A Study of Serum Lipid Profile Changes in Children with Dengue Haemorrhagic Fever and its Correlation with Severity in a Tertiary Care Hospital

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ABSTRACT

Introduction: Dengue Virus (DENV) infection now remains as the most rapidly spreading viral disease worldwide. Lower lipid level occurs in critically ill patients with DENV infection and is postulated as an independent predictor of the clinical outcome.

Aim: To find the correlation between serum lipid levels and severity in children admitted with various grades of Dengue Haemorrhagic Fever (DHF) and secondarily to correlate serum lipid levels with factors like platelet count and duration of hospital stay.

Materials and Methods: This was a prospective descriptive study done in the Paediatric ward from October 2019 to December 2019. Febrile Children with a platelet count of less than 1 lakh/cu.mm and positive for DENV IgM antibodies by Enzyme Linked Immuno Sorbent Assay (ELISA) formed the study group. Demographic details, number of days of fever on admission, duration of stay in hospital and severity grading of DENV infection as per the National guidelines were collected. These children were evaluated with Complete Blood Counts (CBC), fasting lipid profile, X-ray chest and ultrasound abdomen.

Mean and standard deviation was used for categorical variables. Chi-square test was used to assess the strength of association between lipids and severity of DENV infections. Pearson correlation coefficient was used to assess R value between length of stay and serum lipid levels.

Results: The Total Cholesterol, Low Density Lipoprotein (LDL), Triglyceride and Very Low Density Lipoprotein (VLDL) showed a simple linear decline as the severity grade advanced from grade I to grade IV. However, statistical significance was found only for total cholesterol and LDL with p-values of 0.046 and 0.041 respectively. The distribution of High Density Lipoprotein (HDL) across the grades of severity did not follow any pattern and was randomly distributed. On analysis of Pearson correlation between number of days of hospital stay and lipid profile, statistical significance was observed for LDL alone (p=0.023).

Conclusion: The serum levels of total cholesterol and LDL were significantly reduced in severe grades of DHF. Serum levels of total cholesterol and LDL can be used as an economical surrogate marker to predict the severity of DHF at the time of admission.

Keywords: Dengue virus, Dengue shock syndrome, Total cholesterol

INTRODUCTION

DENV is a flavivirus of global importance, with about 4 billion people getting infected annually [1]. It now remains as the most rapidly spreading viral disease worldwide and in recent years its re-emergence in severe forms has been a major threat to children [2]. Of those infected, about 50 thousand develop severe forms of the disease and are hospitalised annually and about 2.5% of them die [3].

Laboratory studies of the pathophysiology of DENV infection suggest that lipids and lipoproteins play a major role in modifying virus infectivity of the target cells [4,5]. After infection, DENV mimic lipid metabolic pathways by increasing lipid raft formation, increasing intracellular levels of total cholesterol, and increasing LDL receptors on the surface of the infected cells. These studies suggest that the lipids are beneficial for DENV multiplication and DENV alters cholesterol metabolism [6-8]. Lower lipid level occurs in critically ill patients with DENV infection and are postulated as independent predictors of the clinical outcome [9]. In most viral infections lipoproteins are thought to bind to the viruses and neutralise their activities [10].

A study in 2002 demonstrated that the levels of total cholesterol, HDL and LDL were decreased in patients with the severe dengue [11]. Biswas HH et al., formed statistical models to analyse the effects of serum cholesterol levels at the time of admission and development of severe forms of dengue infection and concluded that for every 10 mg/dl drop in the total serum cholesterol and LDL-C at the time of admission, the risk of development of DHF and DSS increased

by about 9% and 12%, respectively [12]. Though many studies are available in the literature documenting an association between lipids and DENV infection in adults, there has been a paucity of such studies in children.

The primary aim of the study was to find out the correlation between serum lipid levels on admission and severity in children admitted with various grades of DHF and secondarily to correlate serum lipid levels with factors like platelet count and duration of hospital stay.

MATERIALS AND METHODS

The descriptive cross sectional study was carried out in the Paediatric ward of the hospital, from October 2019 to December 2019. The study period was chosen to coincide with annual surge in dengue fever cases. Written informed consent from the parents was obtained for participation in the study. Institutional ethical committee approval (GMKMC&H/4341/2019) was also obtained.

Inclusion criteria: All children with Dengue IgM antibodies tested by ELISA during the study period.

Exclusion criteria: Children with Dengue fever without DHF, children with negative IgM ELISA for dengue, children with co-morbid conditions like nephrotic syndrome and children who left against medical advice were excluded.

Sample Size Calculation

The sample size was calculated using the below formula. $N=Z^2pq/$

D^2, where, Z is Z score (which is 1.96 for 95% confidence interval), p is prevalence (which is 3%), q is 100-p (which is 97) and D is margin of error (which is 5% with 95% confidence interval) [13]. A minimum sample size of 44.71 was calculated assuming a confidence level of 95%, power of 80%, and prevalence of 3%. But authors have included all the children who fulfilled the inclusion criteria during the study period.

Methodology

As per the hospital policy, all febrile children with a platelet count of less than 1,00,000/cu.mm were admitted to the hospital during the study period. On admission, these children were evaluated with CBC and fasting lipid profile, X-ray chest, ultrasound abdomen and other investigations if necessary. Dengue IgM antibodies were tested by ELISA on 6th day of fever or on admission whichever was earlier. Platelet and total White Blood Cell (WBC) counts were monitored serially and the trough values were recorded for analysis. Serum was separated by standard methods and instructions of the manufacturers were followed meticulously while performing these tests and results were interpreted by qualified personnel. CBC was done using three-part analyser Sysmex KX21. Serum Cholesterol, HDL, LDL, VLDL and Triglycerides were analysed using fully automated analyser BIOSYSTEMS BA 400. Thrombocytopenia was defined as the platelet count of less than 1,00,000/cu.mm and Leucopenia was defined as the leucocyte count of less than 5,000/cu.mm.

For all the participants demographic details including name, age and sex were collected. The number of days of fever on admission and duration of stay in hospital were noted. The severity of DENV infection on discharge was graded from I to IV as per the National guidelines [14].

Grading of Dengue Fever/Dengue Haemorhagic Fever

Dengue Fever

Fever of 2 to 7 days with two or more of the following- Headache, Retro orbital pain, Myalgia, Arthralgia±leukopenia, thrombocytopenia and without evidence of any plasma leakage.

Dengue Haemorrhagic Fever Grade I

Above criteria with positive tourniquet test and evidence of plasma leakage. Thrombocytopenia with a platelet count less than 1,00,000/cu.mm and a Haematocrit increase more than 20% above baseline.

Dengue Haemorrhagic Fever Grade II

Above with the evidence of spontaneous bleeding in skin or other organs and with abdominal pain. Thrombocytopenia with platelet count less than 1,00,000/cu.mm and a Haematocrit increase of more than 20% above baseline.

Dengue Haemorrhagic Fever Grade III (Dengue Shock Syndrome)

Above with circulatory failure (weak and rapid pulse, narrow pulse pressure less than 20 mm Hg, Hypotension, cold and clammy skin and/or restlessness). Thrombocytopenia with platelet count less than 1,00,000/cu.mm and a Haematocrit increase of more than 20% above baseline.

Dengue Haemorrhagic Fever Grade IV (Dengue Shock Syndrome)

Profound shock and undetectable blood pressure or pulses. Thrombocytopenia with platelet count less than 1,00,000/cu.mm and a Haematocrit increase of more than 20% over baseline.

STATISTICAL ANALYSIS

Data was analysed statistically using Statistical Package for Social Studies Version 21.0. Mean and standard deviation was used for categorical variables. Chi-square test was used to assess the strength of association between the lipids and severity of DENV infections. The p-value which is less than 0.05 was considered as statistically significant. Pearson correlation coefficient was used to assess R value between length of stay and serum lipid levels.

RESULTS

After excluding two children that left against medical advice, there were 90 children in the study group; Female children were more in number in the study group with the male:female ratio of 0.73:1. Nearly, half of the study group was comprised of children between 6 to 10 years of age. About 83% of children had fever of less than 5 days in duration, on admission to the hospital.

There were 30, 17, 33 and 10 children in the DHF grades I, II, III and IV, respectively [Table/Fig-1]. About 48% of children were discharged from the hospital after 6 to 10 days of admission and 4.4% of children were discharged after 11 days of admission. The mean duration of stay in the hospital showed a linear progression with increasing grades of DHF as shown in [Table/Fig-1] and was found to be statistically significant (p=0.001).

Around 57% of children in the study group had leucopenia. The mean platelet counts had a linear decline with increasing grades of DHF as shown in [Table/Fig-1] and was statistically significant (p=0.002). Total 25% of children had pleural effusion in their chest X-rays, 29% children had gall bladder wall oedema and ascites in their ultrasonogram abdomen and 19% had gall bladder wall oedema alone. There was no mortality observed during the study period.

Lipid profiles including total cholesterol, HDL, LDL, VLDL and Triglyceride were analysed [Table/Fig-2]. The total cholesterol, LDL, Triglyceride and VLDL showed a simple linear decline as the severity grade advanced from grade I to grade IV. However, statistical significance was found only for total cholesterol and LDL, with p-values of 0.046 and 0.041, respectively. The distribution of HDL across the grades of severity did not follow any pattern and was randomly distributed. On analysis of Pearson correlation [Table/Fig-3] between number of days of hospital stay and lipid profile, statistical significance was observed for LDL alone (p=0.023).

DISCUSSION

In the present study about 48% of children presented with grade III or IV DHF. The incidence of severity varies from study to study [12,13,15]. The study hospital being a referral institution, referral of sick children from secondary care hospitals can be attributed for this high proportion of severe cases. The male: female ratio in this study was 0.7:1 while in other studies an inverse ratio was observed [15,16]. Understanding the gender variations in disease epidemiology will be helpful in public health prevention programs. In the present study a statistically significant negative association was observed between LDL and number of days of hospital stay. Similar negative association was observed in another study by Dunham C et al., [16]. In a study by Siddiqui MSS et al., the negative association was observed only between HDL and number of days of hospital stay which may be a random error due to a small sample size of 50 participants [13].

This study has demonstrated a significant difference in lipid levels between various grades of DHF, lowest levels being observed in grade IV DHF. Many studies have also shown a similar decline as the severity grade increases [15-18]. Though all the studies have observed uniformly lower levels of all the 5 lipids as the severity increases, a study by Ray G et al., did not demonstrate such a decline in lipid levels [19]. Present study has demonstrated a lower level of total cholesterol and LDL on admission. A study by Biswas HH et al., monitored the lipid levels daily and found that the total cholesterol and LDL remained at lower levels between third and eighth days of fever [12].

Factor		Grade of DHF				Total (n=90)
		I (n=30)	II (n=17)	III (n=33)	IV (n=10)	
Gender	Male	12	5	17	4	38 (42%)
	Female	18	12	16	6	52 (58%)
Age	1-5 years	6	5	12	3	26 (29%)
	6-10 years	15	8	14	7	44 (49%)
	>11 years	9	4	7	0	20 (22%)
Days of fever on admission	<5 days	22	17	27	9	75 (83%)
	>6 days	8	0	6	1	15 (17%)
Duration of stay	<5 days	23	10	10	0	43 (48%)
	6-10 days	7	7	22	7	43 (48%)
	>11 days	0	0	1	3	4 (4.4%)
	Mean (days)	4.53	5	6.67	9.7	p=0.001
Trough total WBC count	<5000/cu.mm	18	10	16	7	51 (57%)
	5001-10000/cu.mm	10	6	12	3	31 (34%)
	>10001/cu.mm	2	1	5	0	8 (9%)
Trough platelet count	<10,000/cu.mm	1	1	5	1	8 (9%)
	10,001-50000/cu.mm	16	5	13	7	41 (46%)
	50001-100000/cu.mm	13	11	15	0	39 (43%)
	>100001/cu.mm	0	0	0	2	2 (2%)
	Mean platelet count/cu.mm	63,766	55,894	47,293	18,700	p=0.002
X-ray chest	Normal	29	16	22	0	67 (74%)
	Pleural effusion	1	1	10	10	22 (25%)
	Pulmonary oedema	0	0	1	0	1 (1%)
Ultrasound abdomen	Normal	26	12	8	0	46 (51%)
	Gall bladder wall oedema	3	4	10	0	17 (19%)
	Ascites	0	0	1	0	1 (1%)
	Both gall bladder wall oedema and ascites	1	1	14	10	26 (29%)

[Table/Fig-1]: Clinical profile of the study group.

Grades		Total cholesterol (mg%)	HDL (mg%)	LDL (mg%)	VLDL (mg%)	Triglycerides (mg%)
Grade I	Mean	126.30	30.30	52.67	48.00	243.97
	Standard deviation	35.46	7.2	29.04	21.76	115.92
Grade II	Mean	113.00	27.00	46.11	43.88	220.41
	Standard deviation	23.31	5.79	17.23	17.65	87.53
Grade III	Mean	105.21	31.64	37.27	42.55	214.33
	Standard deviation	24.04	11.33	20.06	9.9	63.11
Grade IV	Mean	97.30	29.80	30.70	42.20	212.40
	Standard deviation	21.52	5.53	15.24	0	73.28
	p-value	0.046	0.360	0.041	0.634	0.562

[Table/Fig-2]: Lipid profile distribution and analysis.

Lipid profile	R value	p-value
TC	-0.157	0.140
HDL	0.102	0.341
LDL	-0.128	0.023
Trigyceride	-0.043	0.689
VLDL	-0.035	0.743

[Table/Fig-3]: Pearson correlation between lipid profile and hospital stay.
TC: Total Cholesterol; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein; VLDL: Very Low Density Lipoprotein

Biswas HH in the year 2015, constructed multivariable models and examined the effect of serum cholesterol levels at presentation on the risk of development of severe dengue later. Using the WHO 1997 DENV infection/disease severity classification, found that for every 10 mg/dL decrease in the total serum cholesterol and LDL-C levels at presentation, risk of development of DHF and DSS increased by about 9% (95% Confidence Interval: 0-19%) and 12% (95% CI: 0-26%), respectively [12]. But such a 10 mg/dL decrease in the HDL-C levels at presentation was not associated with risk of development of the DHF or DSS. A study by Van Gorp ECM et al., also demonstrated the lipid level changes in DENV infection and suggested that it can be used as a marker for severity of the disease [11]. Duran A et al., demonstrated a progressive decline in platelet count with decreasing serum lipid levels [20].

A meta-analysis of seven studies on the association between lipid levels and DENV was done by Lima WG et al., [21]. It is the most elaborate meta-analysis done till date confirming to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement standards. In all the seven studies total cholesterol and LDL levels were significantly lower in severe grades of DHF. But a similar association was not found for other lipids HDL, VLDL and Triglyceride. The authors have concluded that total serum cholesterol and serum LDL levels can be used as markers for DENV severity grade, as they are significantly well associated with the severity in DENV infected patients and are economical.

The exact mechanism behind the lowering of lipid levels and its association is still under research. Many postulates have been put forward for this association. One such postulate proposes the liver cell injury as a major cause for the lowering LDL-C levels [22,23]. The second postulate is that the increased capillary permeability seen with severe dengue disease could possibly enable leakage of cholesterol molecules, resulting in lower serum cholesterol levels [24]. Many other studies have suggested that the lipids are beneficial for DENV multiplication and DENV alters the cholesterol metabolism [6-8].

In the recent years, DENV infections pose a great challenge for the treating Paediatricians. The parents and the general public are also panic driven and pose enormous pressure on the health system during the DENV outbreaks. The search for predictors of severity or mortality like Interleukin-6 or serum nitric oxide early in the course of the DENV infection was done by many researchers [3,25]. In developing countries and in resource limited settings these markers are not readily available and are expensive. Hence, the search for a readily available and economical predictor or marker for dengue severity continues. It is in this situation serum lipid profile done at the time of admission can be used to predict the severity and the physician can place treatment measures appropriately. Further research in a larger sample to estimate the cut-off values for LDL and total cholesterol to predict the severity of DENV infections is necessary.

Limitation(s)

The present study did not correlate the serum lipid levels with the outcome as there were no deaths observed. This was a major limitation of this study.

CONCLUSION(S)

The serum levels of total cholesterol and LDL were significantly reduced in severe grades of DHF. The lower level of LDL is also associated with increased hospital stay of children with DHF. Serum levels of total cholesterol and LDL can be used as a cost-efficient surrogate marker to predict the severity of DHF at the time of admission in resource limited settings.

Acknowledgement

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