Original Article

Pathology Section

Pattern Analysis of Thrombocytopenia in Dengue versus Non-Dengue Patients

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ABSTRACT

Introduction: Dengue fever is one of the most prevalent and fastest spreading mosquito-borne arboviral infection occurring in tropical and sub-tropical regions around the world and leads to explosive outbreaks in urban areas influenced strongly by rainfall and temperature.

Aim: Evaluation of extent of thrombocytopenia in dengue positive patients.

To compare the pattern of fall and rise in platelet counts for five consecutive days in dengue positive cases and dengue negative controls.

Materials and Methods: The present study was a case control study undertaken in a tertiary care centre in Mangalore for three months. A total of 149 cases of thrombocytopenia were studied, out of which 85 were laboratory confirmed cases of dengue. Dengue cases with platelet value >1 lac/µL and < 1 lac/µL were noted. The remaining 64 cases of thrombocytopenia (platelet< 1 lac/µL) with dengue negative serology tests were used as controls. The pattern of fall and rise in platelet counts in five consecutive days in both dengue positive cases and dengue negative controls were studied.

Result: Among the 64 dengue positive thrombocytopenia cases, 34(40%) cases had mild thrombocytopenia (platelet count 51000/µL -1 lac/µL), 25 (29.41%) cases had moderate (20000/µL -50000/µL) thrombocytopenia and 5 cases(5.88%) had severe (< 20000/µL) thrombocytopenia. Among the dengue negative thrombocytopenia control group 39(60.93%) cases had mild thrombocytopenia, 16(25%) patients had moderate thrombocytopenia and 9 (14.06%) patients had severe thrombocytopenia. A graph is plotted comparing the median platelet values in five consecutive days in dengue positive thrombocytopenia cases and dengue negative thrombocytopenia controls. Z-test was used to compare the difference in the platelet values among case and control groups and to know the statistical significance between them.

Conclusion: The median platelet values on consecutive days in dengue positive cases shows a gradual increase in platelet values around the time of recovery i.e. 5th day, even without platelet transfusion. In dengue negative thrombocytopenia graph, the median values of platelet count on consecutive days is almost constant till 5th day without any marked rise.

INTRODUCTION

Dengue fever is one of the most prevalent and fastest spreading mosquito-borne arboviral infection occurring in tropical and sub-tropical regions around the world and leads to explosive outbreaks in urban areas influenced strongly by rainfall and temperature [1]. According to WHO, dengue is endemic in more than 100 countries. Dengue Viruses (DENVs) are the most important human arboviruses worldwide and are transmitted by mosquitoes of the genus Aedes in the form of four distinct serotypes (DENV-1, DENV-2, DENV-3 and DENV-4). Dengue virus belongs to the family Flaviviridae and predominantly transmitted by *Aedes aegypti* and few other members of Aedes species [2]. It is characterized by biphasic fever, headache, body pain, rash, lymphadenopathy and leucopenia [1]. In most cases, dengue fever is self-limited.

The four DENV can cause a varied spectrum of diseases, even though DENV infections may be asymptomatic. The spectrum includes the undifferentiated acute febrile illness, classical Dengue Fever (DF), to the life-threatening conditions Dengue Haemorrhagic Fever (DHF)/Dengue Shock Syndrome (DSS). The classical dengue fever is defined by the presence of acute febrile illness and ≥2 of the following symptoms: headache, retro-orbital pain, myalgia, arthralgia, rash [3].

Dengue haemorrhagic fever is defined as fever with haemorrhagic manifestations, thrombocytopenia (platelet count <100,000/µL) and haemoconcentration or other signs of plasma leakage; DSS is defined as DHF with either hypotension for age (systolic pressure <80 mm of Hg for those <5 years of age and < 90 mm of Hg for those >5 years of age) or narrow pulse pressure (<20 mm of Hg) in the

presence of clinical signs of shock (e.g., slow capillary filling, cold clammy skin) [4].

Thrombocytopenia is a major clinical manifestation associated with dengue patients. In majority of patients, thrombocytopenia is transient and asymptomatic but in significant number of cases, there is bleeding manifestations [5]. Spontaneous bleeding is noted in platelet count of <20,000/µL in majority of patients. Petichae/purpura is seen in platelet count in the range of 20,000-40,000/µL. This signifies the need to evaluate platelet count in Dengue Fever.

Not all patients with platelet<20000/µL will have bleeding. Studies have found that bleeding score did not correlate with platelet count [6]. Unnecessary platelet transfusion without following guidelines leads to wastage of resources. Although, there is a wide literature on platelet counts in dengue haemorrhagic fever and dengue shock syndrome, there is few published data regarding the follow up of platelets in uncomplicated dengue infections. The present study is unique as it includes only uncomplicated dengue fever cases, and highlights the pattern of fall and rise in platelet counts, in comparison with dengue negative thrombocytopenia controls. As the average duration of hospital stay in uncomplicated dengue fever patient was five days, follow up in the present study was for five days [7].

The current study was undertaken to evaluate the extent of thrombocytopenia in dengue positive patients and to compare the pattern of fall and rise in platelet counts for five consecutive days in dengue positive patients and dengue negative controls.

MATERIALS AND METHODS

The present retrospective study was undertaken in a tertiary care centre in Mangalore. This was a case control study for duration of three months from June 2016 to August 2016. Cases included all 85 patients who were tested positive for Dengue during this period. The platelet values at the time of admission (Day 1) were noted and categorized as 2 groups: A) Dengue positive with thrombocytopenia (64); B) Dengue positive without thrombocytopenia (21). Laboratory confirmation of dengue was done using Dengue DAY 1 Test, a rapid visual test based on solid phase immune-chromatography for the detection of Dengue NS1 Antigen and differential detection of IgM & IgG antibodies in Human Serum/Plasma. Platelet values were obtained using automated haematology analyser Sysmex XN-1000 based on impedance technology. Platelet values were evaluated daily for five consecutive days. Platelet values <100000/µl were also calculated manually on a peripheral smear. Whole blood was collected in EDTA vacutainer. Smears were made and stained using Leishman stain. The smear was viewed under oil immersion field in an ideal zone and number of platelets in 10 fields were counted. These 10 fields were selected on a monolayer zone of the smear, where RBCs were equidistantly spaced. The average number of platelets per field were

calculated and multiplied by 20000. This gives the platelet count. Controls were 64 cases of thrombocytopenia i.e., platelet <100000/µL who were tested negative for Dengue.

Exclusion Criteria:

1. Patients classified as dengue haemorrhagic fever and dengue shock syndrome;

2. Patients who were given blood transfusion;

3. Patients with haemorrhagic tendency.

The pattern of fall and rise in platelet counts in five consecutive days in both dengue positive and dengue negative cases were studied. The present study was time bound study for a duration of three months, included all dengue confirmed cases during the study period June 2016 to august 2016. Hence, the power was not used in the estimation of sample size. Patient details were noted from requisition forms. As it was a laboratory based study with no direct patient interaction and data was collected from records, patient consent was not necessary.

RESULTS

A total of 85 cases were found to be seropositive for dengue. Of the seropositive cases, 64 (75.29%) had thrombocytopenia (platelet count <1 lac/µL) while the remaining, 21(24.7%) had platelet counts >1 lac/µL. Majority of the dengue cases were noted in the age group of 21-30 years. Among the dengue cases, 55 (64.7%) were males and 30 (35.29%) were females.

A total of 64 samples having thrombocytopenia which were tested negative for dengue were used as controls.

Platelet	Male	Female	Total
<20000	2	3	5
20-50000	14	11	25
50-100000	25	9	34
>100000	14	7	21

[Table/Fig-1]: Dengue positive cases distributed according to platelet values.

Platelet values at the time of diagnosis of dengue positive cases is given in [Table/Fig-1].

Of the 64 dengue positive thrombocytopenia cases, 34(40%) cases had mild thrombocytopenia (platelet count 51000/µL-1 lac/ µL), 25 (29.41%) cases had moderate (20000/µL - 50000/µL) thrombocytopenia and five cases (5.88%) had severe (< 20000/µL) thrombocytopenia Platelet values of dengue negative controls given in [Table/Fig-2].

The median platelet values of dengue positive thrombocytopenia cases and dengue negative thrombocytopenia controls on five consecutive days is given in [Table/Fig-3].
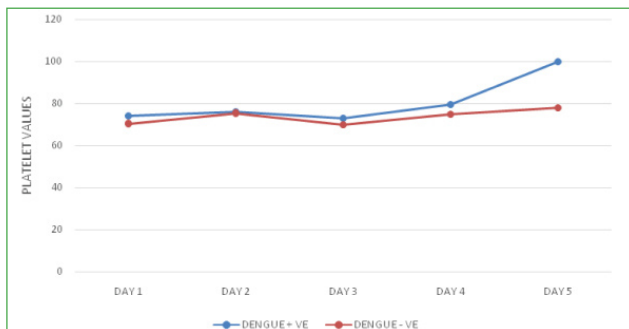
A graph is plotted comparing the median platelet values in five consecutive days in dengue positive Thrombocytopenia cases and dengue negative thrombocytopenia controls,

Platelet	Male	Female	Total
< 20000	4	5	9
20000-50000	12	4	16
50000-100000	26	13	39

[Table/Fig-2]: Dengue negative controls distributed according to platelet values.

Days	Median Platelet Values in Dengue Positive Thrombocytopenia	Median Platelet Values in Dengue Negative Thrombocytopenia
Day 1	74	70.5
Day 2	76	75.5
Day 3	73	70
Day 4	79.5	75
Day 5	100	78

[Table/Fig-3]: Median platelet values on five consecutive days in dengue positive thrombocytopenia cases and dengue negative thrombocytopenia controls.

[Table/Fig-4]: Graph comparing the median platelet values in five consecutive days in dengue positive Thrombocytopenia cases and dengue negative thrombocytopenia controls.

shown in [Table/Fig-4]. Days were plotted on X axis, and median platelet values plotted on Y axis.

Statistical analysis was done using Z-test and p-values were calculated. [Table/Fig-5] shows the difference in p-values in dengue positive thrombocytopenia cases and dengue negative thrombocytopenia controls on day 1 and day five. Statistically very high significant difference in the platelet values was observed between 1st and 5th day of dengue positive thrombocytopenia cases (p-value <0.001).

	p-value (day 1 and day 5)	Significance
Dengue positive thrombo-cytopenia cases (Day 1 and day 5)	<0.001	Very high significant difference
Dengue negative thrombo-cytopenia controls (Day 1 and day 5)	>0.05	Non-significant difference

[Table/Fig-5]: Table comparing the p-values on day 1 and day 5 in dengue positive thrombocytopenia cases and dengue negative thrombocytopenia controls.

Statistically non-significant difference (p-value >0.05) was observed in the platelet values between 1st and 5th day of dengue negative thrombocytopenia controls.

Common Age Group	Present study		Makroo RN[8]
	21-30		21-30
Percentage of Thrombocytopenia in Dengue Positive Cases	Present study		Khan DM[9]
	75.29%		71%
Thrombocytopenia		Present study	Chaudhary PB[10]
	Mild	40%	65.7%
	Moderate	29.41%	21%
	Severe	5.88%	13.1%

[Table/Fig-6]: Comparison of different parameters with other studies.

DISCUSSION

The common age group of affected patients, percentage of thrombocytopenia in dengue positive cases, and severity of thrombocytopenia in dengue cases of the present study with other studies is compared in [Table/Fig-6].

No platelet transfusion was given in any of these cases. Two cases were diagnosed as AML after performing bone marrow aspiration as the platelet values did not increase despite 15 days of supportive therapy. The cancer cells in haematological malignancies undergo accelerated apoptosis, releasing intracellular proteins. These proteins have similarities with dengue NS1 antigen and have been proposed as the reason for the false positive NS1 antigen positivity [11]. Although, an exact underlying reason for thrombocytopenia in dengue fever is not known but it has been suggested that dengue virus inhibits in vitro megakaryopoiesis and induces apoptotic cell death in a subpopulation of early megakaryocytic progenitors which may contribute to thrombocytopenia in dengue cases [12].

Among the dengue negative thrombocytopenia control group, 42 (67.29%) were males and 22 (24.84%) were females. A total of 39(60.93%) cases had mild thrombocytopenia, 16(25%) patients had moderate thrombocytopenia and 9(14.06%) patients had severe thrombocytopenia. None of these patients received platelet transfusion.

The median platelet values on consecutive days in dengue positive cases shows a gradual Increase in platelet values around the time of recovery i.e., 5th day, even without platelet transfusion. In dengue negative thrombocytopenia graph, the median values of platelet count on consecutive days is almost constant till 5th day without any marked rise.

In the study done by Jyothi P et al., the association of thrombocytopenia in dengue parameter positive cases was highly significant, when compared to thrombocytopenia in dengue parameter negative patients [13]. Kulkarni RD et al., showed that association of thrombocytopenia and dengue parameter was significantly higher compared to

thrombocytopenia in dengue negative cases [14]. In the study done by Gita N et al., on correlation of platelet counts and dengue infection, it was found that there was significant increase in platelet counts after complete treatment [15].

In a study done by Lye DC et al., showed that prophylactic platelet transfusion did not improve relevant outcome measures, such as clinical bleeding, platelet increment and platelet recovery [16].

In the study by Lum LC et al., on paediatric patients with dengue shock syndrome and platelet count of <30,000/mm^3, preventive transfusion did not reduce bleeding [17].

LIMITATION

1. The other parameters associated with bleeding in dengue patients like low WBC count, higher neutrophil proportion were not included in the present study

2. The PDW (Platelet Distribution Width) and MPV (Mean Platelet Volume) were not accessible in the study, as the automated analyser calculated platelets on impedance technology and not fluorescence based method.

CONCLUSION

Prophylactic platelet transfusion is given because of the fear of severe bleeding in patients with acute dengue and thrombocytopenia.

Platelet concentrates and other blood components have a fixed lifespan and are usually in limited supply [18]. Most of the dengue patients recover without platelet/blood transfusion within a week. Hence, unnecessary blood/platelet transfusion can be avoided in dengue patients.

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