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Serological and Biochemical Profiling of Pandemic Dengue Virus in Clinical Isolates During An Outbreak in Dehradun Region

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ABSTRACT

Being the most prevalent, Dengue infection or Dengue fever is a mosquito-borne endemic-epidemic arboviral infection majorly found in the subtropical or tropical regions across the globe. With any of its 4 serotypes (DENV-1, DENV-2, DENV-3, & DENV-4), the dengue virus replicates within the vector; Aedes mosquito and is then transmitted to the human host through the bite. Past few decades have witnessed the escalation in the incidence of dengue infection cases by almost 30 fold. The present study involves the profiling from 60 clinical isolates during the outbreak in the dengue region. The study reveals serological and biochemical profiling of the clinical patient's data. Out of the total studied 60 cases, 68.33% were males and 31.66% were females. The majority i.e. 40% of the cases belonged to the 21-30 years age group. The serological tests for the positive reactivity for antigen NS1 and antibodies IgG and IgM resulted out to be; 63%(NS1), 8%(IgG), 0(IgM), 7%(IgG-IgM), 17%(IgM-NS1), 3%(IgG-NS1), and 2%(IgG-IgM-NS1). The confirmed cases depicted thrombocytopenia.

Keywords: Dengue, DENV serotype, serological, IgG-IgM antibody, NS1 antigen, diagnosis, dengue infection, thrombocytopenia.

INTRODUCTION

Dengue virus, whose transmission is done by mosquitoes, is a menace to nearly half of the global population. The past 50 years have witnessed an increase in the incidence of dengue virus fever by 30-fold with the increase in the expansion of the geographical areas to many new tropical countries (Dengue Bulletin 2016). Beyond 70% (counting around 1.8 billion) of the worldwide population is at a risk of dengue virus living presently in the South-East Asia region & the Western Pacific Region, enduring around 75%burden of the dengue disease worldwide (WHO Guidelines 2009). According to WHO, a Bi-Regional Dengue Strategy (2008-2015) for the prevention and control of the illness was implemented across the South-East Asia & Western Pacific regions; containing major six elements including, integrated vector management, the

surveillance of dengue, social mobilization, managing the cases, the response to the outbreak, & communication for dengue and its research (WHO/SEARO 2008).

In the year 2020, dengue has been found to affect many countries, with a large increase of cases in Bangladesh, Cook Islands, Brazil, Ecuador, Indonesia, India, Maldives, Mauritania, Nepal, Mayotte (Fr), Sri Lanka, Singapore, Sudan, Thailand, Timor-Leste & Yemen. In 2021, the effects of dengue continued in Brazil, Colombia, Cook Islands, Kenya, Fiji, Paraguay, Peru & Reunion Island. In 2019, the largest number of dengue cases have been ever reported worldwide. About 3.1 million cases were alone reported in the American Region, with more than 25,000 categorized as severe although the deaths associated with were lesser than in the previous year. (WHO 2019)

Being a small single-stranded RNA virus, the endemic tropical dengue virus (DENV) consists of 4 distinct serotypes which are DENV-1, DENV-2, DENV-3, and DENV-4. These serotypes associated with the genus Flavivirus and family Flaviviridae, are closely linked to each other. Amongst these, all serotypes, DENV-2 and DENV-3 also known as 'Asian Genotypes' are linked to the severe diseases convoying the secondary dengue infections (Leitmeyer, 1999; Lanciotti et al., 1994; Messer, 2003). Dengue fever is a vector-borne illness, the transmission of these viral serotypes to humans is caused by the bite of tropical mosquito *Aedes aegypti*. Possessing all together with a different ecology, behavior, geographical distribution, other species like *Aedes albopictus*, *Aedes polynesiensis* and species of the *Aedes scutellaris* complex have been reported to be contributing to the dengue virus outbreak (Halsey et al., 2012; Guzman et al., 2010). In past decades, outspread of *Aedes albopictus,* has been found from Asia to Africa, Europe, and America. They are borne in usually manmade products in which some amount of water can stay stagnant such as tyres, their tubes, flower vases, water tank lids, and tubs or mugs in homes. Once eggs are laid, they supposedly stay viable for several months even in the water absence. When these infected mosquitoes bite humans for their blood meal, they transmit the virus into the person through their saliva. As soon as the virus enters into the body, it causes infection of immune cells in the skin tissue & enters in the lymphatic system, which then elicits a strong inflammatory response (Halstead, 1974). After this reaction, Viremia takes place; which refers to the spread of infection into full bloodstream which was initially local virus replication at the site of infection.

Having a wide range spectrum of clinical profiling, dengue has found to be often unpredictable clinical conclusions. Though maximum patients attain recovery by following a non-severe clinical course, a few percent of patients forge ahead to severe illness, basically characterized by plasma leakage which can be with or without haemorrhage. This is then referred to as Dengue haemorrhagic fever (DHF). When the mosquito bites a pre-infected person, it draws in the dengue virus contained in the blood. By biting the other person, it transmits the virus and hence spreads the disease (WHO 1997; Azin et al., 2012; Deubel et al., 2001).

The laboratory tests that can be accessed for diagnosing dengue fever &DHF include Virus isolation (serotypic/ genotypic characterization), detection of viral nucleic acid, detection of viral antigen (NS1 Antigen), Immunological response-based tests (IgM & IgG antibody assays), Analysis for haematological parameters (Peeling et al., 2010; Vorndam and Kuno 2001; Shu et al., 2003). The present investigation aims to study dengue virus patients in an outbreak in the Dehradun region by the serological and biochemical profiling in clinical isolates.

MATERIALS AND METHODOLOGY

Study Design

This study was conducted at DNA Lab – A Centre for Applied Sciences (DLCAS), Patthribagh, Dehradun, Uttarakhand. This analytical study of 60 patients suffering from Dengue infection was carried out in the outbreak of the endemic season of dengue fever in the Dehradun region. Ethical Clearance was attained from the laboratory and informed consent was taken from the patients. Patients belonging to almost all age groups and belonging to both sexes were selected and included in the study group. In the present scenario, the serological test is performed, for the confirmed diagnosis of dengue infection such as detection of NS1 antigen of dengue which has 76% sensitivity and 98% specificity, or either the dengue antibody which is IgG and IgM which has 90% sensitivity and 93% specificity by the ELISA method (Prevention C.F.D.C.A., 2012) (Kuno et al., 1991). Several CBC parameters such as Total Leukocyte Count (TLC), White Blood Cell (WBC) Count, Red Blood Count (RBC) Count, Haemoglobin (Hb) were analyzed in the patients which were dengue infection positive.

Simultaneous detection of Dengue NS1 Antigen, & IgG and IgM antibody test were performed to diagnose the dengue viral infection in the patients using SD BIOLINE Dengue Duo; Dengue NS1+Ab Combo (11FK45, 11FK46) test kit. The SD BIOLINE Dengue Duo Rapid Test is an *invitro* immune-chromatographic, in which detection of virus antigen (NS1), and antibody IgG & IgM is performed through a one-step assay with the dengue virus in human serum, plasma or whole blood (Falconar et al., 2006; Chanama et al., 2004). The test is proposed to

be used professionally, in order to utilize the presumptive diagnosis of primary &secondary dengue infections. The SD BIOLINE Dengue Duo issues a preliminary test result. Specific substitute diagnostic methods are used to attain confirmation of dengue virus infection. This may include virus isolation, detection of antigen in fixed tissues, RT-PCR &serological testing such as hemagglutination inhibition (Saxsena et al., 2008).

When serum is added to the specimen well, NS1 Antigen present in the specimen reacts with the mouse monoclonal anti-dengue Ab-colloidal gold conjugates, forming a complex of antibodies and antigens. The complex relocates and is detected by the test device through chromatography.

Specimens for the respective tests are collected separately such that if the specimen is whole blood it is been collected in a tube carrying anticoagulants including heparin, EDTA & sodium citrate (stored up to 3 days only at 2-8˚C); and this sample can be centrifuged to obtain plasma. If the test that is to be performed is serum-based, then the serum sample is collected in a tube containing no anticoagulants. It is then left to coagulate for 30 mins and centrifuge to obtain serum supernatant and can be stored up to2 weeks below -20˚C.

Clinical Profile

The rapid card test is performed in order to detect the antigen NS1 and the antibodies IgG and IgM, which indicates the dengue infection. For Dengue NS1 antigen, 3 drops (100µl) of sample is subjected to well S. For Dengue IgG/IgM antibodies, 10µl of specimen with capillary tube is subjected in well S. About 90-120 µl of assay diluent is added to round assay diluent well. Results are elucidated after 15-20 minutes. Furthermore, haematological parameters involving haemoglobin (Hb), total leukocyte count (TLC), haematocrit (HCT), platelet count was also recorded.

RESULTS AND DISCUSSION

With the changing climatic conditions, poor living habits, inefficient ways of waste management, and the factors that involve industrialization and urbanization, the rates of findings of vector-borne diseases such as dengue fever have become more common. The dengue-infected patients exhibit some localized signs & symptoms and their clinical profile may seem like some other infections. Therefore, it becomes a bit challenging for the clinician to distinguish between the infections and make it an essential way to do a differential diagnosis. The clinical symptoms that were observed frequently included fever, body ache, headache, rashes, edginess, anorexia abdominal pain, and nausea (Patel et al., 2018; Chaloemwong et al., 2018).

The present study reveals that the Dehradun region was more prone to dengue infection during the course of the study along with the maximum patient population found to be in the 21-30 years age group. Along with this, the study data reports about the thrombocytopenia found in 56.67% cases. As the constant findings in dengue infection basically involves thrombocytopenia and haemoconcentration. During the early febrile phase, the white blood cells(WBC) or Total leuckocyte Count(TLC) maybe normal inclusive of the predominant neutorphils, which is gradually decreased with increasing infection stages and reaching to the end of the febrile phase (World Health Organisation, 2011; Muller et al., 2017). Following this, towards the end of febrile phase, sudden drop in the levels of platelet count below the normal range of 1.50-4.00 Lac/mm3, which usually stays normal in the early febrile phase. In our study, the majority of pateints diagnosed with dengue in the data depicted had platelet count levels dropping to 0.95 Lac/mm3, 0.89 Lac/mm3, 0.88 Lac/mm3, 0.5 Lac/mm3, etc. which clearly supports the context mentioned.

Patients diagnosed had clinical symptoms such as bleeding diathesis, headache, loss of appetite, & nausea, wherein the dengue was being serologically confirmed. The number of male patients were 41 out of 60 studied cases (68.33%) which were higher than the female with a number of 19 patients (31.66%) (Figure 1; Table 1).

Table 1:Number of male and female patients.

Total Cases n(%)	Males n(%)	Females n (%)
60(100%)	41(68.33%)	19(31.66%)

The study comprised of dengue patients of age groups ranging from 1-10 age group to 51-60 age group. The maximum number of patients i.e. 24 dengue patients (40% cases) lied in the 21-30 age group. Other age groups included, 1 patient (1.66%) in 1-10 age group, 15 patients (25%) in 11-20 age group, 9 patients (15%) in 31-40 age group, 6 patients (10%) in 41-50 age group and 5 patients (8.33%) in 51-60 age group (Figure 2; Table 2).

Table 2:Total number of cases following in different age groups.

Total Cases	Number of Case	Percentage
01-10 yrs.	1	1.66%
11-20 yrs.	15	25%
21-30 yrs.	24	40%
31-40 yrs.	9	15%
41-50 yrs.	6	10%
51-60 yrs.	5	8.33%

Table 3:Confirmed laboratory cases based on haemoglobin values.

Total Cases	Number of Cases	Percentage
Hb (< 12-15g/dl)	11	18.33%
Hb-Normal (12-15g/dl)	45	75%
Hb (> 12-15g/dl)	4	6.66%

The study covered analysis made on Haemoglobin values in dengue patients. The normal range for the haemoglobin value ranges between 12-15g/dl. It was learnt from the data that the maximum number of patients (45 patients; 75% patients of total cases) had a normal ranging haemoglolin value. Whereby, 18.33% of total cases (11 patients) had the Hb values below the normal range and 6.66% cases (4 patients) had Hb values above the normal range (Figure 3; Table 3).

The serologic result for dengue patients was found positive for NS1antigen in 63% (38/60), dengue IgG antibody in 8% (5/60), and dengue IgM antibody in no case. Whereby, the positivity for the antibody IgM and antigen NS1 was observed in 17% (10/60), for IgG and antigen NS1 in 3% (2/60), IgM and antibody IgG in 7% (4/60) and positive for all the three parameters in 2% (1/60) (Figure 4; Table 4).

Table 4:Number of cases based on IgG, IgM, NS1 positivity.

Reactivity of cases	IgG Reactive	IgM Reactive	NS1 Reactive	IgG-IgM Reactive	IgG-NS1 Reactive	IgM-NS1 Reactive	IgG-IgM-NS1 Reactive
Number of cases	5	0	38	4	2	10	1

Table 5. Clinical profiling including TLC count and platelet count of total confirmed cases.

Age/Gender	Clinical Profiling		Age/Gender	Clinical Profiling	
	TLC (4000-11000/ mm3)	PLT (1.50-4.00 Lac/ mm3)		TLC (4000-11000/ mm3)	PLT (1.50-4.00 Lac/ mm3)
21/M	3600	1.21	28/M	3600	2.05
21/M	3500	2.11	06/F	8700	1.32
23/F	4000	1.67	20/M	4400	1.2
25/F	7200	1.97	35/M	4800	0.89
45/M	2400	0.97	20/M	8700	2.23
30/M	3200	1.58	15/F	3200	1.95
27/M	5000	0.5	36/M	4000	1.51
34/M	3400	1.4	17/M	4600	0.97
27/F	3200	1.93	17/F	3800	1.4
23/F	7300	2.31	22/M	7700	2.02
23/M	6100	2.04	45/M	2500	1.15
15/M	5200	1.35	52/F	4700	2.36
58/F	4600	1.4	23/M	4900	1.92
53/M	3500	0.88	17/M	3000	1.1
23/F	3800	1.38	38/M	3500	1.5
40/M	6000	1.3	17/M	5900	2.45
45/F	4200	2.13	23/F	4400	2.19
24/M	4200	1.2	30/F	4300	2.76
35/M	3500	1.48	43/F	4200	1.66
37/M	4600	1.4	18/F	4400	1.75
47/M	3300	1.2	19/M	4200	2.38
38/M	3600	0.96	21/M	3000	1.3
28/F	3000	1.34	60/F	3000	0.96
46/M	3800	1.5	26/M	3500	1.5
20/M	6500	1.81	20/M	3200	1.43
17/M	2800	1.34	59/M	3500	1.1
20/M	4600	1.16	12/F	2500	0.95
23/M	5700	1.2	27/M	12000	2.42

In the present study, around 56.67% of patients (34 cases) out of 60 were found suffering from thrombocytopenia out of which 26 cases were males and 8 cases were female; the rest i.e. 26 cases (43.33%) had platelet count (PLT) between the normal range 1.50-4.00 Lac/mm3 (Table 5). The variation in the Platelet count value in males and females represented different graph lines (Figure 5 A; 5 B). The total leukocyte count (TLC) in the patients data was observed below the normal range 4000-11000/mm3 in 46.67% cases (28 patients) and the majority i.e. 31 cases lied in the normal range (Table 5). The variation in the total leukocyte count (TLC) value in males and females represented different graph lines (Figure 6 A; 6 B).

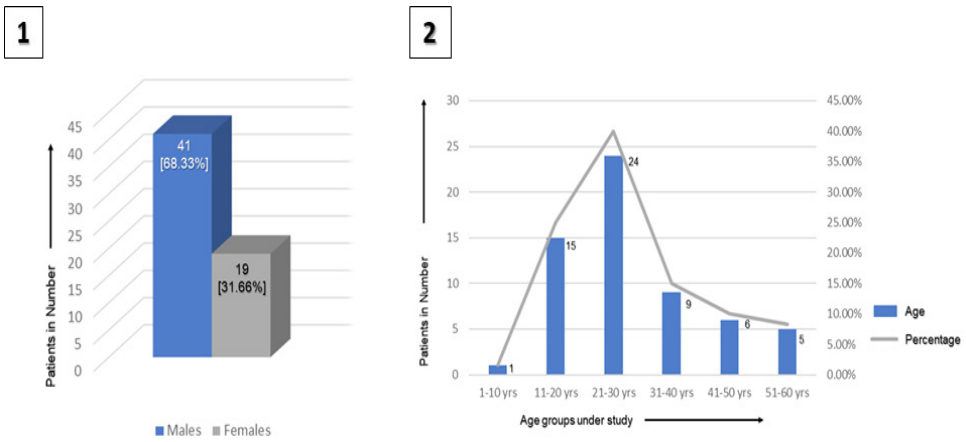
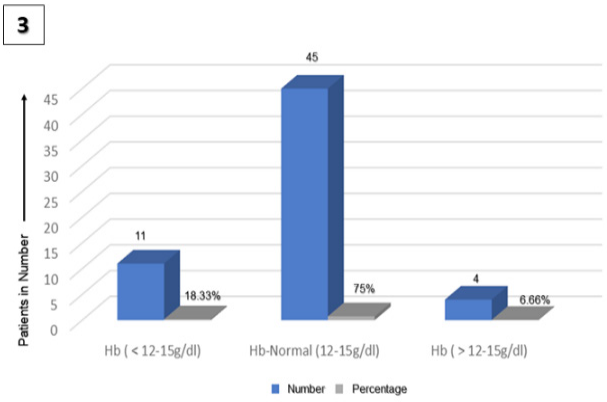


Figure 1. Graphical representation of confirmed cases in males and females.

Figure 2: Graphical representation of diagnosed dengue patients according to age group variation.



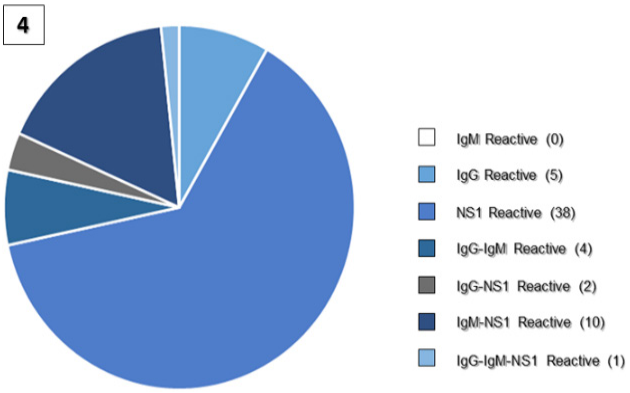
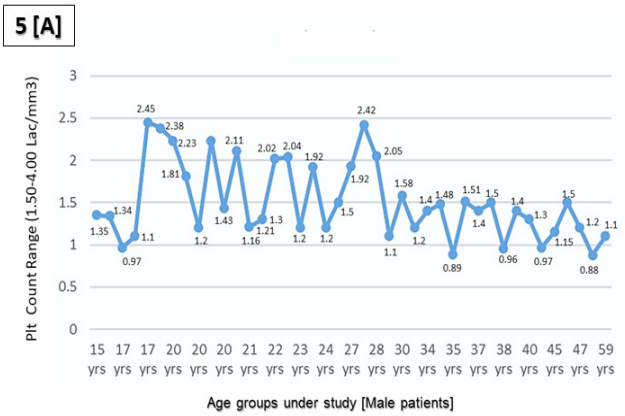


Figure 3: Graphical representation depicting the maximum number of cases in the normal haemoglobin range.

Figure 4: Illustration of the serological result of dengue patients based on NS1 antigen, IgG antibody, and IgM antibody.



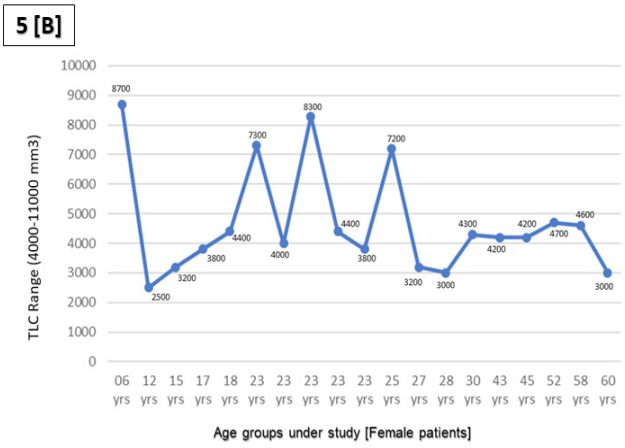


Figure 5: Graphical Representation showing variation in platelet count (A: Male patients; B: Female Patients).

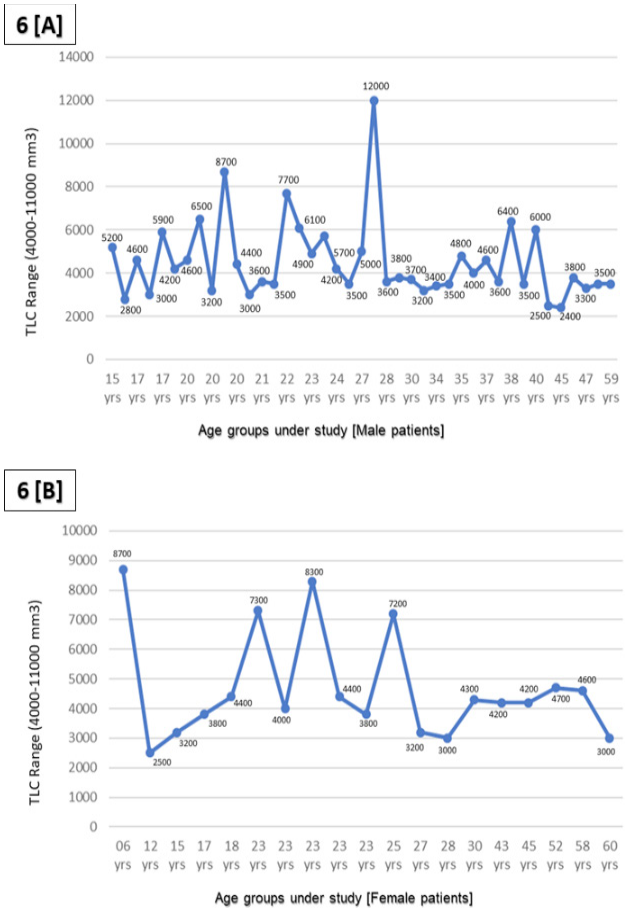


Figure 6: Graphical Representation showing variation in TLC Values (A: Male patients; B: Female Patients).

CONCLUSION

Being a mosquito-born illness, dengue infection is more subjected to areas that belong to subtropical or tropical regions, wherein mosquitoes are found quite more than often. Other factors that could lead to such viral infection involve poor health and hygiene practices, damp areas, globalization, and dumping of waste and wastewaters, etc. In order to control such viral infection several precautions such as making a clear check on wastewater, stagnant waters in surrounding and majorly practicing good health and hygiene; should be taken. From the present study, we can conclude about the significance of the proper diagnosis of dengue infection based on different diagnostic markers. The findings reveal the significance of dropping TLC values and Platelet count (i.e. thrombocytopenia). Along with thrombocytopenia, the patients suffering from dengue virus should be more aware of the levels of the haematocrit that gradually increases during the infection, as increasing heamocrit values and decreasing platelet count (i.e. thrombocytopenia) are the two major factors that are seen in dengue infection. The future holds more opportunities based on technology advancements for the further analysis of dengue virus infection and its treatment on the basis of different diagnostic tests.

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