International Journal of Contemporary Pediatrics
Matti M et al. Int J Contemp Pediatr. 2017 Sep;4(5):1866-1870
http://www.ijpediatrics.com
pISSN 2349-3283 | eISSN 2349-3291

DOI: http://dx.doi.org/10.18203/2349-3291.ijcp20173801

Original Research Article

Comparison of morbidity and mortality between serologically positive and serologically negative dengue cases

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Received: 11 May 2017
Accepted: 05 August 2017

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ABSTRACT

Background: Dengue is most common arboviral disease caused by 4 types of dengue viruses. In most of the hospitals laboratory diagnosis is made by dengue serology using Rapid Immunochromatographic Test for NS1Ag, IgM and IgG antibodies. We found more morbidity and mortality in suspected dengue cases whose serology was negative for dengue.
Methods: Children with clinical features suggestive for case definition for dengue, who belonged to group C of World Health organisation (WHO) guidelines for dengue fever (severe dengue), were included. They were categorised into dengue positive and dengue negative groups. The clinical features and complications of the disease between the two groups were compared.
Results: 98 cases (77.8%) were serologically positive for dengue and 28 (22.2%) were negative. 39% of the patients with dengue negative serology developed hypotensive shock in comparison to 18% of dengue positive cases, mortality in serology negative cases (21%) was significantly higher than positive cases (8%).
Conclusions: We could not find the reasons for more morbidity and mortality in seronegative dengue group, we stress up on improving the sensitivity and specificity of rapid diagnostic tests. More caution need to be taken for serology negative dengue cases. Epidemiological studies directed to find circulation of other viruses which causes dengue like symptoms should be made.

Keywords: Dengue, Rapid diagnostic tests, Serology

INTRODUCTION

Dengue is the most rapidly spreading mosquito-borne viral disease in the world, with 30 fold increase in the incidence since last 50 years. Reported case fatality rates in south-east Asia is approximately 1%, but in India, Indonesia and Myanmar, focal outbreaks away from the urban areas have reported case-fatality rates of 3-5%.[1] Dengue is a disease caused by an arbovirus, which has four related virus serotypes (DENV-1, DENV-2, DENV-3, and DENV-4). Dengue is a systemic and dynamic infectious disease. The infection may be asymptomatic or present itself with a broad clinical spectrum that includes both severe and non-severe clinical manifestations. There are other viral infections, bacterial infections like leptospirosis which clinically mimic dengue fever.In most of the hospitals clinical diagnosis is confirmed solely by dengue serology using rapid Immunochromatographic test or Rapid diagnostic tests (RDT) for NS1Ag, IgM and IgG antibodies against dengue serotypes, which have good sensitivity and specificity.[2] In our intensive care unit we have noted children with clinically diagnosed severe dengue (Group C of WHO)cases, who were tested negative for dengue

serology (using rapid diagnostic tests) had more severe form of disease. These cases were then tested for malaria, typhoid fever, rickettsia, viral hepatitis and leptospirosis and found negative for the same. These serology negative dengue children required more supportive measures and they had more mortality. We compared morbidity and mortality among these serology negative dengue cases with serology positive dengue cases who were admitted in intensive care unit for the severe dengue disease.

METHODS

This study was retrospective study conducted at Pediatric Intensive Care Unit of a tertiary health care centre, over a period of six month during dengue epidemic season.

Inclusion criteria

- Children with clinical features who meets WHO case definition for dengue.
- Only Dengue cases who belonged to Group C of WHO (Severe dengue).

Exclusion criteria

- Group A and Group B dengue cases
- Children diagnosed with other diseases like Malaria, Typhoid fever, Rickettsial fever, viral hepatitis, leptospirosis and sepsis cases with high CRP titre.

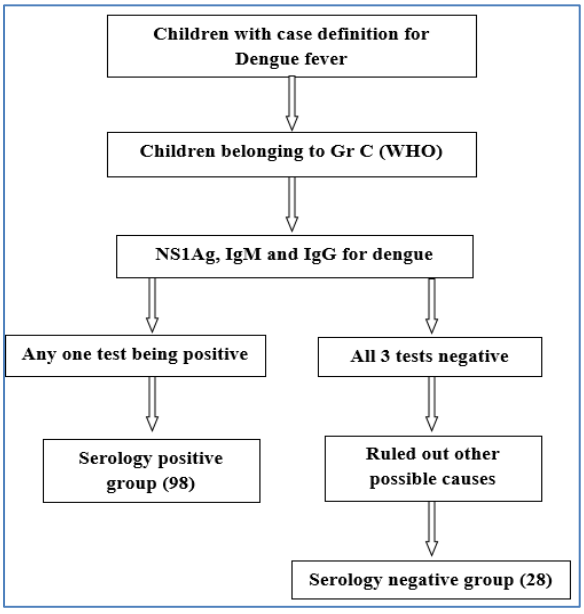

Figure 1: Methodology.

A total of 126 severe dengue cases (Group C WHO) admitted in PICU, who were having symptoms suggestive of dengue fever with thrombocytopenia, raised haematocrit (HCT) and leucopoenia as their initial presentation were included in the study. Following rapid diagnostic tests for Dengue, they were categorised into dengue positive and dengue negative groups and dengue negative children were further investigated for other possible diagnosis like Malaria, Typhoid fever, Rickettsial fever, viral hepatitis, leptospirosis and sepsis. The clinical features and complications of the disease between the two groups were compared. A p-value of ≤0.05 is considered statistically significant.

RESULTS

Of the 126 cases, 98 (77.8%) were serologically positive for dengue and 28 (22.2%) were negative. There was no significant difference in the symptoms and signs between the two groups (Table 1) except for vomiting which was more common in serology negative group.

Table 1: Symptoms and signs.

	Sero-positive dengue cases	Sero-negative dengue cases	P value
No children studied	98 (77.8%)	28 (22.2%)	
Vomiting	61	10	0.0125*
Pain abdomen	41	10	0.56
Rash	7	1	0.42
Bleeding	39	6	0.0736
Hepatomegaly	70	16	0.152

There were no differences among the two groups with respects to haematological parameters like Haemoglobin (Hb%), haematocrit (HCT), total leucocyte count (TLC) and platelet counts (Table 2).

Table 2: Haematological parameters.

	Sero-positive dengue cases (98)	Sero-negative dengue cases (28)	P value
Hb%			
5-7	0	1	
7-10	17	7	0.104
>10	81	20	
HCT			
<25	1	1	
25-30	9	6	0.241
30-35	26	6	
>35	62	15	
TLC			
<4000	19	5	
4000-11000	66	17	0.567
>11000	13	6	
Platelet			
<25000	29	10	
25000-50000	44	8	0.474
50000-1lakh	18	7	
1-1.5 lakh	7	3	

39% of the patients with dengue negative serology developed hypotensive shock in comparison to 18% of dengue positive cases (p value 0.0203), compensated

shock was comparable among two groups (p 0.137) (Table 3).

Table 3: Shock among the two groups.

	Sero-positive dengue cases (98)	Sero-negative dengue cases (28)	P value
Compensated shock	47	9	0.137
Hypotensive shock	18	11	0.0203

The need for resuscitative measures such as oxygen supplementation (39%), inotropes (39%) and diuretic infusion (32%) was more in children with dengue negative serology which was statistically significant (Table 4).

Table 4: Various support measures required.

	Sero-positive dengue cases (98)	Sero-negative dengue cases (28)	P value
Inotropes	13	11	0.0019
Frusemide infusion	15	9	0.045
Oxygen	11	11	0.00056
IV fluids			
1-3 days	53	16	0.399
4-7 days	40	9	
>7 days	5	2	

The mortality in serology negative cases (21%) was significantly higher than positive cases (8%).The duration of hospital stay was comparable among two groups (Table 5).

Table 5: PICU outcome.

	Sero-positive dengue cases (98)	Sero-negative dengue cases (28)	P value
Duration of ICU stay			
1-3 days	53	16	0.732
4-7 days	40	9	
>7 days	5	2	
Mortality	8	6	0.048

DISCUSSION

Viral haemorrhagic fevers (VHFs) are a group of febrile illnesses caused by RNA viruses from several viral families. The four viral families known to cause VHF disease in humans include the Arenaviridae, Bunyaviridae, Filoviridae and Flaviviridae. These highly infectious viruses lead to a potentially lethal disease syndrome characterized by fever, malaise, vomiting, mucosal and gastrointestinal bleeding, edema, and hypotension.[3] Dengue fever is predominant cause of viral haemorrhagic fevers especially in south-east Asia. There are at least 4 distinct antigenic types of dengue virus (DEN-1, DEN-2, DEN-3 and DEN-4), members of the family Flaviviridae.[4] WHO defines Probable dengue as patient who lives in /travel to dengue endemic area with fever and 2 of the following criteria: Nausea, vomiting, rash, aches and pains, tourniquet test positive, leukopenia, any warning signs. And severe dengue (WHO group C) as (i) plasma leakage that may lead to shock (dengue shock) and/or fluid accumulation, with or without respiratory distress, and/or (ii) severe bleeding, and/or (iii) severe organ impairment.[1]

Laboratory diagnosis methods for confirming dengue virus infection may involve detection of the virus, viral nucleic acid, antigens or antibodies, or a combination of these techniques. Virus isolation by cell culture, nucleic acid detection by reverse transcriptase-polymerase chain reaction (RT-PCR), Real-time RT-PCR, or Isothermal amplification methods are more accurate but seldom available in hospitals and are expensive. Hemagglutination-inhibition assay (HAI) and enzyme immunoassay (EIA) for immunoglobulin M (IgM) and IgG antibodies to dengue virus are more superior tests but they require an overnight incubation and are not freely available in every hospital, in such situations rapid test would be advantageous and cost effective. In a study by Vaughn et al, the rapid test demonstrated 100% sensitivity in the diagnosis of dengue virus infection and was able to distinguish between primary and secondary dengue virus infections through the separate determinations of IgM and IgG.[5] Hunsperger et al compared RDTs vs ELISA, study showed that NS1 ELISA sensitivity was 60-75% and specificity 71-80%; NS1 RDT sensitivity was 38-71% and specificity 76-80%; the IgM anti-DENV RDTs sensitivity was 30-96%, with a specificity of 86-92%, and IgM anti-DENV ELISA sensitivity was 96-98% and specificity 78-91%. He concludes that RDTs for both NS1 and anti-DENV IgM continue to have relatively poor performance, even with their lower sensitivity but with comparable specificities compared the ELISAs, they could be used to identify dengue outbreaks and considers acceptable for routine clinical diagnostics.[6] We did not test for convalescent sera for the negative samples to reconfirm, that was a limitation of the study.

Interesting thing observed in our hospital intensive care unit was that, patients who were tested negative for dengue NS1Ag, IgM and IgG (using rapid diagnostic immunochromatographic test kits) were becoming more sick. Hypotensive shock was more among them compared to serology positive cases. More cases required inotropes, supplemental oxygen, some cases were given frusemide infusion for early ARDS and some required artificial ventilation in serology negative cases. Hence, we compared two groups and noted statistically significant difference with respect to morbidity and

mortality. We don't have answer for the above said finding; we try to find some possible reasons.

One possible explanation is false negative dengue cases. Acosta et al in his study, analysed serum samples from 150 NS1-negative patients presumptively diagnosed with dengue. They were probed with real-time reverse transcription PCR, Molecular detection methods showed 33 (22%) positive samples out of 150 NS1-antigen negative samples. He suggests a lower sensitivity of the NS1 test, mainly when DENV-4 is the predominant serotype. Hence Health care providers should therefore be aware of samples tested negative by NS1 antigen assays, especially when clinical symptoms and other laboratory data results show evidence of dengue infection.[7] Similar study found Underreporting of dengue-4 in Brazil due to low sensitivity of the NS1 Ag test in routine control programs.[8]

In a study by Felix AC et al total of 379 RNA-positive samples were selected for thorough evaluation. NS1 was reactive only in 37.7% of cases and IgM in 53.5% (203/379) of patients. Most of the cases were characterized as a secondary infection by dengue 2 virus.[9] As per Indian data DEN-2 is the predominant circulating strain.[10] Dengue virus serotype 2 (DEN-2) is known to cause high morbidity and mortality in Southeast Asia.[11] This could be possible explanation for higher morbidity and mortality in serology negative dengue group in our study.

Another possible expiation is that other organisms causing dengue like illness. Bruce MG et al noted leptospirosis among patients presenting with dengue-like illness. Among 730 dengue-negative sera, they noted 36 (5%) were positive for leptospirosis.[12] Sanders EJ et al noted similar findings in their study.[13] We investigated them with IgM for leptospira and none of them turned positive.

Pessôa R et al investigated outbreak of dengue like illness (77 suspected dengue patients) in Brazil and found DENV in 9 patients (11.7%), ZIKV in 31 patients (40.2%), CHIKV in 1 patient (1.3%), and coinfection of DENV and ZIKV was detected in 2 patients (2.6%).[14] Velasco JM et al found many Chikungunya Virus among Patients with Dengue-Like Illness.[15] This emphasizes the need for a routine and differential diagnosis of arboviruses in patients with dengue-like illness. Unfortunately, laboratory assays, such as real-time reverse transcription polymerase chain reaction, virus cDNA sequencing, and enzyme-linked immunosorbent assay are not easily available.

This evaluation had several limitations. Rapid diagnostic Test for dengue whose performance was not compared with reference laboratory assay results. In patients who were tested negative, repeat IgM and IgG 14 to 21 days after first tests (convalescent sera) were not done. We did not do PCR or RT-PCR to find other organisms. We did not include ward patients who were relatively stable (belonging to WHO group B).

This study stresses on improving sensitivity and specificity of rapid diagnostic tests, ELISA and PCR should be made available at least in bigger hospitals. More epidemiological studies need to be conducted to find out other viruses which clinically mimic dengue. Finally treating doctor should be more careful in treating serology negative dengue cases as they have relatively higher morbidity and mortality.

ACKNOWLEDGEMENTS

Authors would like to thank Head, Department of Pediatrics Dr S.N Joshi, children and their parents, postgraduate students, nursing staff for their support during the study.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Matti M, Maralihalli MB, Dinesh S. Comparison of morbidity and mortality between serologically positive and serologically negative dengue cases. Int J Contemp Pediatr 2017;4:1866-70.