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BIOCHEMICAL INDICATORS FOR THE EARLY DIAGNOSIS OF SEVERE DENGUE FEVER: AN INDIAN STUDY

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ABSTRACT

BACKGROUND

Dengue also known as breakbone fever is a viral infection which is one of the most important mosquito borne diseases of the Indian subcontinent. It has become a major public health problem. Spread of the disease has lead to the recognition of atypical manifestations apart from the classical clinical features of dengue infection.

Aim

To determine the biochemical indicators for the early diagnosis of severe dengue fever.

Methods

A cross sectional study of dengue patients admitted as well as reporting to Medical OPD with confirmed infection with dengue virus was conducted in the year 2014 during the monsoon and post monsoon seasons. Patients with serological confirmation of dengue infection were classified according to the World Health Organization 2009 definitions of dengue fever without warning signs (DF) and dengue fever with warning signs or severe dengue (SD). All patients were evaluated prospectively for the first twelve days of the disease to determine their clinical and biochemical parameters. We evaluated the biochemical parameters as predictors of severe dengue fever (SD).

Results

Out of the 398 patients, 60 developed severe dengue fever (SD). Patients with severe dengue fever had higher levels of lactate dehydrogenase (LDH), creatine kinase (CK), blood glucose and aspartate aminotransferase, and lower levels of albumin, total cholesterol, triglycerides and calcium. Multivariate analysis showed that early alterations of CK (hazard ratio [HR] =6.98, 95% confidence interval [CI] = 2.34-20.85, P =0.001), LDH (hazard ratio [HR] =3.19, 95% confidence interval [CI] = 1.01-10.12, P <0.05), albumin (hazard ratio [HR] =2.54, 95% confidence interval [CI] = 1.09-5.92, P =0.03) and calcium (hazard ratio [HR] =1.49, 95% confidence interval [CI] = 0.62-3.56, P <0.37) were associated with severe dengue fever (SD). Triglyceride levels > 160mg/dl were negatively associated with developing severe dengue fever (SD) (hazard ratio [HR] =0.07, 95% confidence interval [CI] =0.01-0.59, P= 0.01).

Conclusion

The spectrum of dengue fever ranges from mild self limiting illness to severe life threatening infection. Dengue poses a huge burden on the public health care system. Biochemical alterations may be associated with severe forms of dengue infection and can be early prognostic markers for monitoring the illness and identifying patients who can benefit from the future therapies.

KEYWORDS

Dengue fever (DF), severe dengue fever (SD), Thrombocytopenia, Biochemical parameters

ARTICLE HISTORY

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INTRODUCTION

Dengue is the arboviral infection with the largest incidence worldwide[1]. Dengue virus infection causes a wide spectrum of illness from mild asymptomatic illness to severe fatal dengue hemorrhagic fever (DHF)/dengue shock syndrome (DSS). Approximately 2.5 billion people live in dengue risk regions with nearly 100 million cases of DF and between 250,000 and 500,000 cases of severe dengue (SD) are annually reported to the World Health Organization [1,2]. The cumulative dengue diseases burden has attained an unprecedented proportion in recent times with sharp increase in the size of human population at risk. Dengue disease presents highly complex pathophysiological, economic and ecologic problems.

The most dramatic increase in dengue in the past decade occurred in Indian subcontinent, mainly in India, Pakistan, Bangladesh and Srilanka. In India, dengue infection is endemic with cyclical outbreaks in almost all major cities with an at-risk population of 160 million persons. The incidence of dengue in Pune metropolitan city has increased in the recent years posing a major health hazard [3-7].

Severe dengue is characterized by thrombocytopenia, spontaneous

hemorrhages and gradual plasma leakage that can lead to shock1. 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 [1,8]. Thus acute dengue infection is often not recognized until the appearance of the more severe forms of the disease. This observation leads to underestimation of the actual incidence, as well as inadequate or late treatment of a disabling and potentially lethal medical condition [9].

There is direct and indirect evidence of biochemical indicators related to severity of dengue. Studies have reported that patients with DHF have elevated serum levels of transaminases (aspartate aminotransferase [AST] and alanine aminotransferase [ALT][8-12], amylase, lactate dehydrogenase (LDH) [14-16] and creatine kinase (CK)[16] .

Cross-sectional studies have shown differences in serum levels of cholesterol and triglycerides associated with severe forms of dengue studies [13]. However, these potential biochemical markers have not been evaluated prospectively in early stages of dengue. Thus, the information available regarding the utility of biochemical alterations for timely identification of patients who will develop severe dengue fever (SD) is limited. The objective of this study was to evaluate biochemical markers as predictors of dengue severity.

MATERIALS AND METHODS

Study population: - This prospective cross-sectional study was conducted in a tertiary care hospital in Pune metropolitan city, from the period from July to December 2014. All subjects had given informed consent for study inclusion.

Patients who had confirmed serological diagnosis of dengue viral infection by detection of dengue NS1 antigen or dengue viral specific immunoglobulin (Anti dengue IgM and Anti dengue IgG antibody) by Immunochromatographic (ICT) method were included in the study and were further classified as dengue fever without warning signs (DF) and dengue fever with warning signs/ severe dengue (SD) according to WHO guidelines 2009 [1].

Patients who were serological positive for dengue with history of diabetes, acquired immunodeficiency syndrome, hematological disorders, cancer or cardiac disease and those reporting 96 hours after the onset of fever were excluded from the study.

METHODOLOGY: -

A detailed history as well as a thorough general and systemic clinical examination (including the tourniquet test) was recorded. Haematological profiles and biochemical investigations were done at the time of admission and were followed by daily (or bi-daily) investigations as required until discharge. Blood samples were obtained to determine haemoglobin, hematocrit, and platelet count. Peripheral Blood Smear, Blood Glucose, Liver Function Tests (LFT), Renal Function Tests (RFT), Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT), Lipid Profile and Creatine Kinase (CK).

Patients were followed daily until the twelfth day of disease. Data collected included signs and symptoms and daily microhematocrit determinations to facilitate recognition of onset of severe dengue. Platelet counts were repeated daily in cases with counts less than 1,00,000/mm3 or when the patient had spontaneous hemorrhages, signs of effusion, edema or a change in the hematocrit greater than 10%.

A variation of hematocrit value greater than 10% was chosen. Signs of plasma leakage were also assessed by chest radiograph and abdominal ultrasonography.

Statistical analysis: -

Biochemical test results were compared using Student's t-test. We determined the frequency of biochemical alterations using accepted normal upper limits for Blood Glucose (Fasting =100 mg/dl, Post Prandial =140mg/dl); lipids (HDL=35 mg/dl, LDL=80 mg/dl, triglycerides=160mg/dl), and LDH (570 U/L); Bilirubin (0.9 mg/dl) and a three-fold normal upper limit for transaminases (105 U/L); albumin (4g/dl); Calcium (9 mg/dl) and CK (70U/L in women and 90U/L in men), as cut-off values. Cox multivariate regression analysis was used to identify biochemical markers independently associated with severe dengue (SD). We computed hazard ratios (HRs) with their 95% confidence intervals (CIs) for each marker, adjusting for other biochemical markers, age (>15 yrs), sex, time of disease, and use of intramuscular medications during present illness. An association was considered statistically significant when $P < 0.05$. Analysis were computed using STATA software package version 8.0.

RESULTS

A total of 398 patients clinically as well as serologically diagnosed dengue infections were studied. Study participants were five to seventy years of age and had acute febrile syndrome of less than 96 hours duration caused by dengue viral infection.

Out of 398; 338 (84.9%) of these patients developed dengue fever (DF); 60 (15.1%) of these patients developed severe dengue (SD), of which 6 (10 %) patients succumbed to their illness. Ninety-eight dengue positive patients were hospitalized including sixty four with DF and thirty four with severe dengue (SD).

There was no significant difference in age, sex, and duration of symptoms between patients with DF and those with severe dengue (SD). Patients with DF and a severity sign frequently did not meet all criteria for severe dengue (SD) [Table 1].

Patients with severe dengue (SD) had higher levels of LDH, CK and AST as compared to patients with DF. Levels of calcium, albumin, total cholesterol and triglycerides were significantly lower in patients with severe dengue (SD) [Table 2].

Increased levels of CK in severe dengue (SD) was independent of other biochemical alterations (HR=6.98%, CI=2.34-20.85, P=0.001). An early increase in LDH (three times the normal value) was an independent predictor of severe dengue (SD) (HR=3.19, 95% CI=1.01-10.12, P<0.05).

Alterations of other biochemical markers were not associated with severe dengue (SD) by multivariate analysis at the level of power of this study [Table 3].

DISCUSSION

In the present study we primarily focused on the biochemical indices to determine the disease severity. Although WHO severity parameter does not include biochemical changes, several studies suggest that WHO criteria of severity, alone, may not be sufficient to categorize and treat the patients of dengue, particularly those receiving tertiary level care, which primarily deals with complicated cases [17,18]. A variation of hematocrit value greater than 10% was chosen (instead of 20%) because initial hematocrits have greater sensitivity in identifying complications in dengue and have been associated with serious symptoms in patients from areas endemic for dengue [19,20].

This study supports the association between development of severe dengue (SD) and early alterations of the serum levels of CK, LDH, triglycerides, albumin, blood glucose and calcium. These biomarkers have been proposed as indicators of severity in dengue in retrospective and cross sectional studies [12,13-16].

In the present study, the risk of severe dengue (SD) was seven times greater among patients with elevated levels of CK within the first 96 hours of the onset of fever. Increased levels of CK were significantly associated with previous intramuscular drug use (odds ratio=21.95% CI=1.2-3.8, P=0.008). However the association between increased levels of CK and severe dengue (SD) was independent of this intervention and other biochemical alterations (HR=6.98%, CI=2.34-20.85, P=0.001). Additionally, we observed an increase in levels of LDH in severe dengue (SD) patients when compared with DF patients.

These alterations may be caused by subclinical damage of the skeletal muscle in severe dengue (SD) patients, followed by increases in levels of LDH and CK [12-15,21-22]. Moreover liver damage is a frequent problem in dengue that can also be associated with increased levels of LDH [8,12]. Our results suggest that these biomarkers can predict a more severe form of dengue and could also be indicators of early tissue injury in the acute phase of dengue infection.

Levels of AST and ALT in patients with dengue hemorrhagic fever were higher than those in patients with classical dengue fever. However, these biomarkers were not independent predictors of severity among patients with dengue viral infection. However in other studies, these biochemical markers have been associated with severe dengue. These alterations may appear later in the disease course and may not be useful as early predictors of severe dengue. In most cases, the high level of transaminases indicates the degree of hepatocellular injury,

prolonging the clinical course of the disease, often associated with greater morbidity and mortality irrespective of severity of dengue [24].

Serum Bilirubin was not raised significantly in our study, although the transaminases levels were raised, which was a very peculiar finding. Alkaline phosphatase level was also not elevated significantly.

Plasma leakage, which indicates that dengue causes hypoalbuminemia is an indicator of severity of the disease [1,2]. In our study serum albumin levels was an indicator of severity, albuminemia greater than 4g/dl was associated with lower risk of severe dengue (SD) whereas levels greater than than 4g/dl was negatively associated with DHF. It is probable that high values of albuminemia may reflect the integrity of the vascular endothelium, whereas albumin levels less than 4g/dl may be an early indicator of vascular permeability alteration. Therefore this parameter may be an early indicator of plasma leakage and a useful prognostic marker.

Another interesting finding was the negative association between triglyceride levels and DHF. Low levels of triglyceride have been described in patients with dengue hemorrhagic fever [23,25]. Triglycerides levels greater than 160mg/dl were negatively associated with severe dengue (SD).

Within the several biochemical derangements found in this study, the detection of hypocalcemia demands special consideration. There is scarcity of literature reporting hypocalcemia as a complicating factor of dengue. Interestingly a significant number of patients in this series had hypocalcemia, and some of them were symptomatic as well. Hypocalcemia was correlated with conventional severity parameter i.e mean calcium level was lowest in severe dengue (SD) patients.

In our study blood glucose was found higher in more severe cases. Control of blood sugar in diabetic dengue patients needs special consideration, as diabetes has been shown to be a complicating factor of dengue [26]. There were several cases in which glucose intolerance developed with dengue.

The relationship between each biomarker and disease outcome was compatible with the proportional hazard assumption. Among DF cases, levels of biomarkers were not significantly associated with hemorrhagic manifestations or hemoconcentration (P>0.05 for all comparisons).

CONCLUSION

Dengue fever evolves with laboratory alterations starting on the third day and becoming most evident on the fifth day with values restored to normal by the twelfth day. The disease is more severe in individuals aged 15 years and older with a pronounced and persistent presence of liver abnormalities. Hypocalcemia is commonly associated with dengue and correlates with disease severity. Subclinical muscle damage is often associated with severe dengue resulting in elevated levels of CK and LDH. Several cases with severe dengue develop glucose intolerance.

This study suggests that some biochemical alterations detected between 48-96 hours after symptoms can predict a more severe form of dengue infection. These results also imply that early pathogenic changes occur before complications develop. Therefore these biochemical alterations, could be associated with severe stages of the disease and may act as early prognostic markers for monitoring the illness and identifying those who will benefit from future therapies.

Application of these findings may help optimize resource allocation, leading to a more opportune and effective care of dengue patients in disease endemic areas. Thus establishment of these predictor biomarkers for dengue severity will help decrease morbidity and mortality related to the disease.

Tables: Table 1: Characteristics of dengue patients

Characteristic	Total (N=398)	DF (n=338)	SD (n=60)	P value
Male, no. (%)	198 (49.8)	166 (49.1)	32 (53.3)	0.67
Female, no. (%)	200 (50.2)	172 (50.9)	28 (46.7)	0.74
Mean +/- SD age,years	26.8+/- 17.1	26.4+/- 17.5	26.3+/- 14.6	0.96
Adults (15 yrs of age), no. (%)	294 (73.9)	246 (72.8)	48 (80)	0.41
Severity criteria. no. (%)				
Thrombocytopenia	94 (23.6)	34 (10.1)	60 (100)	<0.0001
Hemoconcentration	204 (51.3)	146 (43.2)	58 (96.7)	<0.0001
Pleural effusion or ascitis	12 (3)	2 (0.6)	10 (16.7)	<0.0001
Spontaneous hemorrhage	184 (46.2)	142 (42)	42 (70)	0.005
Positive tourniquet test result	318 (80.3)	262 (78)	56 (93.3)	0.05

Table 2: Results of Biochemical investigations (n= 398)

Biomarker	DF (n= 338)	SD(n=60)	P (DF vs SD)
Blood Glucose(mg/dL) F	105.3 (95.2-116.4)	125.3 (115.2-136.4)	0.09
Bilirubin (mg/dL)	1.0 (0.7-0.9)	1.5 (0.9-1.9)	0.25
AST (U/L)	90.2 (78.3-102.1)	142.7 (106.3-179.2)	0.001
ALT (U/L)	69.7 (58-81.4)	90.8 (57.3-124.5)	0.18
Albumin (g/dL)	4.11 (4.05 -4.17)	3.95 (3.79-4.11)	0.049
Cholesterol (mg/dL)	144.8 (139.2-150.4)	128.9 (114.8-143.2)	0.03
HDL(mg/dL)	36.1 (34.6-37.7)	36.5 (32.8-40.2)	0.86
LDL(mg/dL)	83.1 (78.3-87.8)	73.4 (60.1-86.7)	0.13
TG(mg/dL)	128.9 (117.1-140.8)	96 (82.1-109.9)	0.03
LDH (U/L)	562.3 (524.9-599.7)	711.6 (612.6-810.6)	0.003
CK (U/L)	298.7 (223.1-374.3)	549.6 (267.5-831.6)	0.02
Calcium (mg/dL)	8.6 (7.9-9.4)	7.6 (6.8-8.2)	0.03

Table 3: Early Biochemical test markers associated with severity of Dengue

Characteristic	DF (n=338) no. (%)	SD(n=60) no. (%)	Hazard ratio	95% Confidence Interval	P value
Glucose >100 mg/dL	180 (53.3)	28 (46.7)	0.92	0.42-2.02	0.84
LDH >570 U/L	118 (34.9)	40 (66.7)	3.19	1.01-10.12	<0.05
Elevated CK	176 (52.1)	50 (83.3)	6.98	2.34-20.85	0.001
Bilirubin >1mg/dL	56 (16.6)	18 (30)	0.99	0.35-2.84	0.99
AST > 105 U/L	86 (25.4)	32 (53.3)	1.61	0.48-5.4	0.44
ALT > 105 U/L	56 (16.6)	18 (30)	0.99	0.35-2.84	0.99
Albumin>4g/dL	208 (61.5)	26 (43.3)	0.39	0.17-0.92	0.03
Cholesterol>140 mg/dL	176 (52.1)	24 (40)	2.11	0.56-7.91	0.27
HDL>35mg/dL	170 (50.3)	32 (53.3)	1.23	0.54-2.76	0.62
LDL>80mg/dL	176 (52.1)	24 (40)	0.45	0.12-1.63	0.23
TG>160mg/dL	82 (24.3)	2 (3.3)	0.07	0.01-0.59	0.01
Calcium<9mg/dL	210 (62.1)	44 (73.3)	1.49	0.62-3.56	0.37

REFERENCES

1. World Health Organization: Dengue: Guidelines for diagnosis, treatment, prevention and control. Geneva: WHO; 2009.
2. Gubler D. Dengue and dengue hemorrhagic fever. J Clinical Microbiology Review 1998; 11: 480-496.
3. Kabra SK, Jain Y, Pandey RM, Singhal T, Tripathi P. Dengue hemorrhagic fever in children in the 1996 Delhi epidemic. Trans R Soc Trop Med Hyg 1999; 73: 435–440.
4. Narayan M, Aravind MA, Thilothammal N, Prema R, Sargunam CS. Dengue fever epidemic in Chennai. J Indian Pediatric 2002; 39: 1027–1033.
5. Shah I, Deshpande GC, Tardeja PN. Outbreak of dengue in Mumbai and predictive markers of dengue shock syndrome. J Trop Pediatric 2002; 50: 301–305.
6. Gupta P, Khare V, Tripathi S, Nag VL, Kumar R. Assessment of WHO definition of dengue hemorrhagic fever in North india. J Infect Dev Ctries 2010; 4: 150–155.

7. Kumar A, Sharma SK, Padbidri VS, Thakrey JP, Jain DC. An outbreak of dengue fever in rural areas of northern India. J Commun Dis 2001; 233: 274–281.
8. Kalayanarooj S, Vaughn DW, Nimmannitya S, Green S, Suntayakorn S et al. Early clinical and laboratory indicators of acute dengue illness. J Infect Dis 1997; 176: 313–321.
9. Monath TP. Early indicators in acute dengue infection. Lancet 1997; 350: 1719–1720.
10. Wang LY, Chang WY, Lu SN, Chen TP. Sequential changes of serum transaminase and abdominal sonography in patients with suspected dengue fever. Kao Hsiung I Hsueh Tsa Chih 1990; 6: 483–489.
11. Nguyen TL, Nguyen TH, Tieu NT. The impact of dengue hemorrhagic fever on liver function. Res Virol 1997; 148: 273–277.
12. Kuo CH, Tai DI, Chang-Chien CS, Lan CK, Chiou SS, Liaw YF. Liver biochemical tests and dengue fever. Am J Trop Med Hyg 1992; 47: 265–270.
13. Ray G, Kumar V, Kapoor AK, Dutta AK, Batra S. Status of antioxidants and other biochemical abnormalities in children with dengue fever. J Trop Pediatr 1999; 45: 4–7.
14. Jusuf H, Sudjana P, Djumhana A, Abdurachman SA. DHF with complication of acute pancreatitis related hyperglycemia. Southeast Asian J Trop Med Public Health 1998; 29: 367–369.
15. Krippner R, Hanish G, Kretschmer H. Dengue fever with hemorrhagic manifestations after a stay in Thailand. Disch Med Wochenschr 1990; 115: 858–862.
16. Gascon J, Giner V, Vidal J, Jou JM, Mas E, Corachan M. Dengue: a re-emerging disease. A clinical and epidemiological study in 57 Spanish travelers. Med Clin (Barc) 1998; 111: 583–586.
17. Balmaseda A, Hammond SN, Perez MA, et al. Assessment of the World Health Organization Scheme for classification of Dengue Severity in Nicaragua. Am J Trop Med Hyg 2005; 73(6): 1059–1062.
18. Phuong CX, Nhan NT, Kneen R, et al. Clinical diagnosis and assessment of severity of confirmed dengue infections in Vietnamese children: is the World Health Organization classification system helpful? Am J Trop Med Hyg 2004; 70(2): 172–179.
19. Rigau-Perej JG, Bonilla GL. An evaluation of modified case definitions for the detection of dengue hemorrhagic fever. Puerto Rico Health Sci J 1999; 18: 347–352.
20. Diaj-Quijano FA, Martinez- Vega RA, Villar-Centeno LA. Indicators of severity of dengue fever. Enferm Infect Microbiol Clin 2005; 23: 529–532.
21. Agarwal DK, Tandon P, Chaturvedi UC, Kumar A. Biochemical study of certain enzymes and metabolites of the carbohydrate metabolism in the skeletal muscle of the dengue virus-infected mice. J Gen Virol 1978; 40: 399–408.
22. Korones DN, Brown MR, Palis J. Liver function tests are not always tests of liver function. Am J Hematol 2001; 66: 46–48.
23. Van Gorp EC, Suharti C, Mairuhu AT et al. Changes in the plasma lipid profile as a potential predictor of clinical outcome in dengue hemorrhagic fever. Clin Infect Dis 2002; 34: 1150–1153.
24. Souja LJ, Alves JG, Nogueira RM et al. Aminotransferase changes and acute hepatitis in patients with dengue fever: analysis of 1585 cases. Braz j Infect Dis 2004; 8 (2): 156–63.
25. Alvarez ME, Ramirez-Ronda CH. Dengue and hepatic failure. Am J Med 1985; 79: 670–674.
26. Lee MS, Hwang KP, Chen TC et al. Clinical characteristics of dengue and dengue hemorrhagic fever in a medical center of southern Taiwan during the 2002 epidemic. J Microbiol Immunol Infect 2006; 39 (2): 121–9.