorrhagic symptoms reported in Indian subjects can be of chikungunya infection.

Conflict of Interest:

Nil

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TABLES

Symptoms	Confirmed viral hemorrhagic fever (n=32)				Dengue and Chikungunya negative (n=70) (%)	p
	Chikungunya n=2 (%)	Dengue and chikungunya (n=6) (%)	Dengue (n=24) (%)	Total =32 (%)		
Pleural effusion	0	2 (33.3)	10 (41.6)	12 (37.5)	12 (17.14)	0.46
Ascites	0	2 (33.3)	10 (41.6)	12 (37.5)	2 (2.85)	0.54
Conjunctival suffusion	0	2 (33.3)	8 (33.3)	10(31.25)	14 (20)	0.68
Malena	0	0	6 (25)	6 (18.75)	10 (14.28)	0.89
Bleeding gums	0	0	8 (33.3)	8 (25)	12 (17.14)	0.79
Petechial rashes	2 (100)	6 (100)	14 (58.3)	22 (68.75)	48 (68.57)	0.96
Breathlessness	2 (100)	2 (33.3)	2 (8.33)	4 (12.5)	20 (28.57)	0.67
Diarrhea	0	0	10 (41.6)	10 (31.25)	12 (17.14)	0.57
Sore throat	2 (100)	2 (33.3)	6 (25)	10 (31.25)	34 (48.57)	0.52
Arthralgia	2 (100)	4 (66.6)	6 (25)	12 (37.5)	16 (22.85)	0.58
Myalgia	0	6 (100)	20 (83.3)	26 (81.25)	54 (77.14)	0.77
Retro-orbital pain	0	6 (100)	20 (83.3)	26 (81.25)	26 (37.14)	**0.02**
Headache	2 (100)	6 (100)	22 (91.6)	30 (93.75)	54 (77.14)	0.19
Fever	2 (100)	6 (100)	24 (100)	32 (100)	70 (100)	-

Table 1: Distribution of study subjects based on chikungunya and dengue negative and other viral hemorrhagic fever

Biochemical variables	Dengue and chikungunya negative (n=70)	N	Confirmed VHF (n=32)	N	p-value
ALP (IU/L)	136.6±96.5	50	84.6±37.7	28	0.07
ALT (IU/L)	235.4±488.4	56	183.4±233.3	28	0.5
AST (IU/L)	132.2±130.02	56	125.5±140.3	28	0.7
ApTT (seconds)	40.6±10.2	58	37.2±8.4	28	0.3
PT (seconds)	16.1±14.1	58	15.7±3.3	28	0.6
Platelet count (cells/mm3)	73932±47966	70	96123±43867	32	0.1
TLC (cells/mm3)	10175±12354.1	62	6956.4±6800.5	32	0.2

Hematocrit (%)	35.3±10.3	58	42.3±10.8	30	**0.03**
Hb (gm%)	10.4±2.7	62	11.7±1.7	32	0.06

Table 2: Comparison of the biochemical parameters in dengue and chikungunya-negative subjects and subjects with confirmed VHF

DHF severity	**Subgroup**	**%**	**n=30**
Tourniquet test	Positive	73.33	22
	Not done	26.66	8
Spontaneous bleeding	Yes	73.33	22
	No	26.66	8
Narrow pulse pressure	Yes	20	6
	No	66.6	20
	Not detected	13.3	4
Profound shock	Yes	13.3	4
	No	86.6	26
DHF grade	I	26.66	8
	II	40	12
	III	20	6
	IV	13.3	4
Outcomes	Discharged	20	6
	Expired	13.3	4
	Not assessed	66.6	20

Table 3: DHF severity and grading in study subjects

Laboratory tests	**Subgroup**	**%**	**N=32**
Dengue NS1 Ag (ELISA)	Positive	37.5	12
	Negative	6.25	2
	Not done	56.25	18
Dengue IgM (ELISA)	Positive	75	24
	Negative	25	8
Chikungunya IgM ELISA	Positive	25	8
	Negative	75	24
Dengue RT-PCR	Positive	43.75	14
	Negative	56.25	18
Chikungunya RT-PCR	Positive	0	0
	Negative	100	32
Diagnosis	I with chikungunya	18.75	6
	I	6.25	2
	II	37.5	12
	III	18.75	6
	IV	12.5	4
	Chikungunya	6.25	2

Table 4: Laboratory tests in the study subjects with a confirmed diagnosis of viral hemorrhagic fever