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Research Article

Evaluation of Biochemical Markers of Hemophagocytic Lymphohistiocytosis with Severity and Outcome of Dengue Fever

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Abstract

Background: The aim of the present study was to identify an association between biochemical markers of hemophagocytic lymphohistiocytosis such as serum ferritin, serum triglycerides, serum fibrinogen and serum lactate dehydrogenase with the severity of dengue fever and disease outcome.

Material and Methods: Ninety patients aged ≥ 18 years with dengue fever confirmed with a serology- dengue non-structural protein 1 antigen-positive were included for this prospective observational study. The patients underwent tests that included serum ferritin, serum triglycerides, serum lactate dehydrogenase, and serum fibrinogen. The primary outcome measure was to find an association of serum triglycerides, serum lactate dehydrogenase,, serum ferritin and serum fibrinogen with the severity of dengue disease.

Results: The median serum ferritin (870.0 ng/mL Vs 95.0 ng/mL), the mean serum lactate dehydrogenase (235 ± 36 U/l Vs 192 ± 24 U/l) and mean serum triglyceride (230 ± 33 mg/dL Vs 192 ± 24 mg/dL) were significantly higher in severe dengue fever patients as compared to dengue fever patients. The mean fibrinogen was 180 ± 45 mg/dL and 197 ± 46 mg/dL in severe dengue fever patients and dengue fever patients respectively (p-value = 0.149).

Conclusions: Serum ferritin, serum lactate dehydrogenase and serum triglyceride levels were found to have a significant association with dengue disease severity.

Keywords: Dengue Fever; Serum Ferritin; Serum Fibrinogen; Serum Lactate Dehydrogenase; Serum Triglycerides

Introduction

It is estimated that 390 million dengue virus infections occur per year, of which 96 million manifests clinically, putting over 3.6 billion people living in tropical regions at risk and causing 20,000 deaths annually [1].Dengue viral illness ranges from mild fever to severe conditions like dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS).Early detection and accurate diagnosis are required for appropriate management. Dengue viral illness is diagnosed by identifying biomarkers which include virus products like Non-structural protein 1(NS-1) antigen capture, the virus itself by isolation in culture, mosquitoes or the direct detection of viral genomic ribonucleic acid, or the host immune response to virus infection (through measurement of virus-specific immunoglobulin M and immunoglobulin G) [2]. Endothelial injury and the vascular leak is the hallmark of severe dengue fever [3]. This vascular dysfunction in turn leads to the release of several inflammatory mediators which contribute to the illness. Multiple organ systems can be involved during dengue illness ranging from mild organ dysfunction to severe organ dysfunction [4]. The human innate immune system is the first line of defence against the dengue virus [5]. This will, in turn leads to a cascade of events that establishes an antiviral state at the cell level and leads to an immunological response.

Hemophagocytic lymphohistiocytosis (HLH) is a rare life-threatening disorder of immune dysfunction which is increasingly reported in severe dengue fever patients [6]. It can progress rapidly leading to multiorgan dysfunction and death. Thus, early identification and prompt treatment of these patients is important. It is diagnosed if the clinico-laboratory parameters fulfil the HLH-2004 criteria [7].

Severe dengue fever is associated with raised levels of serum ferritin, serum triglycerides, serum lactate dehydrogenase (LDH) and reduced serum fibrinogen [8-12]. These are also the biochemical markers that are raised in HLH [8].There were case reports that showed that these markers could predict the disease progression into severe dengue fever [8]. There are very few studies published to establish a relation between dengue and HLH biomarkers for dengue severity [13-16].

HLH criteria include above mentioned characteristic biochemical markers, bone marrow biopsy, natural killer cell activity, soluble cluster determinant (CD) 25. But, except biochemical markers, the other criteria cannot be assayed in every clinical setup and would require sophisticated infrastructure. There are no studies that analysed all these parameters together. There is a need to identify the marker which can be correlated with dengue fever severity in the early course of the disease as this can help in identifying severe cases and treating them promptly. The aim of the present study was to identify an association between biochemical markers of HLH such as serum ferritin, serum triglycerides, serum fibrinogen and serum LDH with the severity of dengue fever and disease outcome.

Material and Methods

This prospective observational study was conducted between April 2019 and October 2020. After approval from the institutional ethics committee (Letter No: RECH/EC/2019-20/0067), a written informed consent was obtained from all the patients prior to enrolment explaining the risks and benefits of the procedure. Patients above 18 years of age admitted in Poona Hospital and Research Centre with dengue fever confirmed with a serology-dengue NS1 antigen positive were included. Patients who had haematological conditions like iron overload, malignancies, thalassemias and viral infections other than dengue were excluded.

Baseline demographic data were collected. The patients underwent general physical examination, systemic examination and a group of tests that included serum ferritin (21-274 ng/mL), serum triglycerides (<150 mg/dL), serum LDH (125-243 U/L), serum fibrinogen (220-496 mg/dL), haemogram and ultrasonography of the abdomen and pelvis.

Patient's serum ferritin was measured by ARCHITECT Ferritin 7K59 kit, Abbott, United States of America (USA), serum triglycerides by triglyceride Flex® reagent cartridge, Cat No DF69A, using Dimension® clinical chemistry system, Siemens, Germany, serum LDH by Flex® reagent cartridge, lactate dehydrogenase method, using Dimension® clinical chemistry system, Siemens, Germany and serum fibrinogen levels by Fibrinogen-C kit 0020301100 on HemosIL®, USA. Daily haemogram by SYSMEX XS 800i/ XN1000- sodium lauryl sulphate Hemoglobin method was done. The patients were grouped into dengue fever and severe dengue fever according to World Health Organisation 2009 criteria [17].

Criteria for dengue fever, Lived in/travel to the dengue-endemic area, fever and two of the following - nausea, vomiting/rash/

aches and pains/tourniquet test positive/leucopenia/any warning sign such as abdominal pain or tenderness, persistent vomiting, clinical fluid accumulation – generalised oedema, pedal oedema, abdominal ascites, pleural effusion, mucosal bleed, lethargy, restlessness, liver enlargement >2cm on palpation, increase in haematocrit concurrent with a rapid decrease in platelet count.

Criteria for severe dengue fever, Severe plasma leakage, shock – systolic blood pressure <100 mm of Hg, fluid accumulation with respiratory distress, severe bleeding, severe organ impairment-liver: Aspartate transaminase, or Alanine transaminase ≥ 1000 international units (IU)/L, central nervous system: Impaired consciousness, Heart: Sinus tachycardia, sinus bradycardia, non-specific ST- wave changes, inverted T waves, first-degree heart block and right bundle branch block.

The primary outcome measure was to find the association of biochemical markers of HLH (serum triglycerides, serum LDH, serum ferritin and serum fibrinogen) levels with the severity of dengue fever, whereas the secondary outcome measure was to find the outcome according to the severity of dengue fever.

Switala JR., *et al*. reported that hyperferritinemia was a consistently reliable finding in 93.0% of patients [18]. The sample size was calculated by a formula N [19] = $(Z_{\alpha})^2 p(1-p)/d^2$ We have taken Z_{α} a standard normal variate at 5% type 1 error (1.96). A total sample size of 90 was calculated by the above method.

Data collected were entered in Excel 2007 and analysis of data was done using Statistical Package for Social Sciences for Windows, Version 20.0 from International Business Machines Corporation, Armonk, New York, USA. The data on categorical variables are shown as n (% of cases). The data on continuous variables are presented as mean and standard deviation (SD). Comparison of the distribution of categorical variables was done using the Chi-Square or Fisher's exact test. Comparison of continuous variables was done by unpaired t-test. The underlying normality assumption was tested before subjecting the study variables to an unpaired t-test. The statistical significance of the inter-group difference of medians of continuous variables was tested using the Mann-Whitney U test. The confidence limit for significance was fixed at 95% level with a p-value < 0.05.

Results

Of 90 patients, 24 (27.0%), 24 (27.0%), 19 (21.0%), 18 (20.0%) and 5 (6.0%) were < 30 years, 30 - 39 years, 40 - 49 years, 50 - 59 years, and ≥ 60 years respectively. The mean ± SD of age of the patients was 39.0 ± 13.0 years. Of 90 patients, 47 (52.2%) were males and 43 (47.8%) were females. Of 90 patients, 70 (77.7%) had dengue fever and 20 (22.3%) had severe dengue fever.

There was no statistically significant difference between age group, gender and type of dengue fever (Table 1). The median serum ferritin level, the mean serum LDH and mean serum triglyceride levels were significantly higher in severe dengue fever patients as compared to dengue fever patients. There was no statistically significant difference between mean fibrinogen levels in relation to the type of dengue fever (Table 2).

Criteria	Dengue fever n (%)	Severe Dengue fever n (%)	Total n (%)	p-value
Age in years				
<30	23 (95.8)	1 (4.2)	24 (100.0)	0.349**
30-39	24 (100.0)	0 (0.0)	24 (100.0)	
40-49	14 (73.7)	5 (26.3)	19 (100.0)	
50-59	8 (44.4)	10 (55.6)	18 (100.0)	
≥60	1 (20.0)	4 (80.0)	5 (100.0)	
Gender				
Male	37 (78.7)	10 (21.3)	47 (100.0)	0.821*
Female	33 (76.7)	10 (23.3)	43 (100.0)	

Table 1: Distribution of dengue fever according to age group and gender.

*Chi-square test was used

**Fisher's exact test was used.

Parameters	Dengue fever	Severe Dengue fever	p-value
Median serum ferritin (ng/mL)	95.0	870.0	0.0001*
Mean serum LDH (U/l) ± SD	192.0 ± 24.0	235.0 ± 36.0	0.0001**

Mean serum triglycerides (mg/dL) ± SD	192.0 ± 24.0	230.0 ± 33.0	0.0001**
Mean serum fibrinogen (mg/dL) ± SD	197.0 ± 46.0	180.0 ± 45.0	0.1490**

Table 2: Distribution of dengue fever according to serum ferritin, serum triglycerides, serum LDH and serum fibrinogen levels.

* Mann-Whitney U test was used

**Unpaired t-test was used

SD - Standard deviation

LDH - Lactate dehydrogenase.

Of 90 patients, 4 (4.4%) patients died. The median serum ferritin level was significantly higher in patients who died as compared to patients who survived. There was no statistically significant difference between mean serum LDH, mean serum triglyceride and mean fibrinogen levels between patients who died and survived (Table 3). Of 90 patients, 70 had dengue fever, all of them survived. Of 20 patients who had severe dengue fever, 16 (80.0%) survived, and 4 (20.0%) died. A significantly higher percentage of patients died who had severe dengue fever (p-value = 0.0019).

Parameters	Survived n = 86	Died n = 4	p-value
Median serum ferritin (ng/mL)	233.8	1750.0	0.0001*
Mean serum LDH (U/l) ± SD	201.2 ± 32.60	227.0 ± 9.89	0.268**
Mean serum triglycerides (mg/dL) ± SD	180.2 ± 38.2	233.0 ± 4.24	0.055**
Mean serum fibrinogen (mg/dL) ± SD	193.9 ± 46.07	157 ± 9.89	0.262**

Table 3: Comparison of serum ferritin, serum LDH, serum triglycerides and serum fibrinogen levels with the outcome.

* Mann - Whitney U test was used

**Unpaired t - test was used

SD - Standard deviation

LDH - Lactate dehydrogenase.

Discussion

The present study was conducted to find the association between serum ferritin, serum triglycerides, serum fibrinogen and serum LDH with the severity of dengue fever and disease outcome.

In the present study, 52.2% were males and 47.8% were females. A study conducted in North India reported that males were found to be more affected than females [20]. In the present study, the elderly age group, ≥ 60 years had 80.0% severe dengue fever. These results were similar to other studies that reported elderly age groups have more risk of severe dengue illness [21]. A study conducted in Singapore reported that elderly patients were found to have more risk of severe dengue fever as compared to younger adults. The study further stated that of 6989 cases, 295 (4.4%) were elderly; the elderly suffered more severe disease with a higher percentage of patients with DHF (29.2% vs. 21.4%) and severe dengue fever (20.3% vs. 14.6%) [p-value <0.05] [22].

The increased incidence among the elderly age group might be due to pre-existing comorbidities, or due to immune hyperactivation in elderly individuals [23]. Compared to younger individuals, elderly adults are at increased risk of developing severe dengue fever, DHF and DSS. Several reasons could have contributed to severe disease in the elderly. The process of aging impairs physiological functions and negatively impacts the function of the immune system [24]. In the elderly, monocytes appear to have a lower antioxidant response against oxidative stress induced by the dengue virus [25,26]. T cell response and cytokine production in response to dengue virus infection have also been observed to be impaired in the elderly. Other causes such as the presence of pre-existing comorbidities, like hypertension, diabetes mellitus (DM), chronic kidney disease (CKD), ischaemic heart disease (IHD) may predispose elderly patients to severe dengue fever. Comorbidities such as hypertension, DM, IHD, CKD were shown to have a significant association with severe dengue. A study conducted in Jeddah, Saudi Arabia, reported that comorbidities such as DM, hypertension were found to have an association with severe dengue fever (p-value =0.009) [27]. An unmatched case-control study was conducted on 123 severe cases and 245 controls (non-severe cases) diagnosed during 2014–2016. Risk factors for severe dengue fever were secondary infection (p-value = 0.02), and co-morbidities, particularly DM and hypertension (p-value < 0.001) [27].

In recent times a phenomenon called HLH is being frequently reported in patients with severe dengue fever. HLH is a severe systemic inflammatory condition due to excessive activation and proliferation of T cells and well-differentiated macrophage that leads to hyperactivated but dysregulated immune responses. This result in an overwhelming inflammatory response leading to an unremitting high rise of temperature, organomegaly (involving liver and spleen), haemorrhage, lymphadenopathy and central nervous system dysfunction. Hyperferritinemia (levels above >550 ng/L) is a flagship sign of HLH; however, hypoalbuminemia, cytopenia, coagulopathy, abnormal liver function tests, hypertriglyceridemia, hemophagocytic, and elevated serum soluble CD25 and soluble CD16 levels also serve as adjunct markers of HLH [16]. Serum ferritin was found to be an early predictor of severe dengue illness and it is also a marker of HLH which was reported in some cases of dengue. Hence, measuring serum ferritin in dengue patients may help in the early identification of at-risk groups for severe disease and HLH.

In the present study, the median serum ferritin levels were significantly higher among severe dengue fever as compared to dengue fever patients. Soundravally R., *et al*. reported that the mean serum ferritin levels were significantly higher in severe dengue fever as compared to dengue fever 1264.71 ± 492.59 ng/mL Vs 940.09 ± 568.31 ng/mL (p-value 0.033) [28]. These results are similar tothe present study. A study conducted in Karnataka, India reported that serum ferritin was associated with severe dengue fever (p-value <0.009). The mean ferritin levels in dengue without warning signs, dengue with warning signs and severe dengue were 2,304.48, 12,431.00, and 54655.25ng/mL respectively. The study further recommended that serial measurements of serum ferritin should be done to identify disease progression [29].

In the present study, the mean serum LDH was significantly higher in severe dengue fever as compared to dengue fever. A study conducted in Pakistan reported that serum LDH can predict the severity of dengue fever (p-value <0.001) [30]. A raising serum LDH levels, above 200mg/dL in the early course of dengue illness may help in identifying patients who are likely to progress to severe disease when measured along with serum ferritin.

In the present study, the mean serum triglyceride levels were significantly higher in severe dengue fever patients as compared to dengue fever patients. Chen C-Y., *et al.* reported that the severity of dengue fever was associated with old age and hypertriglyceridemia [31].

Serum fibrinogen, a marker of coagulation, is raised in HLH. A recent multivariate logistic regression analysis reported that serum fibrinogen level can predict severe dengue fever at day 0. The study further identified seven haematological biomarkers (albumin, aspartate aminotransferase, fibrinogen, D-dimer and thrombin anti-thrombin-complex concentrations, activated partial thromboplastin time and thrombin time coagulation tests) as the prognostic markers of severe dengue fever at day 0 [32]. These findings suggest an exacerbation of the dengue-related coagulation disorders in patients experiencing severe dengue fever. In the present study, however, the mean serum fibrinogen levels among severe dengue fever and dengue fever did not differ significantly.

In the present study, 4 (4.4%) patients died. The median serum ferritin level was significantly higher in patients who died as compared to patients who survived. However, other parameters such as serum LDH, serum fibrinogen and serum triglycerides did not show a significant association with death. Chen C-Y., *et al*. reported that 11/644 (1.7%) patients died [31].

In the present study, the mortality was significantly higher in severe dengue fever patients (80.0%) as compared with dengue fever patients (0.0%). Higher mortality in severe dengue fever patients may be attributed to their advanced age and pre-existing comorbidities.

Limitations

The spectrum of dengue infection is wide, and the present study was conducted only in the hospitalized patients with dengue fever and severe dengue fever. It was conducted in a tertiary care centre, so referral bias could not be avoided. A large set of patients with dengue fever treated on an out-patient basis were not included in this study. We have not analysed comorbidities of the patients and the treatment received. Treatment details were not recorded, so there could be a treatment bias. A major limitation is that this study was conducted with a small sample size. Multicentric studies with a large sample size should be undertaken to substantiate the research findings described in this paper.

Conclusions

The serum ferritin, serum lactate dehydrogenase and serum triglyceride levels may be considered as a tool to predict severe dengue fever. The serum ferritin level may be considered as a predictor of mortality. The serum ferritin, serum lactate dehydrogenase and serum triglyceride levels should be tested in each patient with dengue fever.

Conflict of Interest

Dr. Tarun Betha, Dr. Ajit Tambolkar, Dr. Vihita Kulkarni-Nivargi, Dr. Arun Bahulikar and Dr. Deepak Phalgune declare that they have no conflict of interest.

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