Hyperferritinemia in Dengue Fever- Correlation between Serum Ferritin and Thrombocytopenia

S JYOTHI VISALAKSHY[1], SELVA SARAVANAN SAMINATHAN[2], SANDEEP SURENDRAN[3], MGK PILLAI[4]

ABSTRACT

Introduction: Dengue fever is a widely prevalent viral infection in the tropical countries. Many patients with dengue fever are known to have severe cytopenias and the mechanism for the same is not known. Macrophage Activation Syndrome (MAS) is a life threatening complication that is known to be triggered by viral infections. This study attempts to examine the possibility of MAS as an underlying cause of cytopenias in dengue fever. Hyperferritinemia which is an important diagnostic criteria for MAS is known to occur in dengue fever also.

Aim: The primary objective of the study was to assess the correlation between ferritin levels and mean platelet drop among patients admitted with dengue fever. Secondary objective was to do a subgroup analysis and compare the patients treated according to WHO standard protocols in dengue fever with those who were also given steroids and compare outcomes.

Materials and Methods: This was a retrospective study done over a period of 18 months. Patients with dengue fever who satisfied the criteria for MAS were selected and the mean change in platelet count between the time of admission and 48 hours later was calculated. This was compared with the ferritin levels. Subgroup analysis was also done and the groups were correlated on two important clinical outcomes-the mean platelet drop and length of stay in hospital.

Results: Total of 26 patients were enrolled in the study (n=26), of whom 18 (69.2%) were male. Pearson's correlation coefficient (-0.231) was calculated to compare Ferritin levels with mean platelet drop and it showed that there was no correlation. There was no difference between the two groups with respect to mean platelet drop (p=0.08) and length of stay (p=0.443) in hospital.

Conclusion: Hence, it can be concluded that hyperferritinemia does not correlate with the severity of platelet drop. There was no significant change in outcomes in patients with dengue fever associated MAS who were treated with steroids or with only standard treatment protocol.

INTRODUCTION

Dengue fever is a common viral infection whose incidence in India has increased in the last 10 years. Case Fatality Rate (CFR) of dengue fever is 1%, with certain areas in rural India having CFR of 3-5% [1]. The clinical manifestations of dengue fever can be explained by the increased capillary permeability and endothelial damage. However, it is well known that there is significant cytokine stimulation during dengue [2]. Like other viral infections, the treatment is usually supportive management with Intravenous (IV) fluids and blood product transfusions as necessary.

However, for a small subset of patients, this infection is life threatening with severe cytopenias and significant systemic response. The presence of macrophage activation in dengue fever is well reported and could be one possible reason for mortality and morbidity in dengue fever [3]. MAS is a life-threatening haematological syndrome that is associated with cytopenias and fever. This is a complex entity that results from inappropriate activation of tissue macrophages leading to histiocytic proliferation, hypercytokinemia and T-cell immunosuppression. MAS can be rapidly fatal unless it is identified early and treated [4].

The treatment that has been advised for macrophage activation is mainly with steroids and other immunosuppressive agents like cyclosporine. IV immunoglobulins has been reported to be of benefit [4].

Of the overlapping features, what stand of interest for us is the serum ferritin levels. Ferritin is a protein that takes part in iron storage in the body. It is an acute phase reactant. Ferritin levels are important in MAS because it can be diagnostic for the disease as ferritin level >10,000 mcg/L is 90% sensitive and 96% specific for MAS [5]. Dengue being an infective condition, a modest rise in ferritin levels is expected in dengue fever as well. A South Indian study by Soundravally R et al., done on 48 patients with dengue fever shows that the mean ferritin levels at the time of admission was 1028.01±562.89 ng/mL. In this study hyperferritinemia (Ferritin >500 ng/mL) emerged a strong predictor of severe dengue and worsening thrombocytopenia [6]. However, there is not much evidence correlating ferritin levels and the platelet drop.

The use of intravenous steroids in severe dengue is a common clinical practice in many centres. However, there is no evidence for this usage and it is not known whether steroids have a role in this disease. Although intravenous steroids are broadly given in macrophage activation, their role with respect to dengue fever has not been studied. This study was thus done with the primary aim of assessing whether hyperferritinemia correlates with severity of thrombocytopenia. As secondary objective, we have compared the group of patients who were treated with steroids in addition to the standard treatment protocol against patients who were treated according to standard treatment protocols alone. The parameters that have been used to compare were the mean platelet drop and length of hospital stay of the patients.

MATERIALS AND METHODS

It was a retrospective observational study conducted at a Tertiary Care Hospital in Kochi, South India over a period of 18 months from January 2015 to July 2016. The study was commenced after obtaining clearance from both the Institutional Ethics Committee and the Scientific Review Committee.

The study population consisted of adults admitted to the Department of General Medicine with a diagnosis of dengue fever. Only patients who were seropositive for Dengue Fever (Dengue NS 1 antigen or Dengue IgM ELISA-tested after 7 days of onset of fever) were recruited for the study. Patients included in the study also satisfied five out of eight of the Haemophagocytic Lymphohistiocytosis Diagnostic Criteria, 2004 [7]. The included study patients were those who had fever [8], hepatomegaly or splenomegaly, bicytopenia (Total leucocyte count <4000/dL, Haemoglobin <19 gm/dL, Platelet count <1,00,000/dL), elevated ferritin >500 ng/mL and triglyceride level >150 mg/dL [8-10]. Patients who were on antiplatelets, prior steroid therapy or other immunosuppressive therapy, known autoimmune disorders were excluded from the study.

During the study period, 26 patients admitted with dengue fever were found to satisfy both the inclusion and exclusion criteria.

For all 26 patients, the following clinical and biochemical data were collected as per our study protocol:

1) History of the present febrile illness, patient demographics, presence of WHO warning signs for severe dengue, associated co morbidities and length of hospital stay.

2) Complete blood count at the time of admission and at 48 hours along with ferritin and triglyceride levels at admission. From this the change in platelet value from baseline and 48 hours was specifically calculated. Complete blood count was done on Impedance spectrometry and ferritin was estimated by chemiluminescence Immunoassay method.

3) Few of the patients had been treated with steroids at the discretion of the treating teams. Hence, those patients who received steroids at the discretion of the treating physician were taken as a sub-group and were analysed separately.

The following analysis was done on the patients who were included in the study:

1) Demographic analysis;

2) Correlation between serum ferritin levels with the mean platelet drop after 48 hours;

3) The subgroups were compared with each other with respect to clinical outcomes namely, mean platelet drop and length of hospital stay.

STATISTICAL ANALYSIS

Data were compiled in Excel sheets and were analysed using LOTUS IPSS 2.0 software. All numerical variables were expressed as mean and standard deviations. All categorical variables were expressed as percentage. Haematological and biochemical parameters of the patients at admission and after 48 hours were compared using paired t-test. Mann-Whitney U test was used to test for significance in sub group analysis of patients managed according to standard protocol versus those who have received steroids. Pearsons' coefficient was calculated to analyse if serum ferritin levels were proportional to the mean platelet drop. Unpaired t-test was used to compare the ferritin levels of the two sub groups to ensure that they were comparable.

RESULTS

Demographic Data

Of the 26 patients included in the study, 18 (69.2%) patients were male. The mean age of the patients studied was 45.77 years±16.286

years (Range: 21-75 years). The various comorbidities in this patient cohort are as follows [Table/Fig-1].

Comorbidities	Number (%)
Type 2 Diabetes Mellitus	5 (19.2)
Systemic Hypertension	9 (34.6)
Dyslipidemia	5 (19.2)
Atherosclerotic Diseases (Prior CAD/CVA)	1 (3.8)

[Table/Fig-1]: Comorbidities of the patients.

Key Biochemical Parameters

The mean and standard deviation of the important biochemical and haematological parameters are tabulated as follows. The standard deviation is more than the mean of most variables because of the considerable range over which the values were distributed [Table/Fig-2,3].

Parameters	At Admission		48 Hours Post Admission		p-value
	Mean	SD	Mean	SD	
Haemoglobin gm/dL	14.41	2.01	14.09	2.15	0.5818
White Cell Count (cells/dL)	4836.9	3457.9	4500.4	3079.3	0.7125
Neutrophils (cells/dL)	2793.8	876.9	2207.4	714.8	0.0109
Lymphocytes (cells/dL)	1315.1	654.6	1480.1	613.4	0.3528
Platelets (×10³ cells/dL)	78.12	63.70	51.69	56.75	0.1205

[Table/Fig-2]: Comparison of haematological parameters at admission and after 48 hours.
Statistical test used: Paired t-test

Parameters	Mean	SD
C Reactive protein (mg/dL)	19.87	31.32
Erythrocyte sedimentation rate (mm/hour)	9.91	11.33
Aspartate Transaminase (AST) (IU/mL)	232.83	243.29
Alanine Transaminase (ALT) (IU/mL)	245.95	548.38
Ferritin (ng/mL)	11409.02	13249.96
Triglyceride (mg/dL)	210.98	115.12

[Table/Fig-3]: Biochemical parameters.
*Obtained mean values of the parameters are smaller than the standard deviation because the values were distributed over a large range.

Relationship between Ferritin and Platelets

Pearsons' coefficient was calculated to assess the relation between ferritin levels and the mean platelet drop and it showed a negative correlation which was however not statistically significant (r=-0.243, p=0.231).

Subgroup analysis done between patients on steroids versus those managed conservatively without steroids: Of the 26 patients in our study 15 of the patients had been managed with steroids and 11 without steroids. The steroid used was hydrocortisone given intravenously at a dose of 300 mg in three divided doses. The mean ferritin level of the steroid group was 11109.72 (SD=13212.05) and the mean ferritin level of the group managed without steroids was 11817.17 (SD=13935.73). The p-value for the difference between the means as determined by an unpaired t-test was 0.89. This was not significant.

Comparison between the change in platelet count and length of stay in both groups [Table/Fig-4]. Mann-Whitney U test was used as a test of significance and it showed that there was no significant difference in either the length of stay (p=0.443) or mean platelet drop (p=0.087) between the two groups.

Groups	n	Change in platelet			Length of stay		
		Mean (×10³ Cells/dL)	SD	p-value	Mean (no. of days)	SD	p-value
Treated with steroids	15	36.8	12.35	0.087	7.67	2.87	0.443
Treated without steroids	11	35.7	14.10		8.09	2.77	

[Table/Fig-4]: Platelet drop and length of stay between the two subgroups.
Statistical test used: Mann-Whitney U test

DISCUSSION

Before embarking on a discussion of the results of our study it should be noted that there have been no previous studies that have looked at the characteristics of MAS triggered by dengue fever. Macrophage activation in dengue fever is an entity that has not been studied much. However given the increasing prevalence of dengue fever, it is an important entity. Serum ferritin level is the most important biomarker for this condition and our study primarily looks at whether this correlates with the degree of cytopenia [11]. Even though the mean ferritin level was more than 10,000 ng/mL (which is highly sensitive and specific for MAS it did not correlate with the change in platelet levels at 48 hours. Thus, a grossly high ferritin levels were not a predictor of the degree of platelet drop. This is clinically important as the treating physician should not be alarmed by the hyperferritinemia and should continue to follow standard protocol as advised by the WHO.

Of interesting note is the subgroup analysis between those treated with steroids and those treated without steroids. There was no statistical difference between the mean ferritin levels of both groups suggesting that the levels of cytokine activation due to macrophage activation is similar in both groups. It can be seen that there is a statistically significant larger drop in platelet counts in the group treated with steroids. Drastic fall in platelet counts is known to complicate the course of treatment by producing bleeding manifestations which is one of the criteria for severe dengue. While a Cochrane database systematic review which showed there is no evidence for steroids in dengue fever, more analysis should be done as to whether steroids have a negative impact on platelet counts [12]. Steroid use has not shown to affect the length of stay of the patient in hospital as well.

MAS secondary to viral infections is well described in literature. One of the earliest studies is by Risadall RJ et al., in 1979 and with low threshold of clinical suspicion this is being diagnosed with increasing frequency [13]. The most common and well known virus types to trigger this condition are Epstein barr virus, Herpes virus, HIV and cytomegalovirus. The treatment in these conditions is supportive and withdrawal of aggressive immunosuppression. Our study in patients with dengue fever who have associated MAS also conforms to this treatment principle.

LIMITATION

However, a number of limitations do exist in present study especially the lack of mortality and patients with severe dengue, retrospective collection of data, lack of histological evidence of macrophage activation and relatively small sample size.

CONCLUSION

From this study, we conclude that there was no statistical correlation between the mean platelet drop and the ferritin levels in patients with dengue fever. The present study did not show any beneficial effect of steroids in dengue fever.

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PARTICULARS OF CONTRIBUTORS:
1. Associate Professor, Department of Internal Medicine, Amrita Institute of Medical Sciences, Amrita University, Kochi, Kerala, India.
2. Junior Resident, Department of Internal Medicine, Amrita Institute of Medical Sciences, Amrita University, Kochi, Kerala, India.
3. Senior Resident, Department of Internal Medicine, Amrita Institute of Medical Sciences, Amrita University, Kochi, Kerala, India.
4. Professor and Head, Department of Internal Medicine, Amrita Institute of Medical Sciences, Amrita University, Kochi, Kerala, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Selva Saravanan Saminathan,
Junior Resident, Department of Internal Medicine, Amrita Institute of Medical Sciences, Amrita University,
Ponekkara-682041, Kochi, India.
E-mail: selvas22016@aims.amrita.edu

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