Original Research Article

Biomarkers as a diagnostic tool in primary and secondary dengue Infections

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Abstract

Introduction: Dengue virus infection presents with a diverse clinical picture ranging from asymptomatic illness to Dengue fever to dengue hemorrhagic fever/dengue shock syndrome. Because of its varied clinical presentation, accurate diagnosis is difficult and relies largely on laboratory confirmation. We therefore designed a prospective study to ascertain the role of biomarkers in cases of serologically confirmed primary and secondary dengue infections. **Material & Methods:** A total of 2165 patients presenting to the hospital with acute febrile illness were serologically confirmed to be suffering from dengue infection. Hematological examinationand Liver function test were carried out for all these cases. **Results:** A total of 1942 (89.69%) patients were classified as having primary dengue infection and only 182(8.4%) were classified as having secondary dengue infection out of the total seropositive cases (2165).13.16% of these patients had platelet count below 50,000/mm^3. Majority of the patients showed deranged hepatic function. Amongst the hepatic biomarkers AST and GGT were found to be significantly deranged as compared to other parameters.Also it was found that Alkaline phosphatase levels &platelet count may serve as early predictors for the differentiation of primary and secondary dengue infection.**Conclusion:** The study highlights the importance of biochemical markers in distinguishing dengue from other febrile illness and their role in differentiating primary and secondary dengue cases.

Introduction

The World Health Organization (WHO) considers dengue as a major global public health challenge in the tropic and subtropic nations. Dengue has seen a major upsurge worldwide due to increased population growth rate, global warming, unplanned urbanization, inefficient mosquito control, frequent air travel, and lack of health care facilities [1,2]. Two and a half billion people reside in dengue-endemic regions and roughly 400 million infections occurring per year, with a mortality rate surpassing 5–20% in some areas [2,3].

Dengue virus infection presents with a diverse clinical picture ranging from asymptomatic illness to DF to the severe illness of dengue hemorrhagic fever/ dengue shock syndrome (DHF/DSS) [1]. Because of its varied clinical presentation, accurate diagnosis is difficult and relies largely on laboratory confirmation [4]. Primary infection is often asymptomatic but may result in dengue fever (DF). However secondary infection can lead to life threatening dengue hemorrhagic fever (DHF) / Dengue shock syndrome.

Primary infections are characterized by an increase in dengue specific IgM antibodies 4-5 days after the onset of fever and increased IgG antibodies after 7-10 days. In secondary infections, IgG antibodies rise rapidly even during acute phase. However, there are a number of atypical forms of dengue infection; hepatic dysfunction is a well-recognized feature of dengue infection[5,6].

We designed a prospective study to determine the status of hepatic and hematological parameters in cases of serologically confirmed primary and secondary dengue infections Additionally we also tried to determine which

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of these parameters can serve as early laboratory predictors to differentiate primary from secondary dengue infection before the critical stage is reached.

Material and Methods

Place of Study: The present study was undertaken at Shri Guru Ram Rai Institute of Medical and Health Sciences by the department of Microbiology from June to September 2018. During this period, 2165 patients presenting to the hospital with acute febrile illness were serologically confirmed to be suffering from dengue infection. Case definition criteria for DF were high fever, fever with rash, retro-orbital pain, myalgia, arthralgia and conjunctival congestion as per WHO guidelines [7].

Type of Study : Prospective cross-sectional study
Inclusion Criteria : Case definition of dengue fever as per WHO (2009)[7]

Exclusion Criteria

1. All the cases which showed negative serology, (or) positive for other causes of fever (malaria, widal, PUO), outpatient cases were excluded from the study.
2. Patients presenting with other co morbid infections along with dengue fever were not included into the study.
3. All the other causes of thrombocytopenia were also excluded from the study.

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Seropositivity for dengue fever was determined using Microwell ELISA test for NS1, IgM and IgG using Panbio Dengue Early ELISA, Panbio Dengue Capture, Republic of Korea. Hematological profiles and biochemical investigations of seropositive dengue cases were carried out at the time of admission and were correlated.

Based on the serological markers patients were classified as having primary or secondary dengue infection as follows:
Early primary infection: NS1+++
Late primary infection: IgM++, IgG+/-
Early secondary infection: IgG++, IgM+/-, NS1++
Late secondary infection: IgG++, IgM+, NS1-

Hematological examination was done using automated analyser DxH500 by Beckman Coulter, California, USA. Hemoconcentration was seen as raised Hb or RBC count. Leukopenia was defined as less than 4000/cubic mm and thrombocytopenia as less than 1.0 lakh/cubic mm. Liver function test was done using Vitros E Cianalyser (Ortho clinical diagnostics, Rochester, NY). Altered liver function was considered if total bilirubin >2 mg/dl, AST > 60IU/L, ALT >45 IU/L, ALP>147 U/L, GGT >48U/L, or albumin< 3.5mg/dl.

Statistical Analysis- Statistical analysis was done using Sofa Stats software (open source statistics, analysis and reporting software from Paton-Simpson& Associates Ltd).Results were presented as mean and SD for continuous variables while frequency and percentage are given for qualitative variables. Unpaired t-test used for P values and 95% confidence interval (CI) were calculated from the mean, SD and number using Med Calc easy to use statistical software. A p<=0.05 was considered statistically significant

Results

Of the 2165 patients found seropositive for DF, 75% (n=1627) were found positive for NS1Ag alone, 2.9% (n=63) only IgM positive, 4.67% (n=101) only IgG positive, 11.5% (n=249) were found positive for both NS1 & IgM, 1.52% (n=33) both IgM & IgG and 3.3%(n=73) NS1 & IgG positive and 0.87% (n=11) positive for all three NS1, IgM, IgG.

Table-1: Distribution of serological markers among dengue positive cases

Serological marker	Number	Percentage
NS1 Ag	1627	75
IgM	63	2.9
IgG	101	4.67
NS1 and IgM	249	11.5
IgM & IgG	33	1.52
NS1& IgG	73	3.3
NS1, IgM, IgG	11	0.87

The mean age of patients in our study was 37.2 years with a range of 5-80 yrs.

Table-2: Age wise distribution of patients

Age	Total Cases +VE	Percentage
1 - 10 YRS	108	5
11-20 YRS	394	18.2
21-30 YRS	709	32.74
31-40 YRS	414	19.12
41-50 YRS	262	12.1
51-60 YRS	168	7.76
61-70 YRS	83	3.83
> 70 YRS	27	1.25

Maximum number of patients belonged to the age group of 21-30 yrs. There were 62.31% (n=1349) males and 37.69% (n=816) females.

Hematological Profile- The mean (±SE) of Hemoglobin, Hematocrit, TLC and platelet count at admission were 13.2±0.20gm/dl, 40.8±0.5%, 5945±280 cells/mm^3 and 14.5±0.5x1000/mm3 respectively

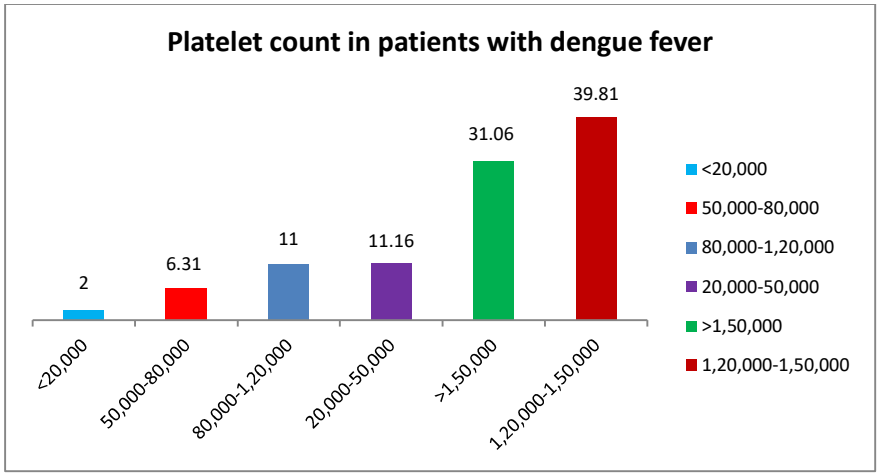

Chart-1: Hematological parameters in patients with dengue fever

Majority of patients (70.87%) had platelet counts above 1 lac while only 13.16% of the patients had platelet count below 50,000 /mm^3.

Liver function test- Hepatic dysfunction, in the form of deranged total bliirubin, AST, ALT, ALP, GGT, albumin and altered albumin globulin ratio was present in 4.12% (n=89),78.3% (n=1695), 55.6%(n=1203), 8.2% (n=187), 36.1% (n=781), 32% (n=692), 1.03% (n=22) of patients respectively.

Table-3: Liver function tests in patients with dengue fever

Liver biochemical test	Mean ± SE*	Number of patients with>ULN**	P value
Total Bilirubin	1.05±0.09mg/dl	4.12% (89)	0.21
AST	356.6±51.2U/L	78.3% (1695)	0.02
ALT	166.2±31.2U/L	55.6% (1203)	0.031
ALP	90.9±8.7U/L	8.24% (187)	0.62
GGT	83.8±6.5U/L	36.1% (781)	0.54
Albumin	3.5±0.5mg/dl	32% (692)	0.032
NOTE: *SE=Standard error **ULN=Upper Limit of Normal			

The mean (±SE) of total bilirubin, AST, ALT, ALP, GGT, albumin was 1.05±0.09mg/dl, 356.6±51.2U/L, 166.2±31.2U/L, 90.9±8.7U/L, 83.8±6.5U/L, 3.5±0.5mg/dl respectively. The number of patients with DF showing rise in AST levels was significantly higher than those showing rise in ALT ($p<0.0001$).

Also the number of patients showing elevated levels of GGT was significantly higher than those with elevated levels of ALP ($p<0.001$). No statistically significant difference was observed in LFT's between male and female patient

Table-4: Comparison of platelet count and liver function tests in patients with primary and secondary dengue fever.

Parameters	Primary Dengue Fever (n=1942)		Secondary Dengue Fever(n=182)		Statistical analysis	
	Number	%	Number	%	P value	95%CI
Low platelet count (<50,000mm^3)	44/182	24.1	19/24	79.16	0.002	1.119 to 2.6797
Total bilirubin raised (>2mg/dl)	9/74	2.1	8/14	57.14	0.001	3.89 to 21.8382
AST raised (60 U/L)	67/79	84.8	5/10	50.0	0.0985	0.9063 to 3.1746
ALT raised (>45 U/L)	40/87	4.5	5/11	45.5	0.974	0.5092 to 2.0091
ALP raised (147 U/L)	7/63	11.1	3/6	50.0	0.0055	0.0768 to 0.6428
GGT raised (>48 U/L)	27/63	42.9	4/7	57.1	0.4219	0.3717 to 1.5134
Albumin decreased (<3.5mg/dl)	0/64	0.0 0.0	0/7	0.0	0.2863	0.0026 to 5.7872

A comparison was also made of the hematological and hepatic functional derangements in patients with primary and secondary dengue infection. Based on the serological finding patients were classified as suffering from primary or secondary DF. A total of 1942(89.69%) patients were classified as having primary dengue infection and only 182(8.4%) were classified as having secondary dengue infection out of the total seropositive cases (2165).

Comparison of platelet count and hepatic markers in patients with primary and secondary dengue infection reveals that platelet counts were significantly deranged ($p<0.05$; 95% CI 1.119 to 2.679.7) and Alkaline phosphatase levels were significantly higher in patients with secondary dengue infection than those having infection for the first time ($p<0.05$; 95% CI 0.0768 to 0.6428). For all other parameters the difference in positivity between primary and secondary dengue infections was not statistically significant.

Discussion

Dengue viral infections are one of the most rapidly evolving vectors borne infections, which now affects 125 countries, causing approximately 100 million apparent infections each year [8]. Infection with DENV results in either undifferentiated viral fever, DF, DHF or DSS. Involvement of the liver leading to hepatic dysfunction is a well-recognized complication of dengue [9-11]. Dengue associated acute liver failure has a high mortality due to complications such as encephalopathy, severe bleeding, renal failure and metabolic acidosis [9,10].

In our study the male to female ratio of dengue patients was 1.65:1 and the largest number of positive samples (32.74%) were from the age-group 21-30 years. These findings are similar to those reported by NishatHussain Ahmed et al in their study wherein they have also reported male female ratio of 1.6:1 and preponderance of infection in the 20-30 years age group [12].

In this study, we have determined the changes in liver enzymes in patients suffering from acute dengue infection. ALT and AST are considered as indicators of

lever cell injury as they are released into the circulation following liver cell injury [13]. Although ALT is also found in low concentrations in skeltol muscles, brain and intestinal tissue, it is predominantly considered to be a liver specific enzyme [25]. In contrast, AST is released following damage to liver, cardiac and skeletal muscles [13]. The important characteristic of hepatic involvement with dengue infection is greater elevation in AST than ALT levels, and distinguishes lever failure caused by dengue infection from that caused by other causes of infectious hepatitis [14].

We have also found rise in AST levels significantly higher than ALT in dengue patients and it is likely to have resulted from other sources apart from liver contributing to rise in serum AST levels [15].

In our study raised AST levels were fund in 78.3% of the patients and raised ALT levels in 55.6% of the patients. In majority of the studies, elevation of AST is more than ALT [16]. The increased AST /ALT ratio is useful for differential diagnosis from acute hepatitis caused by Hepatitis A,B or C viruses where it is rarely observed [17].

Hypoalbunemia has been seen in 32% of our patients while it ranges from 16.5-76% in various other studies[5,18,19]. The heterogeneity of the population and severity of disease may be responsible for such a wide range observed in various studies.

In this study we also assessed the changes in GGT, ALP, serum bilirubin and serum albumin levels. The levels of these were not found to be significantly deranged in dengue patients ($p>0.05$). These findings suggest that there was minimal cholestasis in patients and similar findings have been reported by S. Fernando et al[15].

Further in the study we have also compared the difference in liver enzymes in primary and secondary dengue infection. Transaminases (ALT, AST) were found to be abnormally increased in both primary and secondary dengue infection. However, there was no significant difference in the levels between primary and secondary dengue infected cases. Similar findings have also been reported by Wong et al [5].

The incidence of raised total bilirubin, raised ALP and thrombocytopenia was more significant in secondary dengue infection than in primary dengue infection. Total bilrubin was raised in 57% of the secondary dengue infected cases as compared to just 2% in primary cases ($p<0.05$) while ALP was raised in 50% of secondary dengue cases as against 11% of primary dengue cases ($p<0.05$). Similarly, thrombocytopenia was observed in 79% of secondary dengue cases as against 24% of primary dengue cases ($p<0.05$). These findings corroborated with those reported by Bandaru et al [14].

Conclusion

Considering the above significant values, raised transaminases levels particularly AST may differentiate dengue infection from other febrile illness but may not differentiate primary dengue infection from secondary dengue infection. However, parameters such as total bilirubin, Alkaline phosphatase and platelet count may serve as early predictors for the differentiation of primary and secondary dengue infection Wahid et al[20].

To conclude the present study highlights the importance of biochemical markers in distinguishing dengue from other febrile illness and their role in identifying secondary and primary dengue cases. These findings have important public health implications in managing a case of dengue fever and handling of epidemics.

Contribution of authors

1. Dr. Iva Chandola: Principal Investigator
2. Dr. Brahmnish Sitara: helped with data collection
3. Dr. Nidhi Negi: prepared outline for the project
4. Dr. V. K. Kataria: guidance and proof reading

Findings: Nil; **Conflict of Interest**: None initiated
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