

Predictors for Severity of Dengue Infection during Early Days of Infection

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Abstract

Hepatorenal dysfunction has been implicated in dengue virus infection due to the obvious effects of dengue virus infection on the hepatorenal organs. This study was undertaken to establish the possible role of different serum biomarkers involved to have roles in the liver and kidney to see the pathology and prognosis of dengue virus infection. It is an observational, descriptive and retrospective study conducted on 74 sero positive cases during the early days of dengue virus infection (1 - 7 days) confirmed by Real time PCR (CDC Atlanta) who, in the period of August to November 2011 visited a tertiary care hospital in Lahore. Patients of both genders and all age groups were included. Patients were divided into 3 age groups i.e. 11 - 30, 31 - 50, 51 - 70 years. The tests analysed were platelet count, serum alkaline phosphatase (ALP), alanine aminotransferase (ALT), urea, creatinine and albumin, protein concentrations. This article assesses the value of these serum biomarkers and its association with age, gender, platelet count and bleeding tendencies in dengue patients. Most of these parameters were normal in dengue infected patients except for albumin, urea and creatinine. Hypoalbuminemia was observed in (54.05 %) of patients, low creatinine (95.94 %) and raised urea (41.89 %) values were observed in patients with dengue virus infection. No association was observed between these serum biomarkers and bleeding. More dengue patients had declined platelet counts ($< 50,000/\mu\text{l}$). Dengue infection was more in males and in age group 11 - 30 years but hemorrhagic signs were more in females and in older patients of more than 50 years. From the study, impaired hepatorenal function, found to be common in dengue infected patients, was associated with declining levels of serum albumin, creatinine and raised levels of urea. Hence serum albumin, creatinine and urea can be used as predictive markers for the severity of dengue virus infection during early infection while it cannot help in differentiating dengue fever from dengue hemorrhagic fever. Our study is among the few to report involvement of the kidney along with liver during dengue infection.

Keywords: Predictors of dengue infection, dengue, serum biomarkers, liver function tests, renal function tests

Introduction

Dengue virus infection is caused by four serotypes of dengue virus (DEN1, DEN2, DEN3 and DEN 4) [1] which is a mosquito (*Aedes aegypti*) borne virus. It is a single stranded, non-segmented RNA virus [1,2], belonging to the genus *Flavivirus* of the family *flaviviridae*. Dengue

fever is a disease with wide clinical spectrum and ranges from asymptomatic to undifferentiated fever [3] and more severe forms present hemorrhagic signs, petechiae, ascites, pleural effusion, vascular permeability and marked thrombocytopenia as in Dengue Hemorrhagic

Fever (DHF) [1,4-5]. Incubation period is from 8 - 12 days, after which the virus is capable of transmitting the virus to susceptible hosts.

Lahore was hit by severe dengue attacks during the post-monsoon season in 2011 and less severely in 2012. The current study was undertaken to observe any association of serum biomarkers involved in hepatic and renal functions with severity of the disease. The diagnosis of dengue infection is mostly based on clinical, biochemical (Liver function and renal function tests), hematological (platelet count, blood count, prothrombin time (PT), activated partial thromboplastin time (APTT) and epidemiological parameters [6]. Urea is a non-protein compound present in highest concentration in blood. It is a major excretory product of protein metabolism. Creatinine is formed in muscles and excreted in plasma at a constant rate. It is directly related to glomerular filtration rate. Urea and creatinine are commonly used to assess renal function.

Serum albumin and protein are markers of proper functioning of liver and kidneys. Serum protein measures the total amount of protein in blood plasma or serum. It also measures the amounts of two major groups of proteins in the blood: albumin and globulin. Proteins serve important functions of circulatory transport molecules for lipids, hormones, vitamins, metal enzymes, complement components, protease inhibitors and also in immune system. All the plasma proteins are synthesized in liver except gamma globulins. Protein albumin constitutes 60 %, globulins make up 35 %, fibrinogen 4 % and regulatory proteins (enzymes, proenzymes and hormones) less than 1 % of plasma proteins. Reference range of serum protein is 6 - 8 g/dl or 60 - 80 g/L [7].

Albumin is essential for maintaining the pH, osmotic pressure needed for proper distribution of body fluids between intravascular compartments and body tissues and helps keep the blood from leaking out of blood vessels [8]. It is important for tissue growth and healing, and indicates if the diet contains enough protein, helps determining the cause of edema or ascites or of pleural effusion. Albumin is a negative acute phase protein because its level declines at least by 25 % during the acute phase. The reason may be an increase in the need of immune mediators during stress and inflammation and decreased need for other

proteins that are not essential during immune function.

Liver function tests such as serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), Gamma-glutamyl transpeptidase and alkaline phosphatase levels, and serum albumin levels are the mostly variable biochemical parameters in dengue infected patients in different studies [9,10]. The present study was conducted to assess different biochemical and hematological parameters in patients suffering from dengue infection to evaluate the efficacy of these diagnostic tests.

Materials and methods

3 - 5 ml of venous blood was collected from 74 patients within the first 7 days of infection, with clinical presentation of Dengue fever (DF) (high grade fever, rash, retro-orbital pain and bleeding) during August - November 2011 (active phase of outbreak), at the laboratories of the Pathology Department of King Edward Medical University, Lahore, in collaboration with the Emergency and Accidents Department of Mayo Hospital, Lahore. The samples were collected with proper protocol and consents were taken from the patients or their guardians. All other ethical issues were considered during the process of sampling. Moreover, an approval was taken from the Ethical Consideration board of KEMU. Samples were allowed to clot for half an hour, then samples were centrifuged and serum was separated. Data on sex, age and the presence or absence of bleeding was taken. The inclusion criteria for the study was patients of both sexes and all age groups, with high grade fever i.e. more than 100 °C, a positive tourniquet test, severe headache, having low platelet count i.e. 150,000 μ l, patients without prior treatment, who were quite healthy before the dengue infection during that outbreak.

The samples were sent to the Center of Disease Control (CDC), Atlanta, Georgia, for the purpose of PCR based molecular assays for the determination of the serotypes. All measures were taken for the proper preservation of the samples during their transport to the laboratories of the CDC. Liver and renal function tests were performed on 74 seropositive samples confirmed by CDC. ALT, ALP, albumin, urea, creatinine, total serum protein levels were tested. Patients were divided into 3 age groups; 11 - 30 years, 31 -

50 years and 51 - 70 years. Age group of 1 - 10 and more than 70 years was not included because there were no patients in this range. Liver function tests and renal function tests were performed photometrically having standard kits with a routine chemical analyzer. The principle of the photometer is to measure the light intensity of the solution, the amount of light absorbed is directly proportional to the presence of a particular substance in the solution. The kits of ROCHE Company were used for the performance of liver and renal function tests. Data was analyzed by using SPSS version 17.

Results

All the dengue positive patients were infected by DEN-2 serotype which was confirmed from CDC Atlanta. The majority of the patients who were tested for ALT, urea, creatinine, protein, albumin and ALP were in the age group of 11 - 30 years (45/74; 60 %) 23 patients (31.08 %) in age group of 31 - 50 years, 6 patients (8.10 %) in age range of 51 - 70 years (**Table 1**).

More patients showed normal values for serum ALT, serum total proteins and ALP (**Tables**

1 and **2**), while the levels of serum albumin, creatinine and urea were found to be altered (highlighted in **Table 1** and **2**). Reference values of the mentioned biomarkers are shown in **Table 3**. Albumin and Creatinine were decreased and urea was raised in dengue infected patients (**Table 1** and **2**). Urea was found to be raised in 31/74 (41.89 %) patients, albumin declined in 40/74 (54.05 %) patients and creatinine also declined in 71/74 (95.94 %) patients (Highlighted in **Table 1** and **2**). Most alterations in these parameters, either increased or decreased, were also observed in the age group of 11 - 30 years (**Table 1**). A total of 52 (70.27 %) of these patients were males (**Table 2**) and mostly didn't show any hemorrhagic signs (**Figure 1**). Platelet count was less than 50,000 μ l in infected patients (**Figure 2**). There was no association between bleeding tendency and dengue infection (**Figure 1**). The younger age group 11 - 30 years was infected more (**Table 1**). Hemorrhagic signs were not observed in most of the patients (**Figure 1**), but bleeding if present was more in females and in older patients (more than 50 years).

Table 1 Age wise distribution of dengue patients (highlighted columns show deranged levels).

Age years	ALT				Total	Urea				Total	Creatinine				Total	Protein				Total	Albumin				Total	ALP				Total
	H	N	L			H	N	L			H	N	L			H	N	L			H	N	L			H	N	L		
11-30	10	35	0		45	17	26	2	45	0	1	44		45	12	16	17		45	2	22	21		45	2	41	2		45	
31-50	3	20	0		23	12	10	1	23	0	1	22		23	5	13	5		23	0	8	15		23	2	20	1		23	
51-70	3	3	0		6	2	4	0	6	0	1	5		6	0	5	1		6	0	2	4		6	2	4	0		6	
Total	16	58	0		74	31	40	3	74	0	3	71		74	17	34	23		74	2	32	40		74	6	65	3		74	

Table 2 Gender wise distribution of dengue patients (highlighted columns show deranged levels).

Gender	ALT				Total	Urea				Total	Creatinine				Total	Protein				Total	Albumin				Total	ALP				Total
	H	N	L			H	N	L			H	N	L			H	N	L			H	N	L			H	N	L		
Males	9	43	0		52	19	31	2		52	0	2		50	52	12	26	14		52	2	23	27		52	5	44	3		52
Females	7	15	0		22	12	9	1		22	0	1		21	22	5	8	9		22	0	9	13		22	1	21	0		22
Total	16	58	0		74	31	40	3		74	0	3		71	74	17	34	23		74	2	32	40		74	6	65	3		74

Table 3 Normal ranges of serum biomarkers (LFT's and RFT's).

Range	ALT	Urea	Creatinine	Albumin	Protein	ALP
Normal	10 - 45 U/l	15 - 45 mg/dl	< 1.4 mg/dl	3.5 - 5.3 mg/dl	6.3 - 8.3 g/dl	110 - 130 U/l

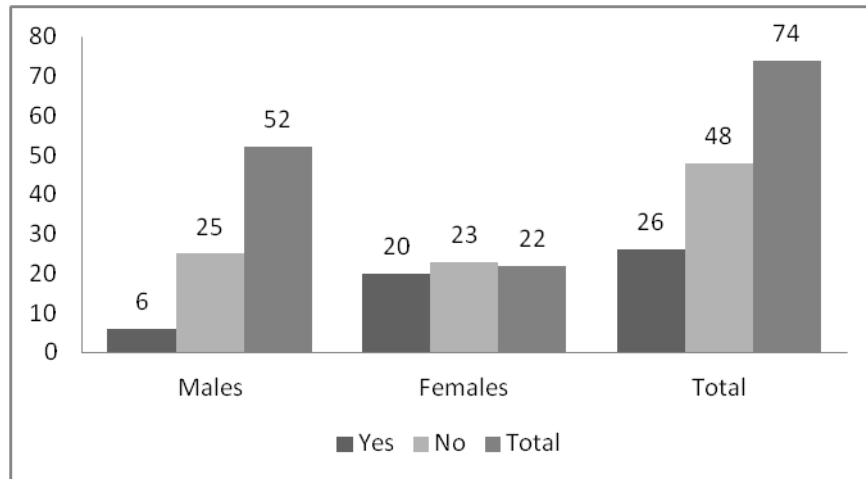


Figure 1 Distribution of patients according to bleeding tendency.

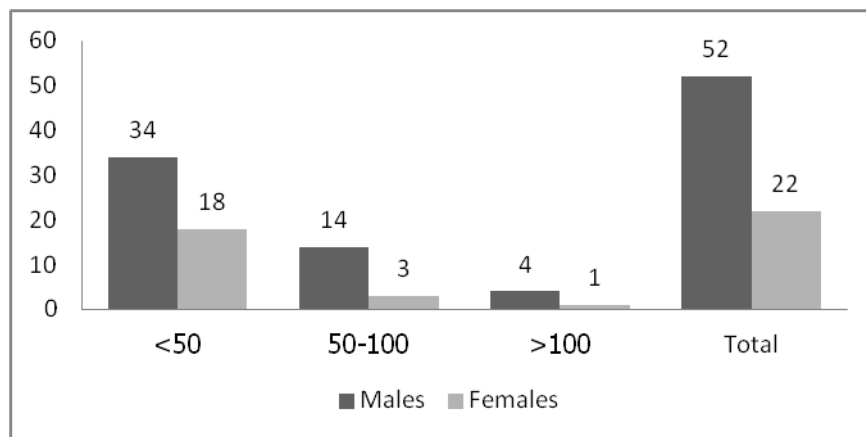


Figure 2 Distribution of patients according to platelet count.

Discussion

The dengue virus is an important public health issue in various tropical regions of the world [11]. The demographic and clinical features of dengue infections have changed rapidly in the last three decades. As symptoms of dengue infection are highly variable and it's difficult to diagnose during early infection, it poses serious health concerns.

In one study, more females (52.27 %) were infected by dengue virus infection as compared to males (47.73 %). Mean age of patients of both the

genders was same (31.38 ± 18.37 years) [12]. Dengue infection was found to be more common in children less than 15 years of age in a study conducted in Kolkata, India [13]. Children and elderly people were found to be infected more with dengue infection as compared to young adults in a previous study [14] while in our study the affected patients were mainly young adults of 15 - 30 years of age. No children were affected and elderly people of more than 50 years of age were also least affected.

Among different laboratory tests, liver and renal function alterations are the widely used diagnostic tests. These tests are performed to assess the function of the liver and kidney during the diseased state. Liver function tests that we performed were Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), albumin, serum total protein and the renal function tests performed were urea and creatinine.

Many variables, including inflammation, are known to affect serum protein markers [15]. Serum proteins are affected by capillary permeability, drugs, impaired liver function and inflammation [15,16]. Serum proteins are involved in repair and maintenance of immune system along with other body tissues. Albumin is an established indicator of morbidity and mortality [17,18]. Liver injury is a common finding in dengue infections [10] and it is mediated by direct infection of hepatocytes and Kupffer cells.

Serum aspartate transaminase (AST) levels are found to be raised in many studies [9,10]. Serum ALT and AST was also found elevated in a study conducted in Thailand. Mahmuduzzaman *et al.* also suggested ALT and AST as diagnostic markers for dengue fever [9]. But our results are contradictory to these findings and we didn't find any raised values of ALT or ALP in our study.

The values of serum total protein are normal while values of albumin are decreased in dengue patients in the current study. Hypoalbuminaemia has been reported in other infections as well [19]. This may, however, be as a result of enhanced impairment of the synthetic function of the liver by dengue infection since it has liver as its target organ. The majority of the proteins are the products of the synthetic function of the liver [20]. Hence, dengue infection adversely affects the liver as shown by this study. So, the synthetic ability of the liver is affected by dengue infection.

A declination in serum albumin and protein levels has been observed in various studies in which hepatic function is altered due to some infection [21] but decline in protein levels is not observed in our study. All proteins are covalently linked polymers of amino acid, made of peptide bonds and plasma and tissue proteins share the same amino acid pool, and thus alterations in one group will eventually affect the other [20]. These alterations could be secondary to infections like dengue. Protein levels in the body may reflect the immunity status of an individual; hence, any

adverse influence may increase the vulnerability to dengue infection.

Urea and Creatinine values are also altered in this study. Urea was raised in dengue patients while creatinine declined. Deranged serum urea and creatinine levels are indicative of renal dysfunction. Previously renal function alterations have not been reported in many studies, only liver function alteration has previously been reported in dengue patients [10]. Our study is among a few to report the involvement of the kidney during dengue infection.

Conclusions

Most of the serum biomarkers in dengue patients appeared to be normal except for serum albumin, urea and creatinine during the first 7 days of infection. However, we can't say that liver function tests are not important to predict the severity of dengue infection.

Conflict of interests

The authors report no conflict of interests.

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