RESEARCH ARTICLE

Role of granulocyte colony stimulating factor (G-CSF) in dengue patients with severe thrombocytopenia

Kamran Amir Khan, Sameed Ullah Qureshi

Abstract

Objective: To determine the effects of granulocyte colony-stimulating factor in improving platelet count in patients with dengue fever.

Method: The retrospective, cross-sectional study was conducted at Northwest General Hospital and Research Centre, Peshawar, Pakistan, between January 2021 and October 2022, and comprised dengue fever inpatients regardless of age and gender who received granulocyte colony-stimulating factor subcutaneously. The impact of colony-stimulating factor on platelet and white blood cell counts as well as any unfavourable consequences was assessed. Convenient sampling was used and a structured format was used for data collection. Data was analysed using SPSS 21.

Results: Of the 100 patients, 67(67%) were males and 33(33%) were females. The largest age group was that of >55 years 31(31%), fever was present in all the 100(100%) cases, bleeding in 18(18%) and platelet count <30,000 in 83(83%) cases. Dengue fever was confirmed by rapid dengue nonstructural protein 1 antigen in 76(76%) cases, dengue immunoglobulin G antibody test 28(28%), and immunoglobulin M antibody test in 31(31%) cases. Overall, 72(72%) patients received only one dose of granulocyte colony-stimulating factor. Post-administration, a substantial rise in the median platelet and white blood cell counts was seen compared to the baseline ($p<0.05$) on day 2.

Conclusion: Granulocyte colony-stimulating factor helped increase platelet and white blood cell counts quickly in dengue fever patients.

Key Words: Granulocyte colony-stimulating factor, Dengue, Thrombocytopenia.

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Introduction

Dengue virus is now one of the most common arthropod-borne diseases both in terms of public health and medicine, with countries like Pakistan and India facing a spike in incidence. Household mosquito Aedes aegypti is the primary vector of transmission to humans. There is no vaccination for dengue at the moment. Clinical investigations for multiple vaccines, though, are now being carried out at different developmental stages.[1] Dengue fever (DF) first emerged as a pandemic in Africa, Asia and North America in 1779-1780. Afterwards, it continued varying till the 19th century. After World War II, there were significant changes in the pattern of dengue virus infections that have persisted to this day[2]. Dr Paul Reiter, of the Entomology Section of the Centers for Disease Control and Prevention (CDC) Dengue Laboratories, said at the 1999 Dengue Symposium in Cairns emphasised that people who were carriers of the dengue virus were roaming the world, infecting mosquitoes[3]. Similar to malaria, dengue virus is found all over the world, and an estimated 2.5 billion people live in areas where pandemic expansion is possible[3].

Dengue has recently grown to be a significant global public health issue. The majority of the world's tropical and subtropical regions are home to this mosquito-borne disease, which is a primary cause of hospitalisation and death. DF was discovered in Thailand, China, Indonesia and Malaysia in 1980. In addition, outbreaks have been reported from Bangladesh in 2005 and from India in 1990[4]. DF has been prevalent in Pakistan for more than two decades. The first substantial pandemic in Pakistan was observed in 1994-95. According to a study, DF was rife in southern Pakistan for two years in a row.[5]

However, the country saw an unprecedented rise in DF cases in 2005 and 2006, with more than 3,640 individuals getting hospitalised with DF-like symptoms and signs. With 40 fatalities, it was the nation's largest and most severe DF epidemic. The 2011 dengue pandemic in Pakistan's Punjab province led to at least 365 deaths and 21,597 dengue cases, making it the worst DF pandemic in history[6].

Department of Internal Medicine, Northwest General Hospital and Research Center, Peshawar, Pakistan.

Correspondence: Sameed Ullah Qureshi.

Email: Sameedqureshi92@gmail.com

ORCID ID. 0000-0002-9189-135X

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The symptoms of DF, an acute febrile infectious illness, typically include headache, discomfort in the muscles, joints and bones, a rash, and leukopenia. It also goes by the name "break bone fever."[4] The four main clinical features of dengue haemorrhagic fever (DHF) are high-grade fever, haemorrhagic abnormalities, frequently accompanied by hepatomegaly, and, in extreme instances, evidence of hypervolaemia. These individuals may experience plasma leakage-related hypervolaemic shock. The condition is lethal, and is known as dengue shock syndrome (DSS)[7].

In the majority of tropical countries of the Western Pacific and South-East Asia, DHF is now a serious public health issue[8]. In at least 8 tropical Asian nations, the illness is one of the top 10 reasons for hospitalisation and mortality[9]. About 50 million individuals worldwide contract dengue, the most common mosquito-borne viral illness, every year. Clinical signs range from a low-grade fever to shock that poses a life-frightening risk[10]. The most noticeable symptom of DF is thrombocytopenia[11]. Among the diagnosing standards for DHF is a platelet count of <100,000/µl. However, both DHF and DF can exhibit severe thrombocytopenia[12]. When human haematopoietic cells are infected, changes in megakaryocytopoiesis occur, which impair progenitor cell proliferation and cause platelet malfunction, like platelet stimulation and clumping, increased breakdown, or overconsumption, like peripheral sequestration and consumption. There is a dearth of research on the impact of platelet transfusions on platelet count in thrombocytopenia caused by DF[13].

Elderly individuals with dengue disease and platelet counts <30,000/µl were the focus of a single-centre, controlled, non-blinded study. Treatment and control groups were randomly generated. Single donor platelets were given to the treated group. Patients who had a post-transfusion platelet increase (PPI) of at least 10,000/µl and/or a corrective count augmentation (CCI) of maximum 5,000/µl one hour after the transfusions were deemed to have responded. Increases in platelet count at 24 and 72 hours were the main outcomes. A high-dose platelet transfusion was ineffective in nearly half of the patients. In addition to having major adverse effects, platelet infusion did not stop the onset of excessive bleeding or speed up the time it took for bleeding to stop. As a result, regular platelet infusions in the treatment of DF are not recommended[14].

Granulocyte colony-stimulating factor (G-CSF), called filgrastim, has been utilised as adjunctive treatment in pneumonia individuals suffering from sepsis[15]. Case study of a DHF patient showed improvement post-G-CSF as a rescue medication. The subject also had myocarditis, chronic pulmonary distress disorder, and febrile neutropenia. The subject's clinical condition had been steadily deteriorating, and normal care, such as antibiotics and mechanical ventilator assistance, had not been effective. She needed inotrope support and had sepsis-like symptoms. In some high-risk patients who have developed febrile neutropenia due to pneumonia, hypotension, sepsis episode, multiorgan failure, fungal infection, untreated main illness, or severe neutropenia, G-CSF has been considered as a rescue medication. G-CSF encouraged the development and maturing of myeloid cells, notably the expansion and diversification of neutrophils. The administration of G-CSF caused the patient's fever to go away and improved her clinical syndrome [16].

In DHF, there is proof of both immune deficiency and immunological-mediated target cell death. This was likely due to the fact that it reversed bone marrow stagnation, enhanced leukocyte and lymphocyte count, and allowed the individual to initiate an immune system response, which, in turn, caused the fever to go away[17].

The current study was planned to determine the effects of granulocyte colony-stimulating factor in improving platelet count in DF patients.

Patients and Methods

The retrospective, cross-sectional study was conducted at Northwest General Hospital and Research Centre, Peshawar, Pakistan, between January 2021 and October 2022. After approval from the institutional ethics review committee, the sample size was determined using the formula[18]: $n=(z\alpha+z\beta)2(\delta/\sigma)2$, where $\delta = |\mu 0 - \mu 1|$ was the detectable difference in the mean, σ was population variance, zα was standardised value associated with alpha (α) being the type I error, and zβ was the standardised value associated with β, the type II error. Non-probability convenient sampling technique was used.

Those included were DF inpatients regardless of age and gender, who received G-CSF 300g/mL subcutaneously, had a confirmed diagnosis of dengue virus, and whose baseline blood samples had been taken within 1-7 days of the beginning of symptoms suggestive of dengue infection. The confirmatory laboratory tests were rapid dengue nonstructural protein 1 (NS1) antigen, and dengue immunoglobulin G (IgG)/IgM antibody testing.

Patients who had not received G-CSF were excluded.

Retrospective evaluation of G-CSF's impact on platelet and white blood cell (WBC) counts, as well as any unfavourable consequences, was done on the basis of

medical records of the patients.

Enhancement in platelet count on day 2 following G-CSF injection was the main outcome, while the secondary outcomes were improvement in WBC count on day 2 following G-CSF treatment.

Data was analysed using SPSS 21. Categorical variables were summarised using percentages and frequencies. Continuous data was expressed as mean +/- standard deviation and median with minimum-maximum range. The data was not checked for normality. P<0.05 was considered statistically significant.

Results

Of the 100 patients, 67(67%) were males and 33(33%) were females. The largest age group was that of >55 years 31(31%), fever was present in all the 100(100%) cases, bleeding in 18(18%) and platelet count was <30,000/µl in 83(83%) cases. DF was confirmed by rapid dengue NS1 antigen in 76(76%) cases, dengue IgG antibody test in 28(28%), and IgM antibody test in 31(31%) cases (Table 1).

Table-1: Demographic and clinical presentation at baseline (N=100),

Parameters	Patient who receives G-CSF (Filgrastim) (N=100)
Age (years)	N (%)
15-24 years	13 (13)
25-34 years	20(20)
35-44 years	19 (19)
45-54 years	17 (17)
55+ years	31 (31)
Gender	
Male	67 (67)
Female	33 (33)
Dengue NS1	
Positive	76 (76)
Negative	24 (24)
IgG	
Positive	28 (28)
Negative	72 (72)
IgM	
Positive	31(31)
Negative	69 (69)
Clinical presentations	
Fever	100 (100)
Bleeding	
Yes	18 (18)
No	82 (82)
Platelet counts at baseline visit (per mm3)	
<10,000	9(9)
10001-20000	37(37)
20001-30000	37(37)
>30000	17(17)

NS1: Nonstructural protein 1, G-CSF: Granulocyte colony-stimulating factor, Ig: Immunoglobulin.

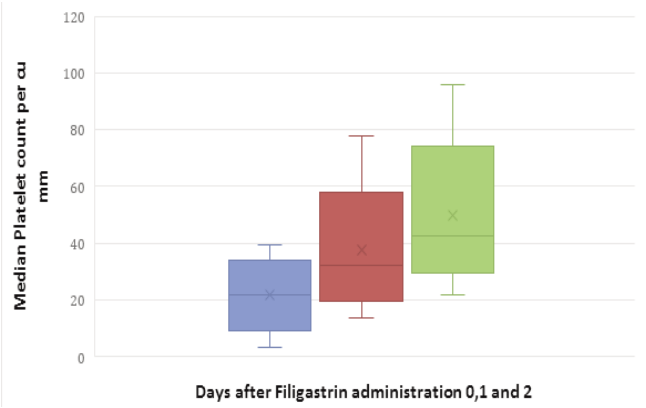

Figure-1: Median platelets count on days 1 and 2 after G-CSF (Filgrastim) administration.
G-CSF: Granulocyte colony-stimulating factor.

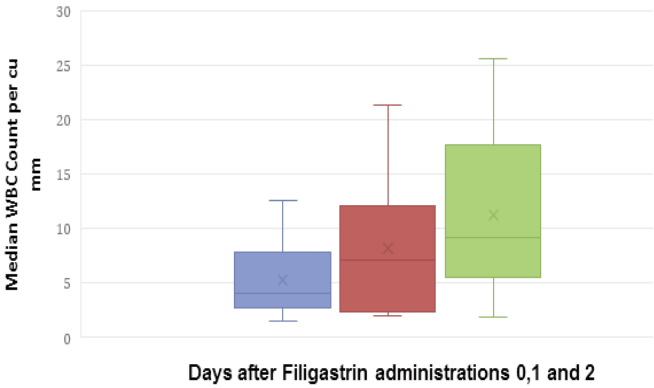

Figure-2: Median WBC count on days 1 and 2 after G-CSF (Filgrastim) administration.
WBC: White blood cells, G-CSF: Granulocyte colony-stimulating factor.

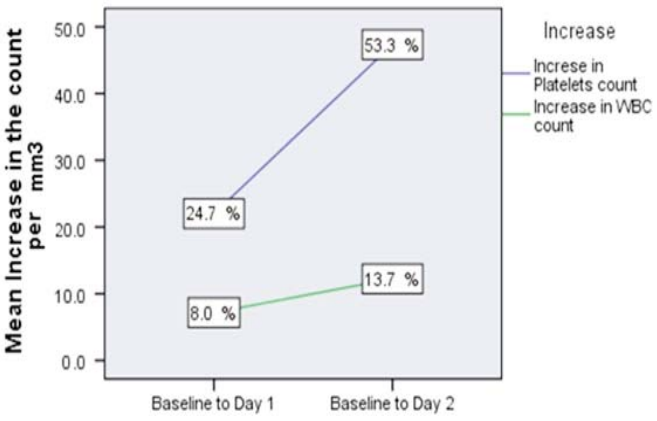

Figure-3: Platelet and WBC counts after G-CSF (Filgrastim) administration.
WBC: White blood cells, G-XSF: Granulocyte colony-stimulating factor.

Table-2: Comparison of platelet count at admission and discharge after the administration of G-CSF (Filgrastim).

Platelet Count per mm3	n	Median platelet count at admission per mm3	Median platelet count at discharge per mm3	Difference (%)
<10000	4	9.6	42.0	33.7
10001 – 20000	25	17.5	53.3	20.3
20001 – 30000	18	23.7	65.9	17.8
>30000	3	34.0	74.0	11.7

G-CSF: Granulocyte colony-stimulating factor.

Overall, 72(72%) patients received only one dose of G-CSF.

Post-administration, a significant rise in the median platelet ($p<0.0001$) (Figure 1) and WBC ($p<0.05$) (Figure 2) counts was seen compared to the baseline ($p<0.05$) on day 2.

Correlation analysis indicated a greater increase in platelets count than WBC counts on days 1 and 2 post-G-CSF administration (Figure 3).

Hospitalisation period of patients was 7 days, and patients who recovered from dengue infection were discharged at 5 days post-G-CSF administration.

G-CSF administration also caused increase in the median platelet count at the time of discharge (Table 2).

Discussion

The dengue virus is becoming a serious public health problem on a global scale. It is the most prevalent mosquito-borne illness in Asia, and epidemic outbreaks are common, particularly during the rainy season[19]. Dengue virus is characterised by haematological disorders, such as thrombocytopenia, leukopenia, hypovolaemia, neutropenia, lymphocytosis, hypotension, increased vascular permeability, or shock. One of the most frequent haematological complications of dengue virus is thrombocytopenia, which can result in life-threatening circumstances, including shock or unexpected bleeding[20].

Individuals who have severe thrombocytopenia and a high risk of bleeding may get platelet transfusions. Individuals at high risk for bleeding who have a platelet count below <20,000/mm3 need an immediate platelet infusion[21].

Although it has been demonstrated that platelet infusion can effectively stop bleeding, it is not constantly useful, and can result in problems related to infection, allergies, or recurrent bleeding. In addition, several groups are barred from receiving blood transfusions or other blood-related products because of their religious convictions [22].

Consequently, there is a need for substances that might boost endogenous platelet synthesis. The current study evaluated how G-CSF affected the increases in platelet and WBC counts.

A G-CSF, called filgrastim, has been suggested as a rescue medication for some high-risk individuals who have formed feverish neutropenia because it encourages the formation and development of myeloid cells[16].

A typical person's peripheral blood may be mobilised by the G-CSF on its own to release dedicated precursors, such as colony-forming unit-granulocytes, macrophages, burst-forming unit-erythroid, and mix-colony-forming units as well as long-term culture starting cells.

In a study, 26 healthy autologous transplant candidates aged 21-41 years were mobilised using G-CSF (filgrastim, =13, or lenograstim 13) at a dosage of 7.5g/kg/day subcutaneously for 5 days. Compared to baseline readings, G-CSF treatment increased the peripheral WBC count by 6 times, the absolute neutrophil count (ANC) by 9 times, the actual amount of lymphocytes by 2 times, and the overall number of monocytes (lymphocytes) by 3 times on day[23].

Recombinant G-CSF has been found to mobilise myeloid, erythroid, megakaryocyte, and multi-potent progenitor cells when given in long-term doses[24].

In the current study, data related to 100 patients who had dengue infection with thrombocytopenia or dengue-like fever with thrombocytopenia (persons in which a drop in fever was followed by a rise in leukocytes and platelets). The majority (46%) of patients had extreme thrombocytopenia at start (platelet count: <20,000/mm3). The platelet and WBC count on days 1 and 2 post-G-CSF injections showed a substantial rise ($p=0.001$). Within two days of using G-CSF, the platelet count doubled in 41 individuals, and the WBC did so within a day. Patients having a starting platelet count <100,000/mm^3 saw the greatest rise in platelet count after discharge.

The percentage of patients getting platelets transfusions were less frequent after G-CSF treatment compared to previously[25]. Use of G-CSF was also connected with the majority of people's fever state improving. Hence, in individuals with dengue, G-CSF usage consistently raised platelet and WBC counts within a day. G-CSF may improve the prognosis of the disease by lowering the need for transfusions and feverish spells during dengue therapy[26].

The current retrospective study analysed the data of 100 patients who were diagnosed with DF. The diagnosis was further confirmed by rapid dengue NS1 antigen, dengue IgG and IgM antibody tests. Overall, 18 patients showed signs of bleeding and platelet transfusion procedure was used to prevent bleeding in patients with low platelet count. Also, 46% participants had experienced serious thrombocytopenia (platelet count <20,000/mm^3). After G-CSF administration, there was a significant increase in platelet count from baseline (21,250/mm^3) to day 2 (42,000/mm^3). This improvement was also observed in WBC count from baseline (6.20/mm^3) to day 2 (10.16/mm^3). G-CSF, as such, proved to be a good alternative for dengue patients.

The current study has limitations owing to a small sample size, and platelet transfusion had been performed in some patients. Larger, prospective randomised controlled trials (RCTs) are needed to validate the findings.

Conclusion

G-CSF administration in DF patients raised platelet and WBC counts within a day, shortened the duration of fever, and treated dengue.

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Conflict of Interest: None.

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References

1. Khan U, Azeem S. The rising toll of dengue cases in Pakistan every year: An incipient crisis. Ann Med Surg. 2022; 76:103549 doi: 10.1016/j.amsu.2022.103549.
2. Basurko C, Matheus S, Hildéral H, Everhard S, Restrepo M, Cuadro-Alvarez E, et al. Estimating the risk of vertical transmission of dengue: a prospective study. The Am J Trop Med Hyg. 2018; 98:1826-32. doi: 10.4269/ajtmh.16-0794
3. McGuigan H. Global Health and emerging infectious diseases. J Emerg Nurs. 2016; 42:272-5. doi: 10.1016/j.jen.2016.03.006.
4. Messina JP, Brady OJ, Golding N, Kraemer MU, Wint GW, Ray SE, et al. The current and future global distribution and population at risk of dengue. Nat Microbiol. 2019; 4:1508-15. doi: 10.1038/s41564-019-0476-8.
5. Paul RE , Patel AY, Mirza S, Hoch SPF, Luby SP. Expansion of epidemic dengue viral infections to Pakistan. Int J Infect Dis. 1998; 2:197-201. doi: 10.1016/s1201-9712(98)90052-2.
6. Choudary A. Inadequate'diagnosis Dengue patients, dept left vulnerable. [Online] [2011 December 12]. Available from: URL: https://www.dawn.com/news/679877/inadequate-diagnosis-dengue-patients-dept-left-vulnerable
7. Yuan K, Chen Y, Zhong M, Lin Y, Liu L. Risk and predictive factors for severe dengue infection: A systematic review and meta-analysis. Plos One. 2022; 17:e0267186. doi: 10.1371/journal.pone.0267186.
8. Roy SK, Bhattacharjee S. Dengue virus: epidemiology, biology, and disease aetiology. Can J Microbiol. 2021; 67:687-702. doi: 10.1139/cjm-2020-0572.
9. Jahan F. Dengue fever (DF) in Pakistan. Asia Pac Fam Med. 2011; 10:1-4.
10. Teixeira MG, Barreto ML. Diagnosis and management of dengue. BMJ. 2009. 339. doi: 10.1136/bmj.b4338.
11. Halstead SB. Dengue. Lancet. 2007; 370:1644-52. doi: 10.1016/S0140-6736(07)61687-0.
12. Wang WH, Urbina AN, Chang MR, Assavalapsakul W, Lu PL, Chen YH, et al. Dengue hemorrhagic fever–A systemic literature review of current perspectives on pathogenesis, prevention and control. J Microbiol Immunol Infect. 2020; 53:963-78.
13. Jayanthi HK, Tulasi SK. Correlation study between platelet count, leukocyte count, nonhemorrhagic complications, and duration of hospital stay in dengue fever with thrombocytopenia. J Fam Med Prim Care. 2016; 5:120-3. doi: 10.4103/2249-4863.184635.
14. Assir MZK, Kamran U, Ahmad HI, Bashir S, Mansoor H, Anee SB, et al. Effectiveness of platelet transfusion in dengue fever: a randomized controlled trial. Transfus Med Hemother. 2013; 40:362-8.doi: 10.1159/000354837.
15. Wunderink R, Leeper KJr, Schein R, Nelson S, DeBoisblanc B, Fotheringham N, et al. Filgrastim in patients with pneumonia and severe sepsis or septic shock. Chest. 2001; 119: 523-9.
16. Deepak D, Garg R, Pawar M, Banerjee N, Solanki R, Maurya I. Filgrastim as a rescue therapy for persistent neutropenia in a case of dengue hemorrhagic Fever with acute respiratory distress syndrome and myocarditis. Case Rep Anesthesiol. 2011; 2011:896783. doi: 10.1155/2011/896783.
17. Bhatt P, Sabeena SP, Varma M, Arunkumar G. Current understanding of the pathogenesis of dengue virus infection. Curr Microbiol. 2021; 78:17-32. doi: 10.1007/s00284-020-02284-w.
18. Charan J, Biswas T. How to calculate sample size for different study designs in medical research? Indian J Psychol Med. 2013; 35:121-6. doi: 10.4103/0253-7176.116232.
19. Balkam FA. Hematological Changes Among Patients With Dengue Fever. Abhath J Basic Appl Sci. 2022; 1:72-82.
20. Yuan K, Chen Y, Zhong M, Lin Y, Liu L. Risk and predictive factors for severe dengue infection: A systematic review and meta-analysis. Plos One. 2022; 17:e0267186. Plos One. 2022; 17:e0267186. doi: 10.1371/journal.pone.0267186.
21. Makroo, R. N., Raina, V., Kumar, P., & Kanth, R. K. Role of platelet transfusion in the management of dengue patients in a tertiary care hospital. Asian J Transfus Sci. 2007; 1:4-7.
22. Chand NK, Subramanya HB , Rao GV. Management of patients who refuse blood transfusion. Indian J Anaesth. 2014; 58:658-64. doi: 10.4103/0019-5049.144680.
23. Chaisiripoomkere W, Jootar S, Ungkanone A. Effect of G-CSF on peripheral blood progenitor cell mobilization and collection from healthy donors. Asian Pac J Allergy Immunol. 2001; 19: 183-90.
24. Sonoda Y , Ohno Y, Fujii H, Takahashi T, Nakayama S, Haruyama H, et al. Multilineage response in aplastic anemia patients following long-term administration of filgrastim (recombinant human granulocyte colony stimulating factor). Stem Cells. 1993; 11: 543-54. doi: 10.1002/stem.5530110624.
25. Price, TH, Michael B, Ryan WH, Jeffrey M, Ness PM, Strauss RG, et al. "Efficacy of transfusion with granulocytes from G-CSF/dexamethasone–treated donors in neutropenic patients with infection." Blood. 2015; 126: 2153-61. doi: 10.1182/blood-2015-05-645986
26. Baid DD, V Jain. Effects of Filgrastim on Thrombocytopenia in Patients with Dengue Fever: A Retrospective Study. [Online] [Cited 2018 June 15]. Available from URL: https://medwinpublishers.com/VIJ/VIJ16000174.pdf

Author's Contributions

KAK: Primary writer, design and conception.

SUQ: Literature review, data analysis, final revision.