Safety and Efficacy of a Single Dose of Anti-D (WinRho®) in Severe Thrombocytopenia Secondary to Dengue Virus Infection

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Abstract

Objective: To evaluate the efficacy of a single intravenous (IV) dose of anti-D in severe thrombocytopenia (<20,000) due to dengue virus (DEV) infection. **Materials and Methods:** An open label, investigator-initiated, randomized interventional study was conducted that included thirty dengue patients (all positive for IgM enzyme-linked immunosorbent assay) with severe thrombocytopenia (<20,000/mm³). Patients were randomized to receive anti-D (50 μg/kg single IV dose) plus supportive therapy or supportive therapy alone. **Results:** The rate of rise in platelet count was significantly high in the intervention group at 24, 36, and 48 h. At the end of 48 h, 60% patients in the intervention group achieved a platelet count of ≥50,000/mm³ as compared to 6.7% in the control group ($P = 0.0019$). The requirement of the platelet concentrate infusion in the control group was significantly higher, i.e. 342 ml (±193) as compared to the intervention group requiring only 187 ml (±79). The intervention group showed a significant improvement in bleeding manifestations in all the patients by 24 h in Grade 2 bleed ($P = 0.032$) and by 48 h in Grade 1 bleed ($P = 0.014$). **Conclusions:** Severe thrombocytopenia (≤20,000/mm³) secondary to DEV infection was rapidly and safely reversed by administration of a single dose of 50 μg/kg (250 IU/kg) anti-D IV.

Keywords: Anti-D, dengue, thrombocytopenia

INTRODUCTION

Dengue is a self-limited viral illness caused by an arbovirus, the dengue virus (DEV), a member of the *Flavivirus* family. It has four serotypes, i.e., DEV 1–4 and is transmitted by the bite of the Aedes mosquito. Infection results in a spectrum of clinical manifestations ranging from asymptomatic infection, dengue fever (DF) occurring in 50%–90% of cases, to dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) with the latter two being life threatening. Early recognition, adequate bed rest, and intravenous (IV) fluid therapy in DHF and DSS decrease fatality rates to 1% or less.[1] Capillary leak and hemorrhagic diathesis/thrombocytopenia are the clinical hallmarks that differentiate DHF from DF. Factors contributing to the hemorrhagic diathesis/thrombocytopenia are hemostatic disequilibrium, increased capillary fragility, thrombocytopenia, abnormal platelet function, and consumptive coagulopathy.[2,3] Thrombocytopenia is a predominant feature of dengue infection. While the virus-induced bone-marrow suppression causes reduced platelet synthesis, immune-mediated platelet destruction also contributes to thrombocytopenia.[4] Saito *et al.* showed an inverse correlation between platelet-associated IgG and IgM antibody levels with the thrombocytopenia, accounting for immune-mediated platelet destruction.[2] Dengue virions or virus-antibody immune complexes adhere to the thrombocyte surface, with subsequent complement activation leading to platelet destruction. Lei *et al.* showed IgM but not IgG anti-NS1Ag antibodies to cross-react with platelets and cause immune-mediated platelet destruction.[5,6] There is no evidence till date to support prophylactic platelet transfusions even in profound thrombocytopenia without bleeding.[7] Adjunctive therapies including high-dose dexamethasone and IV immunoglobulin (IVIG) have been evaluated in DHF but did not prove to be beneficial.[8,9] Anti-D immune globulin has been studied recently in Rh+ DHF patients to improve thrombocytopenia based on its role in blocking Fcγ receptor and reducing immune destruction as already demonstrated in immune thrombocytopenic purpura (ITP) patients.[10]

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Anti-D immune globulin is the IgG fraction of plasma prepared from D-positive-red-cell-immunized individuals and likely rich in polyclonal anti-D specificities. It is used to prevent alloimmunization in Rh − mothers giving birth to Rh+ infants.[11] de Castro *et al.* in their placebo-controlled trial reported ≥20,000/mm³ increase in platelets with anti-D therapy in DHF patients.[12] Kharya *et al.* similarly reported a significant improvement in 5 DHF children with refractory thrombocytopenia.[13] The earlier study was not randomized; thrombocytopenia was not severe in all their patients. The later was only a case series and did not have any control group. In the current study, we evaluate the efficacy of anti-D in DHF with severe thrombocytopenia (≤20,000/mm³).

MATERIALS AND METHODS

One hundred and fifty patients with fever and thrombocytopenia with or without bleeding manifestations who attended the emergency department were screened, and 30 Rh+ patients with serology-proven dengue positivity, severe thrombocytopenia (platelet count ≤20,000/mm³), and willing to consent were included in the study. Institutional Ethics Committee clearance was obtained, and informed consent was obtained from all the study participants. Diagnosis of dengue at screening was made with NS1Ag IgM and IgG RAPID STRIP TEST and confirmed subsequently with enzyme-linked immunosorbent assay (ELISA) for IgM and IgG. Patients with signs of capillary leak and platelet count ≤20,000/mm³ were classified as DHF. Thrombocytopenia ≤20,000/mm³ was considered severe. DHF patients with platelet count ≥20,000/mm³, Rh−, and pregnant females were excluded from the study.

Study design

It was an open label, randomized interventional study. Thirty patients were enrolled into the study and randomized into two groups 15 participants each based on computer-generated codes. One group (intervention group) received injection anti-D, 50 µg/kg (250 IU/kg) IV along with the standard supportive care and platelet support, whereas the other (control group) received standard care and platelet support only. The baseline parameters of all the patients in the study are shown in Table 1. Patients were followed up at 12, 24, 36, and 48 h and at discharge for comparing the level of platelet rise, bleeding, volume of the platelet concentrate transfused, and the duration of hospital stay. Severity of bleeding was classified using the WHO bleeding scale [Table 2].

Study drug

Anti-D (Rh0-D) immune globulin (WinRho®) is a sterile, freeze-dried gamma globulin (IgG) fraction of human plasma containing antibodies to Rh0 (D). It is being manufactured by Cangene Corporation (Winnipeg, Canada) using an anion exchange column chromatographic method. The drug is licensed for the treatment of ITP in many countries including the Philippines, Canada, the United States of America, and in India too.

Table 1: Demographic, clinical, and paraclinical baseline characteristics of the population

Categories	Baseline parameters n (%)/SD		P
	Intervention group (n=15)	Control group (n=15)	
Age (years)			
<20	2 (13.3)	2 (13.3)	1.000
20-50	11 (73.3)	11 (73.3)	1.000
<50	2 (13.3)	2 (13.3)	1.000
Sex (%)			
Male/female	15/0 (100/0)	13/2 (93.3/6.7)	0.143
Blood group			
A+	2 (13.3)	3 (20)	0.624
B+	7 (46.7)	6 (40)	0.713
O+	4 (26.7)	4 (26.7)	1.000
AB+	2 (13.3)	2 (13.3)	1.000
Duration of symptom			
<1 week	15 (100)	14 (93.33)	0.309
>1 week	0	1 (6.67)	0.309
Number of days	4.2 (1.14)	5.53 (1.92)	0.029
Previous history of dengue	No	No	
Prior history of PC transfusion	No	No	
Presenting complaint			
Fever	15 (100)	15 (100)	1.000
Pain abdomen	10 (66.67)	10 (66.67)	1.000
Petechial rash	5 (33.3)	6 (40)	0.705
Purpura/ecchymosis	0	2 (13.3)	0.143
Gum bleed	2 (13.3)	4 (26.7)	0.361
UGI bleed	2 (13.3)	3 (20)	1.000
LGI bleed	1 (6.7)	1 (6.7)	1.000
I/C bleed	-	-	-
Epistaxis	1 (6.7)	1 (6.7)	1.000
Bleeding grades			
Grade 0	5 (33.3)	2 (13.3)	0.195
Grade 1	5 (33.3)	6 (40)	0.705
Grade 2	5 (33.3)	7 (46.7)	0.456
Hematological parameters			
Platelets/mm³	12,267 (3614)	14,333 (3579)	0.127
Hb (g/dl)	14.32 (1.54)	13.93 (2.32)	0.596

SD: Standard deviation; UGI: Upper gastrointestinal; LGI: Lower gastrointestinal

Statistical analysis

The two groups were compared with regard to their response in the form of rise in platelet count. Primary outcome was rise in platelet count ≥50,000/mm³ above the baseline after a period of 48 h of administering anti-D injection in the intervention group. Other outcomes measured were the mean volume of platelet concentrate transfused, improvement in bleeding grades, and difference in the duration of hospital stay. Continuous data were expressed as mean (standard deviation) and/or median (range). Categorical data were expressed as *n* (%). Wilcoxon sum rank test was used for comparison. Chi-square

test or Fisher's exact test was used for categorical data. *P* <0.05 was considered statistically significant.

RESULTS

Patients

This study was conducted from July 2010 to December 2010. A total of thirty dengue (Ns1Ag positive using Rapid strip test) cases were included after screening 150 cases of fever and thrombocytopenia with or without bleeding manifestations for inclusion and exclusion criteria. Diagnosis of all the study participants was confirmed by ELISA. The median age of the patients was 35 years; 93% were men and 7% were women; mean platelet count at baseline was 13,300/mm^3. The mean duration of illness before admission was 4.8 days. Since our institute is a tertiary level facility, all the patients were referred from other hospitals, in view of the development of danger signs and severe thrombocytopenia, but none had received platelet transfusion before the enrollment into the study, and there was no history or documentation of any prior episode of dengue infection.

Efficacy

At the end of 48 h, there were 9 (60%) patients from the intervention arm who had achieved platelet counts of ≥50,000/mm^3 as compared to 1 (6.7%) patient from the control arm (*P* = 0.0019) [Table 3 and Figure 1]. The baseline mean platelet count of the intervention group and control group was 12,266/mm^3 and 14,333/mm^3, respectively. The rise in platelets was more rapid in the intervention group and was significant at 24, 36, and 48 h (*P* = 0.0001, <0.0001, and <0.0001, respectively). However, the difference was not

statistically significant at the time of discharge; average was 6 days postadmission (*P* = 0.21).

The mean volume of platelet transfused to intervention and control group was 187 ml and 342 ml, respectively (*P* = 0.010). The intervention group showed a significant improvement in bleeding manifestations in all the patients, by 24 h in Grade 2 bleed (*P* = 0.032) and by 48 h in Grade 1 bleed (*P* = 0.014) as shown in Figure 2. The mean duration of hospital stay did not show any statistically significant difference between the two groups; 5.7 and 5.8 days for the intervention and control group, respectively (*P* = 0.896).

Adverse effects

Mean Hb of the study population was 14.1 (1.95) g/dl, 14.3 in the intervention group, and 13.9 in the control group. After 48 h of anti-D, the mean Hb of the intervention group dropped to 13.7 which was insignificant (*P* = 0.253). Injection site pain and tenderness were the only adverse drug events reported in one patient. No mortality was noted in the study population.

DISCUSSION

DHF and DSS are a life-threatening complication to DEV infection. Adequate bed rest, IV fluid replacement, antipyretics, and analgesics form the main stem of therapy.[14] Thrombocytopenia is the key parameter determining the severity and the prognosis. Minimizing immune platelet destruction and preventing associated complications are additional goals in treating DHF/DSS. Efficacy of empirical and therapeutic platelet transfusion is not proven in DHF/DSS patients with severe thrombocytopenia without bleeding manifestations.[7]

Over the years, there is a tendency to use minimal platelet/blood products to reduce the risk of alloimmunization. Since the risk of blood borne viruses being transmitted has declined, this has forced us to lower the threshold for platelets transfusion in thrombocytopenic patients. The current recommendations are not to transfuse platelets if the platelet count is above

Table 2: WHO bleeding classification

WHO bleeding scale	
Grade 0	No bleeding
Grade 1	Petechial bleed
Grade 2	Mild blood loss (clinically significant)
Grade 3	Gross blood loss requires transfusion (severe)
Grade 4	Debilitating blood loss, retinal/cerebral bleed (associated with fatality)

Table 3: Improvement in platelet count at 12 hourly interval

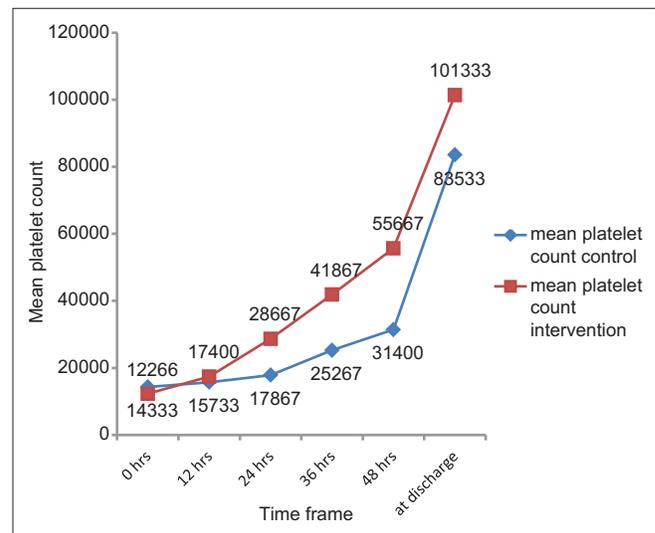| Platelet count rise			
Time duration (h)	Treatment groups Platelet count (/mm^3)		
	Platelet Mean (SD) (*n*=15)	Platelet + Anti-D Mean (SD) (*n*=15)	*P*
0	14,333 (3579)	12,266 (3614)	0.127
12	15,733 (5457)	17,400 (4896)	0.386
24	17,866 (6706)	28,666 (8925)	0.001
36	25,266 (9960)	41,866 (10,315)	≤0.001
48	31,400 (11,343)	55,666 (12,697)	≤0.001
At discharge	83,533 (42,318)	101,333 (33,167)	0.210

SD: Standard deviation

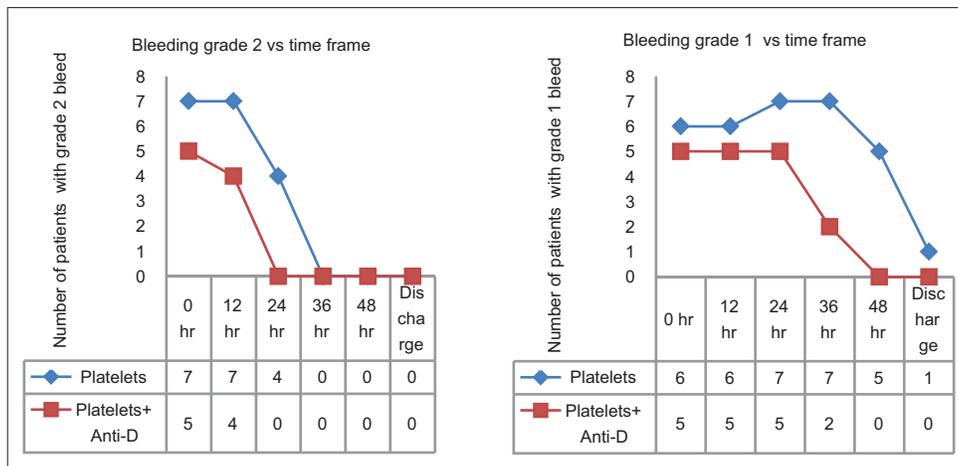**Figure 1:** Rise in platelet count for both study arms.

Figure 2: Improvement in bleeding Grade 2 and Grade 1.

10,000/mm^3. However, risk factors for bleeding at higher platelet counts are disseminated intravascular coagulation with contributory clotting factor deficiencies, structural lesions with loss of vascular integrity, and refractoriness to platelet transfusions. Since DHF/DSS are associated with compromised endothelial integrity resulting in capillary leak, the threshold for supportive therapy in severely ill patients should be slightly lower.

Anti-D immune globulin is studied in DHF/DSS to improve platelet counts because it can effectively block the IgFcγ receptor, thus preventing opsonization of platelets and reducing their immune destruction. Anti-D is the IgG fraction of plasma prepared from D-positive red cell-immunized individuals, likely rich in polyclonal anti-D specificities and originally used in preventing alloimmunization of Rh− woman delivering Rh+ infants.[11] Moreover, anti-D is particularly interesting drug because of its relatively low cost when compared with immune globulin fractions therapies such as IVIG. The limited availability of IVIG, large dose, and the risk of viral infections makes anti-D a better alternative.

The previous studies have shown benefits of anti-D administration in patients with proven DEV infection and thrombocytopenia. Kharya *et al*. described mean platelet count rise of 37,800/mm^3, 48 h after anti-D administration in five DHF patients with refractory platelet counts ≤10,000/mm^3.[13] However, this was only a retrospective case series involving five children; did not have any control group and anti-D was given only after thrombocytopenia was refractory to platelet transfusions. The mean time for an increase in platelets ≥20,000/mm^3 in our study was 43 h after anti-D.

de Castro *et al*. in their randomized controlled trial involving 47 participants with DHF and thrombocytopenia ≤50,000/mm^3 observed that 75% of the anti-D group had an elevation in platelet counts against 58% in the placebo group though they did not mention the statistical significance of this rise.[12] The authors had not described the mean time interval taken for platelet count elevation. Since the chances of bleeding

at a platelet count above 20,000/mm^3 are minimal, there is no rationale to administer platelets or drugs at a platelet count of >20,000/mm^3.

Our study results have documented that a single dose can be safely administered and would result in a rapid rise in platelet count above 50,000/mm^3 without any serious adverse effect.

The strength of this study is that it is a randomized control trial and all participants had severe thrombocytopenia (platelet count ≤20,000/mm^3). In the current study, platelets were increased by 29,000/mm^3 after a mean time of 36 h. We found that the rise in platelet count was statistically significant. This study highlights the efficacy of anti-D in reducing the severity of all grades of bleeding in DHF. We also found that the requirement for therapeutic platelet transfusions was significantly low post anti-D therapy compared to the control group. Thus, there was a significant decrease in the morbidity due to bleeding in patients receiving anti-D and platelets. However, the final platelet count achieved did not significantly differ. The duration of hospital stay for both the groups did not differ significantly which is probably due to the prolonged admission of the intervention group participations for the purpose of monitoring the platelet counts for a period of 7 days.

An important limitation of our study is that anti-D cannot be used in Rh− patients. Another pitfall is the small sample size and no follow-up of the participations after discharge. Since the majority of the study participants were males and aged between 20 and 50 years, the efficacy shown here may not hold true for females and the elderly age group (>50 years). We have also not studied the immune modulatory effects of anti-D, which may have a bearing on the ultimate outcome in severely ill dengue patients.

Anti-D seems to be an effective, promising therapy in Rh+ DHF with severe thrombocytopenia. In future studies, the effect of anti-D on the inflammatory cytokine and a possible antivirus role can be studied in addition to the above involving a larger study group.

CONCLUSIONS

Anti-D in addition to conventional treatment in patients with DHF with severe thrombocytopenia ($\leq$20,000/mm^3) resulted in a rapid reversal of thrombocytopenia at 48 h, improvement of bleeding grades, reduced requirement of platelet transfusions, and showed no adverse reaction. The convenience, efficacy, and safety of anti-D make it an appealing alternative to other currently available modalities of treatment for dengue fever in Rh+ patients with DHF/DSS.

Conflicts of interest

There are no conflicts of interest.

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