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Original Research Article

ASSOCIATION OF SEROLOGICAL TESTS WITH CLINICAL AND BIOCHEMICAL PROFILE IN DENGUE PATIENTS AT A TERTIARY CARE HOSPITAL, MAHARASHTRA

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Abstract

Background: Dengue virus infection is a major source of illness and mortality in the majority of tropical and subtropical nations. With the rising incidence of dengue infection, an early diagnostic confirmation of dengue infection in patients promotes timely clinical intervention, etiological investigation, and disease control. The purpose of this study is to characterize the laboratory and clinical features of dengue patients who appear at a tertiary care hospital in order to observe the presenting trend. The aim of this research was to evaluate the seroprevalence of NS1, IgM, and IgG using commercially available serological test kit (Rapid diagnostic test) and to study the association of seropositivity with clinical and biochemical profile in dengue patients. **Materials and Methods:** Present study was prospective study. It included 37 seropositive dengue patients that were verified by commercially available dengue rapid diagnostic assays. Cases are classified as either dengue negative (NS1, IgM, and IgG all negative or only IgG positive) or dengue positive (NS1 and/or IgM positive). Three groups were created from the dengue-positive cases: only NS1 positive, only IgM positive, and both NS1 and IgM positive. The clinical, biochemical, and hematological characteristics of all patients were examined and contrasted across the three groups. **Result:** The seropositivity rate for NS1/IgM was 100% while seropositivity rate for NS1 alone was 86.5%, for IgM alone was 18.9% and for IgG alone was 5.4%. The mean age of patients was 29.86 ± 13.292 indicating a higher incidence of dengue fever in the 4th decade of life. A predominance of males (70.3%) was reported in seropositivity as compared to females (29.7%). No significant difference in the clinical manifestations, warning signs of dengue and most of biochemical parameters was found when the seropositivity was analyzed for the NS1/IgM, NSI alone and IgM alone. Bilirubin and BUN was found to be significantly higher in IgM positive cases as compared to NS1/IgM or NSI alone. **Conclusion:** The results of this study indicated that a combination of IgM antibody and dengue NS1 antigen testing might improve the detection rate of dengue infection. This combination might improve the effectiveness and help detect dengue infections early. Clinical manifestations, warning signs of dengue and biochemical parameters remain comparable when the seropositivity is analyzed based upon combined NS1/IgM or alone NSI or IgM.

INTRODUCTION

Dengue is a common arboviral disease. A flavivirus from the Flaviviridae family is the cause of this acute viral illness.[1] Dengue virus has a positive sense single stranded RNA viral genome.[2] Humans get dengue through the bite of an infected Aedes mosquito, with Aedes aegypti being the most prevalent vector.[3] In recent decades, dengue has become much more common worldwide. Rapid urbanization and development, which give A. aegypti breeding grounds, are among the main causes of this

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rise in incidence. Modern air travel and foreign trade, such as motor vehicle tires, also contribute to the spread of illness by making it easier for infected people and mosquito larvae to travel to non-affected areas. This poses a risk of spreading the virus and its vector. More than 100 nations in Africa, the Americas, the Eastern Mediterranean, the Western Pacific, and especially South East Asia today have endemic cases of the dengue.[4]

In many regions of India, dengue is endemic, and outbreaks are often reported both domestically and internationally. According to estimates from the World Health Organization (WHO), 390 million cases of dengue occur each year, putting over 2.5 billion individuals at risk of contracting the disease.[5] In India, dengue is the primary cause of hospitalization for individuals in the majority of states. Dengue was mostly found in cities a few decades ago, but it is now also prevalent in rural regions.[6] Human diseases ranging from self-limiting to potentially fatal dengue hemorrhagic fever and dengue shock syndrome (DHF/DSS) can be caused by any of the four dengue serotypes (DENV-1, DENV-2, DENV-3, and DENV-4). About 20% of infections exhibited the typical dengue fever pattern, while the majority of DENV infections remain asymptomatic. Fever and a number of ambiguous signs, including headache, malaise, weakness, rash, and body pains, were involved.[7] A small percentage of dengue infections develop into its more severe variants, such as DHF and DSS, which are distinguished by petechiae, hypovolemia, and increased microvascular permeability.[8]

The onset of fever causes a change in the blood profile of dengue patients. Thrombocytopenia frequently occurs in 3–8 days as a result of plasma leakage, and is followed by leukopenia and hemoconcentration.[9] Compared to influenza, enteroviruses, and leptospirosis, dengue is more commonly linked to acute febrile leukopenia and positive tourniquet tests.[10] One of the least researched side effects of dengue is acute kidney damage (AKI), which is associated with a lengthy hospital stay and a high death rate. Hemodynamic changes brought on by cytokine storm throughout the clinical phase of dengue fever are probably what cause renal damage.[11] In addition, this cytokine storm causes endothelium damage and complement system activation, which raise vascular permeability and cause hemoconcentration, which results in shock, decreased renal perfusion, and kidney damage. For an accurate diagnosis and effective patient management, a precise clinical and laboratory profile is necessary.[12]

Given the rising prevalence of dengue infection, prompt clinical intervention, etiological investigation, and illness management are made possible by early diagnostic confirmation of dengue infection in patients. Therefore, early dengue diagnosis is essential for the recovery of patients. Not only is early dengue virus diagnosis essential for reducing illness burden, but it also helps restrict disease propagation. RT-PCR, non-structural protein 1 (NS1) antigen detection, and IgM detection were popular early detection techniques.[13] The NS1 is a highly conserved glycoprotein and is produced in both a membrane-associated form and in secreted forms.[14]

From Day 1 to Day 9 following the beginning of fever in a sample of primary or secondary dengue-infected patients, the NS1 is detected in high amounts in the sera of patients with dengue during the early clinical phase of the illness.[15] In the event of a primary dengue infection, IgM becomes detectable on Days 3 to 5 of sickness and lasts for two to three months, whereas IgG appears by Day 14 and lasts for life. When secondary infection occurs, IgG and IgM antibodies rise one to two days after symptoms start. As a result, individuals who have secondary infections will typically have positive IgG results but not necessarily positive IgM results. [16,17] This study assessed the potential use of a commercially available Dengue Day 1 Test, a rapid solid phase immuno-chromatographic test, for the early diagnosis of acute dengue virus infection based on a single acute serum sample. The test is intended for the detection of dengue NS1 antigen and the differential detection of IgM and IgG antibodies.

MATERIALS AND METHODS

Study design: Present study was prospective study. The study was conducted from January 2021 to December 2021. It included 37 seropositive dengue patients of tertiary care hospital of Maharashtra. Patients were verified by commercially available dengue rapid diagnostic assays. Cases are classified as either dengue negative (NS1, IgM, and IgG all negative or only IgG positive) or dengue positive (NS1 and/or IgM positive). Three groups were created from the dengue-positive cases: only NS1 positive, only IgM positive, and both NS1 and IgM positive. The clinical, biochemical, and hematological characteristics of all patients were examined and contrasted across the three groups.

Data collection: The findings of the liver function, renal function, and hematological tests were taken from the laboratory databank. The clinical profile information was gathered from the medical records of patients. Patients with known chronic renal disease, liver illness, or fever of another known infectious cause were excluded from the research. A standardized proforma that was self-designed was utilized to record patient demographic information, clinical symptoms, and hematological and biochemical measurements.

Laboratory Analysis: The DengueNS1Ag + Ab combination kit, an ICT-based rapid diagnostic test kit with 96.5% sensitivity and 100% specificity for IgM and 96.7% sensitivity and 100% specificity for IgG, was used to diagnose dengue in laboratories. The autoanalyzer analyzed hematological parameters, liver function, and renal function tests.

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The aseptic method and standard protocol were followed for handling and processing the samples.

Statistical analyses: The data was entered in the spreadsheet and then analyzed using the SPSS software. Mean and standard deviation was calculated for quantitative variables whereas qualitative variables are represented in number and percentage. Chi-square test was used to compare two qualititative variables while one way ANOVA was used to compare means. All test were performed by taking the p value <0.05 as statistically significant.

RESULTS

Total 37 patients included in the study who exhibited the seropositivity on NS1/IgM. The seropositivity rate for NS1/IgM was 100% while seropositivity rate for NS1 alone was 86.5%, for IgM alone was 18.9% and for IgG alone was 5.4% [Table 1].

Table 1: Seropositivity rate for NS1/IgM, NS1, IgM, and IgG.

Seropositivity	Number	Percentage
NS1/IgM	37	100
NS1	32	86.5
IgM	7	18.9
IgG	2	5.4

The mean age of total 37 patients who are positive on NS1/IgM was 29.86 ± 13.29 years indicating a higher incidence of dengue fever in the 4th decade of life. A predominance of males was reported in seropositivity as compared to females. Total 70.3% males were seropositive while 29.7% females were found to be seropositive indicating a higher incidence of seropositivity among male gender. No significant difference in the age and gender distribution was found when the seropositivity was analyzed for the NS1/IgM, NSI alone and IgM alone [Table 2].

Table 2: Age and gender distribution in dengue seropositive cases.

Variable	Domain	NS1/IgM positive		NS1 positive		IgM positive		P Value
		Mean or N	SD or %	Mean or N	SD or %	Mean or N	SD or %	
Age	Mean age	29.86	13.29	29.34	12.83	32.57	17.86	0.849
Gender	Male	26	70.3	23	71.9	4	57.1	0.740
	Female	11	29.7	9	28.1	3	42.9	

Among total 37 patients who are positive on NS1/IgM, clinical manifestation includes orbital pain (62.2%), rash (35.1%), nausea (64.9%), vomiting (78.4%), headache (97.3%), fever (100%), joint pain (35.1%), and muscular pain (24.3%). No significant difference in the clinical manifestations was found when the seropositivity was analyzed for the NS1/IgM, NSI alone and IgM alone [Table 3].

Table 3: Clinical manifestation in dengue seropositive cases.

Clinical Manifestation	NS1/IgM positive		NS1 positive		IgM positive		P Value
	N	%	N	%	N	%	
Orbital pain	23	62.2	20	62.5	4	57.1	0.946
Rash	13	35.1	12	37.5	2	28.6	0.902
Nausea	24	64.9	20	62.5	6	85.7	0.495
Vomiting	29	78.4	24	75	7	100	0.337
Headache	36	97.3	31	96.9	7	100	0.895
Fever	37	100	32	100	7	100	NA
Joint pain	13	35.1	9	28.1	5	71.4	0.095
Muscle pain	9	24.3	7	21.9	2	28.6	0.923

Warning signs of dengue fever in 37 patients who are positive on NS1/IgM includes abdominal pain (35.1%), persistent vomiting (29.7%), respiratory distress (40.5%), restlessness (2.7%), lethargy (54.1%), hepatomegaly (8.1%), and fatigue (8.1%). Mucosal bleed or hematemesis was not reported in any case. No significant difference in the warning signs of dengue was found when the seropositivity was analyzed for the NS1/IgM, NSI alone and IgM alone [Table 4].

Table 4: Warning signs in dengue seropositive cases.

Warning Signs	NS1/IgM positive		NS1 positive		IgM positive		P Value
	N	%	N	%	N	%	
Abdominal Pain	13	35.1	10	31.3	4	57.1	0.430
Persistent vomiting	11	29.7	8	25	4	57.1	0.244
Respiratory distress	15	40.5	12	37.5	4	57.1	0.631
Restlessness	1	2.7	1	3.1	0	0	0.895
Lethargy	20	54.1	16	50	5	71.4	0.588
Hepatomegaly	3	8.1	3	9.4	3	42.9	0.280

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Mucosal bleed	0	0	0	0	0	0	NA
Fatigue	3	8.1	1	3.1	2	28.6	0.077
Hematemesis	0	0	0	0	0	0	NA

Biochemical parameters in 37 patients who are positive on NS1/IgM indicate mean platelets was 20960.98 ± 36212.46, WBC was 5402.09 ± 6109.47, neutrophils were 62.32 ± 13.75, lymphocytes were 32.08 ± 13.16, hemoglobin was 12.31 ± 1.79, hematocrit was 38.72 ± 5.51, AST was 94.95 ± 94.17, ALT was 82.89 ± 72.84, ALP was 95.51 ± 40.74, bilirubin was 0.64 ± 1.04, creatinine was 0.68 ± 0.15, and BUN was 25.35 ± 8.61. Bilirubin and BUN was found to be significantly higher in IgM positive cases as compared to NS1/IgM or NSI alone while rest of the biochemical parameters showed no significant difference among three groups [Table 5].

Table 5: Biochemical parameters in dengue seropositive cases.

Biochemical parameters	NS1/IgM positive		NS1 positive		IgM positive		P Value
	Mean	SD	Mean	SD	Mean	SD	
Platelets	20960.98	36212.46	21673.41	36680.48	24574	42026	0.972
WBC	5402.09	6109.47	6245.00	6157.81	492.3	1282	0.070
Neutrophils	62.32	13.75	60.47	13.51	66.00	19.46	0.626
Lymphocytes	32.08	13.16	32.97	12.39	33.43	21.09	0.950
Hemoglobin	12.31	1.79	12.51	1.76	11.69	1.85	0.539
Hematocrit	38.72	5.51	39.45	5.30	36.63	5.93	0.460
AST	94.95	94.17	88.75	75.22	114.00	154.40	0.809
ALT	82.89	72.84	72.28	59.78	120.60	107.70	0.272
ALP	95.51	40.74	90.66	33.60	115.10	60.79	0.346
Bilirubin	0.64	1.04	0.45	0.27	1.54	2.24	0.036*
Creatinine	0.68	0.15	0.67	0.15	0.74	0.11	0.557
BUN	25.35	8.61	24.19	7.39	34.57	12.91	0.017*

DISCUSSION

In tropical and subtropical regions of the world, including India, dengue infection has become a serious public health concern due to its rising incidence. Timely clinical intervention and illness control are made possible by early diagnostic confirmation of dengue infection in patients. To diagnose dengue infection, a number of laboratory techniques are available, including NS1 Antigen detection, IgM and IgG antibody detection, viral isolation, and RNA detection. However, techniques like RT-PCR and viral isolation require a specialized facility and skilled workers. However, a lot of labs with little funding don't have RT-PCR or viral culture capabilities. The potential use of NS1 antigen and IgM antibody detection for the early diagnosis of dengue fever has been examined in this study.[5]

From the first day following the beginning of fever until day nine, the patient serum contains a lot of the NS1 antigen, which is thought to be a biomarker for early dengue diagnosis. In the event of a primary dengue infection, the IgM antibodies are detected on Days 3 to 5 of sickness and last for two to three months. The quick detection of dengue NS1 antigen and the differential identification of IgM and IgG antibodies were accomplished in this study using a commercially available rapid solid phase immuno-chromatographic technique. This test may be conducted in an outpatient clinic, is easy to use, and aids in quick diagnosis. Notifying public health authorities and managing patients are aided by early detection.[5]

In this study, the seropositivity rate for NS1/IgM was 100% while seropositivity rate for NS1 alone was 86.5%, for IgM alone was 18.9% and for IgG alone was 5.4% s. This is similar to a prior study that shown a combination of tests would improve the rate of dengue disease diagnosis.[5,18] Previous study by Tuladhar et al. involved assessment of dengue seropositivity indicated that 184 patients were seropositive for NS1, 75 were seropositive only for IgM, and 87 were seropositive for both NS1 and IgM.[12]

In this study, we found that the mean age group affected was 29.86 ± 13.29 years. This was consistent with the other studies on dengue in India.[5,19,20] Other studies have also found a greater prevalence of dengue in the same age range.[21-23] In present study, A predominance of males (70.3%) was reported in seropositivity as compared to females (29.7%). There were somewhat more females (n = 190, 54.9%) with dengue infection than men (n = 156, 45.1%) in the earlier research by Tuladhar et al.[12] In present study, clinical manifestation includes orbital pain (62.2%), rash (35.1%), nausea (64.9%), vomiting (78.4%), headache (97.3%), fever (100%), joint pain (35.1%), and muscle pain (24.3%). This was in accordance to the study reported by Murmu et al,[24] Mangudkar et al,[25] and Agrawal et al.[26] Fever was the most common symptom (94.5%), followed by headache (79.8%), myalgia (74.7%), and nausea (58.5%), according to the study by Tuladhar et al.[12]

In present study, Bilirubin and BUN was found to be significantly higher in IgM positive cases as compared to NS1/IgM or NSI alone while rest of the biochemical parameters showed no significant difference among three groups. According to Tuladhar et al. study, dengue-positive patients were substantially correlated with thrombocytopenia, reduced leukocyte counts with neutropenia, lymphocytosis and monocytosis, higher SGPT and

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SGOT, increased urea and decreased albumin, and increased prothrombin time.[12]

The RT-PCR test is sensitive and capable of differentiating between dengue virus serotypes. Nevertheless, this test cannot differentiate between several dengue virus serotypes. Since most labs lack the funding necessary to establish a PCR lab, the NS1 antigen need to be taken into consideration as an extra diagnostic method for early dengue virus infection. The main limitation of this study is that it used a quick diagnostic procedure based on ICT for testing. Resource limitations prevented the use of more precise and sensitive techniques, such as molecular and enzyme-linked immunosorbent assays. We were unable to classify the patients based on their severity because we lacked the necessary patient data to classify dengue fever. We recommend more study to determine which viral serotypes are associated with dengue complications throughout the outbreak season.

CONCLUSION

Serological tests and clinical characteristics are significantly important in the evaluation of the current diagnostic and therapeutic strategies developed for the prevention of dengue outbreak. A nationwide awareness campaign is need to be conducted to promptly visit the hospital and do laboratory tests in order to be alert for any potential dengue infection complications.

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