



Degree of liver injury in Dengue virus infection

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Abstract

This study evaluated the degree of liver damage during an extensive Dengue Virus epidemic in Port Sudan, Sudan, extending from July to December 2009. In 633 confirmed Dengue cases, the degree of hepatic injury was assessed as follows: Grade 0 - normal levels of liver enzymes; Grade 1 - mild elevation in the liver enzymes, not exceeding the double of the reference value; Grade 2 - elevated liver enzymes, with the levels of the enzymes increased to more than three times the reference values; Grade 3 - acute hepatitis, with liver enzymes levels increased to at least 10 times their normal values; Grade 4 - evidence of hepatic failure (high prothrombin time) or renal involvement (high creatinin). It was observed that 63.8% of Grade 1, 17.9% of Grade 2, 3.9% of Grade 3 and 1.1% of Grade 4 had liver damage. In this study, the severe degree of liver injury existed with the presence of the complications. So an aspartate aminotransferase (AST), at least, should be done regularly in the follow up of Dengue patients.

Keywords: Dengue virus, hepatitis, liver failure, Port Sudan.

INTRODUCTION

Dengue virus (DENV) is a member of the Flaviviridae family, which include West Nile virus, yellow fever virus, Japanese encephalitis virus, and tick-borne encephalitis virus, among others (Lindenbach and Rice, 2003). The most serious manifestations of the infection are Dengue hemorrhagic fever (DHF) and Dengue shock syndrome (DSS). Meanwhile, no effective vaccine or antiviral drug therapy is currently available against Dengue virus (Gubler, 2002).

Dengue viral infection has been recognized as one of the world's biggest emerging epidemic. Throughout the tropics, this infection has an annual incidence of 100 million cases of DF with another 250,000 cases of DHF and mortality rate of 24,000 to 25,000 per year (Gubler, 2002; Halstead, 1999). In the last years, Port Sudan has faced many outbreaks; one was reported in 2005. After this outbreak, Port Sudan has become an endemic area with Dengue virus (Ageep et al., 2006). Dengue virus type 3 is the common circulating serotype in the region (Amal et al., 2010).

Typically, people infected with Dengue virus are asymptomatic (80%) or have mild symptoms such as an uncomplicated fever (Whitehorn, 2010; Reiter, 2011). Others have more severe illness (5%), and in a small proportion it is life-threatening (Whitehorn, 2010; Reiter, 2011). The incubation period ranges from 3 to 14 days,

but most often it is 4 to 7 days (Chen et al., 2010). The characteristic symptoms of Dengue are: a sudden-onset fever, headache (typically behind the eyes), muscle and joint pains, and a rash. The alternative name for Dengue, "break-bone fever", comes from the associated muscle and joints pains (Varatharaj, 2010). Severe disease is marked by two problems: dysfunction of endothelium and disordered blood clotting (Martina et al., 2009). Endothelial dysfunction leads to the leakage of fluid from the blood vessels into the chest and abdominal cavities, while coagulation disorder is responsible for the bleeding complications. Higher levels of virus in the blood and involvement of other organs (such as the liver) are also associated with more severe disease (Seneviratne et al., 2006).

Dengue may occasionally affect several other body systems (George, 1997). This may be either in isolation or along with the classic Dengue symptoms (Seneviratne et al., 2006). Hepatic dysfunction is common in Dengue infection, and is attributed to a direct viral effect on liver cells or as a consequence of dysregulated host immune responses against the virus. Other contributing factors include race, diabetes, hemoglobinopathies, pre-existing liver damage and the use of hepatotoxic drugs (George, 1997; Chen et al., 2004). Although there are isolated case reports of fulminant hepatic failure, the derange-

ments in the transaminases are usually mild and self-limiting (George, 1997).

Although, the number of patients affected by the virus is increasing each year, little work has been done in the studied area (regarding the pathogenesis, the liver changes and the complications of Dengue infection). Hence, this study was aimed at evaluating the degree of liver injury by measuring the level of the liver enzymes, prothrombin time (PT) and creatinine in Dengue-infected patients. These parameters were compared with the clinical presentations of the patients evaluate how the degree of liver damage is related to the complications of the disease.

This study has many significant values. First, it will increase the awareness of the local health staff about the importance of evaluating the degree of liver damage in Dengue infected patients. It also highlighted the importance of measuring the liver enzymes (at least the aspartate aminotransferase (AST)) in the follow-up of Dengue virus infection. Finally, this research form a base for future studies in the region regarding the outcome, the mortality, the hospital stay and the prognosis of Dengue infection according to the level of liver damage.

METHODOLOGY

Study area/setting

This study was conducted in the outpatient and inpatient departments of Port Sudan Teaching Hospital, Port Sudan, Sudan, during the outbreak of Dengue infection in the period from July to December, 2009. Port Sudan city is the capital of the Red sea state and it is the major sea port of the Sudan. The total number of the whole population was (739,300) according to the national census of 2002, with adjusted growth rate. There are four localities in this area (Port Sudan, Sinkat, Tokar and Halayib). Port Sudan Teaching Hospital is a governmental hospital, which is regarded as a tertiary care hospital. The total number of beds is about 380, and the medical services are opened for all population with the aid of the best professional staff in the region.

Study design

This is a descriptive, hospital-based study.

Study subjects

This included all confirmed cases during the outbreak of Dengue infection in the period from July to December 2009, who were seen in Port Sudan Teaching Hospital. This study constituted all age groups from infants to old patients.

Exclusion criteria

All of the patients were tested first for malaria, typhoid, leptospira and brucellosis. Any patient infected with these diseases was excluded from the research. Patient with positive tests of hepatitis A, B, C, E or even had recent history of infection with these hepatotropic viruses was also excluded from this research.

Data collection

The clinical information were taken from the patients and registered in predesigned questionnaires. The following information was included:

1. Clinical presentation: classic or not.
2. Hemorrhage: present or not.
3. Dengue shock: present or not.
4. Features of encephalopathy: present or not.
5. Features of renal impairment: present or not.
6. Gastrointestinal bleeding: present or not.
7. Features of cholecystitis: present or not.

Ethical clearance

Informed consent was taken from all patients participating in this research. Ethical clearance was approved from the local ethical review committee (ERC).

Enzyme-linked immunosorbent assay (ELISA) technique

Antibodies against Dengue virus antigens were test by ELISA technique (ELISA test NovaTec Germany). This test had 98% sensitivity and 95% specificity. In the procedure of test the following steps was taken:

1. Microtiter strip wells were pre-coated with Dengue virus antigen type 2 to bind to the corresponding antibodies of the specimens.
2. Afterward, the wells were washed to remove all the unbound sample material.
3. Then, horseradish peroxidase (HRP) labeled anti-human IgM conjugate was added. The conjugate bound to the captured Dengue virus-specific antibodies.
4. A second step of washing was formed.
5. The immune complex formed by the bound conjugate was visualized by adding tetramethylbenzidine (TMB) substrate, which gave blue reaction product. The intensity of the product was proportional to the amount of Dengue virus-specific antibodies in the specimen.
6. Sulfuric acid was added to stop the reaction. This produced a yellow end point color.
7. The test was read using ELISA micro-well plate reader at 450 nm.

Biochemical and coagulation tests

To study the degree of liver damage, samples were collected in to two blood containers. The first was a plain container into which serum was extracted for the assessment of the liver enzymes - aspartate aminotransferase (AST) and alanine transaminase (ALT), and creatinine levels. The second was tri-sodium citrate container from which plasma was used to detect the prothrombin time (PT) level. A semi-automated spectrophotometer (Bio-system) was used for the measurement of these biochemical tests.

Liver injury scoring system

The degree of liver damage was assessed according to the levels of the liver enzymes, PT and creatinine as follows: Grade 0 - normal levels of liver enzymes; Grade 1 - mild elevation in the liver enzymes, not exceeding the double of reference value; Grade 2 - elevated liver enzymes, with the levels of the enzymes increased to more than three times the reference values; Grade 3 - acute

Table 1. Relation between clinical presentation and the degree of liver injury in patients infected with Dengue virus.

Clinical presentation	N	F (%)	Males (%)	Females (%)	Grade 0 (%)	Grade 1 (%)	Grade 2 (%)	Grade 3 (%)	Grade 4 (%)
Dengue fever	567	89.6	248(39.2)	319 (50.4)	82 (12.9)	394 (62.2)	91 (14.4)	0	0
Dengue hemorrhage	28	4.5	15(2.3)	13 (2.2)	2 (0.3)	6 (0.9)	12 (1.9)	8 (1.3)	0
Dengue shock syndrome	10	1.6	4 (0.6)	6 (0.9)	0	2 (0.3)	3 (0.5)	5 (0.8)	0
Gastro-intestinal bleeding	16	2.5	10(1.6)	6 (0.9)	0	1 (0.2)	6 (0.9)	9 (1.4)	0
Encephalopathy	4	0.6	2 (0.3)	2 (0.3)	0	0	0	0	4 (0.6)
Cholecystitis	2	0.3	1 (0.2)	1 (0.2)	0	1 (0.2)	1 (0.2)	0	0
Renal impairment	6	0.9	0	6 (0.9)	0	0	0	3 (0.5)	3 (0.5)
Total (%)	633	100	280(44.2)	353 (55.8)	84 (13.2)	404 (63.8)	113 (17.9)	25 (4.0)	7 (1.1)

N, Number of the patients; F, frequency (%).

hepatitis, with liver enzymes levels increased to at least 10 times their normal values; Grade 4: evidence of hepatic failure (high PT) or hepato-renal involvement (high creatinine).

The reference values for the tests

The normal ranges for the blood samples according to control normal people in the area, were as follows:

1. ALT: 7 to 56 Unit/L
2. AST: 5 to 40 Unit/L
3. Creatinine: 0 to 1 mg/dl
4. PT: 11 to 15 s

Statistical analysis

Statistical analysis was done using SPSS program. Chi square test was used to compare categorical variables and Fischer exact test were applicable. For none normally distributed quantitative variables, median and Inter quartile ranges (IQR) were used. Chi square test was used to compare categorical variables and Fischer exact test were used when numbers were too small to perform the Chi-square testing. Results were presented as frequency and percentage.

RESULTS

Six hundred and thirty three confirmed Dengue

patients were included in this study. 248 were male and 319 were female (male to female ratio was approximately 3:4).

Degree of liver injury

Table 1 shows that, 13.2% of the patients had no increase in the transaminases level (Grade 0), 63.8% presented mild alterations in the liver enzymes levels (Grade 1), 17.9% presented (Grade 2) liver involvement, 3.9% of the patients had progressed to acute hepatitis (Grade 3) and 1.1% had severe liver damage with fulminant hepatic failure (Grade 4).

Changes in the liver enzymes

In 86% of the patients there was elevation of the liver enzymes. All of them (549 patients) had increase in the AST level. The change in the ALT was seen in 82% of the patients.

Uncomplicated Dengue infection

Most of the patients (567 patients) had features of

Dengue fever without complications. In this group, the common degree of liver damage (69%) was Grade 1 (that is. mild elevation in the liver enzymes).

Complicated Dengue infections

Dengue hemorrhage

Twenty eight patients presented with Dengue hemorrhage and 12 of them (43%) had Grade 2 liver injury.

Dengue shock syndrome

Ten patients presented with Dengue shock syndrome. In this group, 5 patients (50%) had (Grade 3) liver injury.

Gastro-intestinal bleeding

From the 16 patients presented with gastro-intestinal bleeding, 56% had (Grade 3) liver injury.

Encephalopathy

4 patients presented with encephalopathy and all of them had (Grade 4) liver damage.

Renal impairment

Of the 6 patients who were complicated by renal impairment, 50% had Grade 3 and 50% had (Grade 4) liver damage.

DISCUSSION

Dengue virus is among the commonest causes of febrile illnesses in Port Sudan, Sudan (Ageep et al., 2006). The importance of this study lies in the fact that it is the first documented research in this region of Africa, which studied the severity of liver damage in Dengue infection. In this research, we included Dengue patients with mild symptoms seen in the outpatient department and severe cases who were admitted in the inpatient units; so this study covers the mild as well as the severe cases of Dengue virus infections.

To date, there are two hypotheses that explain the damage of the liver in Dengue patients. The first is the immune enhancement hypothesis. Chen et al. (2004) reported that strong correlation was found between T cell activation and hepatic cellular infiltration in immunocompetent mice infected with Dengue virus. They noted that the kinetics of liver enzyme elevation also correlated with that of T cell activation and suggested a relationship between T cell infiltration and elevation of liver enzymes. Chaturvedi et al. (1999) in their study detected the appearance of different helper cells cytokines in human white blood cells cultures infected *in vitro* with Dengue virus type 2. In their study, they have reported that during Dengue infection, monocytes, B cells, T cells and mast cells produce large amounts of cytokines. Despite all this, the role of host immunity in Dengue infection is still very unclear. Unregulated host immune response may play a role in severity of Dengue infection by modifying the immune response; severe infection can be prevented. The second hypothesis relates the damage in the liver to direct virulence of the virus (Seneviratne et al., 2006). According to these studies, we can hypothesize the same mechanism responsible for the liver damage that occurred in our patients.

Liver damage with elevation of aminotransferases and reactive hepatitis is a common complication of dengue virus infection. Hence, measurement of AST and ALT is mandatory to ascertain the liver involvement (Souza et al., 2004). In this study, 86% of our patients had high AST level and 82% had high ALT level. However, Wong and Shen (2008) reported that AST abnormality was predominantly higher compared to ALT; 91 and 72%, respectively. Another study done by Kuo et al. (1992) has

shown approximately 90% of the AST abnormality in Dengue patients. A different retrospective study from Wichman et al. (2004) among Thai patients in 2001 outbreak, reported liver dysfunction in 20 from 347 patients (5.8%) with Dengue infection. Our study is consistent with the results from previous studies. The difference from Wichman et al. (2004) results may be explained by the difference in the Dengue virus type involved in their outbreak, or its hepatotoxicity. Other differences may be in the immune status of their patients or the days of collection of their serum samples (Wichman et al., 2004).

In this study, we noticed relation between the degree of liver damage and the presence of the complications. In 71% of the patients having Dengue hemorrhage, severe degree of liver damage (Grade 2 and 3) occurred. The deranged liver functions may participate in the causation of bleeding in these patients. Severe degree of liver injury (Grade 2 and 3) also was found in 80% of Dengue shock syndrome. All of the patients having encephalopathy had (Grade 4) liver damage. Encephalopathy in our patients may be due to fulminant hepatic failure or a high level of the virus that directly damage the brain. Involvement of the kidneys was also related to the severity of liver damage; 50% with Grade 3 and 50% with Grade 4. Again, this may be a part from hepato-renal syndrome or direct virus virulence. Similar results to our work, in complicated Dengue infection, were also seen in other countries. In Saudi Arabia, Khan et al. (2008) had made an association between high AST level and complications of Dengue virus. In Taiwan, Kuo et al. (1992) also reported higher bleeding episodes in those who had high levels of AST and ALT. In Vietnam, Nguyen et al. (1997) reported that DHF may cause mild to moderate liver dysfunction in most cases; only few patients may suffer from acute liver failure leading to encephalopathy and death. Additionally, a report from India done by Shah (2008) pointed to a high mortality in Dengue patients with hepatitis and encephalopathy.

Since Port Sudan is one of the endemic areas with Dengue infection, Dengue virus should be added to the differential diagnosis of hepatitis in the local hospitals protocol. AST can be a useful surrogate marker to predict disease severity and bleeding outcome in Dengue infection, therefore it should be measured in all Dengue patients. The level of the other liver enzymes, PT and creatinine should also be assessed in severe cases of Dengue infection. We suggest the grading system presented in this study to be applied in Dengue virus management protocol.

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