ORIGINAL ARTICLE

A Randomized Clinical Trial of Human Interleukin-11 in Dengue Fever-Associated Thrombocytopenia

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Abstract

Objective: To assess the effectiveness of recombinant human (rh) IL-11 to increase platelets count in patients suffering from Dengue fever (DF).

Study Design: Randomized double blind placebo control study.

Place and Duration of Study: Farooq Hospital, Lahore, from July to October 2011.

Methodology: Forty hospitalized patients suffering from Dengue fever having platelets count ≤ 30000 per micro liter were randomly categorized into two groups, rhIL-11 (test) and distilled water (placebo) groups. The efficacy outcomes (as indicated by step up in platelets count at 48 hours) for the treatment group were compared with the outcomes for the placebo group.

Results: The data revealed that the increase in platelet response with recombinant human interleukin 11, 1.5 mg subcutaneously is significantly more brisk than the placebo group. The platelets response in patients with severe thrombocytopenia was greater in the treatment group (50%) at 48 hours as compared to the placebo group (20%) (p=0.047). Response rate was slightly greater among males (6/10, 60%) than females (8/16, 50%); moreover, three-fourth (75%) female responders were in the placebo group, compared to half (50%) male responders in the treatment group.

Conclusion: Results of the study suggest that treatment of severe thrombocytopenia accompanying DF with recombinant human interleukin11 may be a useful therapeutic option.

Key Words: *Dengue fever. Thrombocytopenia. Interleukin-11. Bone marrow.*

INTRODUCTION

Since 1940's when first proven case of Dengue fever was reported in the subcontinent, the disease has emerged as a major social, fiscal and health burden to our society.[1,2] At present, Lahore is facing a Dengue epidemic.

Dengue virus may lead to thrombocytopenia either via direct bone marrow suppression or by means of raising antibodies to destroy platelets in the peripheral circulation.[3] The levels of positive humoral regulators of megakaryopoiesis were not increased during the thrombocytopenic phase of disease (3rd to 8th days) despite low platelet counts. It is likely that dengue viruses trigger temporary changes in the humoral immune control of platelets production perhaps mediated through lymphoid tissue damage.[4]

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IL-11 levels increase in response to the reduced circulating platelets (thrombocytopenia) and it promotes rapid platelets recovery.[5] Most likely it encourages proliferation of early progenitor cells and helps in the maturation of megakaryocytes.[6,7] This proliferation seems to be due to recruitment of G_o phase population to active cycle along with reducing the cell-cycle duration in cells.[8] IL-11 along with thrombopoietin (TPO) stimulate megakaryocytopoiesis in bone marrow cells at different stages.[7,9,10] There is a growing evidence from the literature to support the synergistic role of IL-11 in production, differentiation and maturation of megakaryocytes.[11,12]

Despite the increased levels of thrombopoietin (TPO) in the serum levels among Dengue disease suggesting its possible potential early clinical marker, no detectable levels of IL-11 were found.[13] Because of the controversies generated out of multiple studies weighing the levels of TPO and IL-11 and acceleration of megakaryopoiesis, researchers believe some other reasons behind megakaryocyte mass with reduced production and maturation.[14]

There are few unpublished hearsays trying IL-11 based on the assumption that IL-11 play a role in thrombogenesis; physicians have been claiming mixed results in treating Dengue thrombocytopenia. Likewise we could not find any published data outside Pakistan on the possible role of interleukin-11 to enhance levels of thrombocytes levels in Dengue fever.

METHODOLOGY

Approval for this study was taken from the Ethical Review Board, Farooq Hospital attached with Akhter Saeed Medical College, Lahore, Pakistan. This was a pilot trial study. Patients with Dengue fever were enrolled through non-probability consecutive sampling. Informed written consent was obtained from all study participants according to the study protocol. Then the patients were randomly allocated to two groups, either in treatment or placebo. Possible diagnosis of Dengue infection was carried out at screening with a Dengue Duo IgM and IgG Rapid Strip Test (SD , Standard Diagnostics, Inc. Suwon city, Kyonggi province, Korea), and confirmed by an IgM and IgG capture enzyme-linked immunosorbent assay (HUMAREADER SINGLE PLUS' Semi Automated Elisa Strip Reader Human Germany, Cat no 18000). Thrombocytopenia was confirmed when platelet count through a Japanese Sysmex K series KX21 (Cellpack +Lyse) was 100,000 per micro liter of blood, and severe thrombocytopenia was defined for this study when the platelet count was < 30,000/ per micro liter of blood.

Inclusion criteria were adults under 50 years of age, with Dengue fever (Dengue IgM antibody positive) hypertension, ischaemic heart disease, allergies, bronchial asthma, orthopnea and paroxysmal nocturnal dyspnea and palpitations. Moreover, these patients had no abnormal findings on ECG, ophthalmoscopic examination, prothrombin time and APTT and no signs of disseminated intravascular coagulation.

Any patient with a drop in platelet counts to less than 10,000 per micro liter of blood or who developed epistaxis, heavy menstruation, microscopic hematuria, a positive test of stool for occult blood were excluded from this study. All those patients whose conditions warranted transfusion of any blood products, or with serum creatinine more than 1.1 g/dl were excluded. Likewise every pregnant or breastfeeding mothers were removed from the study.

rhIL-11 was administered in a single dose of 1.5 mg by subcutaneous route. To maintain the blind, a black covered vial system was used because rhIL-11 was in off-white powder form. Placebo consisted of an empty vial labeled with a black cover. To make an injection an equal volume of distilled water was provided in the system. Both the investigational medicine (rhIL-11) and the placebo were prepared by a pharmacist who maintained the blind and allocated study drug/placebo with a predetermined randomization code.

The efficacy outcomes in the form of increase in the platelet counts for the treatment group were compared with the outcomes for the placebo group. Levene's test was used for equality of variance, Independent t-test to compare the equality of means and for calculation of the 95% two-sided confidence intervals for the means. Repeated measures ANOVA was used separately for each parameter in order to compare the mean in relation to treatment hours and by gender groups. The analysis among the subgroups was carried out on the basis of patients' baseline platelet count. All analyses were done in Statistical Package for Social Sciences (SPSS), version 21 (SPSS Inc., Chicago, USA).

RESULTS

Forty adults (male 16, female 24) between the ages of 17 and 47 years (mean=29.6 ± 8.199 years) were enrolled. Results showed a progressive increase in mean platelet counts in both genders with continuation of treatment from zero hour to 48 hours (Table I and Figure 1). When statistical analysis was done to see the significance in platelets response for patients in the test group as compared to placebo group over the treatment hours; highly significant changes occurred in the platelets response in the test group over this time period of therapy. There was marginal improvement in the treatment group as compared to the placebo but statistically the test group showed significant recovery as compared to the placebo group in platelet at 12 (p=0.007) and 24, 36 and 48 hours (p < 0.001), after starting the treatment (Table II).

Figure 1 shows the progressive changes in the mean platelet counts over treatment hours in the test and placebo groups. There is a sustained increase in the test group mean platelets starting from 12 hours, maintained over 48 hours; it is statistically significant with p = 0.007 - 0.001 starting from 12 hours.

Differences in response rates by gender are shown in Table III. Although both males and females showed comparative responses, the females achieved statistical

Table II: Comparison of platelet by treatment.

Treatment hours	Placebo group (n=20)	Test group (n=20)	p-value
0	22150 ± 4283.01	22550 ± 6660.76	0.822
12	20650 ± 3963.10	26150 ± 7513.32	0.007
24	21400 ± 7576.10	30550 ± 8009.70	0.001
36	25700 ± 9073.40	35700 ± 8442.40	0.001
48	30000 ± 10125.53	41050 ± 8629.66	0.001

Independent samples t-test was used.

Table I: Distribution of mean platelet in relation to treatment hours and by gender groups in subjects with profound thrombocytopenia (n=40).

Treatment hours	0	12	24	36	48	p-value
Mean platelet counts						
Males (n=16)	22812.50 ± 6273.95	24562.50 ± 6879.62	26312.50 ± 9090.06	32125.00 ± 10512.69	35625.00 ± 10726.13	< 0.0001
Females (n=24)	22041.67 ± 5094.57	22625.00 ± 6350.71	25750.00 ± 9099.69	29750.00 ± 9794.63	35458.30 ± 11147.15	< 0.0001
Total (n=40)	22350.00 ± 5531.03	23400.00 ± 6550.78	25975.00 ± 8982.85	30700.00 ± 10023.56	35525.00 ± 10841.48	< 0.0001

Repeated measures ANOVA was used separately for each parameter.

Table III: Differences in the mean (SD) platelet response rates by gender among the placebo and test groups (n=40, independent sample t-test).

Treatment hours	Males (n=16)		p-value (0 to 48 hurs)	Females (n=24)		p-value (0 to 48 hours)
	Placebo (n=8)	Test (n=8)		Placebo (n=12)	Test (n=12)	
0	22250 ± 3918.82	23375 ± 8262.44	0.733	22083.33 ± 4679.90	22000 ± 5688.90	0.969
12	22500 ± 3023.72	26625 ± 9085.90	0.243	19416.67 ± 4144.18	25833.33 ± 6685.58	0.01
24	22000 ± 6546.54	30625 ± 9575.83	0.054	21000 ± 8453.10	30500 ± 7242.55	0.007
36	27375 ± 8601.29	36875 ± 10548.36	0.068	24583.33 ± 9577.04	34917.67 ± 7115.39	0.007
48	30500 ± 8366.60	40750 ± 10793.52	0.052	29666.67 ± 11499.67	41250 ± 7374.71	0.008

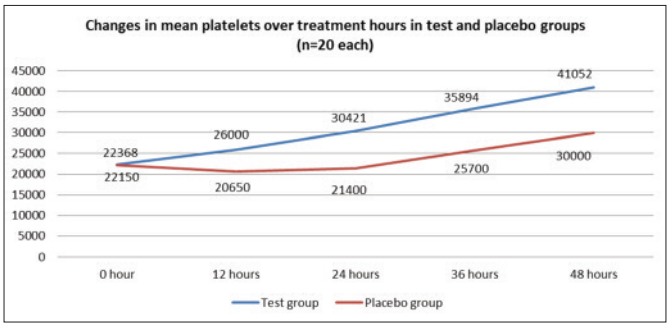

Figure 1: Graphical representation of the progressive and sustained increase in mean platelets of the test group compared to the placebo group over the 48 hours of treatment (p = 0.007 to 0.001 at different treatment hours, starting from 12 hours).

significant recovery much earlier than the males, who achieved near significance, at best.

In this study the response was identified as an increase in platelets of more than 20,000 over baseline at 48 hours of the institution of the drug/placebo. As such, there were 14 patients (6 males and 8 females) who were labeled as 'responders'. Four responders belonged to the placebo group and 10 to the test group (p=0.047); 3 patients in the treatment group responded within 36 hours after administration of IL11. Response rate was slightly greater in males (6/10, 60%) than females (8/16, 50%); moreover, 3/4 (75%) female responders were in the placebo group, compared to 5/10 (50%) male responders in the treatment group.

DISCUSSION

Most of the patients with severe Dengue fever develop thrombocytopenia between 3rd to 8th days into the illness.[15] There may be a need of treatment for low platelet count towards the end of the febrile phase due to expectant drop in the subsequent 2 days. Prophylactic platelet transfusions are still in practice despite condemnation in the literature against such strategies. Moreover, platelets transfusion is expensive and time-consuming in an outbreak situation when compared with the cost of IL-11 injection.

Pathology behind the drop in platelet count is not well understood, but some suggest it may be due to the suppression of the bone marrow directly by the Dengue virus.[16] According to some researchers from Thailand, bone-marrow examination of Dengue patients revealed hypocellularity in the critical stage, subsequently hypercellularity during recovery and later on normocellularity in the convalescence.[17] So it was hypothesized that rhIL-11 may hasten the recovery of platelets because it directly stimulates the proliferation of hematopoietic stem cells and megakaryocyte progenitor cells and induce megakaryocytes maturation.

Interleukin eleven has established its role in the prevention of severe thrombocytopenia and reduction in the need for platelet transfusion following myelo-suppressive chemotherapy.[18] To-date there are no published studies regarding its use in the treatment of thrombocytopenia in Dengue fever.

Although at the time of inclusion, the patients showed no hemorrhagic manifestation but later in the study period 7 of them (3 from the test group and 4 in the placebo) developed signs of minor bleeding like mild nose and gum bleeding and microscopic hematuria. While serious bleeding in this disease is rare, even clinical bleeding does not correlate sufficiently with the platelet count.[19,20]

In the past decade, studies have shown a rising trend in platelet counts while treating these patients with anti-D immunoglobulin but many studies could not find significant differences in the kinetics of the platelet count in randomized controlled trials.[7,10] Similarly, prophylactic platelet transfusions were ineffective in preventing bleeding in Dengue cases with severe thrombocytopenia.[21,22]

Subsequent to the intervention, there was statistically significant increase in the mean platelet levels between placebo and treatment groups at 12 hours (p= 0.007). Sellahewa reported similar results when he tried Fresh Frozen Plasma to check its efficacy in Dengue associated thrombocytopenia at 12 hours with (p=0.04). But the incremental pattern in platelet counts in intervention group than in controls at 24 and 48 hours after administration, was not statistically significant.[23] Whereas we found significant contribution of IL-11 regimen towards increasing platelets steadily against placebo group over time at 24, 36 and 48 hours.

Headache, fatigue, nausea and generalized body aches and pains are the main side effects of IL-11.[24] Sometimes, trivial conjunctival bleeding or slight oedema is found. Although increased heart rate and drop in blood pressure are of major concern but they seldom occur when IL-11 is administered in patients with chemotherapy.[24] In addition, it was recently showed that interleukin-11 administration induced capillary leak

syndrome in primary hepatic carcinoma patients.[25] Vascular leakage is one of the hallmarks of severe Dengue infection and such report raises concern on the safety of IL-11 in the treatment of Dengue patients.

The fact that every patient received a single and identical dose (1.5 mg) may have hampered the efficacy of the treatment schedule. Ideally, drug dose should be obtained based on the weight of the patient. The finding that female showed significant response rates, but not males (Table III), suggest that it may be due to low dosage in male patients. It may be interesting to state the IL-11 dosage was in mg/kg between male and female groups or measuring IL-11 levels in the patients.

Though it is likely that the responses observed in our patients were by chance and not because of the injection of rhIL-11. The adult Dengue patients included in the study presented with platelet counts > 10,000 and no clinically significant bleeding. Consequently, these patients didn't need any therapeutic intervention. However, this study suggests that IL-11 may hasten the recovery of Dengue associated thrombocytopenia. This is of some pathophysiological interest. This study may motivate the researchers to use it in a larger trial on the basis of robust recovery of platelets. These findings should stimulate further studies to analyze the effectiveness of its actual and possible role as an alternative treatment.

CONCLUSION

While bleeding manifestations are only partially associated with severe thrombocytopenia in severe Dengue fever, the role of bone marrow suppression by virus remains unclear. The results of this study have shown a tendency in the platelet count to rise signifi-cantly after rhIL-11 administration in patients with severe Dengue thrombocytopenia compared to placebo. More research must be conducted using rhIL-11, which may explain its possible role.

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