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Article

Study of Hepatic Dysfunction Associated with Dengue Epidemiology in a Tertiary Care Hospital, Kolkata

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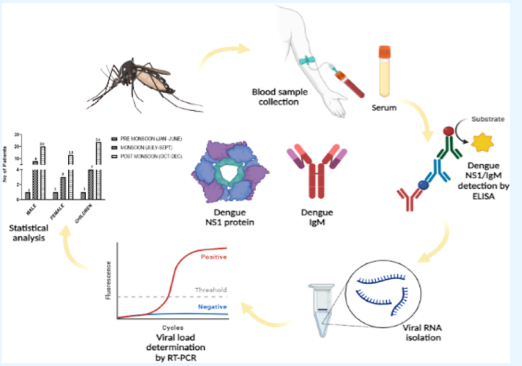
ABSTRACT: Dengue is a common arthropod-borne life-threatening febrile illness. This disease affects liver functions with an imbalance of liver enzymes followed by other clinical manifestations. The dengue serotypes can cause asymptomatic infection to more severe versions of hemorrhagic fever and dengue shock syndrome in West Bengal and around the globe. The main aim of this study is to establish how different liver enzymes act in identifying markers for dengue prognosis for the early detection of severe dengue fever (DF). The diagnosis of dengue patients was confirmed by enzyme-linked immunosorbent assay, and associated clinical parameters [aspartate transaminase (AST), alanine aminotransferase (ALT), alkaline phosphatase, total bilirubin, total albumin, total protein, packed cell volume, and platelet count] were analyzed. Furthermore, the viral load estimation was also carried out by RT PCR analysis. The majority of these patients had elevated AST and ALT levels; ALT levels were higher than AST levels, which were partially observed in all non-structural protein 1 antigen- and dengue immunoglobulin M antibody-reactive patients. Almost 25% of patients had very low platelet count or thrombocytopenia. Furthermore, the viral load shows a significant association with all the clinical parameters with a *p*-value of <0.0001. All these liver enzymes are significantly correlated with an increased level of T.BIL, ALT, and AST. This study depicts that the intensity of hepatic involvement may play a critical role in the morbidity and mortality of DF patients. As a result, all of these liver parameters can be useful early markers for determining the severity of the disease, allowing for early detection of high-risk cases.



INTRODUCTION

Recently, dengue fever (DF) is the most common vector-borne disease worldwide. In tropical and subtropical countries, this febrile illness prevails as a major health issue. According to the World Health Organization (WHO), 2.5 billion people and 124 countries are in peril and there is an estimation of over 100 million cases and 30,000 deaths worldwide.[1] Generally, in tropical and subtropical countries, outbreak of DF occurs after every 3−4 years. India has also encountered this outbreak every year for the last 10 years. It is the cause of arboviral infection across the world. There are some factors responsible for the emerging and re-emerging of DF such as the lack of hygiene, unorganized health care systems, lack of awareness, and increasing international travel. DF cases have been documented in 129 countries in the WHO's Africa, Americas, Southeast Asia, and Western Pacific regions. Globally, 390 million dengue infections are projected based on modeling data.[1] The number of reported dengue cases at the WHO increased sixfold from 505,430 in 2000 to 3,312,040 in 2015. The National Center for Vector Born Diseases Control Programme (NVBDCP) data reported 1,10,473 cases of dengue and 86 deaths reported in India until 31st October, 2022.[2] However, a majority of asymptomatic infections and differing national standards for recording and reporting dengue cases would contribute to an overestimate of the global dengue burden. Previously, inoculation of serum from patients in suckling mice was used for isolating the dengue virus in Japan in 1943, and serum samples from US soldiers were used to isolate the virus in Calcutta (now Kolkata) in 1944.[3] The first clinical dengue-like sickness pandemic was recorded in Madras (now Chennai) in 1780, and the first virologically proven DF epidemic in India occurred in Calcutta and the Eastern Coast of India between 1963 and 1964. The first large dengue hemorrhagic fever (DHF) epidemic occurred in the Philippines in 1953−1954, followed by a rapid global expansion of DF or DHF outbreaks. DHF was present in neighboring nations; however, it was not present in India for unclear

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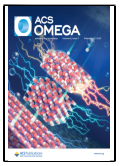

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reasons, despite the presence of all risk factors. There are four serotypes of dengue virus termed DEN-1, DEN-2, DEN-3, and DEN-4, all possessing RNA as the genetic material, and recently, DENV-5 has been discovered by scientists in Malaysia. All four serotypes are potent to cause the disease from a non-severe infection to mild self-limiting disease, DF, and a severe one, DHF or dengue shock syndrome which can cause death. Prompt identification and treatment will aid in the reduction of mortality, particularly among children. These findings are backed up by studies, including one from Ludhiana, India, which found that 81 children with DHF were hospitalized during the epidemic in 2005−2006, and another from Chennai, India, which found that 20% of total dengue cases were detected in infants during an outbreak in October 2012 to December 2012.[4] Because of its cost-effectiveness and greater sensitivity and specificity, the non-structural protein 1 (NS1) antigen and immunoglobulin M (IgM) antibody enzyme-linked immunosorbent assay (ELISA) is the primary diagnostic modality in endemic countries.[5] Therefore, the main objective of this study is to investigate and analyze the significant relationship between different clinical parameters [aspartate transaminase (AST), alanine amino-transferase (ALT), alkaline phosphatase (ALP), total bilirubin (T.BIL), total protein (T.PRO), packed cell volume (PCV), total platelet count (PLT), and albumin (ALB)] associated with patients with DF of different age groups.

RESULTS

A total of 78 patients with DF and associated symptoms were included in this study, out of which 38 patients (48.717%) were NS1-reactive and 40 patients (51.28%) were dengue IgM-reactive. A significant number of patients (60% in males and 36.8% in females) having elevated levels of AST and ALT (46.7% in males and 23.6% in females) are represented in Table 1.

Table 1. Percentage of Dengue-Positive Patients Who Have Differential Expression Levels of AST and ALT[a]

AST (U/L)	male (%)	female (%)
<40	36.7	63.2
41−250	60	36.8
>400	3.3	0
ALT (U/L)	male (%)	female (%)
<40	43.3	73.7
41−250	46.7	26.3
>400	10	0

[a]In the case of AST, 60% in males and 36.8% in females, and for ALT, 46.7% males and 23.6% females showed high levels of serum expression.

The mean value of ALT was significantly higher than that of AST, as shown in Figure 1. The mean ALP level is 110 IU/L in males and 83.5 IU/L in females. The majority of patients had a higher level of bilirubin in blood (mean bilirubin—2.75 and 1.86 mg/dL in male and female patients, respectively). The mean value of albumin levels is 3.49 g/dL in male patients and 3.69 g/dL in female patients. All clinical parameters are depicted in Figure 1.

Almost 25% of patients had very low platelet count or thrombocytopenia. The mean PCV level is 38.96% in males and 33.94% in females. Out of 78 patients, 30 adult male patients and 19 adult female patients were incorporated into this study, and after comparing different parameters, obtained data were found to be insignificant by using the χ^2 test and Student's t-test, as shown in Table 2. Out of 29 children, 17 were male and 12 were female, and there was no significant association between clinical parameters (T.PRO, T.BIL, AST, ALT, ALP, ALB, PCV, and PLT) after analyzing the data by using the χ^2 test and Student's t-test (data not shown).

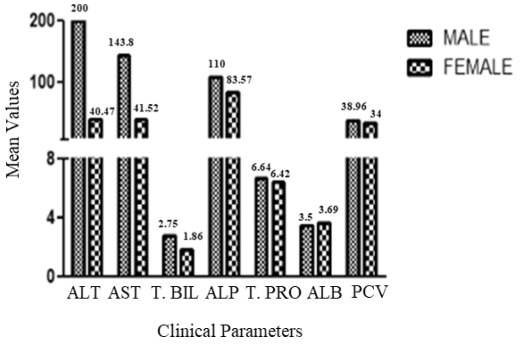

Figure 1. Comparison of mean values of different clinical parameters. High levels of SGPT, SGOT, ALP, and PCV were encountered in males compared to females, while T.BIL, T. PRO, and ALB show a similar pattern of expression.

Table 2. Comparison of T.PRO, T.BIL, AST, ALT, ALP, ALB, PCV, and PLT in Dengue Patients above 18 Years, Which Fails to Reach the Statistically Significant Levels but Shows Elevated Levels in a Large Proportions of Case Cohort

	male	female	
parameters	mean ± SD	mean ± SD	p-value
PLT	133,171.5 ± 62,418	143,315.7 ± 70,954	0.6
T.BIL	2.75 ± 5.5	1.86 ± 4.4	0.54
ALT	200.06 ± 685.8	40.47 ± 34.6	0.2
AST	143.8 ± 401.07	41.52 ± 29.1	0.17
ALP	110 ± 77.1	83.57 ± 38.69	0.11
T.PRO	6.64 ± 1.2	6.42 ± 0.74	0.43
ALB	3.49 ± 0.83	3.69 ± 0.55	0.3
PCV	38.96 ± 23.59	33.94 ± 5.47	0.27

Considering seasonal variation, in this study, it was seen that a good number of NS1- and dengue IgM-reactive cases were detected in the post-monsoon (Oct−Dec) period, and the highest case positivity in the post-monsoon period was 77% followed by the monsoon (July−Sept) period which was 19.23%, as shown in Figure 2. In the pre-monsoon period case, positivity was 3.84%. In winter and summer, it is observed that the case positivity declines readily.

In this study, we found that almost 25% of patients had very low platelet counts or thrombocytopenia and 75% of patients show normal platelet count. Among NS1- and dengue IgM-reactive patients, whose platelet counts were less than 100,000 IU/L showed a high level of liver enzymes (mean value ALP, AST, and ALT levels were 99.73, 318.7, and 203.80 IU/L respectively) shown in Table 3. Liver enzymes showed the mean values (mean values of ALP, AST, and ALT levels were 183.02, 127.85, and 110.77 IU/L, respectively) when the patient's platelet count was more than 100,000 IU/L, as shown in Table 3.

The correlation data show a positive correlation with T.BIL, ALT, and AST with the viral load with a significant value of r^2

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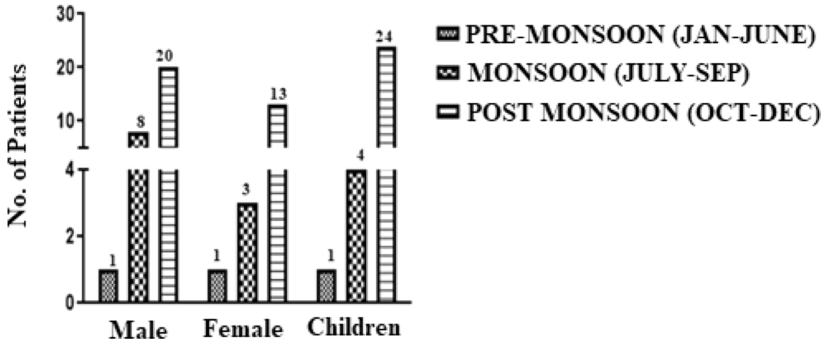

Figure 2. Monthwise representation of dengue-positive patients. Data show that the post-monsoon period (Oct−Dec) was the critical period (77% case) for dengue emergence and the monsoon (July−Sept) (19.23% cases) winter and summer act as the positivity declines phase.

Table 3. Association of ALP, AST, and ALT with Platelet Count[a]

	mean value		
platelet count	ALP (IU/L)	AST (IU/L)	ALT (IU/L)
<1,00,000/μL	99.73	318.7	203.80
>1,00,000/μL	183.02	127.85	110.77

[a]The level of ALP, AST, and ALT is very high for those who have platelet count <100,000 whereas is moderately high for those who have >100,000 platelet count.

of 0.0136, 0.0345, and 0.017 accordingly, but other liver parameters like ALP, T.PRO, and ALB show a negative correlation with viral load, as presented in Table 4.

Table 4. Correlation Analysis of Different Liver Parameters Associated with Viral Load Here T.BIL, ALT, and AST have a Positive Correlation but ALP, T.PRO, and ALB Show a Negative Correlation

liver parameters	r^2	p-value	remark
T.BIL	0.0136	0.316243	positive correlation
ALT	0.0345	0.103384	positive correlation
AST	0.017	0.255884	positive correlation
ALP	0.0046	0.554737	negative correlation
T.PRO	0.0033	0.620116	negative correlation
ALB	0.0146	0.295336	negative correlation

On the other hand, all of these six clinical parameters show significant association with viral load while tested for a t-test with a p-value of <0.0001, as presented in Table 5.

DISCUSSION

A study from January 2014 to December 2017 represents 28.4% of NS1 positivity.[6] According to a study in 2020 in North India, the NS1 positivity rates in 2016, 2017, and 2018 were 12.92, 11.77, and 12.92%, respectively,[7] and in the following three successive years 2018, 2019, and 2020, dengue NS1 positivity was documented as 52.6, 50.2, and 53.1%, respectively in south india, which was similar to the present study.[8] In our study, out of 78 clinically positive dengue samples, the NS1 positivity rate is 48.717%.

It is reported from Bangladesh in 2019 that 0.94% of cases were testified in the pre-monsoon season but most of the cases emerged in the monsoon period (50%) and the post-monsoon season (49%), and the peak season of dengue in Bangladesh was from July to October.[9] Similarly, a study in 2016[10] showed that infection started in the post-monsoon season and case

Table 5. Comparison of Different Liver Parameters with Viral Load by t-test[a]

liver parameters	mean ± SD	p-value
T.BIL vs viral load	2.017 ± 4.185 vs 139,216.97 ± 227,890.00	<0.0001
ALT vs viral load	111.96 ± 431.77 vs 139,216.97 ± 227,890.00	<0.0001
AST vs viral load	132.369 ± 423.120 vs 139,216.97 ± 227,890.00	<0.0001
ALP vs viral load	116.506 ± 86.062 vs 139,216.97 ± 227,890.00	<0.0001
T.PRO vs viral load	6.574 ± 1.102 vs 139,216.97 ± 227,890.00	<0.0001
ALB vs viral load	3.669 ± 0.712 vs 139,216.97 ± 227,890.00	<0.0001

[a]The levels of T.PRO, T.BIL, AST, ALT, ALP, ALB, PCV, and PLT are significantly associated with the elevated level of viral load with a p-value of <0.0001.

positivity increased in October (>70%). In this present study period, due to the COVID-19 pandemic, general awareness about hygiene and environmental sanitation had greatly improved, and due to outdoor activities being prohibited, the number of patients also decreased reportedly. However, our study reported that the post-monsoon (Oct−Dec) period is the most critical period for dengue emergence as 77% of our studied cohort was reported in post-monsoon. Hence, it proved that this febrile illness has seasonal variation. The case positivity has its peak value in October and November (together 61.5%).

The gender distribution of dengue patients is generally determined by a society's socio-economic pattern and work ethic. In our study, 44.73% of NS1-reactive cases were females and 55.26% were males, indicating that males were more commonly impacted by dengue. In a study, it is found that males made up 55.2% of dengue patients while females made up 44.7%.[10] An inadequate platelet count can lead to two different conditions: thrombocytopenia: a low platelet count (<150,000/μL) can cause excessive bleeding and bruising.[11] Thrombocytosis is a condition in which a high platelet count (>450,000/μL) causes inappropriate blood coagulation.[12] In this study, 33.33% of NS1- and dengue IgM-reactive patients had a platelet count of less than 100,000/μL, and the rest 66.66% had a platelet count of more than 100,000/μL. The mean platelet count in reactive patients was found to be 138,033/μL in male patients and 143,315.8/μL in female patients. This finding is discordant with the study,[13] where 64.7% of NS1-reactive patients had less than 100,000/cu mm platelet count. In another study[14] in 2015, 56% of NS1-reactive patients had thrombocytopenia.

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In this study, we found that almost 25% of patients had very low platelet counts such as less than 100,000 IU/L, and showed a high level of liver enzymes (mean values of ALP, AST, and ALT levels were 99.73, 318.7, and 203.80 IU/L, respectively).

These findings suggest that dengue patients are prone to hepatic dysfunction or liver failure. A comparison of the clinical parameters was carried out between male and female patients. The liver function tests show more elevated results in the case of male patients. In research, they discovered that liver injury was more common among females, contrary to our observations.[9]

The majority of dengue patients had a higher level of T.BIL in their blood (mean value of bilirubin is 2.75 and 1.86 mg/dL in male and female patients, respectively), as shown in Figure 1. There were only three patients who have a very high level (20.1−23.9 mg/dL) of T.BIL in their blood. The mean value of the ALB level is 3.49 g/dL in male patients and 3.69 g/dL in female patients, which lie in the normal range shown in Figure 1.

Albumin is normally affected in chronic liver disease, as dengue is an acute illness, and the level of albumin is unaffected in dengue patients. The mean value of T.PRO is 6.64 and 6.42 gm/dL in male and female dengue patients, respectively, which lies in the normal range. The mean value of the PCV level is 38.96% in males and 33.94% in females, which are also in the normal range. A reduced PCV indicates that the loss in RBC count is attributable to factors such as blood loss, anemia, cell death, and decreased bone marrow synthesis. Increased PCV often indicates that a person is dehydrated and that RBC production is higher and may have polycythemia, and the higher values of AST, ALT, and T. BIL are seen in male patients, indicating an increased risk of severe dengue infection.

According to the clinical features of the patients, 100% of them had fever and chills and 50% had retro-orbital pain. Arthralgia and myalgia were present in every patient, with 53.33% having rashes, 70% having a headache, 66% having abdominal pain, and 60% having nausea and vomiting. Only 4% of patients have had seizures during their illness. This finding is reinforced by a 2015 study,[15] in which they found that 100% of patients had fever and myalgia, 52.17% had a headache, 42.03% had vomiting, and 11.59% had abdominal pain. DF manifests in a variety of ways. The liver effects are usually asymptomatic, but they might be unusual and of varying intensity. The wide range of symptoms, from asymptomatic high transaminase levels to fulminant hepatic failure, makes treating the illness difficult. When compared to adults, children's hepatic involvement is more common and severe.

In Table 2, the values of specific clinical parameters (T.PRO, T.BIL, AST, ALT, ALP, ALB, PCV, and PLT) are found to be insignificant by using the χ^2 test and Student's t-test due to the small sample size. A large number of samples may resolve this limitation.

This study showed that DF is associated with elevated liver function enzymes. The majority of these patients had elevated AST and ALT levels. It was also observed that ALT levels were higher than AST levels, which was partially depicted in all NS1- and dengue IgM-reactive patients. Also, thrombocytopenia is observed in patients. On the other hand, association between viral load and liver enzymes shows a positive correlation with an increased level of T.BIL, ALT, and AST, while all other parameters also show a significant association with a p-value of less than 0.0001. Therefore, not only the platelet count is the thumb marker for dengue detection and severity but also all of these liver parameters may act as diagnostic tools to test the fatality of this disease with high precession and lead a way out for better treatment.

Limitation. Diagnosis of dengue by the indirect conventional methods is not sufficient enough to distinguish dengue serotypes with high specificity. The severity and asymptomatic nature of dengue cases result in a fatal condition with no prior detection. Therefore, to date, there is no proper screening process of DF and ambiguous clinical parameter correlation makes poor understanding. Thus, genetic screening is the best method that needs to be developed in order to identify and predict the severity of the disease.

Future Perspectives. The severe outcome of the dengue NS1 antigen and viral load is associated with the genetic changes. The viral load was depicted to be correlated with other clinical and genetic parameters and secondary dengue infection. Besides this, the identification of different dengue virus serotypes with host-specific genetic backup will provide a clear map of marker allele distribution in different geographical areas. These efficient and rapid diagnosis approaches will provide more time for a better prognosis while also lowering morbidity and mortality rates.

METHODS

Design of the Study. The prospective hospital-based study was conducted in the Nil Ratan Sircar Medical College and Hospital, Kolkata in the Department of Microbiology. A total of 78 patients with DF and associated symptoms were included in this study over a period from March 2021 to March 2022 for 1 year. All patients were classified into three groups as follows: adult males, adult females, and children (<18 years). Children were also divided into males and females. All the participants provided written informed consent for the investigation. The study was approved by the Institute Ethics Committee of Nil Ratan Sircar Medical College & Hospital, Kolkata (Ethical no/NMC/697 dated 10.02.20) abiding by the declaration of Helsinki of World Medical Council.

Sample Collection. The Venus blood sample (3−5 mL) was collected under aseptic conditions from all patients according to inclusion and exclusion criteria and was divided into two vials, plain clot vial and EDTA vial.

Inclusion Criteria. All the patients in OPD with symptoms of DF were diagnosed with positive serology (dengue NS1-positive and IgM-positive).

Sample Processing. *Hematological Analysis.* Blood in the EDTA vial was subjected after homogenization to a fully automated cell counter to detect hematocrit. Platelet count was determined by a Sysmexauto analyzer. AST, ALT, ALP, and T.BIL are to be determined by the KONELAB autoanalyzer.

Serum Separation. A total of 78 blood samples from plain vials were allowed to clot, and the serum is separated.

Serological Analysis. The diagnosis of dengue was performed and confirmed by two ELISAs, namely, NS1Ag[16] (Bhat Bio-Scan dengue NS1 antigen ELISA kit by Bhat Bio-Tech India Pvt. Ltd., Bangalore-560 100, Karnataka, India) and IgM antibody (MAC)[17] (Dengue MAC IgM Capture Microlisa J. Mitra and Co, New Delhi-110 020, India).

RNA Isolation. Serum was used to isolate RNA by a Q1Amp RNA mini kit (Qiagen).[18] The sample was first lysed under

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highly denaturing conditions to inactivate RNAse and to ensure the isolation of intact viral RNA. Buffering conditions were then adjusted for optimum binding of RNA to the QIAamp membrane as the sample was loaded to the QIAamp mini spin column. The contaminants were washed away by using a wash buffer (AW1 and AW2). High-quality RNA can be eluted after adding elution buffer (AVE) for storage.

RT-PCR. Real-time PCR was carried out by using the HELINI dengue virus real-time kit.[19] The HELINI dengue RT-PCR kit contains reagents and enzymes for specific amplification of a highly conserved region of the dengue virus genome. RT PCR reaction was carried out in a CFX 96 thermal cycler. The amplification protocol was set as stated in the kit literature.

Statistical Analysis. The outcomes of continuous variables were depicted as mean and standard deviation. Some categorical variables are described in percentages. Student's *t*-test and χ^2 test for different variables were employed to check the significance of the tests. A *p*-value of less than 0.05 was included as statistically significant. All the statistical calculations were carried out using Graphpad Prism 9 software.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

DF, dengue fever
WHO, World Health Organization
DHF, dengue hemorrhagic fever
NS1, non-structural protein 1
IgM, immunoglobulin M antibody
ELISA, enzyme-linked immunosorbent assay
AST, aspartate transaminase
ALT, alanine aminotransferase
ALP, alkaline phosphatase
T.BIL, total bilirubin
T.PRO, total protein
PCV, packed cell volume
PLT, total platelet count
ALB, albumin

REFERENCES

(1) Organization WH. 2020 Dengue. Dengue South-East Asia [Internet]. 2020;41 (December), Available from: https://www.who.int/publications/i/item/dengue-bulletin-vol-41?sequence=1&isAllowed=y (accessed Nov, 01 22).

(2) Ministry of Health and Family Welfare. National Vector Borne Disease Control Programme, Government of India. http://nvbdcp.gov.in (accessed Dec, 1 22).

(3) Naing, C.; Ren, W. Y.; Man, C. Y.; Fern, K. P.; Qiqi, C.; Ning, C. N.; Ee, C. W. Awareness of Dengue and Practice of Dengue Control Among the Semi-Urban Community: A Cross Sectional Survey. *J. Community Health* **2011**, *36*, 1044−1049.

(4) Itrat, A.; Khan, A.; Javaid, S.; Kamal, M.; Khan, H.; Javed, S.; Kalia, S.; Khan, A. H.; Sethi, M. I.; Jehan, I. Knowledge, awareness and practices regarding dengue fever among the adult population of dengue hit cosmopolitan. *PLoS One* **2008**, *3*, No. e2620.

(5) Malhi, A.; Patel, P. M.; Malhi, N. S.; Avasthi, G. L. Spectrum of Liver Dysfunction in Patients with Dengue Infection and the Markers of Severe Disease: Study from a Tertiary Care Centre in Punjab. *J. Liver Res. Disord Ther.* **2017**, *3*, 95−98.

(6) Murhekar, M.; Joshua, V.; Kanagasabai, K.; Shete, V.; Ravi, M.; Ramachandran, R.; Sabarinathan, R.; Kirubakaran, B.; Gupta, N.; Mehendale, S. Epidemiology of dengue fever in India, based on laboratory surveillance data, 2014-2017. *Int. J. Infect. Dis.* **2019**, *84*, S10−S14.

(7) Agrawal, A.; Kumar, V.; Kumar, S.; Mani, N. A study on prevalence and seasonal distribution of dengue at a tertiary care centre of North India. *Healthline* **2020**, *11*, 100−107.

(8) Rajeshwari, K. G.; Abhinaya, H.; Brunda, M. S.; Mohan, A. Dengue in COVID 19 pandemic year 2020-Enquiry into the unanswered question of 2020? *Indian J. Microbiol. Res.* **2021**, *8*, 123−127.

(9) Mone, F. H.; Hossain, S.; Hasan, M. T.; Tajkia, G.; Ahmed, F. Sustainable actions needed to mitigate dengue outbreak in Bangladesh. *Lancet Infect. Dis.* **2019**, *19*, 1166−1167.

(10) Singla, N.; Chaudhary, P.; Thakur, M.; Chander, J. Dengue: An Analysis of Epidemiological Pattern Over a Six Year Period. *J. Clin. Diagn. Res.* **2016**, *10*, DC12−DC14.

(11) Ralapanawa, U.; Alawattegama, A. T. M.; Gunrathne, M.; Tennakoon, S.; Kularatne, S. A. M.; Jayalath, T. Value of peripheral blood count for dengue severity prediction. *BMC Res. Notes* **2018**, *11*, 400.

(12) Wisanuvej, K.; Boonyawat, K.; Savetamornkul, C.; Virapongsiri, S.; Krongvorakul, J.; Sungkanuparph, S.; Phuphuakrat, A. Comparison between blood hemoglobin concentration determined by point-of-care device and complete blood count in adult patients with dengue. *PLoS Neglected Trop. Dis.* **2021**, *15*, No. e0009692.

(13) Article, O.; Mehta, K. D.; Ghediya, B.; Khandhediya, S.; Shingala, H.; Sinha, M. Study of Correlation Between Platelet Count and Serological Markers of Dengue Infection with Importance of NS1 Antigen in Western Region of India. *Natl. J. Lab. Med.* **2016**, *5*, 55.

(14) Jyothi, P.; Metri, B. C. Correlation of serological markers and platelet count in the diagnosis of Dengue virus infection. *Adv. Biomed. Res.* **2015**, *4*, 26.

(15) Shivkar, R. R.; Padwal, M. K.; Vaidya, A. Correlation of Liver Transaminases with Platelet count in Dengue patients from Tertiary

Care Hospital in Western India. *Int. J. Curr. Res. Rev.* **2018**, *10*, 15−22.
(16) Pal, S.; Dauner, A. L.; Mitra, I.; Forshey, B. M.; Garcia, P.; Morrison, A. C.; Halsey, E. S.; Kochel, T. J.; Wu, S. J. Evaluation of dengue NS1 antigen rapid tests and ELISA kits using clinical samples. *PLoS One* **2014**, *9*, No. e113411.
(17) Martin, D. A.; Muth, D. A.; Brown, T.; Johnson, A. J.; Karabatsos, N.; Roehrig, J. T. Standardization of immunoglobulin M capture enzyme-linked immunosorbent assays for routine diagnosis of arboviral infections. *J. Clin. Microbiol.* **2000**, *38*, 1823−1826.
(18) Fernando, A. N.; Malavige, G. N.; Perera, K. L.; Premawansa, S.; Ogg, G. S.; De Silva, A. D. Polymorphisms of Transporter Associated with Antigen Presentation, Tumor Necrosis Factor-α and Interleukin-10 and their Implications for Protection and Susceptibility to Severe Forms of Dengue Fever in Patients in Sri Lanka. *J. Global Infect. Dis.* **2015**, *7*, 157−164.
(19) Kothandan, S.; Arumugam, S.; Purushothaman, I.; Swaminathan, R. Real time molecular detection of Chikungunya and dengue virus in the female Aedes mosquitoes collected in Chennai, Tamilnadu, India. *Trop. Biomed.* **2017**, *34*, 166−173.

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