Original Article

Seroprevalence and incidence of primary dengue infection and its correlation with fetomaternal prognosis in Western Rajasthan

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Abstract

Background: Viral hemorrhagic fevers are becoming increasingly common in the tropics and subtropics. Dengue fever is currently the most important arthropod-borne viral disease because of its widespread distribution in more than 100 countries and its potential for extensive outbreaks of life-threatening disease. **Material and Methods:** This study was a hospital-based cross-sectional study conducted in the Microbiology Laboratory of Maternal and Child Tertiary Care Hospital in Western Rajasthan, India, between January 2021 and December 2021. Institutional Ethical Committee permission was obtained. All patients with clinical suspicion of dengue-like illness (DLI), attending outpatient department (OPD) or inpatient department (IPD), were included in the study after obtaining their written consent. A blood sample was collected, and the Dengue Duo rapid card test was conducted for the detection of nonstructural protein 1 (NS1) antigen and immunoglobulin (Ig) M or IgG antibody estimation. All positive samples were tested for IgM enzyme-linked immunosorbent assay (ELISA) test using MAC-ELISA. **Results:** Of 250 positive sample, the distribution of cases as per clinical features was as follows: all cases presented with fever (100%) followed by myalgia (24.5%), headache (16.06%), hemorrhagic manifestation (13.25%), rash (8.84%), and bleeding gums (2.01%). Thrombocytopenia was seen in 30.40% (76/250) of dengue fever cases. NS1 antigen was detected in 157 cases (62.80%) followed by IgG in 84 cases (33.60%), IgM in 77 cases (30.80%), NS1+IgG in 27 cases (10.80%), NS1 + IgM in 16 cases (6.40%), and NS1 + IgM + IgG in five cases (2%). Of 250 samples, 77 cases were IgM positive and 173 were IgM negative by the Dengue Duo card test. Among the 173 Dengue Duo IgM card negative, 131 cases (79.39%) were also detected negative by IgM ELISA and 42 cases (49.41%) were detected positive by IgM ELISA. The sensitivity was 50.59%, the specificity was 79.39%, the positive predictive value (PPV) was 55.84%, the negative predictive value (NPV) was 75.72%, and the diagnostic accuracy was 69.90%. The case fatality of the cases was 2.35%. **Conclusion:** Early diagnosis and treatment can prevent mortality in pediatric and pregnant females suffering from dengue and dengue-like illness. Facility and availability of ELISA kits should be adequate for early confirmation of suspected dengue patients by ELISA test.

Keywords: Dengue Duo rapid card, dengue-like illness, dengue virus, ELISA, fetomaternal mortality

Introduction

Dengue is a vector-borne viral disease belonging to the genus Flavivirus, transmitted by Aedes mosquitoes.[1,2] Dengue virus (DENV) has four different serotypes[3]: DENV-1, DENV-2, DENV-3, and DENV-4.

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Globally, each year 390 million people are infected with dengue of which 96 million people have clinical disease.[4]

Primary infection with one type of serotype develops a mild self-limiting illness with life-long immunity to that serotypes, but secondary infection with another type of dengue serotype leads to severe dengue disease because of antibody-dependent enhancement of infection.[5]

Therefore, serological diagnosis by the detection of immunoglobulin (Ig) M and IgG antibodies to dengue in the serum is essential for monitoring the treatment. Commercial kits are available, which can help in differentiating between primary and secondary dengue infections. A rapid dengue detection test kit is used for the preliminary diagnosis. Enzyme-linked immunosorbent assay (ELISA) tests are very useful in dengue serology. They detect IgM and IgG antibodies in the serum and thus are able to distinguish primary and secondary infections.[6,7]

This study aimed to study the prevalence of dengue infections, to evaluate the seropositivity in tertiary care setup, thereby to create awareness about the preventive measures to be taken by the general public and the healthcare system, and to improve our infrastructure for diagnosing and treating dengue infections.

Material and Methods

This study was a hospital-based cross-sectional study conducted in the Microbiology Laboratory of Maternal and Child Tertiary Care Hospital in Western Rajasthan, India, between January 2021 and December 2021. After taking permission from institutional ethical committee (IEC) and taking patient consent, patients of all age groups, with clinical symptoms and signs of dengue-like illness, were included and patients with coinfection of chikungunya, malaria, and other febrile illnesses were excluded from the study. Primary details (age, sex, outpatient department (OPD) or inpatient department (IPD), gyne or pediatric, medical history, and complaints or platelet count level) were noted in the pro forma. Blood samples were collected with proper aseptic precaution. Serum was separated by centrifugation technique. All samples were tested as per standard laboratory protocol and availability of kit. All clinically suspected samples were tested by the Dengue Duo rapid card method (Precision Biomed) for dengue nonstructural protein 1 (NS1) antigen and/or IgG or IgM antibody detection as per manufacturer's instruction and those that came positive by rapid card test were retested with ELISA test for IgM antibody using Panbio Kit according to manufacturer's instruction.

Result

In study time, a total of 1664 cases were tested for dengue fever, among them 897 cases (53.90%) came positive for dengue by the Dengue Duo rapid card test. A total of 250 Dengue Duo card-positive samples were included in this study.

Table 1 shows the distribution of cases as per age group. It was observed that the maximum dengue case was seen in ≤10 year of age (42%) followed by 11 to 20 years (32.4%), 21 to 30 years (17.2%), 31 to 40 years (7.2%), and >40 years (1.2%) of age group.

Of 250 samples, 142 were male (56.80%) and 108 were female (43.20%). 87.60% belong to urban area, and 12.40% were rural area. OPD patients were more with 64.80% than IPD (34.40%) and emergency (0.8%).

Table 2 shows the seasonal distribution of dengue cases for one year (from January 2021 to December 2021). Of 1664 cases, the maximum cases were seen in October (350 card-positive cases) followed by November (225 cases). Most of the cases were between August and December.

Table 3 shows the distribution of cases as per clinical features where all cases were presented with fever (100%) followed by myalgia (24.5%), headache (16.06%), hemorrhagic manifestation (13.25%), rash (8.84%), and bleeding gums (2.01%).

Table 4 shows the distribution of cases as per platelet counts. Thrombocytopenia was seen in 30.40% (76/250) of dengue fever cases.

Of 250 samples, NS1 antigen was detected in 157 cases (62.80%) followed by IgG in 84 cases (33.60%), IgM in 77 cases (30.80%), NS1 + IgG in 27 cases (10.80%), NS1 + IgM in 16 cases (6.40%), and NS1 + IgM + IgG in five cases (2%) [as shown in Table 5].

Table 1: Distribution of the cases according to age groups

Age (in years)	Number	Percentage (%)
≤10	105	42.00
11–20	81	32.40
21–30	43	17.20
31–40	18	7.20
>40	3	1.20
Total	250	100.00

Table 2: Seasonal distribution of dengue cases

	Total no. of suspected dengue fever cases	Total no of rapid card-positive cases	Percentage (%)
January	3	0	0
February	0	0	0
March	0	0	0
April	4	0	0
May	1	0	0
June	11	2	18.18
July	8	3	37.5
August	118	61	51.69
September	441	220	49.88
October	600	350	58.3
November	387	225	56.1
December	91	36	39.56
Total	1664	897	53.9

It was found that of 250 Dengue rapid card test-positive samples, 85 cases (34%) were also detected positive by IgM capture ELISA test and 165 cases (66%) were negative by IgM capture ELISA test [Table 6].

Table 7 depicts the age-wise distribution of dengue-positive cases. It was found that for the age group ≤10 years, 71 cases were NS1 positive, 34 cases were IgG and IgM card positive each, and 30 cases were ELISA IgM positive. Similar findings were observed with other age groups. This is significant as *P*-value is 0.479.

Table 3: Distribution of the cases according to clinical features

Clinical features	Number	Percentage (%)
Fever	249	100.00
Myalgia	61	24.50
Headache	40	16.06
Hemorrhagic manifestation	33	13.25
Rash	22	8.84
Bleeding gums	5	2.01
Petechiae	8	3.21
Hematemesis	8	3.21
Melena	5	2.01
Epistaxis	5	2.01
Vaginal bleeding	1	0.40
Ecchymosis	1	0.40

Table 4: Distribution of the cases according to platelet count

Platelet count/µl of blood	Number	Percentage (%)
<20,000	25	10
21,000–40,000	13	5.22
41,000–50,000	8	3.21
51000–1 lakh	30	12.05
1 lakh–1.5 lakh	34	13.60
>1.5 lakh	140	56.00
Total	250	100.00

Table 5: Dengue Duo rapid card (Precision Biomed) result for the diagnosis of dengue

Methods	Total	Positive	Percentage (%)
NS1	250	157	62.80
IgG	250	84	33.60
IgM	250	77	30.80
NS1 + IgM	250	16	6.40
NS1 + IgG	250	27	10.80
NS1 + IgM + IgG	250	5	2.00

Table 6: IgM capture ELISA test result

IgM ELISA	Number	Percentage (%)
Positive	85	34.00
Negative	165	66.00
Grand total	250	100.00

Of 250 samples, 77 were IgM Dengue Duo card positive and 173 were IgM negative by the Dengue Duo card test. Among 173 IgM Dengue Duo card negative, 131 cases (79.39%) were also detected negative by IgM ELISA and 42 cases (49.41%) were detected positive by IgM ELISA. The sensitivity was 50.59%, the specificity was 79.39%, the positive predictive value (PPV) was 55.84%, the negative predictive value (NPV) was 75.72%, and the diagnostic accuracy was 69.90% [Table 8].

Table 9 depicts the diagnostic values of Dengue Duo card (NS1) with reference to IgM ELISA.

Of 250 samples, 157 cases were dengue NS1 Ag card positive and 93 cases were dengue NS1 Ag negative by card test. Among 157 NS1 Ag card-positive cases, 51 cases (60%) were also detected dengue IgM positive by IgM capture ELISA. Among 93 NS1 Ag card-negative cases, 34 cases (40%) were detected dengue IgM positive by IgM ELISA. The sensitivity was 60%, the specificity was 35.76%, the PPV was 32.48%, the NPV was 63.44%, and the diagnostic accuracy was 44%.

Table 10 depicts the diagnostic values of Dengue Duo card (IgG) with reference to IgM ELISA. Of 250 samples, 84 cases were IgG card positive and 166 cases were IgG card negative. Among 84 IgG card-positive cases, 29 cases (34.12%) were also detected IgM antibody positive by IgM capture ELISA, and among 166 IgG card-negative cases, 56 cases (65.88%) were detected IgM antibody positive by IgM capture ELISA.

Table 11 the sensitivity was 34.12%, the specificity was 66.67%, the PPV was 34.52%, the NPV was 66.27%, and the diagnostic accuracy was 55.60%.

Discussion

In the present study, 1664 patients were having clinical suspicion of dengue-like illness (DLI) and were screened by the Dengue Duo rapid card test (NS1 Ag and/or IgM or IgG antibody).

Of this, 53.90% (897/1664) cases came positive by the Dengue rapid card test. In the present study, a total of 250 Dengue rapid card-positive cases were taken and all were retested by IgM capture ELISA. The seropositivity of dengue cases in this study was 34% (85/250). This is in concordance with the study conducted by other authors such as Ukey PM *et al*.[8] (31.3%), Deshkar ST *et al*.[2] (24.49%), Malik *et al*.[9] (52%), Lakshmi *et al*.[10] (33.64%), and Rathore *et al*.[11] (44.4%).

Differences in seropositivity rates observed by various authors might be due to changes in geographical area and different climatic conditions usually affecting the life of viral vector responsible for disease transmission.[12]

In the present study, 42% of cases were seen in the pediatric age group (0–10 years) and 32.4% in 11–20 years, which is in

Table 7: Age-wise distribution of dengue-positive cases

Age (in years)	Total	NS1 positive		IgG positive		IgM positive		ELISA IgM positive		*P*
		Number	Percentage (%)	Number	Percentage (%)	Number	Percentage (%)	Number	Percentage (%)	
≤10	105	71	45.22	34	40.48	34	44.16	30	35.29	0.47
11–20	81	50	31.85	24	28.57	21	27.27	20	23.53	
21–30	43	25	15.92	19	22.62	15	19.48	22	25.88	
31–40	18	9	5.73	7	8.33	6	7.79	12	14.12	
>40	3	2	1.27	0	0.00	1	1.30	1	1.18	
Total	250	157	100.00	84	100.00	77	100.00	85	100.00	

Chi-square=11.593 with 12 degrees of freedom; *P*=0.479

Table 8: Diagnostic values of Dengue Duo card with reference to IgM ELISA

IgM card	IgM ELISA				Total
	ELSA positive (IgM)		ELISA negative (IgM)		
	Number	Percentage (%)	Number	Percentage (%)	
IgM card positive (77)	43	50.59	34	20.61	77
IgM card negative (173)	42	49.41	131	79.39	173
Total	85	100.00	165	100.00	250

Table 9: Diagnostic values of Dengue Duo card (NS 1) with reference to IgM ELISA

NS1 card	IgM ELISA				Total
	Positive		Negative		
	No	%	No	%	
Positive	51	60.00	106	64.24	157
Negative	34	40.00	59	35.76	93
Total	85	100.00	165	100.00	250

Table 10: Diagnostic values of Dengue Duo card (IgG) with reference to IgM ELISA

IgG	IgM ELISA				Total
	Positive		Negative		
	No	%	No	%	
Positive	29	34.12	55	33.33	84
Negative	56	65.88	110	66.67	166
Total	85	100.00	165	100.00	250

Table 11: Comparative analysis of various Dengue Duo card (NS1/IgG/IgM) with reference to IgM ELISA

	IgM	IgG	NS1
SN	50.59%	34.12	60%
SP	79.39%	66.67	35.76%
PPV	55.84%	34.52	32.48%
NPV	75.72%	66.27	63.44%
Accuracy	69.90%	55.6	44%

Table 12: Case fatality in dengue cases

Total dengue cases	Number of deaths	CFR
85	2	2.35%

concordance with the finding noted by authors such as Garg *et al*.,[13] Patel and Bhatnagar,[14] Kumar *et al*.,[15] and Padhi *et al*.[16]

However, in the study by Nasreen *et al*.[17] and Chakravarti and Kumaria[18] maximum dengue cases were observed in 21–30 years of age group.

Ukey PM *et al*.,[8] Deshkar ST *et al*.,[2] and Srinivas MS *et al*.[19] observed maximum cases in the age group of 0–10 years, which was 43.90%, 40.50%, and 35.84%, respectively, in concordance with the present study.

In India, the first dengue case was reported from Kolkata in 1963,[20] which means DENV has been circulating in the Indian population for 50 years, resulting in immunity in older people and causing more risk of 1° and 2° infection among the pediatric and young adult age group.

The increased rate of infection in children could be due to a lack of appropriate ant–mosquito prevention taken by children. More outdoor activity and shorter height make children prone to mosquito bite.

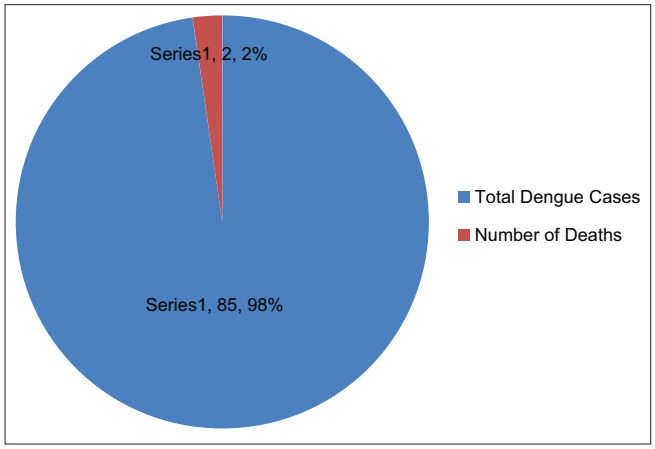
More number of cases in the pediatric population signifies the endemicity of dengue where there is a decline in adult infection and an increase in rate of infection in children.[1,10,14]

However, the present study is only representative of patients coming to tertiary care hospital for treatment rather than the truly infected population.

In this study, 56.80% of cases were male and 43.20% of cases were female. Male preponderance was also observed in the study conducted by Garg *et al*.[13] (67% males and 33% females), Patel

Table 13: Clinical classification of two fatal dengue cases

Case	Sex/age	Underlying conditions	Presentation of symptoms	Classification	Hospital stay (days)	Cause of death
1	9/F	Nil	Fever, cough, pallor, dyspnea	DSS	3	Dengue shock syndrome with acute respiratory distress syndrome, multi-inflammatory syndrome associated with COVID-19 in children.
2	20/F	Antenatal	Fever, pallor, rashes, hematemesis	DHS	4	Dengue hemorrhagic shock syndrome with massive GI bleed with intracerebral bleed.



Graph 1: Case fatality in dengue cases

and Bhatnagar[14] (61.2% males and 38.7% females), Malik *et al.*[9] (62% males and 38% females), and Rathore *et al.*[11] (70.9% males and 29.9% females).

This might be because of difference in sociocultural behavior where males are involved in more outdoor activity and their body is covered less as compared to females.[10,14]

Seasonal variation was seen with 88.62% (795/897) of positive cases observed between September and November. There is an increase in the prevalence of infection soon after the monsoon season. This might be due to high humidity, stagnant water in puddles, and open drainage system making mosquito breeding environment favorable[9,11,19,20] The study conducted by various other authors from different places of India also presented with same seasonal variation trend in concordance with the present study.[15,18,21,22]

In the present study, Thrombocytopenia was seen in 30.40% (76/250) of dengue fever cases. In this study, NS1, IgG, and IgM test results were positive in 157 (62.80%), 84 (33.60%), and 77 (30.80%), respectively, and maximum positivity was seen in NS1 antigen cases, which depict that patient reported to the tertiary level hospital in first five days of fever. IgM positivity was observed in 30.80 patients, which show primary infection, and 33.60% IgG antibodies were observed, which show secondary immune response.

MAC IgM antibody ELISA detection was done in all serum samples that were reported positive by the Dengue Duo card test for NS1, IgG, or IgM test. Of 250 card-positive samples, 85 (34%) were positive by MAC IgM antibody ELISA.

In age-wise distribution of positive cases by NS1, IgM, IgG, and MAC IgM antibody, ELISA maximum positivity was seen in the pediatric age group <10 years of age (105 cases) and was 71, 34, 34, and 30, respectively. Maximum positivity was seen by NS1 antigen. Minimum cases were seen in the elderly population >40 years (3 cases) and were 2, 0, 1, and 1 for NS1, IgM, IgG, and MAC IgM antibody ELISA, respectively.

In the comparative evaluation of IgM positivity by the Dengue Duo card test and MAC-ELISA, there was concordance in 43 (50.59%) cases, which were both positive for IgM by the Dengue Duo card test and MAC-ELISA. While 42 (49.41%) cases of IgM MAC-ELISA-positive cases of dengue card were negative, 34 (20.61%) cases of card-positive IgM MAC-ELISA was negative. Case fatality was seen in 2 cases 2.35% [Tables 12 and 13 and Graph 1].

Conclusion

Early diagnosis and treatment can prevent mortality in pediatric and pregnant females suffering from dengue and dengue-like illnesses. Facility and availability of ELISA kits should be adequate for early confirmation of suspected dengue patients by ELISA test.

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Conflicts of interest

There are no conflicts of interest.

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