Research article

Hemostatic defects in Dengue infection at a tertiary care hospital in Karachi

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ABSTRACT

This study was aimed to investigate haemostatic defects in dengue infection. A cross sectional study was conducted from 2013-2014 at National Institute of Blood Diseases & Bone Marrow Transplantation, Karachi. Total 127 dengue patients of either sex were included. After clinical examination, serology was performed to confirm dengue. The complete blood picture (CBC), prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen, D-Dimer, liver function tests were performed. Out of 127 cases about 95.8% patients presented with myalgia and 88 had headaches. No splenomegaly or hepatomegaly was observed. Serologic antibodies were found in all patients. Average platelet count and white blood cell count were 47.2x10^3 /ul and 5.3x10^3/ul respectively. Eighty-three patients had prolonged PT while 92 patients had prolonged APTT value. Raised total bilirubin, alkaline phosphatase and SGPT were found in 7, 91 and 87 patients respectively. Highly elevated D-Dimer values were recorded in 96% cases while only 12% patients had higher fibrinogen levels. Marked hematological abnormalities were observed among all the patients diagnosed with DF regardless of age, sex and clinical presentation.

Key words: Dengue fever; fibrinolysis; D- Dimer; PT; APTT; hemostatic defects

INTRODUCTION

Dengue infection is a mosquito borne tropical illness caused by any of the four different viral serovers i.e. DEN I-IV. The common vectors for transmission in human are *Aedes aegypti* and *Aedes albopictus*. Clinically apparent disease due to dengue virus varies in severity from mild undifferentiated dengue fever (DF) through to more severe forms such as dengue hemorrhagic fever (DHF) and dengue shock

syndrome (DSS). The DF and DHF are more commonly occurring pathological events due to dengue virus world-wide; accounting to more than 100 million annual cases.[1,2] Acute febrile phase is manifested by headache, body aches, weakness, joint pains, GI discomfort and last upto 3-5 days. Capillary leakage due to increase vascular permeability is a key differentiating feature observed in DHF otherwise absent in DF. Excessive decrease of intravascular volume in DHF may lead to DSS. Common symptoms for DHF include skin petechiae or bruising, minor bleeding episodes but major hemorrhage is unusual.[3] The exact cause of bleeding in dengue infections is still obscured. Most evident physiological changes in DF include thrombocytopenia (platelet counts less than 150,000/l), raised hemoglobin, and hematocrit, and decreased total leucocyte counts. Viral attack on hepatocytes cause deranged liver function tests.[4]

Abnormalities in platelets function are common in dengue infections such as ADP induced impaired platelets aggregation response and increased secretory activities of platelets.[5] Furthermore, hyperfibrinogenemia, decreased fibrin monomers (FM) and slightly prolonged prothrombin (PT) and partial thromboplastin times (APTT) have also been reported in dengue infected patients.[6,7] According to some studies small fraction of DHF patients may exhibit alteration in levels of coagulation factors, such as factor II, V, VII, VIII, IX, X, antithrombin, and α-2–antiplasmin.[8] This study was aimed to investigate haemostatic defects in patients with dengue infection admitted to NIBD.

METHODS

A total of 127 dengue patients of either sex were enrolled to conduct a cross sectional study over a period of two years from 2013-2014 at National Institute of Blood Diseases & Bone Marrow Transplantation, Karachi. The study was conducted after approval by ethical review committee of NIBD. A clinical examination with detailed history was carried out. Serology was performed to confirm dengue using solid-phase immunochromatographic assays (MP Diagnostics MULTISURE dengue Ab/Ag Rapid Test kit). The primary and secondary infections were confirmed by presence or absence of NS-1 antigen and IgA, IgM, IgG. Primary dengue cases were NS-1, IgA or IgM positive while secondary dengue cases also showed positivity for IgG. Complete blood count (CBC) using EDTA blood sample analyzed by hematology analyzer XE- 2100. First line coagulation screening tests were performed to rule out the findings of hemostatic defects. Citrated plasma was used for Prothrombin time (PTT), activated prothrombin time (APTT), fibrinogen and D-Dimer levels using STA Compact R, Diagnostic Stago, France. Serum was used for Liver function test (LFT) using biochemical analyzer Mindray 200, China. The data obtained was subjected to simple descriptive analysis using SPSS version 23.0.

RESULTS

The dengue serological tests confirmed dengue infection in 127 patients. Out of these, 90 were males and 37 were females; mean age of the patients was 24.8(3-41 yrs). Overall, 79 patients had dengue secondary infection based on their serological findings i.e presence of IgG, IgM+IgG or IgA alone (*Figure-1*). Beside fever, vomiting and fatigue; other hematological manifestations observed were epistaxis, petechia, gum bleeding and hematemesis (*Table-1*). The diagnostic findings such as mean hematocrit, platelet count, PT, APTT, and liver function tests (LFTs) for DF patients are shown in *Table-1*. The hematological parameter were elevated with abnormally high count of reactive lymphocytes observed in all cases. Mean platelets count was 80.76±84 x 10^9/L ($p<0.001$) with significantly raised APTT ($p=0.048$) and D-Dimer (<0.001)

values. Raised APTT was observed in 104 (81.88%) patients most of these patients were also presented with bleeding and other coagulopathy symptoms as expected. Deranged biochemical tests including abnormal LFTs were also observed (*Table-1*).

***Table 1*: Laboratory and clinical findings of Dengue infected patients (n=127)**

Variables	Mean ±S.D (range)	p-value
Clinical findings		
Duration of illness (days)	5±2.1 (1-14)	-
Temperature (°F)	101.97±0.87 (100-105)	-
Headache (n%)	34	-
Fatigue(n%)	48.4	-
Epistaxis (n%)	19.82	-
Petechia (n%)	81.62	-
Gastrointestinal discomfort (n%)	23.48	-
Gum bleeding (n%)	11.9	-
Hematemesis (n%)	5.0	-
Hematological parameters		
Hematocrit (%)	38.05±6.22 (18-49)	<0.001
TLC (x 10^9/L)	5.32±3.09 (1.09-16.05)	0.001
Reactive lymphocytes (%)	73 ±9.03 (40-82.03)	0.001
Hemostatic parameters		
Platelets (x 10^9/L)	80.76±84.8 (2-421)	<0.001
PT (seconds)	14.04±2.36 (10-18)	0.99
APTT (seconds)	37.39±7.29 (20-50)	0.048
D-Dimer (mg/L)	2.58±2.72 (0.009-13.08)	<0.001
Fibrinogen (g/L)	2.56±0.78 (1.01-4.39)	0.99
Biochemical parameters		
AST (U/L)	145.12±250.61 (18-1996)	<0.001
SGPT (U/L)	92.10±119.55 (18-940)	<0.001
Gamma GT (U/L)	290.46±157.44 (128-840)	<0.001
Alkaline phosphatase (U/L)	83.49±114.44 (11-664)	<0.001
Direct Bilirubin (mmol/L)	0.63±0.71(0.1-5.8)	0.94
Albumin (g/dL)	4.64±3.63 (3.1-6.4)	<0.001

n%, percentage of patient out of 127; SD, Standard deviation; PT, prothrombin time; APPT, activated partial thromboplastin time; AST, aspartate aminotransferase; SGPT, serum glutamic-pyruvic transaminase;Gamma GT, gammaglutamyl transpepsidase.

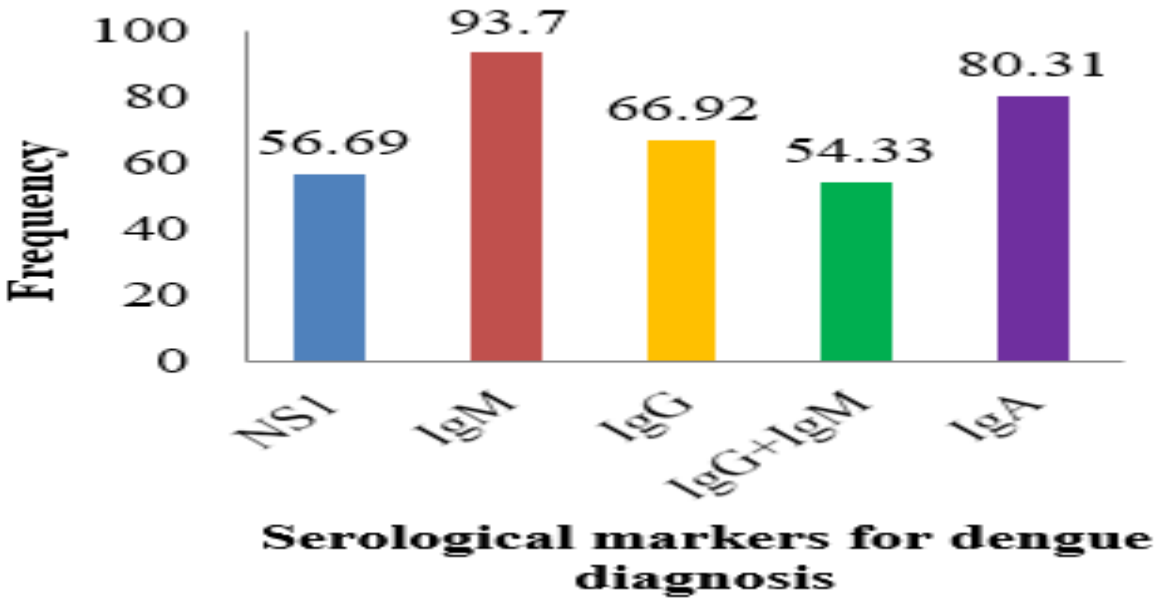

Figure 1: Frequency of serological markers in patients confirmed primary and secondary dengue infection

DISCUSSION

Over the past few decades DF has become one of the major viral infections in Pakistan. Post-monsoon seasonal outbreaks were a common observation throughout the country.[9,10] Untreated or misdiagnosed cases result in death due to hemostatic defects causing increased vascular permeability associated with severe form of DF i.e. DHF/ DSS with serious bleeding manifestations. Occurrence of DHF or DSS is more common in secondary dengue infections or reinfection due to different serover of dengue virus.[5] Past immuno-pathological mechanistic studies of DF suggest direct involvement of both humoral and cell-mediated immune responses.[11,12] Halstead proposed; that beside complement system; the antibodies from previous DF cause increased replication of virus infected macrophages/ mononuclear cells via an antibody dependent mechanism thereby contributing to pathogenesis of DHF.[13] Therefore, the present study was aimed to evaluate the possible association of defects in the extrinsic and intrinsic coagulation pathways with severity of DF. About 62.20% patients in our study population were presented with secondary dengue infections with 5% patients having episodes of hematemesis. The frequency of common symptoms such as elevated body temperature, fatigue and headache is comparable to other local and international studies.[9,7,14] Elevated hematocrit and activated lymphocytes were observed in most DF patients (*Table-1*). To study the cause of bleeding complications in DF; Kurane and Ennis hypothesized that activated lymphocytes such as T-cells along with monocytes release cytokines (IL-2, IFN-γ, TNFα, IL-6, etc.) along with chemical mediators (histamine and complement components etc.) which work synergistically to induce tissue injury due to dysfunctional vascular endothelial cells.[15] The

shock due to plasma leakage causes defects in coagulation cascade resulting in hemorrhagic manifestations.

Transient coagulopathy which last for few days during the course of disease have been reported in previous studies.[8,7] The abnormal PT and APTT correlate with defects in the extrinsic and intrinsic coagulation pathways significantly therefore, raised APTT ($p= 0.048$) and D-Dimer ($p=<0.001$) were observed in 81.88% cases confirm abnormal events in intrinsic pathway in our study population.

Another important factor is the level of fibrinogen, an acute phase protein, which is degraded to thrombin in case of vascular injury. Most of our patients had moderately low fibrinogen concentration which is in-line with previous studies.[7,14] The mechanism of fibrinogen consumption in Dengue is poorly understood. Wills et al., has proposed that the presence of dengue virus in DF directly activates fibrinolysis via molecular mimicry without the need of a usual thrombotic signal.[14] The fibrinogen degradation in turn initiates secondary activation of pro-coagulant homeostatic cascade. Therefore, hemorrhagic manifestations or bleeding episodes in DF does not follow the classical disseminated intravascular coagulation pattern rather it may be a combined consequence of low platelets count, altered platelets function, and augmented lysis of fibrinogen.[16] Increased D-Dimer values also confirmed increased fibrinolysis in our study. Like most viral infections abnormal LFT values were also observed as reported by Giri et al., in a past study.[4]

Conclusion: Significant association of transient coagulopathy was observed in most of the DF patients with suggested altered events in intrinsic coagulation and fibrinolytic pathways i.e. D-Dimer. Understanding the pathogenesis of DF is important and may lead directly to more effective treatment of patients with DF, particularly those with complex hemostatic disorders. Further research with large sample size and more parameters like determination of levels of clotting factors and inhibitors of coagulation pathways is suggested to confirm the clinical relevance of the current findings.

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