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Oxidative Stress Markers and Its Correlation with Aminotransferases in Hepatic Manifestation of Dengue Virus Infection

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ABSTRACT
Objective: *To study the correlation between oxidative stress markers and Aminotransferases in patients of dengue fever with hepatic manifestations.*
Background: *Manifestation of viral infection may be contributed by the alteration of oxidation/reduction (Redox) status in virus infected patients and this alteration starts prior to symptoms of disease appear. Increased level of Aminotransferases may be a useful marker of hepatic insult.*
Method: *We conducted a cross sectional hospital based study of 90 patients serologically confirmed to have dengue virus infection who have hepatic manifestations. Blood samples were collected on 4th day and onwards after the fever and analysed for liver function tests (LFT), prothombin time (PT) and thrombocytes and other haematological parameters. And to measure the oxidative stress blood was analysed for malondihyde (MDA), catalase (CAT), glutathione peroxidase(GPx) and paraoxanase(PON).*
Results: *Among the LFT parameters the level of Aspartate transaminase (AST) was significantly raised followed by alanine transaminase (ALT) and other parameters were either within normal limit or not significant. The level of MDA was significantly raised and level of GPx , CAT and PON was reduced. The significant correlation was found between ALT, AST and oxidative stress markers.*
Conclusion: *So we can conclude that in patients of dengue infection, raised liver enzyme (AST, ALT) may be associated with oxidative stress and AST can be used in a severity index and as prognostic marker in hepatic manifestations.*

INTRODUCTION

Dengue is the most common and widespread arthropod borne viral infection in the world today and is caused by a flavivirus spread by the Aedes aegypti mosquito. It is estimated that worldwide, each year 50-100 million cases of dengue fever occur and in South East Asia it continues to be the major challenge to public health. [1,2]

In India dengue is endemic in 31 states /UTs. During 2011-12 about 18059 cases were reported with around 120 deaths with case fatality rate 0.65%.[3]

Although dengue virus is a non-hepatotropic virus, hepatomegaly is commonly seen in dengue along with a rise in serum aminotransferases. The degree of liver dysfunction varies from mild injury with elevation of aminotransferases to even fulminant hepatic failure. Hepatic dysfunction in dengue infection may be attributed to direct viral effect on liver cells or as a consequence of dysregulated host immune responses against the virus.[2]

There is now much evidence that oxidants play a complex role in viral diseases, starting from influences on host cell metabolism and viral replication and extending to desirable inactivating effects on viruses and less desired toxic effects on host tissue. Oxidative damage may affect all biochemical compounds including lipids, proteins, nucleic acids, carbohydrates, and macromolecules of connective tissue. [4]

Oxidative stress initiates and regulates the transcription and activation of a large series of others mediators in cells, which culminate in common mechanism of damage: apoptosis, necrosis, inflammation, immune response, ischemia, vasculitis, altered gene expression, and regeneration. The prevalence and the persistence of one or more of these aspects may influence the occurrence and manifestation of different types of diseases.[4]

AST and ALT are liver enzymes involved in amino acid metabolism. The inflammatory process resulting from infection by the dengue virus leads to a parenchymatous lesion that releases these markers into the blood. [2]

In the acute phase of the disease, an increase occurs in aminotransferases, the levels of which subsequently decrease as the liver recovers. AST may be found in high concentrations in the heart muscle, liver cells and skeletal muscle and, in lower concentrations, in the kidney and pancreas. Together with other enzymes, this test is useful for the diagnosis of myocardial infarction and liver disease.[5]

MATERIAL AND METHOS

The study was hospital based cross sectional study carried out in department of medicine and central pathology in NSCB Govt. Medical College Jabalpur, during June2015 to Dec 2015. The ethical committee clearance was taken. All clinically suspected dengue infection cases were as per the revised World Health Organization (WHO) guidelines. In patients who either presented in OPD or were admitted in ward with manifestation, were screened and serologically confirmed by ICMR centre, Jabalpur. The study consists of 60 dengue infected patients who were serological IgM positive.

Inclusion criteria

The study included 90 patients serologically IgM positive and had the dengue infection for the first time and any pre existing liver disease or alcohol intake and other concomitant infections affecting the liver such as malaria, typhoid, hepatitis A and B ,was ruled out before including in the study. In our study there were no patient of Dengue haemorrhagic fever (DHF) and Dengue shock syndrome (DSS)

Exclusion criteria

Individuals having the history of pre-existing liver disease or alcohol intake and having the subsequent infection, Dengue shock syndrome (DSS). Children below 14 yrs and person above 70 yrs were not included. Patients having heart disorder, muscle disorder, acute kidney injury and pancreatic disease were not included in study.

A detailed history and thorough clinical examination were performed in all cases. Data was collected in a predesigned proforma. All cases were subjected to the following investigations:, Total Bilirubin, Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP), Serum Albumin, Globulin, Total proteins, Prothrombin time (PT), Thrombocyte count. LFT was done on randox (Imola) fully automated biochemistry analyser and PT and Thrombocyte count was done in pathology department.

Malondialdehyde (MDA)

The MDA concentrations was analyzed with a commercial kit (Randox Ltd.). In this assay, stable chromophore production after incubation for 40 minutes at 45°C is measured at 586 nm using a CHEM 7 (Erba, Transasia) analyser. Lipid peroxidation was expressed as the normalized content of MDA. Values were expressed in nanomoles (nM).

Catalase (CAT)

Catalase catalyses the breakdown of hydrogen peroxide according to the following reaction:

$$2\,H_2O_2 \xrightarrow{\text{Catalase}} 2\,H_2O + O_2$$

In the UV range H2O2 shows a continuous increase in absorbance with decreasing wave length. When H2O2 is decomposed by catalase, the absorbance at 230 nm decreases. ΔA/min at 230 nm is measured (By Aebi. H.) [6]

Glutathione peroxidise (GPx)

Evaluation of GPx activity was determined using a commercial kit (Randox, Ltd.). Briefly, GPx catalyzes the oxidation of GSH by cumene hydroperoxide. In the presence of GSH reductase and NADPH, the oxidized GSH is immediately converted to the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance at 340 nm is measured. [7]

Serum paraoxonase (PON) levels were measured by spectrophotometric method using phenylacetate as substrate.[8]

Statistical Analysis

Statistical analysis was carried out with IBM Statistical Package for the Social Sciences 19.0, (SPSS. Inc., IBM Copyright 1989, U.S.A. 2010 SPSS)

Results

The study group included 90 dengue fever patients with hepatic manifestations of age between 14 years and 70 years during the 6 months study period satisfying the revised WHO 2009 criteria for DF after excluding Malaria, Enteric fever, Hepatitis A and Hepatitis B. Out of these, 48 (53.3%) patients were male and 42(46.7%) were females. 50(55.5%) patients belonged to rural area while 40 (44.5%) came from urban area. Minimum age of patient was 14 years and maximum age was 70 years with mean age of 37.1 ± 1.1. The epidemiological profile and clinical features have been summarized in Table -1

Table -1 Epidemiological and clinical features of study population

parameter	DF patients with raised transaminase (n=60)	DF without raised transaminase (n=30)
Age(years)	36± 1.7	24± 1.8
Sex(male/female)	16:15	4:3
Symptoms		
Fever	60	30
Bodyache	56	21
Vomiting	04	02
Peticheal spots	01	01
Clinical parameters		
Hepatomegaly	58	12
Jaundice	01	0
Bleeding manifestatios	01	0

Table 1 shows various epidemiological and clinical parameters in patients in those who were having raised aminotransferase and those who don't have raise aminotransferase.

Table shows that mean age of patients having raised aminotransferase is higher than those having within reference range. the number of patients having body ache, hepatomegaly and jaundice were also more in patients having raised transaminases

Table -2 Aminotransferases in dengue fever confirmed patients

Aminotransferases	Raised aminotransferases (n=60) (group) l Mean± SD	Within reference range(n=30) (groupll) Mean ± SD
ALT(U/L)	73±2.4	38±1.6
AST(U/L)	130±3.8	46±2.3

Table 2 depicts the mean value of ALT and AST with standard deviation in those patients who were having increased concentration of aminotransferases and in those who were having values within reference values.

Table 3 Oxidative stress markers in DF patients

Oxidative stress markers	In raised aminotransferase patients (Mean + SD)	Without raised aminotransferase patients (Mean +SD)	P value
MDA(nM/ml)	15.8±2.5	8.3±2	.00
PON(U/mL)	41±4	57±4.5	.00
CAT(U/mL)	17.8±2.9	34.8±2.7	.00
GPx(U/mL)	23±3.8	47.9±2.8	.00

Table 3 shows mean values with std. Deviation and its significance (t- test) in patients having raised and in patients having within reference range aminotransferase. this table shows that in patients having raised transaminases, the MDA level was high and the concentration of antioxidants viz PON,CAT and GPx was low.

Discussion

Hepatic involvement in dengue infections is often demonstrated by hepatomegaly and mild-to-moderate increases in transaminase levels. Presentation with jaundice is important as it can simulate acute hepatitis[9].in our study 60 (66.6%) patients out of 90 were having hepatomelay and among 60 patients 58 (96%) also had raised aminotransaminase.

Roy et al. showed in their study that 80.8% had hepatomegaly, which was more common in severe dengue (93.1%) and dengue with warning signs (84.4%) group than in dengue without warning signs group (13.3%) [2]. In our study the male female ratio was 16:15 and 4:3 in respective groups which is quite similar to study done by kalenahalli et. al.[9]

Abnormal hepatic enzymes in dengue infection varies from 36.4% to 96% both in children and adults in different studies [2] but in our study in group l the increase in AST and ALT was around 3 times and 2 times that was found to be consistent with other studies [4,5]. the raised enzyme level was more in two extremes of age group i.e in age group of 14-24 and 55-65 that is consistent with study of roy et.al [2].

Antioxidant enzyme levels are sensitive to oxidative stress. Both increased and decreased levels have been reported in different diseases in which an enhancement of ROS is a cause or a consequence of the illness [5], in our study we observed increase in level of MDA and there there was significant fall in the level of antioxidants in group l patients who also have increased concentration of transaminase.

So we can conclude that in dengue fever patients there is hepatic insult that is caused by oxidative stress that hepatic stress is manifested by increased level of aminotransferases.

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