Research article

Serum lipid profile in dengue patients: a simple laboratory tool to predict disease severity

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ABSTRACT

Introduction and Aim: Serum lipids play a pivotal role in the immune response of the host during dengue. The aim of this study was to evaluate the serum lipid abnormalities in dengue patients and to study the relationship between serum lipids with disease severity and platelet count.

Materials and Methods: This case control study was carried out in 75 cases of dengue of age group $\geq$ 18 years divided into three groups namely dengue without warning symptoms (DNWS), dengue with warning symptoms (DWWS) and severe dengue (SD) and 75 age and gender matched healthy controls. Lipid parameters such as total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides were thereafter measured in all the subjects.

Results: Among 75 patients with dengue fever, 52 (69.4%) were classified as DNWS, 15 (20%) as DWWS and the remaining 8 (10.6%) as SD. Lower levels of serum total cholesterol (TC) levels (118.8 $\pm$ 30.71) (p <0.0001) were observed among patients with DNWS when compared to controls. A strong significant positive correlation was seen between the platelets and serum HDL cholesterol levels among the subjects with SD (r = 0.712, p=.047*) and weak negative correlation was observed between the platelets and triglycerides in patients with dengue with warning signs (r = -0.275, p=.048*).

Conclusion: We observed a strong association of diminished TC, HDL and LDL cholesterol levels with the severity of dengue. Based on our findings, these three lipid parameters could be utilized as a simple laboratory tool to identify dengue severity in resource limited settings.

Keywords: Dengue severity; vector-borne, arbovirus, lipoproteins, lipids.

INTRODUCTION

Dengue is one of the most significant vector-borne viral disease worldwide. It is caused by the bite of infected arthropods such as *Aedes aegypti* and *Aedes albopictus* and is most common in tropical and sub-tropical countries. Dengue being common in South-Eastern Asian countries, Africa and Western Pacific regions grabs more attention in almost all states in India making leading to many admissions in the country (1, 2). While many studies have looked at factors and their correlation with disease severity in dengue infection, currently there is no robust way to predict disease severity (3-5). Furthermore, several studies have also reported an association of alterations of serum lipid profile levels with viral infection (6-8). It is stated that cellular lipids play a vital role at various stages of the cycle of replication during dengue infection (9). Lipid alterations may occur due to elevated cytokine levels, most importantly with the severity of the disease (10). Clinical evidence has shown that in viral infection, binding of lipoproteins to viruses and neutralizations of their toxic effects take place (11, 12). Few viruses enter cells through low density lipoprotein (LDL) receptors and consequently reduce the viral uptake by the cells (7, 13). This shows viral infections have some correlation with the lipoprotein.

The association of lipid parameters with dengue severity (6, 7) and its role in the prognostication of the disease is poorly understood with varying results (14-16). Also there has been an association of thrombocytopenia and hemorrhagic manifestations with severity of dengue, with very few reports showing relationship of lipoprotein alterations with platelet count (6, 15). To strengthen the link between dengue infection and alteration in serum lipid, we aimed to find the impact of severity of dengue infection on lipid profile and the association of the lipid levels with platelet count.

MATERIALS AND MATERIALS

Study design

This case-control study was carried out over a one-year period from September 2015 to September 2016 in the department of Internal Medicine at a tertiary care hospital, in Karnataka, India. The study was approved by the Institutional Ethics Committee (IEC: 566/2015). Written informed consent was taken from the participants.

Participants with selection criteria

75 adult patients of age $\geq$ 18 years who were diagnosed with dengue and willing to volunteer were recruited for this study. Among the cases of dengue, 52 (69.4%) were classified as having dengue without any warning symptoms (DNWS), 15 (20%) as dengue with warning symptoms (DWWS), and 8 (10.6%) as severe dengue (SD) according to the classification by WHO in 2009 (1). The study also included 75 age and gender matched healthy controls. Patients with chronic liver disease, subjects on statin therapy and those with co-infections including malaria, scrub typhus, leptospirosis and typhoid were excluded from the study.

Sample size

The sample size was calculated based on comparison of mean TG between cases and controls to detect a difference of 50 units in TG with SD of 110, 80% power and 5% value of significance. The sample size to be achieved was 75 in each group.

Data collection

History and relevant general physical and systemic examinations were recorded. Basic laboratory investigations included complete blood count, biochemical parameters and relevant radiological investigations. Venous blood samples were obtained on the day of hospitalization for measurement of lipid profile parameters such as total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL). Confirmation of the diagnosis of dengue was made either by non-structural protein 1 (NS1) antigen test using Pan Bio ELISA kit or dengue IgM ELISA using a kit from National Institute of Virology, Pune. Blood samples were also sent for culture to rule out other bacterial infections. Likewise, blood samples were also taken from healthy controls with similar age and gender distribution (n=75).

The normal reference ranges for the serum lipid profiles were taken as per ATP III 2002 guidelines (17). Estimation of TC was done by cholesterol oxidase- peroxidase method, HDL cholesterol by direct homogenous method, LDL cholesterol by Friedwald's formula and TG by GPO- Trinder method.

Statistical analysis

The parametric clinical variables were represented using mean $\pm$ SD and the non-parametric clinical variables were represented using median and interquartile range while frequency and percentages were used to represent categorical variables. The parametric and non-parametric variables were analyzed by Independent Samples t test and Kruskal Wallis test respectively in IBM SPSS 16 version. Pearson's correlation was done to correlate between platelet count and lipid profile in dengue patients. Statistical significance was considered only when p vales were <0.05.

RESULTS

Out of the 75 diagnosed dengue patients, majority (n= 35, 46.6%) were in the age group of 18-29 years and 30-39 years (n= 15, 20%)**.** There were 56 males (75%) and 19 females (25%). The common presenting symptoms were fever 72 (96%) followed by myalgia 68 (90.6%), arthralgia 32 (42.66%) and bleeding 12 (16%). Among the clinical signs, skin rash (n = 43, 57.33%) followed by hepatomegaly (n = 14, 18.66%) and splenomegaly (n = 12, 16%) were commonly found among the cases.

Fig. 1 shows comparison between serum lipoprotein variables of dengue patients and healthy controls. Significant differences in all the parameters of serum lipids were noted among the cases and controls (p <0.0001). Serum TC, HDL and LDL cholesterol levels were low, while the serum TG levels were noted to be high among dengue cases compared to controls. The serum TC levels were lower among patients with DNWS and severe SD, but not in patients with DWWS as compared to controls. HDL and LDL cholesterol levels were decreased in all groups of dengue with significant association with dengue severity (p <0.0001). Increased TG levels were observed among all groups of patients with dengue. However, statistical significance could be established only in those with DNWS (Table 1).
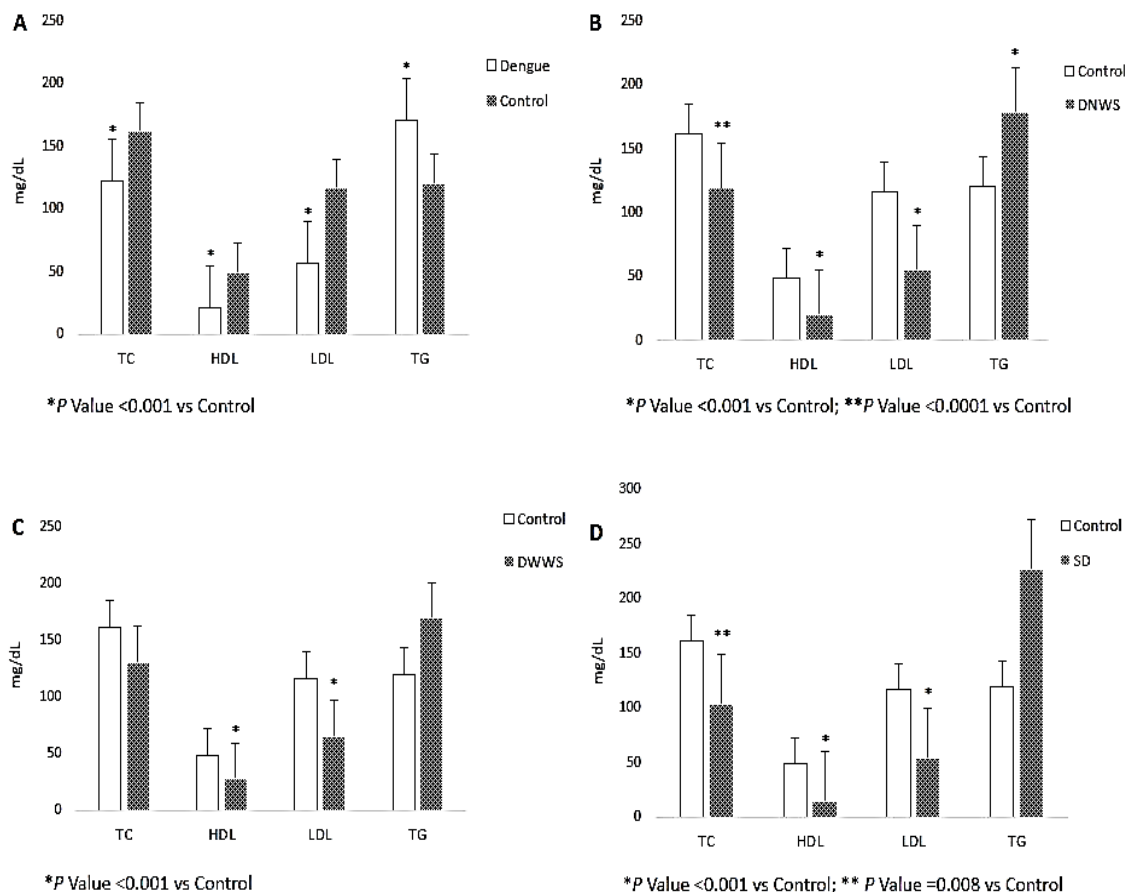
Fig. 1: Serum lipid profile in dengue. Comparison of lipid parameters between controls and cases (A), dengue without warning signs (DNWS) (B), dengue with warning signs (DWWS) (C), severe dengue (SD) (D).

Table 1: Comparison of serum lipid profile variables between different groups of dengue and healthy controls

Lipid profile parameters	Disease severity	Mean ± SD Median (Q1, Q3)	p value
TC (mg/dl)	Control	161.6 ± 44.1	
	DNWS	118.8 ± 30.71	<0.0001*
	DWWS	130.7 ± 54.1	0.8519
	SD	103.1 ± 19.1	0.008*
TG (mg/dl)	Control	120 (88.5, 157)	
	DNWS	178 (128.7, 267.5)	<0.001*
	DWWS	147 (130, 186)	0.1785
	SD	222.7 (100, 294)	0.3807
HDL (mg/dl)	Control	48.9 ± 8.14	
	DNWS	20 ± 11.19	<0.001*
	DWWS	27.86 ± 13.56	<0.001*
	SD	14.12 + 12.34	<0.001*
LDL (mg/dl)	Control	116.6 ± 47.6	
	DNWS	54.7 ± 26.3	<0.001*
	DWWS	65 ± 23.89	<0.001*
	SD	53.87 + 20.16	<0.001*

*p <0.05, DNWS: Dengue without warning signs, DWWS: Dengue with warning signs, SD: Severe dengue, TC: Total cholesterol, TG: Triglyceride, HDL: High density lipoprotein, LDL: Low density lipoprotein.

Thrombocytopenia was noted in 84% (63/75) patients. All the patients with SD (n = 8, 100%) had a platelet count of <20000. Distribution of degree of thrombocytopenia is depicted in Fig. 2. The platelet count and serum lipid levels among dengue patients showed a strong significant positive correlation between HDL levels and platelet counts (r = 0.712, p=.047*) among SD patients, whereas serum triglycerides had weak negative correlation with platelet counts in patients with dengue with warning signs (r = -0.275, p =.048*; Table 2).

Table 2: Correlation between serum lipid profile variables and platelets in dengue patients

Platelet count	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Dengue without warning signs	r = 0.307, p=.265	r = 0.051, p=.857	r = 0.449, p=.093	r = 0.472, p=.089
Dengue with warning signs	r = 0.13, p=.358	r = -0.275, p=.048*	r = 0.229, p= .102	r = 0.172, p= .227
Severe dengue	r = 0.347, p=.4	r = -0.25, p=.953	r = 0.712, p=.047*	r = -0.243, p=.6

* Pearson correlation, TC: Total cholesterol, TG: Triglyceride, HDL: High density lipoprotein, LDL: Low density lipoprotein
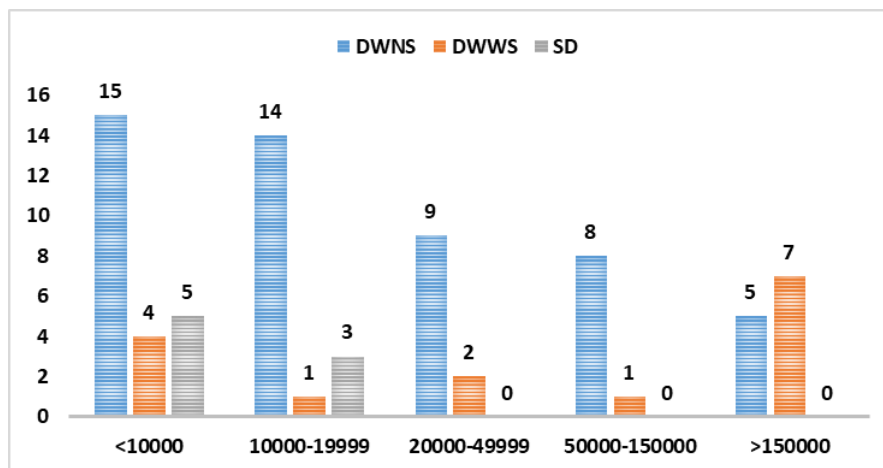
Fig. 2: Distribution of thrombocytopenia in different groups of dengue

DISCUSSION

An increasing intrusiveness is present in the recent times in knowing the possible association between dengue severity and changes in the circulating lipids. Lipids are said to be involved in levels of cytokine regulation and modification of host immune response (18, 19). Some viruses gain entry into the host cell by frequently affecting the receptors assembly in subdomains rich with cholesterol and also by altering lipid microenvironment (20).

In this study, we observed that serum TC, HDL and LDL cholesterol levels were found to be decreased in all groups of dengue. Strong statistical significance could be established for TC among patients with DNWS and SD, while mean HDL and LDL levels were significantly low among all the groups. In a study by Duran *et al.,* significantly low TC, HDL and LDL levels were observed among dengue patients irrespective of their severity (6). The evidence which is available currently specifies that LDL cholesterol may allow cell entry of viruses via corresponding LDL receptors. Viral complexes of LDL cholesterol and receptor interact with each other thereby promoting further cells infection (20, 22). Thus, a low LDL level and its association with disease severity is justified.

It has been shown that tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1) diminish TC levels by affecting the hydroxymethylglutaryl (HMG) Coenzyme A reductase enzyme (23). TNF-α, IL-1 and IL-6 also decreases the secretion of apolipoprotein B which further decrease cholesterol and induce increased infectivity (24). The deposition of

cholesterol in various organs leading to steatosis of liver is also due to diminished levels of TC (14) or increased endothelial permeability resulting in its leakage (25-27). In addition, liver damage seen in dengue infection could further contribute to decreased TC levels (16). In a recent systematic review performed by Lima *et al.,* has pointed out that the developing risk of SD is high among patients with lower circulating levels of TC and LDL cholesterol. This further indicates that the infusion of prophylaxis of these lipid levels in severe dengue affected patients can be scrutinized (28).

Severe dengue patients had diminished HDL cholesterol levels, while previous studies have shown an increase in HDL levels in severe forms of dengue (6). Differences in the study population may explain this variation observed in the relationship between HDL cholesterol and various forms of severity of dengue. The possible explanation for decrease in HDL cholesterol levels are: Firstly, during severe infection, TNF-α is thought to diminish the plasma lecithin cholesterol acyl transferase (LCAT) activity which helps in esterification of free cholesterol in HDL cholesterol to form cholesteryl esters (23). Second, lack of circulating ApoA1, HDL cholesterol binding to DENV has association with increased infection of the virus thereby decreasing HDL cholesterol levels in severe dengue (21).

Though the mean serum TG level was found to be elevated in all forms of dengue compared to controls, statistical significance was found only among patients with DNWS. In contrast, Duran et al., observed that serum TG levels were elevated in severe dengue,

whereas it was not altered among dengue cases with and without warning signs (6). Studies have shown that dengue virus infection changes few components which have association with metabolism of triglycerides. The NS3 protein secreted by the dengue virus increases the activity of fatty acid synthase in triglyceride biosynthesis during the multiplication phase, in human cells (29, 30). Furthermore, triglycerides form complexes of lipoproteins with the NS1 protein that has association with dysfunction of endothelium in severe dengue infection contributing to elevated permeability of vesicles (31, 32). Suvarna *et al.,* in his study showed that the odds of getting dengue shock syndrome are four times more at TG levels <150 mg/dl when compared to TG levels >150 mg/dl (15). A study with larger sample size would probably explain these differences. A previous clinical study by Duran *et al.,* has shown a significant association between low LDL levels and thrombocytopenia with bleeding manifestations among severe dengue patients (6). In the present study, very strong positive correlation was seen between HDL values and platelet counts (r = 0.712, p=.047*)) among SD patients suggesting their role in the decreased number of platelets. However, further studies are required to confirm this finding since the information regarding the relationship between HDL levels and low platelet count is lacking.

CONCLUSION

The changes seen in the serum lipoprotein levels among dengue patients in the current study suggests that serum TC, HDL and LDL cholesterol levels could be utilized as markers for assessing the severity of dengue which might help the clinicians in initiating an accurate patient therapy and thereby help in better and cost-effective management in resource limited settings where the burden of the disease is high. Prospective studies in large scale are required to implement the routine use of lipid parameters to predict dengue severity in clinical practice.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in this study.

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