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Original Research Article

Clinicohematological Review of Dengue Cases in Thrombocytopenia of Infants and Children

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Abstract

Background: Dengue fever is a major public health issue forming a burden of monsoon related illness. There is a need to study the clinical features and laboratory investigations to predict the severity of dengue fever for timely intervention and management to decrease the morbidity and mortality.

Methods: An observational prospective study was conducted in children of less than 12 years of age in hospitalised cases, to study the severity of clinical features and variations in laboratory finding in proved cases of dengue fever. All cases of thrombocytopenia were included in this study and proved cases of dengue fever which were positive for NS1 antigen (done by ELISA using Dengue NS1 Ag microlisa kit) or IgM antibodies to dengue (done by using DEN MAC ELISA kit) or both NS1Ag and IgM antibodies were compared with laboratory finding of other causes of thrombocytopenia.

Results: This study encountered 130 patients with thrombocytopenia during the period of 18 months. Of these 114 cases had infective cause, 12 cases had haematological cause and 4 cases of nutritional deficiency as etiology. Of the 114 cases of infective cause, 47 cases were proved dengue fever. Out of the 47 cases of dengue fever, 22 cases were positive for NS-1 antigen, 14 cases were positive for IgM antibody and 11 cases were positive for both antigen and antibody. Bleeding manifestations were found in 42.55% of cases. In severe thrombocytopenia cases more than three fourth cases had bleeding manifestations. Mortality for dengue was seen in 4.25% of cases. All the patients who expired belonged to Dengue Shock Syndrome.

Conclusion: A significant association was seen between bleeding manifestations and severity of thrombocytopenia. A statistically significant correlation of p value < 0.001 was seen with relation to platelet count of dengue and non dengue viral infections.

Keywords: Dengue Fever; NS-1 Antigen; Thrombocytopenia.

Introduction

Dengue fever is one of the most prevalent and fastest spreading mosquito-borne arboviral infection occurring in tropical and subtropical regions around the world and leads to explosive outbreaks in urban areas influenced strongly by rainfall and temperature. According to WHO, about two-fifths of the world's population (2.5 billion people) are currently at risk of dengue fever. Dengue fever is endemic in more than 100 countries [1]. Once considered an urban problem, it has now penetrated into rural areas also due to high population density and other factors. An estimated 50 million dengue infections occur annually and approximately 2.5 billion people live in dengue endemic

countries [2]. In India the annual incidence is estimated to be 7.5 to 32.5 million [3]. According to the WHO, the case fatality rate for dengue is roughly 5% [5].

Dengue fever presents as common viral fever which causes dangerous complications. Bleeding in Dengue is one of the dreaded complications. Broadly bleeding disorders are the generalised defect in homeostasis due to abnormalities of blood vessels, platelets or coagulation factors. Bleeding manifestations are highly variable and do not always correlate with the laboratory abnormalities in the coagulation profile [4]. The disease progresses in three phases- febrile phase, critical phase and recovery phase.

Criteria for Dengue

Probable Dengue: Live or travel endemic area, fever and two of following criteria - nausea, vomiting, rash, aches and pains, tourniquet test positive, leukopenia.

Laboratory Confirmed Dengue: Warning Signs: Abdominal pain or tenderness, persistent vomiting, fluid accumulation, mucosal bleeding, lethargy, liver enlargement, increase in hematocrit with decrease in platelet count.

Severe Dengue: Severe plasma leakage leading to dengue shock syndrome (DSS), fluid accumulation with respiratory distress. Liver: AST/ALT >1000 U/L, impaired consciousness [5]. Dengue re-infection is observed to be more severe in children due to immunological phenomenon [3].

Thrombocytopenia may be associated with alteration in megakaryopoiesis by the infection of human hematopoietic cells and impaired progenitor cell growth, resulting in platelet dysfunction [platelet activation and aggregation], increased destruction or consumption[peripheral sequestration and consumption]. Hemorrhage may be a consequence of the thrombocytopenia and associated platelet dysfunction or disseminated intravascular coagulation [6].

The objective of this study was to assess the clinical and laboratory profile of the dengue infection in children less than 12 years of age. To determine the relationship between platelet count, the occurrence and the severity of bleeding. To evaluate the outcome of Dengue fever in these children in the largest tertiary care hospital of North Karnataka.

Materials and Methods

The present study was a prospective observational study conducted from 1st December 2014 to 31st May 2016, coming to in-patient, Department of KIMS, Hubli, Karnataka.

Inclusion Citeria

1. All patients in the age group of 28 days to 12 years.

2. All patients with thrombocytopenia of platelet count < 1.5 lakh/cumm

3. The above group of patients who are tested positive for IgM antibodies for dengue virus by ELISA and NS1 antigen in serum also by ELISA.

Exclusion Criteria

1. Patients with abnormal coagulation.

In the patients who fulfilled the criteria detailed demographic profile, history, signs and symptoms were recorded from the patient case files. The type of bleed such as petechiae, purpura, ecchymosis (as cutaneous bleeds), haemetemesis, melena, epistaxis (as mucosal bleeds) and any major bleeds were studied in detail. These patients were followed up with platelet counts till their discharge. All these patients were subjected to laboratory test such as complete hemogram using 3-part differential count (Sysmex XP-100), detailed peripheral smear examination (after Leishman's Stain) including for hemoparasites. For all cases of thrombocytopenia Dengue NS1 antigen done by ELISA using dengue NS1 Ag microlisa kit (J. Mitra and Co. Pvt. Ltd. New Delhi) and IgM antibody done by IgM capture ELISA using DEN MAC ELISA kit prepared and supplied by NIV, Pune, India. Procedure was followed as per manufacturer's instructions and results were interpreted accordingly. Widal test by slide method, liver function tests, blood urea, serum creatinine and serum electrolytes were done in all the cases of thrombocytopenia. In suspected cases Weil- Felix test and Rickettsial IgM antibodies were done.

The study was approved by the Ethical committee, Karnataka Institute of Medical Sciences, Hubli.

Results

In this study, a total of 130 patients with platelet count of less than 1.5 Lakhs/cumm were included.

In our study, 114 patients had infective cause as the commonest etiology for thrombocytopenia, 12 patients had hematological cause while 4 cases had nutritional deficiency as the etiology for thrombocytopenia.

Among haematological causes 4 cases were of immune thrombocytopenia, 3 cases of thalassemia major, 2 cases of aplastic anemia and one case each of acute leukemia and myelofibrosis were encountered. There was one case which was undiagnosed due to bone marrow aspiration failure and later patient went against medical advice.

Among the 114 subjects who had infective etiology, 87 turned out to be viral etiology, 15 cases were of Sepsis, 8

were diagnosed to have Rickettsial Fever, 2 cases were diagnosed as Malaria while 1 each presented with Typhoid Fever and disseminated Tuberculosis.

Among the 87 cases with viral etiology for thrombocytopenia, 47 (54%) had Dengue fever, 33 (38%) had Viral Hemorrhagic Fever (Non Dengue) which was diagnosed by clinical findings and lymphocytosis, while 7 (8%) were infected with HIV, as shown in Table 1.

Out of 47 children diagnosed with Dengue fever, 22 (47%) were boys and 25 (53%) were girls. Majority (49%) of

patients belonged to an age group of 5-10 years of age. Maximum cases were seen during the months of September to November which corresponds to the monsoon period accounting to 36%.

Among the 47 subjects diagnosed with Dengue illness, 12 had Dengue with No Warning Signs, 27 had Dengue with Warning Signs and 8 presented with Dengue Shock Syndrome (DSS), as illustrated in Figure 1.

Out of 47 patients presenting with Dengue, 22 (47%) patients were positive for NS1 antigen, 14 (30%) patients

Table 1: Viral etiology of Thrombocytopenia

Viral Infections	No. of Cases	Percentage (%)
Dengue fever	47	54
VHF (Non Dengue)	33	38
HIV	7	8
Total	87	100

Table 2: Distribution of clinical manifestations of Dengue fever

Symptoms	No. of Patients	Percentage (%)
Fever	47	100
Nausea/Vomiting	27	57.44
Pain abdomen	21	44.6
Headache	20	42.55
Chills and rigor	9	19.14
Myalgia	17	36.17
Arthralgia	8	17.02
Rashes	11	23.40
Edema	7	14.89
Convulsions	4	8.51
Retro-orbital pain	8	17.02
Diarrhea	4	8.51
Hepatomegaly	21	51.06
Epistaxis	5	10.63
Gum bleeding	1	2.12
Petechiae	12	25.53
Melena	6	12.76
Conjunctival hemorrhage	1	2.12

Table 3: Distribution of laboratory investigations in Dengue fever

Lab Test	No. of Patients	Percentage (%)
Hemoglobin (≤10)	19	40.42
Hematocrit (≥40)	10	21.27
Leukocytosis	9	19.14
Leucopenia	6	12.7
Platelet (≤20000)	13	27.65
Deranged LFT	4	8.51
Deranged RFT	5	10.63

Table 4: Mean hemoglobin and mean packed cell volume in different cases of thrombocytopenia

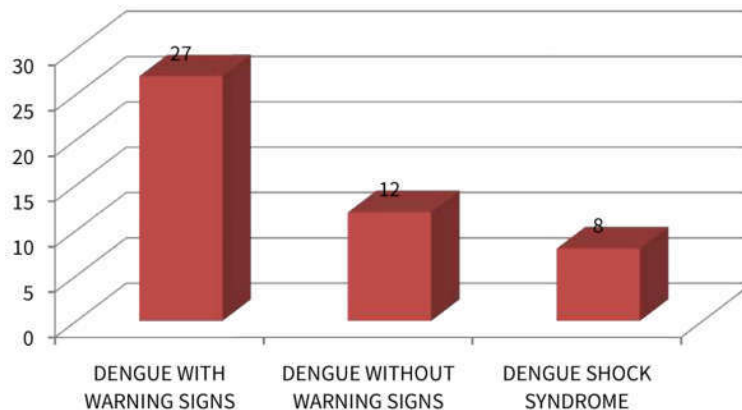| Etiology	No. of cases (n=130)	Mean Hb (g/dl)	Mean PCV (%)
Dengue	47	10.91	35.34
Non-dengue viral	40	8.93	29.21
Other infections	27	9.02	30.4
Hematological	12	6.35	21.55
Others	4	6.57	22.22

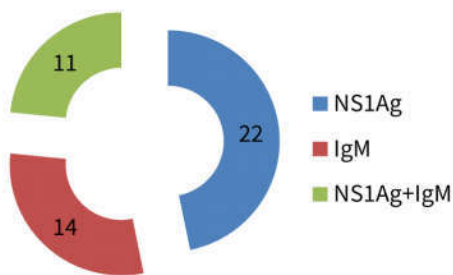**Fig. 1:** Various presentations of Dengue Fever

Fig. 2: Pattern of seropositivity in Dengue Fever

were seropositive for IgM antibody and 11 (23%) patients were positive for both the antigen and the antibody as illustrated in Figure 2.

The commonest presenting clinical symptoms were fever (in all patients, 100%), followed by nausea/vomiting, pain abdomen, headache and myalgia. The other common symptoms included rashes, chills, arthralgia, retro-orbital pain, oedema, diarrhoea and convulsions. Hepatomegaly was seen in 51% of cases.

Bleeding manifestations were found in 20 (42.55%) cases. Overall, melena was present in 6 patients, epistaxis in 5 patients, conjunctival hemorrhage in 1 patient, bleeding gums in 1 patient and petechiae in 12 patients. Five patients had both cutaneous and mucosal bleeds. The most common bleeding manifestation was petechiae, followed by melena, as illustrated in Table 2.

Out of 47 cases reviewed, 19 patients were anemic. Leukocytosis was found more than leukopenia. Mean hemoglobin level was 10.91 g/dl in Dengue fever, mean packed cell volume was 35.34 % and mean platelet count was 46021cells/cumm in dengue fever. Other laboratory findings are illustrated in Table 3.

Out of 47 cases of Dengue, 28% of cases presented with a platelet count of less than 20,000 cells/cumm, 36% of cases presented within the platelet count range of 20,000-50,000 cells/cumm, 28% of cases within the platelet count range of 50,000-1 lakh cells/cumm and 9% of cases had platelet count more than 1 lakh/cumm.

Comparison of mean hemoglobin and mean PCV between dengue and other causes of thrombocytopenia is shown in Table 4.

Comparison of platelets counts between dengue and other causes of thrombocytopenia is shown in Table 5.

The difference in mean platelet count of dengue with non-dengue viral infections was found to be statistically significant as shown in Table 6.

Table 7 illustrates a significant difference found between bleeding manifestations and severity of thrombocytopenia. No bleeding manifestations were seen in all four cases of thrombocytopenia with platelet count of > 1 lakh/Cumm.

In present study, 4 patients (8.5%) presented with altered sensorium, out of which 1 patient had persistent low Glasgow Coma Score and seizures in spite of correction of

Table 5: Mean platelet counts in the spectrum of thrombocytopenia

	n	Mean	SD	Median	Minimum	Maximum
Dengue	47	46021	34258	32000	2000	130000
Non dengue viral	40	75275	44978	72500	14000	270000
Other infections	27	65518	31940	60000	20000	138000
Hematological	12	33166	34212	22500	3000	100000
Others	4	57000	30232	62000	16000	88000

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Table 6: Statistical correlation of platelet count between dengue and other causes of thrombocytopenia

Statistical correlation of platelet count in different diagnostic groups	P value
Dengue v/s non-dengue viral	<0.001
Dengue v/s other infections	0.006
Dengue v/s Hematalogical	0.141
Dengue v/s others	0.344

Table 7: Correlation of bleeding manifestations with Thrombocytopenia

Platelet Count	<20000 (n=13) (Severe)	20000-50000 (n=17)(Moderate)	50000-100000 (n=13)(Mild)
Bleeding manifestations	10 (77%)	7 (41%)	3 (23%)
Without bleeding manifestations	3 (23%)	10 (59%)	10 (77%)

shock. Dengue encephalopathy was suspected in view of normal metabolic profile and CSF examination. Out of 47 cases, 4 patients showed mild elevation of liver enzymes and in 5 patients, blood urea levels were mildly elevated. Mortality for Dengue in the present study was seen in two (4.25%) cases both of these patients who expired had DSS with Adult Respiratory Distress Syndrome.

Discussion

Dengue is an important emerging disease of the tropical and sub-tropical regions today. The first case of dengue in India was reported during 1956 from Vellore [3]. In the present study maximum number of patients were admitted in the rainy season (September to November) that is related to favourable conditions for growth of vector Aedes aegypti. Transmission of dengue increases during monsoon. The correlation between occurrence of dengue and monsoon is clearly evident in this study and study done by Mendez A & Gonzalez G [7]. In this study, maximum number of patients belonged to the age group of 5-10 years with similar findings seen in a study by Samantha Nadia Hammond et al [8] and Nagaram et al [2]. This could be due to proneness of this age group to mosquito bites because of their outdoor activities.

This study showed a slightly more female predominance, which is similar to the data reported by Emily Bomasang et al [9], G. Garcia et al [10] and Anabella Salise Oncog et al [11], in which dengue fever occurred in more females than in males. Again, this finding is contrary to that found in study done by Gurdeep S Dhuria et al [12], in which the proportion of males and females was 65% and 35% respectively.

In the present study, 22 (47%) cases were positive for only NS1 antigen followed by 14 (30%) cases were only IgM positive and 11 (23%) cases were positive for both antigen and antibody. This is in correlation with study done by Subhankar Mishra et al [3] in which 47.42% patients showed positivity for NS1 antigen, 32.98% patients were

seropositive for IgM antibody and 5.14% patients were positive for both the antigen and the antibody. Non structural protein 1 (NS 1) is a highly conserved glycoprotein for all the serotypes. It is detectable in blood from first day after the onset of fever upto day 9 and is also detectable in the presence of IgM antibodies [13]. IgM antibodies are detectable in 50 percent of patients by three to five days after onset of illness, increasing to 80 percent by day-5 and 99 percent by day-10 [5]. Anti-dengue IgG antibodies are detectable from 2 weeks onwards can be present for several months and probably even for life. In primary infection there is high molar fraction of anti dengue IgM antibodies and low molar fraction of anti dengue IgG antibodies. Individuals with immunity due to previous flavivirus infection or immunisation mount to secondary antibody response. In secondary flavivirus infection there is high levels of anti dengue IgG antibodies before or simultaneously with IgM response [5]. Presence of IgG antibodies help to know the disease burden in geographic area [14]. False positive reactions are seen with other flavivirus infections and in rheumatoid fever [14].

According to the present study, fever was the most common presentation (in 100%), followed by vomiting (in 57.44%), pain abdomen (in 44.6%), headache (in 42.55%) and myalgia (in 36.17%). Our findings were similar to the studies done by Jhamb R et al [15], Mendez et al [7] and Kulkarni et al [16] though with variable percentages.

In this study various types of bleeding presentations were seen. Bleeding is one of the dreaded complications. Clinical manifestations of bleed are highly variable from simple skin bleeds, gastrointestinal bleeds and fatal intracranial bleeds. Bleeding manifestations were observed in 42.55% of cases which is similar to study done by M Kulkarni et al [16].

Bleeding manifestations was found to be significantly higher among Dengue shock syndrome patients (seen in 6 out of 8 cases). Overall, melena was present in 6 patients, epistaxis in 5 patients, conjunctival haemorrhage in 1

patient, bleeding gums in 1 patient and petechiae in 12 patients. Hence, the most common bleeding manifestation was petechiae, followed by melena. These findings are similar to the studies done by Subhankar Mishra et al [3] and Gurdeep S Dhooria et al [12].

Children with bleeding tendencies were not necessarily those having low platelet count, indicating a combination of causes like thrombocytopenia, coagulopathy and vasculopathy explaining the bleeding manifestations of the illness. In present study, mean hemoglobin was 10.91 g/dl and mean platelet count was 46,021cells/cumm whereas in a study by Hema Mittal et al [17] these values were 11.5 g/dl and 38,800cells/cumm respectively.

In our study the mean hematocrit at presentation was 35.5% similar to study done by Gurdeep S. Dhooria et al [12]. Only 21.27% cases had hematocrit more than 40% and since preillness hematocrit was not known it was difficult to document hemoconcentration. These observations suggest that hemoconcentration may not be a good indicator for diagnosis and monitoring of fluid if pre-illness hematocrit is not known, particularly because there is a high prevalence of anemia in the population.

Bleeding occured significantly more often in patients with severe thrombocytopenia, most often in patients with platelet count less than 20,000 cells/cumm which is similar to the finding of Shivbalan et al [18] and R.N.Makroo et al [4].

In our study, leucocytosis was seen in 19.14% and leucopenia in 12.76%. Similar findings were seen in the study done by Anju Aggarwal et al [19]. This contrasted with a study done by Shah N et al [20] in which most of the patients presented with Leukopenia (53.33%).

Among all Dengue patients with thrombocytopenia, 43 (91.48%) patients had platelet count < 100000 cells/cumm but Rashmi K.S et al [21] reported 72.77% of patients had platelet count <100000 cells/cumm. Among these 47 children with Dengue fever, 28% of cases presented with a platelet count of less than 20,000cells/cumm, 36% of cases presented within the platelet count range of 20,000-50,000 cells/cumm, and 28% of cases within the platelet count range of 50,000-1 lakh cells/cumm. This is in contrast with a study done by Dost Mohammed Khan et al [1] where out of 76 patients with thrombocytopenia (platelet count <1 lakh), 38 (50.0%) cases had mild thrombocytopenia (platelet count 51,000 – 1 lakh), 28(36.8%) cases had moderate thrombocytopenia (platelet count 20,000 – 50,000) and remaining 10(13.2%) cases had severe thrombocytopenia (platelet count <20,000). So our study reflected that more patients were encountered with higher grade thrombocytopenia.

Mortality in the present study was 4.25 %. All patients who expired belonged to DSS. In the study by Anju Aggarwal et al [19] overall mortality seen was 6%, compared to 8.8% in Shah N et al [20]. This could be due to delay in recognition or delay in seeking medical attention.

Conclusion

Dengue is one of the major causes of undifferentiated fever. It presents as a highly unspecific illness and is hardly recognized as a clinical entity by primary health care physicians. Appropriate investigations, strict monitoring and prompt supportive management can reduce mortality in dengue. This study supports further studies on applying intervention measures to improve the diagnostic accuracy and precision at the primary healthcare level. Clinicians in endemic areas thus should be aware of the extensive clinical profile of the disease for appropriate management to improve the outcome of this potentially morbid and occasionally fatal disease in pediatric population. Thus, for effective management, recognition of the epidemic and taking extensive steps for prevention of transmission by eliminating the mosquito vector is essential. Health education would also play a major role in preventing the disease.

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