Research Article

Biochemical and radiological markers as predictors of dengue severity in children admitted in a tertiary care hospital

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ABSTRACT

Background: Dengue fever is the arboviral infection with the largest incidence worldwide. Clinical expression of dengue virus infection varies from no symptoms to severe dengue with shock. Severe Dengue is characterized by thrombocytopenia, spontaneous haemorrhages, and gradual plasma leakage that can lead to shock. Biochemical and radiological markers have not been evaluated prospectively in early stages of dengue and their utility as predictors of dengue children progressing to severe dengue have not been studied previously in our part of the country. The objective of this study was to evaluate biochemical and radiological markers as predictors of dengue severity.
Methods: This Prospective Cohort study was done in a Tertiary care teaching hospital (Sri Ramachandra Medical College) from January 2010 to June 2011. Children from the age of one month to ≤18 years, who fulfilled the WHO criteria for dengue fever, duration of clinical symptoms < 96 hrs and children with dengue NS 1 antigen positivity with or without IgM antibodies were included in the study. An informed consent was obtained and blood samples were taken for biochemical markers (AST, ALT, CPK and LDH) and radiological markers in the form of plasma leakage was detected by Chest X ray and Ultrasonogram of abdomen.
Results: 103 children with dengue infection were enrolled in the study, of which 50 children had warning signs at the time of admission. Among the 50 children with warning signs, 37 children progressed to severe dengue. Serum AST, ALT, CPK and LDH showed significant elevation in children with dengue with warning signs in comparison to children with dengue fever. Radiological markers Chest X-ray and Ultrasonogram abdomen also predicted plasma leakage significantly in children with warning signs than in children with dengue fever.
Conclusions: This study suggests that elevated AST, ALT, CPK, LDH, and signs of plasma leak detected within 96 hrs of onset of symptoms can predict a more severe form of dengue infection. Hence, these biochemical and radiological markers may be useful in identification and close monitoring of potentially sick children with dengue infection and serve as valuable prognostic markers early in the course of the illness.

Keywords: Dengue fever, Biochemical, Radiological, Markers

INTRODUCTION

Dengue fever is the arboviral infection with the largest incidence worldwide.[1] Clinical expression of dengue virus infection varies from no symptoms to severe dengue with shock. Nearly 100 million cases of Dengue fever and between 250,000 and 500,000 cases of severe dengue are annually reported to the World Health Organization.[2,3] Severe Dengue is characterized by thrombocytopenia, spontaneous hemorrhages, and gradual plasma leakage that can lead to shock.[4]

Despite its clinical variability, the acute phase of dengue begins with fever that is indistinguishable from the initial phase of other acute febrile infectious diseases. Thus, acute dengue infection is often unrecognized until the appearance of the more severe forms of the disease.[1,2,4] This non specificity of clinical features leads to inadequate or late treatment of a potentially lethal medical condition. There is direct and indirect evidence of biochemical and radiological alterations related to severity of dengue.[5,6]

Studies have reported that those patients with Severe Dengue have elevated serum levels of transaminases (aspartate aminotransferase [AST] and alanine aminotransferase [ALT]) lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) along with radiological findings in the form of chest X ray and ultrasonogram abdomen showing features suggestive of fluid leakage.[6-9]

However, these potential biochemical and radiological markers have not been evaluated prospectively in early stages of dengue and their utility as predictors of dengue children progressing to severe dengue have not been studied previously in our part of the country.

METHODS

This prospective cohort study was done in the department of paediatrics, Sri Ramachandra medical college from January 2010 to June 2011. The institutional research & ethical committee approved the study protocol. Analysis period was from July 2011 to October 2011. Children from the age of one month to ≤18 years, who fulfilled the WHO criteria for dengue fever, duration of clinical symptoms < 96 hrs and children with dengue NS 1 antigen positivity with or without IgM antibodies were included in the study. Exclusion criteria included children with diabetes; Immuno compromised state, known bleeding disorders, malignancies, Cardiac diseases and severe dengue fever.

Hundred and three patients from one month to 18 years of age, who met the inclusion criteria, were enrolled in the study before the development of severe dengue symptom. After a meticulous clinical examination, an informed consent was obtained and children underwent the following investigation. Complete blood count, packed cell volume, Dengue NS1 antigen Card test, AST, ALT, LDH, CPK, Chest X-Ray and Ultrasound Abdomen. Biochemical markers used in this study are AST, ALT, LDH, and CPK and the serum levels of these markers were detected in our laboratory using photometric method. The reference ranges of our lab for these parameters were for ALT 0-45U/L, AST 0-45U/L, LDH 0-190U/L, CPK 0-232U/L. A cut off value of three times above the reference range was taken as significant elevation in accordance with previous studies.

All children were diagnosed to have dengue virus infection by doing dengue NS1 antigen card test. NS 1 antigen card test was used to detect dengue antigen, which is done within duration of 30 min in the microbiology laboratory. It is an in-vitro immunochromatographic, one step assay to detect dengue virus NS1 antigen and differential IgG / IgM antibodies to dengue virus in human serum, plasma or whole blood. This test device contains a membrane strip, which is pre-coated with anti-dengue NS 1antigen capture on test band region. The anti-dengue NS 1antigen colloid gold conjugate and serum, plasma or whole blood sample move along the membrane chromatographically to the test region (T) and forms a visible line as the antibody-antigen gold particle complex forms. The dengue NS 1Ag test device result window has 2 pre-coated lines, T (NS 1Ag test line) and C (control line). Both the test line and the control line in result window are not visible before applying any samples. The control line is used for procedural control and should always appear if the test procedure is performed correctly. The dengue NS 1 antigen can identify dengue virus in serum, plasma or whole blood specimens with a high degree of sensitivity and specificity.

Based upon the Indian reference, a haematocrit value of above 36% was considered as raised haematocrit. Platelet count of less than one lakh is defined as thrombocytopenia according to WHO. For the purpose of the study, WHO 2009 guidelines for dengue case definition as dengue with or without warning signs and severe dengue was considered. Radiological markers used in the study were Chest X Ray showing features suggestive of pleural effusion and ultrasonogram of abdomen demonstrating elements evocative of fluid collection like gall bladder wall thickening with pericholecystic oedema, ascites, and perinephric fluid collection.

Participants were followed daily until discharge. The presence of oedema / signs of pleural effusion like the one decreased air entry /signs of ascites like fullness of abdomen have been taken as signs of severe dengue. We studied the correlation between the progression of disease and biochemical and radiological markers obtained during initial evaluation of the children. Windows SPSS software was used for statistical analysis, which included student's t-test and Cox multivariate regression analysis.

RESULTS

During the study period of over 18 months, 186 children were admitted with a clinical diagnosis of dengue fever with and without warning signs. Hundred and three children diagnosed as dengue fever based on the NS1 antigen positivity were enrolled in our study. Out of this, 48 % (50) of children had warning signs at the time of admission. Thirty-seven children (35%) with warning signs progressed to severe dengue (Figure 1).

Table 1: Serum CPK levels in children with dengue fever and dengue fever with warning signs.

CPK	Group			Total	P- value
	Dengue fever Without warning signs	Dengue fever With warning signs			
		Not progressed to severe dengue	Progressed to severe dengue		
Normal	51 96.2%	1 2.0%	0 0%	52 50.5%	<.001
Mild elevation 300 - 600	2 3.8%	14 28.0%	0 0%	16 15.5%	<.001
Significant elevation > 600	0 .0%	0 0%	35 70.0%	35 34.0%	<.001
Total	53 100.0%	15 30.0%	35 70.0%	103 100.0%	<.001

Table 2: Serum LDH levels in children with dengue fever and dengue fever with warning signs.

LDH	Group			Total	P- value
	Dengue fever Without warning signs	Dengue fever With warning signs			
		Not progressed to severe dengue	Progressed to severe dengue		
Normal	49 92.5%	1 2.0%	0 0%	50 48.5%	<.001
Mild elevation 300 - 600	4 7.5%	12 24.0%	0 0.0%	16 15.5%	<.001
Significant elevation >600	0 .0%	0 0%	37 74.0%	37 35.9%	<.001
Total	53 100.0%	13 26.0%	37 74.0%	103 100.0%	<.001

There was no significant difference in age, sex, clinical symptoms, hemoconcentration, and leukopenia between children with dengue fever and those with dengue fever with warning signs. Children with dengue fever with warning signs had higher serum levels of AST, ALT, CPK, and LDH than in children with dengue fever.

In the present study, 70% (35) of children with dengue with warning signs had marked increase in CPK levels, which is significant (p value<0.001) in comparison to children with dengue fever (Table 1). Serum LDL levels were radically increased in children (74%) in dengue fever with warning signs (p value<0.001) in contrast to children with dengue fever (Table 2).

In the current study, amongst the 50 children with dengue with warning signs, 20 (40%) had significant elevation of AST (p value of < 0.005), in comparison to children with dengue fever (Table 3).Serum ALT is drastically raised in children (22%) with dengue fever with warning signs(p value<0.001) in contrast to children with dengue fever (Table 4).

In our analysis, radiological abnormalities were detected in 30% (15) of children with dengue with warning signs and they showed significance (p value 0.003) in correlation to children with dengue fever (Table 5). Ultrasound abdomen revealed abnormalities in 48% (24) of children with dengue fever with warning signs (p value

<0.001) in contrast to children with dengue fever (Table 6).

DISCUSSION

Our study substantiates the correlation between progression of dengue fever with warning signs to severe dengue and raised serum levels of CPK, LDH, AST and ALT. These biomarkers have been projected as indicators of severity in dengue in many retrospective and cross-sectional studies.[4,5,7-9] In the present study, elevation of CPK and LDH levels detected within 96 hrs of onset of symptoms could predict a more severe form of dengue infection. Both these levels were 5-7 times higher in children who progressed to severe dengue during hospital stay.

Table 3: Serum AST levels in children with dengue fever and dengue fever with warning signs.

AST	Group			Total	P- value
	Dengue fever Without warning signs	Dengue fever With warning signs			
		Not progressed to severe dengue	Progressed to severe dengue		
Normal	42 79.2%	7 14.0%	0 0%	49 47.6%	<.005
Mild Elevation 100 -200	11 20.8%	23 46.0%	0 0%	34 33.3%	<.005
Significant elevation >200	0 .0%	0 0%	20 40%	20 19.4%	<.005
Total	53 100.0%	30 60.0%	20 40.0%	103 100.0%	<.005

Table 4: Serum ALT levels in children with dengue fever and dengue fever with warning signs.

ALT	Group			Total	P- value
	Dengue fever Without warning signs	Dengue fever With warning signs			
		Not progressed to severe dengue	Progressed to severe dengue		
Normal < 100	48 90.6%	23 46.0%	0 0%	71 68.9%	<.001
Mild elevation 100 -200	5 9.4%	16 32.0%	0 0%	21 20.4%	<.001
Significant elevation >200	0 0%	0 0%	11 22%	11 22.0%	<.001
Total	53 100.0%	39 78.0%	11 22.0%	103 100.0%	<.001

In children with dengue fever with warning signs, raised LDH and CPK levels can be attributed to skeletal muscle damage.[5,10-15] In addition, liver damage is a frequent problem in dengue that can also be related to increased levels of LDH.[10,11] Our analysis suggests that these biomarkers can predict a more severe form of dengue and could be indicators of early tissue injury in the acute phase of dengue infection.

In the current study, Children with dengue fever with warning signs and severe dengue showed high serum AST and ALT levels in contrast to children with dengue fever.

Amongst 50 children with warning signs 40 %(20) had more than threefold rise in levels of AST (p value < 0.005) and 22 % (11) had more than threefold rise in levels of ALT (p value < 0.001). Hence, normal plasma AST and ALT levels was a strong negative predictor to exclude Dengue fever with warning signs. De Souza and colleagues in their study of 1585 dengue patients observed alterations of AST and ALT levels.

Interestingly, authors noted the levels of AST were elevated significantly in 93.3% of patients and ALT in 82.2 % who progressed to DHF, which is similar to our study.[16]

Table 5: Chest X-ray in children with dengue fever and dengue fever with warning signs.

CXR	Group			Total	P- value
	Dengue fever Without warning signs	Dengue fever With warning signs			
		Not progressed to severe dengue	Progressed to severe dengue		
Present	4 7.5%	0 0%	15 30.0%	19 18.4%	.003
Absent	49 92.5%	35 70.0%	0 0%	84 81.6%	.007
Total	53 100.0%	35 70.0%	15 30.0%	103 100.0%	.003

**Table 6: Ultrasonogram abdomen in children with dengue fever and dengue fever with warning signs.**

USG Abdomen	Group			Total	P- value
	Dengue fever Without warning signs	Dengue fever With warning signs			
		Not progressed to severe dengue	Progressed to severe dengue		
Present	8 15.1%	0 0%	24 48.0%	32 31.1%	<.001
Absent	45 84.9%	26 52.0%	0 0%	71 68.9%	<.001
Total	53 100.0%	26 52.0%	24 48.0%	103 100.0%	<.001

The other component of the study was on radiological markers as indicators of severe dengue. We used Ultrasonogram abdomen and chest x ray to look for evidence of plasma leakage. Plasma leakage in dengue fever with warning signs is usually due to hypoproteinemia, since hypoproteinemia is infrequent and clinical recognition of plasma leakage is difficult in a sick child. Ultrasonography and radiography of the chest can reliably detect presence of pleural effusion and ascites in children with dengue fever with warning signs. 30% of children with warning sign had pleural effusion in chest x ray (p value 0.003) and 48% had features suggestive of fluid leakage in USG abdomen in form of gall bladder thickening with pericholecystic oedema, ascites, perinephric fluid collection (p value < 0.0001). A study of 73 cases of dengue fever by chacko, concluded that chest x-ray and ultrasound abdomen findings was present in around 50% patients who progressed to severe dengue, which is similar to our study In another study by Balasubramaniam, 65 children with dengue fever were enrolled of which 35 children developed dengue hemorrhagic fever.[17] Of the 35 children, with dengue hemorrhagic fever, pleural effusion was detected by ultrasonography in 88.57% (31) and 74.19% (23) had radiographic evidence of the plasma leakage[18].Similar to the above study, our study also demonstrated ultrasonogram to be superior to radiography in detecting plasma leakage. Ultrasonogram would be ideal owing to its safety in that it is non-ionizing and would assist detecting plasma leakage even before it clinically manifests.

In synopsis, biochemical and radiological changes that appear before 96 hours help to predict a likely progression to severe dengue. These results also imply that early pathogenic changes occurred before complications developed. This finding may help to identify and appropriately manage the high-risk children when compared with a large number of children with dengue fever with warning signs, thereby reducing unnecessary hospitalization as well as morbidity and mortality due to dengue fever. Application of these results may help optimize resource allocation, leading to a more opportune and effective care of those patients with dengue in disease-endemic areas.
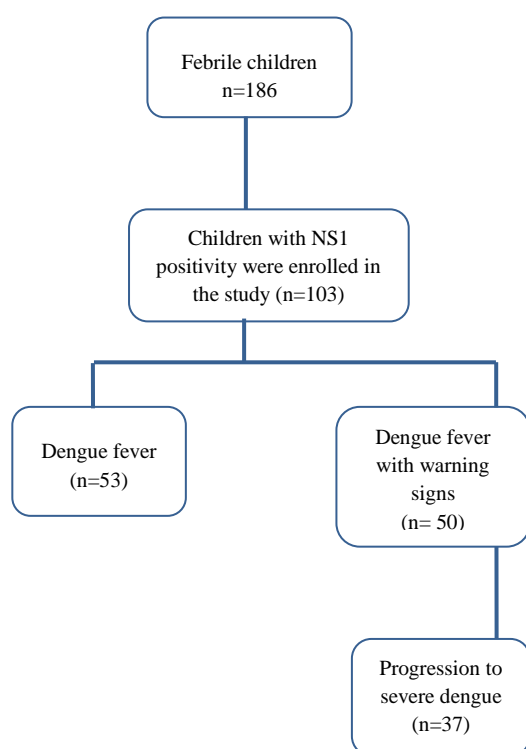
Figure 1: Enrolment and study status of children.

CONCLUSION

1. Our study suggests that three to four-fold elevation of LDH and CPK levels detected within 96 hrs of onset of symptoms was consistently associated with progression of dengue fever with warning signs to severe dengue in children.
2. A four-fold elevation of AST and ALT levels detected within 96 hrs of onset of symptoms was a reliable predictor of progression of dengue with warning signs to severe dengue in children.
3. Radiological findings of fluid leak detected within 96 hrs of onset of symptoms were more commonly associated with children who later developed severe dengue.
4. Hence, these biochemical and radiological markers may be useful in identification and close monitoring of potentially sick children with dengue infection and serve as a valuable prognostic marker early in dengue fever.

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