Effectiveness of Platelet Transfusion in Dengue Fever: A Randomized Controlled Trial

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Key Words

Dengue fever · Dengue hemorrhagic fever ·
Single donor apheresis platelets · Platelet transfusion ·
Corrected count increment · CCI · Transfusion reaction

Summary

Background: Scientific data regarding effects of platelet transfusion on platelet count in dengue-related thrombocytopenia is scanty. **Methods:** A single center, randomized non-blinded trial was conducted on adult patients with dengue fever and platelet counts less than 30,000/ μ l. Patients were randomized to treatment and control group. Treatment group received single donor platelets. Patients with post-transfusion platelet increment (PPI) $\geq 10,000/\mu$ l and/or corrected count increment (CCI) $\geq 5,000/\mu$ l 1 h post-transfusion were considered responders. Primary outcome was platelet count increments at 24 and 72 h. **Results:** 87 patients were enrolled, and 43 (48.2%) received platelet transfusion. Mean PPI and CCI at 1 h post-transfusion in the treatment group were 18,800/ μ l and 7,000/ μ l respectively. 22 (53.6%) patients in the treatment group were non-responders. Mean platelet increments at 24 and 72 h were higher in the treatment group as compared to the control group. Responders showed significantly higher increments when compared to non-responders and the control group at 24 h ($p = 0.004$ and $p < 0.001$, respectively) and 72 h ($p = 0.001$ and $p < 0.001$, respectively). Significant differences were found between non-responders and the control group at 24 h ($p < 0.001$), but not at 72 h ($p = 0.104$). Patients with lower baseline platelet count were more likely to be non-responders. Platelet transfusion neither prevented development of severe bleeding nor

shortened time to cessation of bleeding. Three severe transfusion reactions and two deaths occurred in treatment group. **Conclusion:** In this trial, almost half the patients showed no response to a high-dose platelet transfusion. Platelet transfusion did not prevent development of severe bleeding or shorten time to cessation of bleeding and was associated with significant side effects. Therefore, platelet transfusion should not be routinely done in the management of dengue fever.

Introduction

Dengue is the most prevalent mosquito borne viral disease infecting over 50 million people each year [1]. Its clinical symptoms vary from mild fever to life-threatening shock [2]. Thrombocytopenia is a prominent feature of dengue infection [3]. A platelet count of less than 100,000/ μ l is one of the diagnostic criteria for dengue hemorrhagic fever [4]. However, severe thrombocytopenia can be seen in both dengue fever and dengue hemorrhagic fever. There is a significant negative correlation between disease severity and platelet count [5]. Although low platelet count and hypofibrinogenemia are the two most prominent hemostatic defects responsible for bleeding in dengue infection [6], thrombocytopenia and coagulation abnormalities do not reliably predict bleeding in dengue infection [7, 8]. Causes of thrombocytopenia include both bone marrow suppression and platelet destruction. Immune complex-mediated platelet destruction is probably the most important factor contributing to thrombocytopenia in dengue infection [6].

Fig. 1. Schematic presentation of measurement of platelet increments at different time intervals. P0 is platelet count at baseline, P1 is platelet count within 10 min to 1 h post-transfusion, P24 is platelet count at 24 h and P72 is platelet count at 72 h. PPI is post-transfusion platelet increment within 10 min to 1 h of transfusion. PI24 and PI72 are PPI at 24 and 72 h, respectively. SponI24 is spontaneous increment in platelet count and equals to difference of PI24 and PPI.

Historically, platelet transfusion has been done in patients with thrombocytopenia due to hypoproliferative disorders of bone marrow. The rationale of platelet transfusion therapy in these patients has been that the bleeding risk correlates with severity of thrombocytopenia [9], platelet transfusion increases the platelet count, and this in turn reduces the risk of bleeding. On the other hand, degree of thrombocytopenia in dengue fever does not correlate with the bleeding risk. For example, one study found that the incidence of clinical bleeding was 6% among patients with platelet count $>150 \times 10^3$ platelets/ml, 12% among patients with platelet count of $100\text{--}149 \times 10^3$ platelets/ml, 11% among patients with platelet count of $80\text{--}99 \times 10^3$ platelets/ml, 10% among patients with platelet count of $50\text{--}79 \times 10^3$ platelets/ml, 11% among patients with platelet count of $20\text{--}49 \times 10^3$ platelets/ml, 13% among patients with platelet count of $10\text{--}19 \times 10^3$ platelets/ml, and 0% among patients with platelet count $<10 \times 10^3$ platelets/ml ($p = 0.22$, by test for trend) [10]. This study also found no effect of platelet transfusion on mean platelet count after 24 h. However, this was a retrospective study including only few cases of dengue hemorrhagic fever, and patients received variable doses of platelets in the form of pooled platelet concentrates with a median dose of 4 platelet units [10].

Theoretically, immune-mediated destruction of platelets in dengue fever may lead to refractoriness or poor response to platelet transfusion due to destruction of donor platelets. Although a few retrospective observational studies have been conducted to find frequency of platelet transfusions in dengue patients in various setups [11, 12], there is scanty data on the kinetics of response to platelet transfusion therapy in dengue infection. We conducted this study to understand the response

to platelet transfusion in dengue infection and the effectiveness in dengue-related bleeding.

Material and Methods

Study Design

The study was a single-center, parallel-assignment, randomized, non-blinded prospective analysis of increments in platelet counts in patients with dengue infection who received platelet transfusion therapy versus those who did not. The study was conducted in a high-dependency unit for dengue in Jinnah Hospital Lahore, Pakistan. Ethical review board of the hospital approved the protocol that meets the standards of the Declaration of Helsinki in its revised version of 1975 and its amendments of 1983, 1989, and 1996.

Study Population

Adults of the age 14 years and above presenting with dengue fever or dengue hemorrhagic fever, platelet counts less than $30,000/\mu\text{l}$, and having no bleeding or mild bleeding (WHO grade 1 or 2 bleeding) were enrolled in the study after taking written informed consent. Case definitions based on WHO guidelines were used to diagnose dengue fever and dengue hemorrhagic fever [4]. Patients with other causes of thrombocytopenia (e.g. idiopathic thrombocytopenic purpura, aplastic anemia), chronic ailments (chronic liver disease, chronic kidney disease, cancers), prior history of platelet transfusion and severe bleeding (WHO grade 3 and 4) were excluded from the study. Demographic data, history, and examination findings of all patients were recorded, and blood samples were drawn to measure the platelet counts. Patients were randomized into treatment or control group. The treatment group received single donor platelet (SDP) transfusion while the control group did not receive any platelet transfusion. Filtered apheresis platelets from a single donor (dose fixed at $\geq 5 \times 10^{11}$ platelets) were collected with a Haemonetics model MCS+LN 9000 apheresis machine (Haemonetics Corp., Braintree, MA, USA). All transfusions were done within 1 h of apheresis.

Measurement of Platelet Increments

Blood samples were collected in EDTA anticoagulated vials, and platelet counts were measured by automated count analyzer. In order to avoid pseudo-thrombocytopenia, citrated samples were used to repeat platelet counts if EDTA-induced platelet clumping was seen [13]. Platelet counts were obtained at baseline (P0), 24 h (P24), and 72 h (P72) for all patients. Additionally, platelet counts were also obtained within 10 min to 1 h post transfusion (P1) for the treatment group (fig. 1).

Corrected count increment (CCI) was determined using the following formula:

$$\text{CCI} = (\text{PPI} \times \text{BSA} (\text{m}^2)) \times 10^{11} / \text{number of platelets transfused} \quad (1).$$

PPI represents the post-transfusion platelet increment (post-transfusion platelet count minus pre-transfusion platelet count), and BSA is the body surface area measured in square meters. We used Mosteller formula for calculating BSA.

We measured PPI and CCI at 10 min to 1 h post-transfusion in the treatment group. Based on their responsiveness to platelet transfusion, patients in the treatment group were further divided into responders and non-responders. Patients with $\text{PPI} \geq 10,000/\mu\text{l}$ and/or $\text{CCI} \geq 5,000/\mu\text{l}$ 1 h post-transfusion were considered responders; the rest were considered non-responders.

PPI at 24 and 72 h were calculated using the following formulas (fig. 1):

$$\text{PPI at 24 h} = \text{platelet count at 24 h} - \text{platelet count at baseline} \quad (2),$$

$$\text{PPI at 72 h} = \text{platelet count at 72 h} - \text{platelet count at baseline} \quad (3).$$

For the treatment group, we calculated the spontaneous platelet recovery at 24 h using following formula:

$$\text{Spontaneous platelet recovery at 24 h} = \text{PPI at 24 h} - \text{PPI at 1 h post-transfusion} \quad (4).$$

For patients having bleeding at baseline, we noted site and WHO grade [14]. Patients were assessed every 12 h for bleeding. Any new onset of bleeding, progression to WHO grade ≥ 3 bleeding, and time to cessation of bleeding were documented. Any adverse event (transfusion reaction, anaphylaxis, death) was documented.

Outcome Measures

The primary outcome measure was PPI at 1 h (for treatment group) and at 24 and 72 h for both groups. The secondary outcome measures were progression to severe bleeding (WHO grade 3 and 4), any new onset bleeding, time to cessation of bleeding, and any adverse event including death.

Data Analysis

Data was analyzed using SPSS Statistics software Version 17 (SPSS Inc., Chicago, IL, USA). Patient characteristics were compared using of the chi-square or Fisher's exact test for categorical variables. Mann-Whitney test and Kruskal-Wallis test were used for continuous variables (PPI at 24 and 72 h, duration of bleeding). A one sided $p < 0.05$ was considered to be statistically significant with the confidence interval of 95%.

Results

Characteristics of the Patients

A total of 87 patients were enrolled in the study between August and October 2011 (fig. 2). 43 (48.2%) received single donor platelets. Two patients from the treatment group left before completion of study, and 1 patient was excluded due to later diagnosis of liver cirrhosis. These patients were removed from final analysis. Baseline characteristics of the patients including age, sex, diagnosis (dengue fever, dengue hemorrhagic fever), baseline platelet count, and frequency of bleeding were similar in both groups (table 1).

Results of Platelet Transfusion

All platelet transfusions were ABO-identical with the recipient. Mean PPI and CCI at 1 h post-transfusion were 18,800/ μl (range -7,000 to 77,000/ μl) and 7,000/ μl (range -1,050 to 27,260/ μl), respectively. Only 15 (36.5%) patients showed a 1-hour post-transfusion PPI of more than 20,000/ μl . Three patients (7%) showed a decrement in platelet counts 1 h post-transfusion. 22 (53.6%) patients in the treatment group were non-responders.

Comparison of PPI

Comparison between Treatment and Control Groups

Mean PPI at 24 h was 34,780/ μl in the treatment group

Fig. 2. Profile of the trial.

being significantly higher than in the control group with 4,280/ μl ($p < 0.001$). Similarly, mean PPI at 72 h was 75,430/ μl in the treatment group and 32,840/ μl in the control group ($p < 0.001$) (table 2).

Comparison between Responders, Non-Responders and Controls

At 24 h, responders showed significantly higher PPI (mean 53,310/ μl) as compared to non-responders (mean 18,770/ μl) and controls (mean 4,280/ μl) ($p = 0.007$ and $p < 0.001$, respectively). PPI in non-responders were lower than in responders, but higher than in controls ($p < 0.001$). Similarly, 72-hour post-transfusion PPI were significantly higher in responders (mean 103,440/ μl) as compared to non-responders (mean 51,430/ μl) and controls (mean 32,840/ μl) ($p = 0.002$ and $p < 0.001$, respectively). However, no significant differences were found between non-responders and controls ($p = 0.110$).

Spontaneous platelet recovery at 24 h (PPI at 24 h minus PPI at 1 h post-transfusion) was higher in NR as compared to controls. However, no significant difference was found between R and NR or between R and controls (table 3). Baseline characteristic of R and NR were comparable, except for baseline platelet count. NR had significantly lower baseline platelet counts (mean $\pm$ SD 9,710 $\pm$ 4,410/ μl) as compared to R (14,315 $\pm$ 7,265/ μl) ($p = 0.012$).

Comparison of Bleeding

Amongst patients with bleeding at baseline, progression to WHO grade 3 bleeding was observed in 1 patient (responder) in the treatmentgroup but in none in the control group. The patient received two pints of RBC transfusion. The mean time to cessation of bleeding was 31.6 h in the treatment group and 25.2 h in the control group. However, this difference was not

Table 1. Baseline characteristics of the patients

Characteristics	Treatment group (N = 43)	Control group (N = 44)	Total (N = 87)
Age, years			
Median	33	36	34
Range	15–65	16–78	15–78
Sex, no. (%)			
Male	28 (65)	29 (66)	57 (65)
Females	15 (35)	15 (34)	30 (35)
Diagnosis, no. (%)			
DF	17 (40)	20 (46)	37 (42)
DHF 1	08 (18)	06 (14)	14 (16)
DHF 2	18 (42)	18 (41)	36 (42)
DSS	0	0	0
Baseline platelet count/ μ l			
Median	10,000	10,500	
Interquartile range	8,000–15,000	9,000–17,700	
Bleeding at the time of enrolment, no. (%)			
Yes	19 (44)	20 (45)	39 (45)
Site of bleeding, no. (%)			
Oral and nasal	11 (58)	15 (75)	26 (66)
Gastrointestinal	01 (05)	04 (20)	05 (14)
Genitourinary	04 (21)	00 (00)	04 (10)
Pulmonary	03 (16)	01 (05)	04 (10)
WHO grade of bleeding, no. (%)			
WHO grade 1	09 (47)	06 (30)	15 (38)
WHO grade 2	10 (53)	14 (70)	24 (62)
DF = Dengue fever; DHF = Dengue hemorrhagic fever; DSS = Dengue shock syndrome; WHO = World Health Organization.			

Table 2. Comparison of PPI in treatment and control group

Parameter	Treatment group	Control group	P value
PI24, platelets/ μ l			
Mean $\pm$ SD	34,780 $\pm$ 43,820	4,280 $\pm$ 10,360	<0.001
Median	22,000	3,000	
Interquartile range	12,000–45,750	–2,000 to 9,000	
PI72, platelets/ μ l			
Mean $\pm$ SD	75,430 $\pm$ 69,465	32,840 $\pm$ 30,900	<0.001
Median	53,500	23,000	
Interquartile range	31,750–89,250	9,000–57,000	
Spontaneous increment at 24 h			
Mean $\pm$ SD	4,525 $\pm$ 38,080	4,280 $\pm$ 10,360	0.327
Median	1,500	3,000	
Interquartile range	–5,750 to 10,000	–2,000 to 9,000	
PI24 = PPI at 24 h; PI72 = PPI at 72 h.			

statistically significant ($p = 0.346$). Similarly, no difference was found between responders, non-responders and controls ($p = 0.352$ Kruskal-Wallis test). None of the patients without bleeding at baseline in either group had new onset bleeding during study period.

Group Analysis Based on Baseline Platelet Count

Analysis was also performed with patients further classified into three groups based on baseline platelet count (<10,000/ μ l, 10,000–20,000/ μ l, >20,000/ μ l) (table 4). There

Table 3. Comparison of PPI amongst responders, non-responders and controls

Parameter	Responders (R)	R vs. NR p value	Non-responders (NR)	NR vs. C p value	Controls (C)	C vs. R p value
P0, platelets/ μ l						
Mean $\pm$ SD	14,315 $\pm$ 7,265	0.012	9,710 $\pm$ 4,410	0.019	13,300 $\pm$ 6,400	0.384
Median	14,000		9,000		10,500	
Interquartile range	10,000–20,000		7,500–11,500		9,000–17,700	
PI24, platelets/ μ l						
Mean $\pm$ SD	53,310 $\pm$ 56,760	0.007	18,770 $\pm$ 16,800	<0.001	4,280 $\pm$ 10,360	<0.001
Median	38,000		16,000		3,000	
Interquartile range	18,000–81,000		9,000–31,000		–2,000 to 9,000	
PI72, platelets/ μ l						
Mean $\pm$ SD	103,440 $\pm$ 72,950	0.002	51,430 $\pm$ 58,660	0.110	32,840 $\pm$ 30,900	<0.001
Median	72,000		34,000		23,000	
Interquartile range	52,000–130,000		18,000–65,500		9,000–57,000	
Spontaneous increment at 24 h						
Mean $\pm$ SD	5,050 $\pm$ 53,680	0.456	4,050 $\pm$ 15,275	0.631	4,372 $\pm$ 10,360	0.258
Median	1,500		2,000		3,000	
Interquartile range	–33,000 to 11,000		–5,000 to 11,500		–2,000 to 9,000	
P0 = Baseline platelet count; PI24 = PPI at 24 h; PI72 = PPI at 72 h						

Table 4. Group analysis based on baseline platelet count

Parameter	P0 <10,000	P0 11,000–20,000	P0 21,000–30,000	P value
Number of cases				0.972
Treatment group	21	14	5	
Control group	22	16	6	
Bleeding, yes				0.090
Number of cases	15	18	6	
WHO grade of bleeding, n				0.707
Grade 1	7	6	2	
Grade 2	8	12	4	
Response to platelet transfusion				0.035
Responders	06 (29)	09 (64)	04 (80)	
Non-responders	15 (71)	05 (36)	01 (20)	
Progression to WHO grade 3 or higher bleeding				0.035
Number of cases	0	0	1	
Adverse events,				0.774
Transfusion reactions	2	0	0	
Death	1	0	0	
P0 = Baseline platelet count.				

were significantly more non-responders in patients with baseline platelet count < 10,000/ μ l. On the other hand, progression to WHO grade 3 bleeding occurred in a patient with a baseline platelet count > 20,000/ μ l.

Adverse Events

Three patients in the treatment group (7%) showed severe anaphylactic reaction and hypotension. There were 2 deaths in the treatment group. One death was due to development of transfusion-related acute lung injury (TRALI) while the other

death was in a patient who received platelet transfusion but due to later diagnosis of liver cirrhosis was excluded from the trial. The cause of death in the latter case was multifactorial, including anaphylactic shock, bleeding from esophageal varices due to liver cirrhosis, and old age.

Discussion

Pathogenesis of thrombocytopenia in dengue infection is not fully known and may be multifactorial. Immune-mediated destruction of platelets is considered to be the most important factor. For example, one study found presence of antibodies directed against dengue virus nonstructural protein 1 (NS1) that showed cross-reactivity with human platelets and endothelial cells, which lead to platelet and endothelial cell damage and inflammatory activation [15]. In most patients, bone marrow responds to peripheral platelet destruction with increased production of platelets. Over time, immune-mediated destruction of platelets ends and spontaneous recovery in platelet counts is seen in almost all patients. The degree of thrombocytopenia varies in dengue infection, and platelet count may be extremely low in few patients. Platelet transfusions have been done in many patients with dengue related thrombocytopenia; however, response to platelet transfusion is not fully studied in these patients. The present study provides insight to the response of platelet transfusion in dengue infection.

The normal response to platelet transfusion is an immediate increase in the platelet count that is maximal at about 10 min to 1 h post-transfusion. Following this, there is a steady linear decrease in the platelet count, which usually returns to baseline at about 72 h post-transfusion. Infusion of one apheresis unit or six units of whole blood-derived platelets to an adult with a body surface area of 2.0 m² raises the platelet count by approximately 30,000/ μ l at 1 h after the infusion. CCI is a more qualitative way to determine adequacy of the response to platelet transfusion. The theoretically expected value of the CCI is approximately 20,000/ μ l. However, in practice the observed increase in platelet count following transfusion in many patients is often less than expected, and the CCI value is closer to 10,000/ μ l. The response to platelet transfusion is usually assessed in patients with hematological and oncological disorders by measuring post-transfusion PPI and CCI. Refractoriness or poor response to platelet transfusions is assumed when two consecutive platelet transfusions lead to 10-min to 1-hour post-transfusion CCI values of less than 5,000/ μ l [16]. However, unlike hematological disorders, dengue-related thrombocytopenia lasts for shorter duration, and repeated platelet transfusions are not usually required. Hence, a low CCI on two consecutive transfusions is difficult to document in order to establish refractoriness. Therefore, we avoided using the term refractoriness and divided recipients into responders and non-responders on the basis of response to one single donor platelet transfusion.

Although immune-mediated platelet destruction in dengue fever is likely to destroy transfused platelets at an accelerated rate and lead to suboptimal response to platelet transfusion, this study shows that about half the patients showed a PPI of $\geq 10,000/\mu$ l 1 h post-transfusion. This study also shows that patients who received platelet transfusion showed higher PPI at 24 and 72 h than controls, and these increments were independent of baseline platelet count. This higher increment in the treatment group can be attributed to persistence of transfused platelets in the circulation as spontaneous PPI at 24 h was not significantly different between treatment and control groups.

Almost half the patients in the treatment group were responders. This finding implies that contribution of immune-mediated destruction to thrombocytopenia in dengue infection may be variable in different patients and, even with same degree of thrombocytopenia immune-mediated platelet destruction, may be of lesser severity in responders as compared to non-responders. This hypothesis is further strengthened by the finding that although both responders and non-responders showed higher PPI at 24 h as compared to controls, only responders showed higher PPI at 72 h, and no significant difference was found between non-responders and controls. Similarly, non-responders had significantly lower baseline platelet count as compared to responders; also there was greater proportion of non-responders in patients with baseline platelet count < 10,000/ μ l as compared to those with higher baseline platelet counts. It can be hypothesized that patients with lower platelet counts may have greater degree of immune-mediated platelet destruction that lead to poor response to platelet transfusion. This also implies that, although patients with platelet count < 10,000/ μ l are more likely to receive platelet transfusion, they are less likely to benefit from it.

Sometimes a similar situation is seen in other kinds of autoimmune thrombocytopenia. Like dengue infection, patients with autoimmune thrombocytopenia (i.e., idiopathic thrombocytopenic purpura) or drug-induced thrombocytopenia (e.g., quinidine-induced thrombocytopenia) also have antibodies that are likely to destroy transfused platelets at an accelerated rate. However, acceptable increases in platelet counts occur in some of these patients following platelet transfusion. For example, in one report 13 of 31 (42%) platelet transfusions raised the platelet count to >20,000/ μ l; on the next day platelet counts remained elevated in 5 of the 7 responders [17].

Platelet transfusion, despite increasing platelet count in half the recipients, neither prevented progression to severe bleeding (WHO grade 3) nor appeared to shorten time to cessation of bleeding. WHO grade 3 bleeding occurred in one patient with baseline platelet count > 20,000/ μ l and who was a responder to platelet transfusion. This implies that a high baseline platelet count and a successful platelet transfusion could not prevent progression to severe bleeding.

Platelet transfusion is associated with a risk of severe ad-

verse reactions. In one study, 2% of the platelet transfusions were associated with a severe adverse reaction [18]. In another study, deaths due to platelet transfusion were 0.015% (20 of 1,712 transfusions) [19].

The frequency of severe adverse reactions was higher in this study population, and the cause is unclear although hyperactive immune mechanisms may be implicated. One death due to TRALI is directly attributable to platelet transfusion. However, there was no reported death attributable to platelet transfusion in the other 287 recipients of apheresis platelet transfusion with dengue fever outside the trial in the same hospital.

Conclusion

In this trial, half of the patients showed almost no response to a high-dose platelet transfusion. Platelet transfusion did not prevent development of severe bleeding or shorten time to cessation of bleeding and was associated with significant side effects. Although patients with lower platelet counts are more likely to receive platelet transfusion, they are less likely to show an appropriate response. Therefore, platelet transfusion should not be routinely done in the management of dengue fever.

Disclosure Statement

The authors declare no conflict of interest.

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