ORIGINAL ARTICLE

Thrombocytopenia in Dengue Fever and Its Correlation with Serum Vitamin B12 Level

Muhammad Nadeem Ahmed Khan[1*]

[1]Bahria University Medical and Dental College, Karachi, Pakistan

ABSTRACT

Background: The most prevalent and significant arboviral disease affecting humans is dengue. It is spread by the Aedes genus of mosquitoes, which are common in tropical and subtropical regions of the world. Thrombocytopenia occurs commonly among patients suffering from dengue fever, making them more prone to hemorrhagic complications. The predisposing factors leading to thrombocytopenia and bleeding manifestations after dengue infection are not well-known

Objective: We attempted to determine the frequency of thrombocytopenia and its co-relation with serum vitamin B12 levels among dengue patients.

Methods: This cross-sectional study after taking approval from the hospital ethical committee was carried out at the Department of Medicine PNS Rahat Hospital from June, 2022 to November, 2022. A total of 145 diagnosed patients suffering from dengue fever fulfilling the inclusion criteria were enrolled. Patients with concomitant sepsis, malignancy, autoimmune disorder, hematological disorder, and drugs causing thrombocytopenia were not included in the study. Precise history and biodata were entered in the Pro forma. Patients were investigated for CBC, VIT B12 LEVEL, and other routine investigations. SPSS version 27 was used for data analysis. P-value≤0.05 was considered as statistically significant.

Results: Out of a total of 145 patients, 78 (53.8%) were males and 64 (46.2%) were females With a mean age of 38.22±15.93 years.139 patients (95%) had thrombocytopenia. we found a significant positive correlation of thrombocytopenia with Vitamin B12 deficiency (r=0.731, p<0.001).

Conclusion: Dengue fever patients with thrombocytopenia were found to have vitamin B12 deficiency. Thrombocytopenic patients may require more platelet transfusion and longer hospitalizations.

Keywords: Dengue fever, thrombocytopenia, vitamin B12, hemorrhagic complications, predisposing factors.

INTRODUCTION

Dengue fever also known as break bone fever is an abroviral disease caused by dengue virus (DENV) serotype 1-4 transmitted by the Aedes aegypti mosquito. As per WHO, approximately half of the global population is at risk for dengue, ranging from 100-400 million cases annually [1]. Regardless of serotype, symptoms are identical among infected persons, which include high fever, retro-orbital headache, muscle and joint pain, nausea, lymphadenopathy, vomiting, flue cough, and rash. The patient may present with any combination of symptoms. Severe dengue manifestations include impaired vascular endothelial permeability, plasma leakage, and decline in platelet levels, hemorrhage, low blood pressure, and shock, which may lead to higher morbidity and mortality [2, 3]. The incidence of dengue fever has increased significantly across the globe, 40-50% of the global population is at risk of this disease in tropical, subtropical, and, most recently, more temperate areas [4].

The Epidemiology of dengue fever in the Indian subcontinental is quite complicated and it has transformed over the years as well concerning the strains, affected regions, and disease severity. Investigations for dengue fever include complete blood count with peripheral film, liver fiction tests, renal function, serum vitamin B12 levels, folic acid levels, and coagulation profile. No large epidemiological study has been done to assess serum B12 levels in the population but few studies suggest that serum vitamin B12 deficiency is very common in the Pakistani population [5, 6].

Due to nonspecific symptoms in the majority of cases, many cases of dengue fever are labeled as febrile illness of other etiology and remain undiagnosed [7]. Dengue fever is usually a self-limited disease and if detected early and treated timely its mortality is less than 1%. Treated severe dengue has a mortality of 2% to 5% but mortality is had high as 20% among untreated cases [8].

The etiology of the dengue virus includes replication of viral mainly in macrophages, direct infection of the skin by a virus, and immunological and chemical-mediated mechanisms induced by host–viral interaction [9].

Low platelet count may be linked to alterations in megakaryocytopoiesis, manifested by infection of human hematopoietic cells and impaired progenitor cell growth, leading to platelet dysfunction, damage, or depletion, contributing to significant hemorrhages [10, 11]. Approximately 10% of patients with

108 ISSN: 2708-9134 (Online) Liaquat National Journal of Primary Care 2024; 6(2): 108-112

symptomatic B12 (cobalamin deficiency) have significant thrombocytopenia [12]. Thrombocytopenia is categorized as Mild (1 to 1.5lac/μl) Moderate (50,000 to 1 lac/μl) and Severe (less than 50,000 lac/μl) [13,14]. Likewise, serum vitamin B12 deficiency is classified as normal (>300pg/mL), low normal (200-300 pg/ml) and deficient (200-300 pg/ml) [15].

Infected patients with vitamin B12 deficiency are more likely to develop significant thrombocytopenia. Our study is conducted to determine the correlation between the severity of thrombocytopenia, hospital stay, and hematological, and biochemical parameters with vitamin B12 deficiency and to predict the likely role of vitamin B12 replacement in patients with dengue fever.

METHODOLOGY

After obtaining approval from the hospital ethical committee (ERC/PNR/001/2022), this cross-sectional study was conducted at the Department of Medicine PNS Rahat Hospital, Karachi from June, 2022 to November, 2022. History and clinical examination were done as per proforma. All relevant investigations were carried out and recorded. Patients were labeled to have dengue fever based on positive dengue NS1 or dengue serology (dengue IgM). Platelet counts and serum vitamin B12 levels were checked on admission before starting treatment.

Sample Size was calculated by the wnarifin sample size calculator (available at https://wnarifin.github.io/ssc/ss1prop.html) by taking the prevalence of thrombocytopenia among dengue patients=40.3% [16], margin of error=8% at 95% confidence interval. The calculated sample size was 145 patients. All confirmed cases of dengue fever aged between 18-50 years were included while patients refusing to give consent, pregnant females, those patients suffering from sepsis, malignancy, autoimmune disease, chronic kidney disease, chronic liver disease, on chemotherapy, and hematological disorders were excluded from the study. Patients with co-infections such as malaria, enteric fevers, *etc.* Taking drugs known to cause thrombocytopenia was also not included in the study.

STATISTICAL ANALYSIS

IBM SPSS Statistics version 27 was used for data analysis. Mean and standard deviation were computed for quantitative variables while frequency and percentage were reported for qualitative variables. Fisher exact test was applied to check the association between qualitative variables. Pearson's coefficient correlation was computed to determine the relationship between quantitative variables. P-value≤0.05 was considered as statistically significant.

RESULTS

A total of 145 patients were included in the study out of which 53.8% were males and 46.2% were

Table 1: Descriptive statistics of study population (n=145).

Variables	n (%)
Gender	
Male	78(53.8)
Female	67(46.2)
Age(years); mean± Std. Dev	38.22±15.93
Age Group	
≤30 years	55(37.9)
>30 years	90(62.1)
Hemoglobin(g/dl); mean± Std. Dev	13.20±1.67
Anemia	
Yes	54(37.2)
No	91(62.8)
Mean corpuscular volume (μm³); mean± Std. Dev	86.83±10.99
Mean Corpuscular Volume Group	
Abnormal	58(40)
Normal	87(60)
Total leukocyte count(/cmm); mean± Std. Dev	4.51±2.08
Total Leukocyte Count Group	
Low	80(55.2)
Normal	65(44.8)
Hospital Stay Duration (days); mean± Std. Dev	3.71±1.43
≤3 days	71(49)
>3 days	74(51)
Vitamin B12 Levels (pmol/L); mean± Std. Dev	220.29±119.14
Vitamin B12 Deficiency	
Yes	118(81.4)
No	27(18.6)
Platelets Count (× 109/L); mean± Std. Dev	65.41±40.77
Thrombocytopenia	
Yes	139(95.9)
No	6(4.1)
Thrombocytopenia Severity (n=139)	
Mild	15(10.8)
Moderate	54(38.8)
Severe	70(50.4)

females with a mean age of 38.22±15.93 years. Mean hemoglobin, mean corpuscular volume, total leukocyte count, hospital stay duration, vitamin B12 levels, and platelets count was 13.20±1.67 g/dl, 86.83±10.99 μm3, 4.51±2.08 /cmm, 3.71±1.43 days, 220.29±119.14 pg/ml and 65.41±40.77 × 109/L respectively. We found 81.4% of patients were with vitamin B12 deficiency and 95.9% had thrombocytopenia as presented in Table **1**.
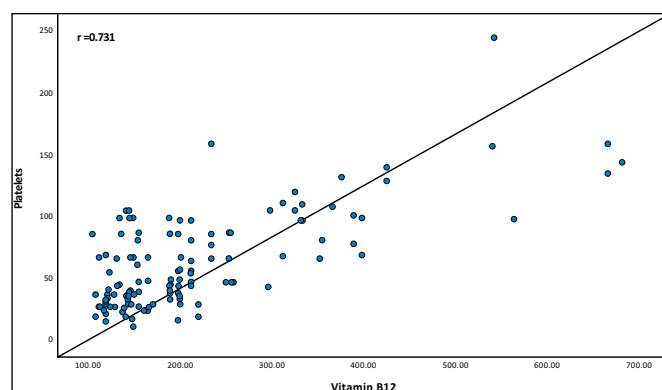
Fig. (1): Relationship between platelets and vitamin B12.

Table 2: Association of thrombocytopenia with population characteristics.

Variables	Thrombocytopenia		p-value
	Yes n(%)	No n(%)	
Gender			
Male	76(54.7)	2(33.3)	0.415
Female	63(45.3)	4(66.7)	
Age Group			
≤30 years	51(36.7)	4(66.7)	0.200
>30 years	88(63.3)	2(33.3)	
Anemia			
Yes	52(37.4)	2(33.3)	1.000
No	87(62.6)	4(66.7)	
Mean Corpuscular Volume Group			
Abnormal	56(40.3)	2(33.3)	1.000
Normal	83(59.7)	4(66.7)	
Total Leukocyte Count Group			
Low TLC	76(54.7)	4(66.7)	0.691
Normal	63(45.3)	2(33.3)	
Hospital Stay Duration			
≤3 days	65(46.8)	5(83.3)	0.107
>3 days	74(53.2)	1(16.7)	
Vitamin B12 Deficiency			
Yes	117(84.2)	1(16.7)	<0.001
No	22(15.8)	5(83.3)	

Fisher exact test was applied, and a P-value≤0.05 was considered significant.

We found a significant and strong relationship between platelet count and serum vitamin B12 level (p<0.001) as presented in **Fig. (1)**, we found a significant association of thrombocytopenia with Vitamin B12 deficiency (r=0.731, p<0.001). No significant association was found with gender (p=0.415), age group (p=0.200), anemia (p=1.000), mean corpuscular volume group (p=1.000), total leukocyte count group (p=0.691) and hospital stay (p=0.107) as presented in Table **2**.

Males are more likely than females to have thrombocytopenia, according to univariate logistic regression (OR=2.413, p=0.318). In addition, it was discovered that patients with anemia were more likely to have thrombocytopenia than non-anemic patients (OR=1.950, p=0.840). Additionally, we discovered that patients with vitamin B12 insufficiency had a higher likelihood of developing thrombocytopenia than those who did not (OR=26.591, p=0.003). Table **3** displays the odds results in detail.

Results also show that the severity of thrombocytopenia is linearly linked to the severity of vitamin B12 deficiency as shown in Table **4** and **Fig. (2)**.

Table 3: Odds ratio for thrombocytopenia.

Variables	Odds Ratio (95% CI)	p-value
Gender		
Male	2.413(0.428-13.608)	0.318
Female®	1	-
Age Group		
≤30 years	0.290(0.051-1.638)	0.161
>30 years®	1	-
Anemia		
Yes	1.195(0.212-6.755)	0.840
No®	1	-
Mean Corpuscular Volume Group		
Abnormal	1.349(0.239-7.618)	0.734
Normal®	1	-
Total Leukocyte Count Group		
Low TLC	0.603(0.107-3.402)	0.567
Normal®	1	-
Hospital Stay Duration		
≤3 days	0.176(0.020-1.543)	0.117
>3 days®	1	-
Vitamin B12 Deficiency		
Yes	26.591(2.962-238.74)	0.003
No®	1	-

CI: Confidence interval, **®:** Reference group, binary logistic regression was applied.
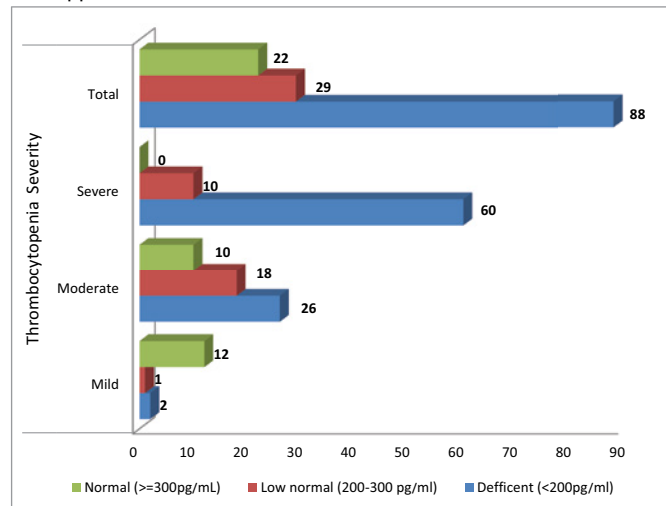
Fig. (2): Relationship According To Severity between Platelets and Vitamin.

DISCUSSION

The etiology of thrombocytopenia is multifactorial; notably, in a misdiagnosis this condition may be due to pre-analytical laboratory artifacts. Knowledge about the common etiology of thrombocytopenia will assist clinicians in decision-making and interpretation of laboratory tests and this may lead to prompt, adequate patient management and cost-saving measures.

Table 4: Thrombocytopenia Associated With Vitamin B12 Deficiency.

Vitamin B12 Severity	Thrombocytopenia Severity				p-value
	Mild n(%)	Moderate n(%)	Severe n(%)	Total n(%)	
Deficient (<200pg/ml)	2(13.3)	26(48.10)	60(85.7)	88(63.3)	
Low normal (200-300 pg/ml)	1(6.7)	18(33.3)	10(14.3)	29(20.9)	<0.001
Normal (>=300pg/mL)	12(80)	10(18.5)	0(0)	22(15.8)	

One of the major reasons leading to hospitalization among patients suffering from dengue fever is thrombocytopenia and its related complications. The etiology of thrombocytopenia is multifactorial. Knowledge of the common etiology of thrombocytopenia helps clinicians make decisions and interpret laboratory tests that can lead to prompt, appropriate patient care and cost-saving measures. Approximately 10% of symptomatic B12 deficient individuals have significant thrombocytopenia [17], thus patients who are already vitamin B12 deficient are more prone to develop significant thrombocytopenia. Our study was carried out with the thought to determine the factors contributing to the severity of thrombocytopenia and its recovery time.

Vitamin B12 is an important co-factor for the synthesis of cells in bone marrow and its deficiency may lead to anemia, leukopenia as well as thrombocytopenia. Patients suffering from thrombocytopenia due to dengue fever are not routinely screened for deficiency of vitamin B12. Deficiency of vitamin 12 is prevalent in our population so our study aimed to find out the correlation between vitamin B12 deficiencies and with severity of thrombocytopenia among dengue patients.

Our study shows that vitamin B12 deficiency is linked to thrombocytopenia. Out of 145 dengue patients enrolled in our study, 118(81.4%) patients were deficient in vitamin B12 and 139 patients (95.9%) had thrombocytopenia. It was also noted that none of the patients with normal vitamin B12 levels had severe thrombocytopenia. Most of the patients (85.7%) with severe thrombocytopenia had vitamin B12 deficiency.

A similar study conducted in India showed among 75 dengue patients, 81% (61 patients) with thrombocytopenia had a deficiency of vitamin B12 (levels less than 300pg/l), and 94% of patients with severe thrombocytopenia were vitamin B12 deficient [18].

Another study carried out in Bangalore India showed among the patients suffering from dengue fever, those without thrombocytopenia and mild thrombocytopenia, 100% had normal Vitamin B12 levels whereas those with Moderate thrombocytopenia, 62.5% had Vitamin B12 Deficiency and among those with severe thrombocytopenia, 94.4% had Vitamin B12 deficiency [19].

Tak *et al.* in their study showed that a deficiency of vitamin B12 is one of the risk factors for the severity of thrombocytopenia among dengue patients specifically in the Indian population [20]. A study conducted among the patients admitted with acute febrile illness demonstrated that in patients with vitamin B12 level of <100 pg/L, 60% had severe thrombocytopenia and 40% had moderate thrombocytopenia whereas, among patients with vitamin B12 101-200 pg/L, 74% had severe thrombocytopenia, moderate thrombocytopenia in 8%, and mild thrombocytopenia in 17% of patients [21].

Another study carried out in India also shows that a deficiency of vitamin B12 is a contributing factor to the development of thrombocytopenia [22].

Thrombocytopenia is a common clinical condition that is associated with multiple systemic diseases [23]. Our study was conducted in a single healthcare setup and only hospitalized patients were included so a larger study is needed for confirmation of our findings. Since we determined a relationship between serum B12 levels and the degree of thrombocytopenia, the next logical step should be to check the patient's response to B12 replacement among dengue patients.

CONCLUSION

Dengue fever patients with moderate to severe thrombocytopenia have Vitamin B12 deficiency and are more prone to have bleeding manifestations leading to the requirement for platelets transfusion and may lead to a higher length of hospital stay. Vitamin B12 replacement may lead to early recovery from thrombocytopenia and less need for platelet transfusion.

ETHICAL APPROVAL

Ethical approval was obtained from the Institutional Review Committee of PNS Rahat Hospital, Karachi (REF letter No. ERC/PNR-001-2022). All procedures performed in studies involving human participants were by the ethical standards of the institutional and/or national research committee and with the Helsinki Declaration.

CONSENT FOR PUBLICATION

Written informed consent was taken from the participants.

AVAILABILITY OF DATA

The data set may be acquired from the corresponding author upon a reasonable request.

FUNDING

Declared none.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

Declared none.

AUTHOR'S CONTRIBUTION

Muhammad Nadeem Ahmed Khan; Data collection Supervised the statistical analysis did a literature search, interpreted the results, drafted the manuscript, and finalized it.

REFERENCES

1. World Health Organization. Dengue and severe dengue 2023. Accessed: 17 March: https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue

2. Shepard DS, Coudeville L, Halasa YA, Zambrano B, Dayan GH. Economic impact of dengue illness in the Americas. Am J

Trop Med Hyg 2011; 84(2): 200-7. DOI: https://doi.org/10.4269/ajtmh.2011.10-0503

3. Chen LH, Wilson ME. Dengue and chikungunya infections in travelers. Curr Opin Infect Dis 2010; 23(5): 438–44. DOI: https://doi.org/10.1097/qco.0b013e32833c1d16

4. World Health Organization. Dengue and severe dengue fact sheet. WHO. Available at http://www.who.int/mediacentre/factsheets/fs117/en/. April 2017; Accessed: September 28, 2017.

5. Yakub M, Iqbal MP, Kakepoto GN, Rafique G, Memon Y, Azam I, *et al.* High prevalence of mild hyperhomocysteinemia and folate, B12 and B6 deficiencies in an urban population in Karachi, Pakistan. Pak J Med Sci 2010; 26(4): 923-9

6. Soofi S, Khan GN, Sadiq K, Ariff S, Habib A, Kureishy S, *et al.* Prevalence and possible factors associated with anaemia, and vitamin B12 and folate deficiencies in women of reproductive age in Pakistan: analysis of national-level secondary survey data. BMJ Open 2017; 7(12): e018007. DOI: https://doi.org/10.1136/bmjopen-2017-018007

7. Waggoner JJ, Lionel G, Vargas MJ, Gabriela B, Tellez Y, Soda KJ, *et al.* Viremia and clinical presentation in Nicaraguan patients infected with Zika Virus, Chikungunya Virus, and Dengue Virus. Clin Infect Dis 2016; 63(12): 1584-90. DOI: https://doi.org/10.1093/cid/ciw589

8. Dengue. Medscape (2022). Accessed: Nov 16, 2022. Available from: https://emedicine.medscape.com/article/215840-overview.

9. Wu SJ, Grouard-Vigel G, Sun W, Mascola JR, Brachel E, Putvatana R, *et al.* Human skin langerhans cells are targets of dengue virus infection. Nat Med 2000; 6(7): 816-20. DOI: https://doi.org/10.1038/77553

10. Guzman MG, Kouri G. Dengue and dengue hemorrhagic fever in the Americas: Lessons and challenges. J Clin Virol. 2003; 27: 1-13. DOI: https://doi.org/10.1016/s1386-6532(03)00010-6

11. Revised and Expanded ed. New Delhi: WHO; 2011. World Health Organization. Comprehensive Guidelines for Prevention and Control of Dengue and Dengue Haemorrhagic Fever.

12. SmitSibinga CTh. Neonatology and Blood Transfusion. 1st Ed. Springer 2010.

13. Jali AGP, Nkambule BB. Prevalence and etiology of moderate and severe thrombocytopenia in a tertiary and quaternary centre in KwaZulu-Natal. Afr J Lab Med 2020; 9(1): 799. DOI: https://doi.org/10.4102/ajlm.v9i1.799

14. Gernsheimer T, James AH, Stasi R. How I treat thrombocytopenia in pregnancy. Blood 2013; 121(1): 38-47. DOI: https://doi.org/10.1182/blood-2012-08-448944

15. Ankar A, Kumar A. Vitamin B12 Deficiency. [Updated 2022 Jun 5]. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2022 Jan. Available from: https://www.ncbi.nlm.nih.gov/books/NBK441923/

16. Castilho BM, Silva MT, Freitas ARR, Fulone I, Lopes LC. Factors associated with thrombocytopenia in patients with dengue fever: a retrospective cohort study. BMJ open 2020; 10(9): e035120. DOI: https://doi.org/10.1136/bmjopen-2019-035120

17. Andres E, Affenberger S, Zimmer J, Vinzio S, Grosu D, Pistol G, *et al.* Current hematological findings in cobalamin deficiency. A study of 201 consecutive patients with documented cobalamin deficiency. Clin Lab Haematol 2006; 28(1): 50-6. DOI: https://doi.org/10.1111/j.1365-2257.2006.00755.x

18. Kansara YK, Sharma E. To study of the correlation between vitamin B12 level and thrombocytopenia in dengue fever and malaria. East Afr Scholars J Med Sci 2022; 5(2): 29-34. DOI: https://doi.org/10.36349/easms.2022.v05i02.001

19. Ramalingaiah MT, Jogihalli Mood JK, Narayanashetty S, Bhuvaneshappa RM. The study of clinical profile and vitamin B12 in dengue fever patients. Asian J Med Sci 2021; 12(7): 47-51. DOI: http://dx.doi.org/10.3126/ajms.v12i8.36311

20. Tak S, Rathore JS, Charan SS, Bijarniya R, Lakhotia M. Severe thrombocytopenia in dengue fever and vitamin B12 level. J Assoc Physicians India 2018; 66(9): 61-63.

21. Gayathri BH, Muttanna R, Seetaram NK, Chandrashekar K. A Study of correlation of vitamin B12 levels in patients of febrile thrombocytopenia. J Cardiovas Dis Res 2022; 13(8): 474-80.

22. Paliwal HP, Mittal C, Kalia A, Jain M, Choudhary S, Rijhwani P, *et al.* Correlation of vitamin B12 levels with degree of thrombocytopenia in dengue patients. J Mahatma Gandhi Univ Med Sci Tech 2022; 7(1): 1-3.

23. Stasi R. How to approach thrombocytopenia. Hematol Am Soc Hematol Educ Program 2012; 2012(1): 191-7. DOI: https://doi.org/10.1182/asheducation.V2012.1.191.3798260