Original Article

A Prospective, Observational Study of Serum Triglyceride and Cholesterol Level as Markers of Dengue Severity in Children in a Tertiary Care Hospital

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Background : Dengue related morbidity and mortality results from shock and hemorrhagic manifestations that occur predominantly in critical phase of illness due to severe capillary leakage. Several clinical and laboratory parameters have been studied to predict possibility of Severe Dengue. This study aims to evaluate serum cholesterol and triglyceride level as markers of Dengue severity in children.

Materials and Methods : This prospective observational study includes confirmed cases of dengue infected children of 1 month to 12 years old. Cases were grouped as Dengue Fever without Warning Signs (DF-WS), Dengue Fever with Warning Signs (DF+WS) and Severe Dengue (SD) fever. Serum triglyceride, Cholesterol and other relevant investigations were recorded with changing clinical severity and at recovery stage.

Result : Eighty four children were included in the study of which 53% belongs to 5 to 10 years age group. Mean duration of fever was 4.4 days. Eleven children (13.1%) were admitted in a critical stage. We had 37(44%), 44(52.4%), 3(3.6%) children with DF+WS, DF-WS and SD respectively at first evaluation, which subsequently progressed to 43(51.1%), 30(35.1%), 9(10.7%) children respectively with 2 death (2.3%). We noticed fall in mean serum cholesterol level in SD (Mean=104.9mg%) compared to D+WS (Mean=140.6 mg%) and DS-WS (Mean=158.4 mg%). However, triglyceride level increased in SD (Mean=214 mg%), compared to D+WS (Mean=97.6 mg%) and D-WS (Mean=60.6mg%).

Conclusion : Decreasing serum cholesterol and increasing triglyceride values can be taken as a surrogate marker of Dengue severity along with the clinical severity classification.

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Key words **: Dengue in Children, Lipid Profile in Dengue, Triglyceride in Dengue, Cholesterol in Dengue.**

Dengue is a mosquito born disease caused by Dengue virus (Flavivirus) which affects people globally across all age groups with a seasonal outbreak. About 5 million people Worldwide gets affected by Dengue infection annually, of which approximately five thousands Dengue related deaths reported in the year 2023 from 80 countries/territories and five WHO regions: Africa, Americas, South-East Asia, Western Pacific and Eastern Mediterranean regions[1,2]. Dengue illness has varied clinical presentations starting from undifferentiated Dengue fever, Classical Dengue fever, and Dengue hemorrhagic fever to Dengue shock syndrome[3]. WHO (2009) classified dengue cases as per clinical severity as Dengue Fever without Warning Signs (DF-WS), Dengue Fever with Warning Signs (DF+WS) and severe Dengue fever (SD)[4]. Mortality in Dengue illness increases when fatal complications like hemorrhagic manifestations and shock develop due to immune mediated vascular damage and altered permeability along with thrombocytopenia, coagulopathy and multi organ dysfunction precipitate. The success in the management of complicated dengue lies on its early detection and initiation of proper therapy. Several biochemical and radiological abnormalities can pick up the early signs of critical phase of Dengue fever before its clinical manifestation. Cholesterol and triglyceride play an important role in the pathophysiology of Dengue fever right from its receptor mediated cellular entry, virus multiplication to fresh invasion to a new target host cell[5,6]. Thus alteration in serum cholesterol and triglyceride is expected in critically ill Dengue patients and probably surrogate

Editor's Comment :

- The success of Dengue fever management lies on early detection of risk factors and judicious fluid management as till today there is no definite chemotherapeutic agents.

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markers for impending adverse outcome. Various studies have found that total cholesterol level decreases in critical phase of Dengue patients[7-9]. Biswas, *et al* observed that every 10 mg/dl drop in serum cholesterol and LDL in Dengue patient since admission risk the development of DHF and DSS by about 9% and 12% respectively[10]. Alteration in triglyceride level is not uniform among the previous studies. There are paucity of studies in children with Severe Dengue and altered cholesterol and triglyceride level. So the aim of our study is to determine the correlations between serum cholesterol and triglyceride level with Dengue severity in a child suffering from Dengue illness. Alteration in serum cholesterol and triglyceride levels can be taken as an additional marker for Dengue severity so that early intervention can be planned.

Materials and Methods

This was a prospective study conducted at Dr B C Roy Postgraduate Institute of Pediatric Sciences from 1st September, 2020 till 30th November, 2021 amid COVID-19 pandemic and covering 2 monsoons at Kolkata. The monsoon was chosen as there is an annual surge during this time at Kolkata, but during 2020 due to COVID-19 outbreak and nationwide closure of schools we got very minimum cases, whereas we got most of the cases of our study from monsoon of 2022 and also we extended our study period from 12 months to 14 months. Written informed consent was obtained from parents willing to participate in the study group after obtaining approval from Institution Ethics Committee (IEC,memo no: BCH/ME/PR/2960 dated 09/12/2020). We have strictly excluded COVID-19 patients from our study group and they had a separate isolation ward.

Inclusion Criteria :

All the febrile children attending OPD and admitted at IPD with following —

(1) Diagnosed as a confirmed case of Dengue as per WHO 2009 case definition[4].

(2) Age : 1 month to 12 years.

Exclusion Criteria :

(1) Children with pre-existing liver diseases & nephrotic syndrome.

(2) Children with prior documented abnormal lipid profile.

(3) Children with history of familial dyslipidemia.

(4) Children with COVID-19 infection or any other co-infections like Malaria, Typhoid, Scrub to minimize bias factor.

Observation

All admitted children satisfying inclusion criteria were included in the study. Dengue infection diagnosed by serological test (NS1 and IgM by Mac ELISA) as per WHO guideline and COVID-19 by (RAT or RT-PCR for SARS COVID-19). Demographical profile of all subjects enrolled was recorded in a self-made printed data collection sheet. On first contact clinical evaluation and necessary investigations done. Blood sent for CBC, LFT, Lipid profile, Chest X-ray and USG abdomen as required case to case and recorded in data sheet. Dengue cases were grouped as per WHO 2009 clinical severity classification guidelines as Dengue Fever without Warning Signs (DF-WS), Dengue Fever with Warning Signs (DF+WS) and Severe Dengue (SD) fever[4]. They were managed as per national Dengue management protocol. Serum lipid and other relevant investigations repeated with changing clinical severity and finally at recovery phase. So, we recorded three sets of investigational reports along with total duration of hospital stay with clinical outcome.

All data were plotted in Microsoft office 365 excel sheet for statistical representation. Categorical variables are expressed as number of patients and percentage of patients. Mean and SD was used for categorical variables. Chi-square test used to assess the strength of association between Cholesterol and Triglycerides with Dengue severity. The statistical software SPSS version 26 has been used for the analysis. Data representation and result analysis done using proper statistical methods. P value <0.05 was considered statistically significant

Results

Out of 7974 children got hospitalized during our study period, 84 (1.05%) children met the inclusion criteria for Dengue illness and were selected for our study. We found that 5 to 10 years age group children (n =45; 53%) were mostly affected by Dengue illness of the cohort of 84 children. Sex ratio of the entire study population was Boys : Girls = 45:39. We recorded maximum admission from rural area 45 (53.6%), followed by urban slum 26 (31%) and urban/suburban 13 (15.5%). Mean duration of fever was 4.4 days.

Out of total 84 subjects, 73 (86.9%) got admitted in febrile stage and 11 (13.1%) was admitted in a critical stage; which were further distributed as per WHO clinical severity. Thus, at the time of first evaluation we had 37 (44%), 44 (52.4%), 3 (3.6%) children with DF+WS, DF-WS and SD respectively. Among the children admitted in febrile stage (n=73), 29 (39.7%)

had warning sign (DF+WS) and out of 11 children admitted in critical stage 3 (27.3%) had Severe Dengue (SD)(Table 1). The baseline serum cholesterol level at the time of admission among children admitted in febrile stage was recorded as 152.5 mg%, 160 mg% and 135.8mg% respectively among D+WS, D-WS, SD. The serum triglyceride was 62mg%, 57.1mg% and 92.2 mg% respectively among D+WS, D-WS and SD (Tables 2 & 3).

The clinical distribution according to disease severity changed subsequently as the disease progressed. Amongst the children with D+WS, 6 (6/37; 16.2%) were developed Severe Dengue and out of 44 children with D-WS, 2 (4.5%) developed Severe Dengue and 12 (27.3%) developed warning signs. Hence, the distribution changed to 43 (51.1%), 30 (35.1%), 11 (13.1%) children with DF+WS, DF-WS and SD respectively. Two (2/84; 2.3%) children with Severe Dengue died in critical stage (Table 1).

We recorded mean serum cholesterol and triglyceride level among these 3 clinical group during critical stage and found that there was significant dip in mean serum cholesterol level in Severe Dengue (Mean= 104.9mg%) group compared to D+WS (Mean=140.6 mg%) and DS-WS (Mean=158.4 mg%). But the changes in triglyceride level among the 3 clinical groups was opposite and the mean triglyceride level increased to maximum level among children with SD (Mean=214 mg%), compared to D+WS (Mean=97.6 mg%) and D-WS (Mean=60.6 mg%).

Table 1 — *Demography clinical severity, and outcome of Dengue patients (n=84)*

Factors		Frequency
Gender	Male: n (%)	45 (55.6%)
	Female: n (%)	39 (46.4%)
Age	1-4 years: n (%)	32 (38%)
	5-10years: n (%)	45 (53%)
	≥ 11 years: n (%)	7 (9%)
Locality	Urban: n (%)	13 (15.6%)
	Urban slum: n (%)	26 (31%)
	Rural: n (%)	45 (53%)
Duration of fever (days): mean (sd)		4.4 (0.8)
Clinical stage at admission	Febrile stage: n (%)	73 (86.9%)
	Critical stage: n (%)	11 (13.1%)
Clinical severity at the time of admission	D+WS: n (%)	37 (44%)
	D-WS: n (%)	44 (52.4%)
	SD: n (%)	3 (3.6%)
Clinical severity at the time of discharge (excluding 2 deaths)	D+WS: n (%)	43 (51.1%)
	D-WS: n (%)	30 (35.1%)
	SD: n (%)	9 (10.7%)
Duration of hospital stay (days): mean (sd)		7.4 (1.3)
Mortality: n (%)	2 (2.3%)	

D= Dengue; WS= Warning Sign; SD= Severe Dengue; sd = standard deviation

Table 2 — *Serum cholesterol level at various stages of Dengue illness (n=84)*

Clinical stage	Number	Mean (mg/dl)	sd	Maximum	Minimum	Median
Analysis of serum Cholesterol at Febrile stage of Dengue illness						
D+WS	37	152.5	12	178	134	155
D-WS	30	160	8.7	180	137	160
SD	6	135.8	6.6	147	128	135
Analysis of serum Cholesterol at Critical stage of Dengue illness						
D+WS	43	140.6	13.4	163	110	140
D-WS	30	158.4	4.7	165	149	158
SD	11	104.9	10.7	116	84	108
Analysis of serum Cholesterol at Recovery stage of Dengue illness						
D+WS	43	147.7	13.9	176	124	149
D-WS	30	159.8	7.9	171	136	162
SD	9	133.2	93	151	122	132

Table 3 — *Serum Triglyceride level at various stages of Dengue illness (n=84)*

Clinical stage	Number	Mean (mg/dl)	sd	Maximum	Minimum	Median
Analysis of serum Triglyceride at Febrile phase of Dengue illness						
D+WS	37	62	13.7	88	30	64
D-WS	30	57.1	12.5	78	30	57
SD	6	92.2	25.4	123	57	92.5
Analysis of serum Triglycerides at Critical phase of Dengue illness						
D+WS	43	97.6	28	186	41	93
D-WS	30	60.6	17	90	35	64
SD	11	214.6	71.8	304	92	236
Analysis of serum Triglyceride at recovery phase of Dengue illness						
D+WS	43	85.1	22.5	176	47	82
D-WS	30	61.3	14.7	84	36	63
SD	9	153.4	48	46	88	128

Following recovery we again recorded serum cholesterol and triglyceride among the 3 groups and found serum cholesterol (Mean=133.2 mg%) and triglyceride (Mean=153.4 mg%) level restored to normal values in Severe Dengue group. (Table 2 &3)

Mean duration of hospital stay was 7.4 days. Two children died from Dengue Shock Syndrome (DSS) and multi organ dysfunction and rest all recovered.

Discussion

In the present study 84 (1.05%) children got enrolled out of 7974 children admitted in pediatric ward during

the study period of 15 months, which was much less compared to previous years, probably due to COVID-19 pandemic, which caused prolonged school closure and restricted public gatherings and events with enhanced health consciousness. Thus there was significant drop in infectious diseases. Children mostly affected belonged to 5-10 years age group which was quite similar to another study from Eastern India by Purakait R, *et al*[11]. The male : female ratio of our study population was 1.15:1 closely matching with another study by Nayak R, *et al* from Odisha[12], however, in another study by Prabhuraj A et al. the female patients outnumbered male patient with a sex ratio of M:F:: 0.7:1[13]. The knowledge of gender demography in disease epidemiology is helpful in executing public health prevention programs[14,15]. We found that 45 children got admitted from rural area which is a reversal of previous concept that Dengue is an urban-centric disease and Nayak, *et al*. At Odisha also had a similar observation. This paradigm shift may be due to rapid urbanization of rural areas.

In our study, 73 patients (86.9 %) got admitted in febrile stage of Dengue and 11 (13.1%) were in critical stage of illness. Among 84 patients, 43 (51.1%) had Dengue Fever with Warning Sign (DF+WS), 30 (35.1%) had Dengue Fever without Warning Sign (DF-WS) and 11 (13.1%) had Severe Dengue (SD) infection (Table 1). A study from Odisha in 2016 by Nayak, *et al* showed out of 97 total hospitalized cases, 84 (86.59%) were non severe and 13 (13.40%) were Severe Dengue[12]. But in our study dengue without warning sign cases were much less in comparison to their study which may be due to pandemic situation where only serious dengue cases sought hospital admission.

In our study the mean (sd) serum cholesterol level at febrile stage was around the normal range in all clinical groups which was 152.5(12), 160 (8.7), 135.8 (6.6) mg/dl respectively among Dengue Patients with Warning Sign (D+WS), without Warning Sign (D-WS) and Severe Dengue (SD). But with changing severity serum cholesterol level drops in critical stage of illness and the mean value was 140.6 (13.4), 158.4 (4.7), 104.9 (10.7) mg/dl among dengue with warning sign (D+WS), Dengue without Warning Sign (D-WS) and Severe Dengue (SD) fever respectively. The drop is highest in patients with Severe Dengue and also the 2 unfortunate children who succumbed. With recovery serum cholesterol level returned to normal range with a slow recovery in Severe Dengue group and the mean (sd) value was 147.7 (13.9), 159.8 (7.9), 133.2 (9.3) mg/dl among Dengue with Warning Sign (D+WS), without Warning Sign (D-WS) and Severe Dengue (SD) fever respectively (Table 2).

On the contrary, serum triglyceride level which was near normal range at the time of first contact, increased 2 to 3 times during critical stage in patients with Severe Dengue and Dengue with Warning Signs. The mean (sd) value of serum triglycerides was 62(13.7), 57.1(12.5), 92.2(25.4) among D+WS, D-WS and Severe Dengue respectively at admission, which increased to 214.6 (71.8) in Severe Dengue group during critical stage with death of 2 children (Table 3).

A meta-analysis of seven studies by Lima WG, *et al*, showed that total cholesterol and LDL were significantly lowered in severe grade of DHF[16]. But they didn't found similar association with other lipid components like Triglycerides, HDL, VLDL whereas the present authors found that serum triglycerides level increases with Dengue severity. The exact mechanism behind this disproportionate lipid components alteration with Dengue severity is not clear, but few research work postulates that damage to liver cells in severe dengue is responsible for lowered serum cholesterol along with increased cholesterol leakage due to enhanced capillary permeability[17,18]. Many other studies suggest excessive lipid utilization by replicating Dengue virus[19]. Serum triglyceride level increases along with other inflammatory markers like Ferritin, LDH, IL-6 as it happens in other clinical conditions like MISC or MAS.

Dengue illness during monsoon is a clinical, challenge to treating pediatrician almost every year. The rapidity of clinical deterioration and sudden death provokes trepidation both to doctor and parents. Thus there is a requirement of early predictors of Severe Dengue which will alert and enable us for early meticulous intervention. Studies found that use of NO/ IL-6 as early predictors but they are not readily available in resource poor settings[20]. However, both serum cholesterol and triglyceride level estimation at admission and its serial progression will help early diagnosis of severe and deadly Dengue.

The strength of the study was, more number of serious ill patients incorporated in the study group, with single primary investigator reducing interpretation bias and all laboratory investigations were done in same institute with strict adherence to national guidelines of Dengue management.

Limitation(s) :

The weakness of the study is small sample size due to COVID-19 pandemic. This study didn't correlate subtypes of cholesterol with Dengue severity and serum lipid levels with the outcome.

Conclusion

This study has demonstrated that serum cholesterol significantly decreases with severity of Dengue fever along with increase in serum triglyceride level which signals impending Dengue illness associated catastrophe.

Conflict of interest : None

Funding : None

Contribution : SP: concept of the study and design data collection; MKM: review of literature, data collection and statistical analysis; SSB: writing manuscript, statistical analysis, guarantor; AD: writing manuscript, editing, manuscript review.

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