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B12 Deficiency: Hidden Player in Dengue-Induced Thrombocytopenia

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ABSTRACT

Dengue fever is a significant health challenge in tropical and subtropical countries. Because it increases the likelihood of thrombocytopenia with hemorrhagic symptoms, treating thrombocytopenia is a serious clinical concern. According to some studies, certain vitamin status may impact platelet deficiency and hence the clinical outcome of the disease. **Objectives:** To determine the association between the severity of thrombocytopenia, bleeding manifestations, and the severity of Dengue fever with Vitamin B12 deficiency. **Methods:** It was a prospective cohort study carried out over 4 months. A total of 139 patients were included in the study. Serial blood counts and Vitamin B12 were checked. Patients were observed for any bleeding manifestations and need for transfusion of Blood components. **Results:** Among 139 patients, 46% exhibited moderate thrombocytopenia, 30.9% showed severe thrombocytopenia and 5.8 % had a very severe drop in platelet count. A large proportion (82.7%) had normal B12 levels and cross-tabulation between serum B12 levels and severity of thrombocytopenia was not significant (p-value=0.34). **Conclusion:** It was concluded that the severity of thrombocytopenia was not associated with underlying Vitamin B12 deficiency.

INTRODUCTION

Dengue Virus is an enveloped RNA, a single-stranded virus that belongs to the flavivirus group. There are four different serotypes. Dengue Virus is responsible for causing Dengue Fever, a vector-borne disease spread by mosquitoes [1]. High-grade fever, retro-orbital discomfort, headache, joint pain, vomiting, abdominal pain, and bleeding manifestations are the hallmarks of dengue fever. Dengue infection can occasionally result in harmful symptoms like dengue shock syndrome and dengue hemorrhagic fever [2, 3]. Recent studies show Pakistan has an endemic Dengue Virus infection, most prevalent during the monsoon season. During 2022, around 78,554 confirmed cases of dengue fever were reported in Pakistan. Three large outbreaks of Dengue Fever in Pakistan in 2006, 2010, and 2011 affected almost 40,000 people [4]. Thrombocytopenia and leucopenia are significant laboratory markers of Dengue Fever. Several mechanisms, including bone marrow suppression, antibody-mediated destruction, and peripheral platelet consumption, have been suggested as potential causes of thrombocytopenia [5]. Platelet counts show a sharp decline during the early days of Dengue infection leading to mucosal bleeding and may need transfusion of platelets or RCC (red cell concentrate) occasionally. Hemorrhagic manifestations are one of the most common serious complications which are associated with high morbidity and mortality in Dengue Hemorrhagic

Fever and Dengue Shock Syndrome [6, 7]. Interestingly the degree of thrombocytopenia in Dengue Fever is correlated with Vitamin B12 deficiency. Several studies have demonstrated a link between marked thrombocytopenia with bleeding symptoms and a prolonged hospital stay with Vitamin B12 deficiency [8]. Vitamin B12 is essential for DNA synthesis and cellular proliferation, particularly in hematopoietic cells of the bone marrow. Animal-based meals can provide Vitamin B12, which is primarily stored in the liver of humans [9]. Before signs of Vitamin B12 insufficiency appear, liver reserves are adequate for three to five years. Dietary insufficiency, chronic gastritis, particularly Helicobacter-associated gastritis, terminal ileum surgery, intrinsic factor deficiency, pancreatitis, and fish tapeworm infestation are a few of the reasons for Vitamin B12 deficiency [10, 11]. In processes necessary for pyrimidine and purine biosynthesis, including the production of DNA nucleotides, folate and cobalamin are both necessary to maintain one-carbon metabolism. Therefore, a deficiency in vitamin B12 leads to a disruption in DNA synthesis, which is followed by less significant alterations in RNA and protein synthesis, which finally results in disturbed cell division and unbalanced cellular proliferation. [12]. Nutritional deficiency of Vitamin B12 is prevalent in the subcontinent and is predominantly caused by poor nutrition or malabsorption [11]. A recent study revealed that a lack of vitamin B12 is the third most common cause of pancytopenia in children in Pakistan [13]. The clinical manifestations of Vitamin B12 deficiency are varied including hematological, gastrointestinal, and neurological. Severe Vitamin B12 deficiency can lead to pancytopenia due to bone marrow suppression [10]. Vitamin B12 deficiency reduces platelet production by about 10% in individuals, mostly due to impaired thrombopoiesis and aberrant platelet function [14]. Several studies have suggested that Vitamin B12 deficiency may have a role in the development of severe thrombocytopenia in people who have Dengue Fever, particularly in the subcontinent [15]. Thrombocytopenia results in mucosal membrane bleeding is the main concern with dengue fever. With Dengue Fever looming around the corner, there are many herbal treatments available to treat thrombocytopenia, which may lead to irrational drug and platelet product use due to a fear of bleeding. In Pakistan, a third-world nation, health facilities are few and inaccessible. Products made from platelets are expensive and not widely accessible for many people.

This study aims to determine whether severe thrombocytopenia in dengue fever patients is caused by concomitant Vitamin B12 insufficiency. If a link exists between Serum Vitamin B12 levels and the degree of thrombocytopenia, treating Vitamin B12 deficiency will prevent marked thrombocytopenia and the subsequent requirement for platelet transfusions.

METHODS

This prospective cohort study was carried out in Department of Medicine Units 1 and 2 of Pakistan Air Force (PAF) Hospital Islamabad from July 2023 to October 2023 over 4 months. Necessary ethical approval was taken before the start of the study with reference number 230601. The study included all individuals with dengue fever who were older than 13 and who visited the PAF hospital's outpatient department or emergency room. Dengue fever was confirmed by non-structural protein 1 (NS1) Antigen and Immunoglobulin M (IgM) serology. Patients with strong clinical suspicion were included in the study in some cases. All individuals with underlying liver disease, malignancy, sepsis, hematological disorders, and those who were using any drugs that could lead to thrombocytopenia were excluded from the study. Following informed consent, demographic data were acquired. All confirmed cases of Dengue Fever fulfilling the admission criterion were admitted and their serum vitamin B12 was checked. Serial blood counts were performed and a baseline complete blood count (CBC) was obtained. The minimum platelet count, length of hospital stay, any bleeding manifestations requiring blood products transfusion, and any complications from Dengue Hemorrhagic Fever and Dengue Shock Syndrome were evaluated for the patient. For Outpatient Department (OPD) cases, Vitamin B12 was checked at presentation. CBC was done initially and patients were called for follow-up with serial CBC. Patients were divided into four groups according to the severity of thrombocytopenia where mild was defined as a platelet count of more than $1 \text{ lacx}10^9/\text{L}$, moderate was a platelet count of 50,000 to $1 \text{ lacx}10^9$, severe 20,000–50,000 and very severe with platelet count less than $20,000 \text{x}109/\text{L}$. The WHO sample size calculator calculated the sample size; keeping the percentage positivity of the study outcome at 52%, a confidence interval of 90%, and 7.1% precision, a Sample size of 139 participants was considered adequate [16]. Participants were included using non-probability convenient sampling. Data were entered and analyzed using SPSS version 23.0. While mean and standard deviation were used to summarize continuous variables, frequencies, and percentages were used to characterize categorical variables. Cross-tabulation was done between vitamin B12 levels and several patient groups based on the decrease in platelets. A p-value of less than 0.05 was deemed noteworthy.

RESULTS

A total number of 139 patients were included, with a mean age of 36.6 + 1.4 years. Among them, 68.3% were male, while 31. 7% were female. Out of 139, the majority 76.3% of

the patients had no co-morbid. Dengue was confirmed by NS1 antigen in 89%. Only 15.8% of patients had a history of prior dengue infection. The majority of patients, (82.7%) had normal Serum B12 levels. The demographics of participants are shown in Table 1.

Table 1: Demographic Characteristics of study participants

| Demographics	Mean+ SD/ n=139 (%)
Age	36.6 + 1.4 Years
Up To 35 Years	83 (59.7)
Gender	
Male	95 (68.3)
Female	44 (31.7)
Comorbidities	
None	106 (76.3)
Diabetes	17 (12.2)
Hypertension	6 (4.3)
COPD	3 (2.2)
Others	7 (5)
Diagnostic Test	
NS1 Antigen	125 (89.9)
IGM Serology	9 (6.5)
Clinical Diagnosis	5 (3.6)
History of Previous Dengue Fever	
Yes	22 (15.8)
No	117 (84.2)
Serum Vitamin B12 Levels	
Normal	115 (82.7)
Deficient	24 (17.3)

COPD: Chronic Obstructive Pulmonary Disease

The mean duration of hospital stay was 4.24 + 1.75 days. Among 139 patients with dengue fever, 16.5% exhibited mild (>100,000x109 /L) thrombocytopenia. Moderate (50,000-100,000x109/L) thrombocytopenia had been observed in 46.8% of patients. However, 30.9% experienced severe (20,000-50,000x109/L) and only 5.8% expressed very severe (<20,000x109/L) thrombocytopenia. Out of 139, 12.2% suffered from Dengue hemorrhagic fever, and only 23 (16.6%) exhibited bleeding manifestations, amongst them 10.1% showed petechial hemorrhages and 2.9% had a Gastrointestinal (GI) bleed. However, only 5 (3.6%) required transfusion of blood product (RCC/Platelet). Only one patient died due to arrhythmia secondary to viral myocarditis. The characteristics of Dengue fever are shown in Table 2.

Table 2: Course of Dengue Fever

Characteristics	Mean+ SD/ n=139 (%)
Length of Hospital Stay	4.24 + 1.75
1-3 Days	70 (50.4)
4-7 Days	56 (40.3)
More Than 7 Days	13 (9.4)

Thrombocytopenia	
Mild	23 (16.5)
Moderate	65 (46.8)
Severe	43 (30.9)
Very Severe	8 (5.8)
Dengue Hemorrhagic Fever	
Yes	17 (12.2)
No	122 (87.8)
Bleeding Manifestation	
None	116 (83.4)
Petechial Hemorrhages	14 (10.1)
Nasal/ Gum Bleed	3 (2.2)
GI Bleed (Melena)	4 (2.9)
Hematuria	2 (1.4)
Transfusion (Platelet/RCC)	
Yes	5 (3.6)
No	134 (96.4)
Outcome Of Dengue Fever	
Recovered	138 (99.3)
Died	1 (0.7)
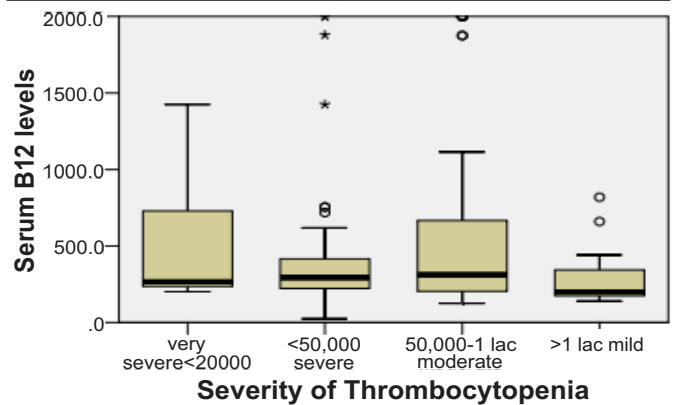
Figure: 1 Association of Thrombocytopenia with Serum B12 Level in Patients with Dengue Fever

However, the study results show that the severity of thrombocytopenia in dengue patients was significantly associated with their comorbid, occurrence of dengue hemorrhagic fever and need for transfusion with p-value <0.00. However, age, gender, previous dengue infection, bleeding manifestations, and duration of illness were not found to be significantly associated with the severity of thrombocytopenia as shown below in Table 3.

Table 3: Association of Severity of Thrombocytopenia in Patients with Dengue Fever

Variables	n=139 (%)	Mild n (%)	Moderate n (%)	Severe n (%)	Very Severe n (%)	p-value
Age						
Up to 35 Years	83 (59.7)	17 (12.2)	39 (28)	23 (16.5)	4 (2.9)	0.272
36 Years or Above	56 (40.3)	6 (4.3)	26 (18.7)	20 (14.4)	4 (2.8)	
Gender						
Male	95 (68.3)	14 (10.1)	45 (32.4)	28 (20.1)	8 (5.7)	0.210
Female	44 (31.7)	9 (6.5)	20 (14.4)	15 (10.8)	0 (0)	
Comorbid						
None	106 (76.3)	21 (15.1)	48 (34.5)	35 (25.2)	2 (1.4)	<0.00
Diabetes	17 (12.2)	1 (0.7)	12 (8.6)	3 (2.2)	1 (0.7)	
Hypertension	6 (4.3)	1 (0.7)	3 (2.2)	1 (0.7)	1 (0.7)	
COPD	3 (2.2%)	0 (0)	0 (0)	1 (0.7)	2 (1.4)	
Other	7 (5)	0 (0)	2 (1.4)	3 (2.2)	2 (1.4)	
History of Previous Dengue Fever						
Yes	22 (15.8)	7 (5)	7 (5)	6 (4.3)	2 (1.4)	0.136
No	117 (84.2%)	16 (11.5%)	58 (41.7%)	37 (26.6%)	6 (4.3%)	
Dengue Hemorrhagic Fever						
Yes	17 (12.2)	2 (1.4)	4 (2.9)	6 (4.3)	5 (3.6)	<0.00
No	122 (87.8)	21 (15.1)	61 (43.9)	37 (26.6)	3 (2.2)	
Bleeding Manifestation						
None	116 (83.5)	21 (15.1)	55 (39.6)	35 (25.2)	5 (3.6)	0.489
Petechial Hemorrhages	14 (10.1)	1 (0.7)	5 (3.6)	6 (4.3)	2 (1.4)	
Nasal/ Gum Bleed	3 (2.2)	1 (0.7)	1 (0.7)	1 (0.7)	0 (0)	
GI bleed (Melena)	4 (2.9)	1 (0.7)	2 (1.4)	1 (0.7)	0 (0)	
Hematuria	2 (1.4)	0 (0)	2 (1.4)	0 (0)	0 (0)	
Transfusion (Platelet/RCC)						
Yes	5 (3.6)	0 (0)	0 (0)	2 (1.4)	3 (2.2)	<0.00
No	134 (96.4)	23 (16.5)	65 (46.8)	41 (29.5)	5 (3.6)	
Serum Vitamin B12 Levels						
Normal	115 (82.7)	17 (12.2)	53 (38.1)	37 (26.6)	8 (5.8)	0.345
Deficient	24 (17.3)	6 (4.3)	12 (8.6)	6 (4.3)	0 (0)	
Duration of Illness						
1–3 Days	70 (50.4)	8 (5.8)	41 (29.5)	17 (12.2)	4 (2.9)	0.010
4–7 Days	56 (40.3)	9 (6.5)	23 (16.5)	21 (15.1)	3 (2.2)	
More Than 7 Days	13 (9.4)	6 (4.3)	1 (0.7)	5 (3.6)	1 (0.7)	

DISCUSSION

The Discussion section of this study engages deeply with the complex interrelationship between thrombocytopenia severity in dengue fever patients and their serum vitamin B12 levels. Thrombocytopenia, marked by a substantial reduction in platelet count, stands as a defining feature of dengue fever, often contributing to bleeding complications that can significantly impact patient prognosis and recovery [16]. The role of vitamin B12 in cellular proliferation and DNA synthesis, particularly in hematopoiesis, emerges as a crucial factor in understanding the potential exacerbation of thrombocytopenia severity among dengue patients [17]. Emerging evidence suggests that a deficiency in this essential vitamin may exacerbate thrombocytopenia, thereby worsening clinical outcomes in dengue fever patients [8]. This holds particular significance in regions like the Indian subcontinent, where vitamin B12 deficiency is prevalent and may compound the challenges posed by dengue fever [15]. The results of current study provide an overview of various demographic and clinical characteristics among the 139 dengue fever patients. These findings offer valuable insights into the complexities surrounding thrombocytopenia severity, and serum B12 level and their interplay within the context of dengue fever [18]. Notably, a substantial proportion (82.7%) of patients exhibited normal serum vitamin B12 levels. Cross tabulation between the drop in platelets and underlying serum B12 was not significant. These findings underscore the complex multifactorial nature of thrombocytopenia in dengue fever, suggesting that factors beyond nutritional

deficiencies may contribute to its pathogenesis [3]. However, this is in contrast to many studies done by Sagar *et al.,* [15] and Kansara and Sharma [17] in India previously which showed a positive association between the severity of platelet drop and underlying B12 deficiency. Numerous Indian studies revealed a favourable correlation between thrombocytopenia and B12 deficiency. However, what current research showed was the exact reverse. This could be explained by the fact that the majority of Indians are likely vegetarians, and vitamin B12 deficiency is common, thus the association may be coincidental. The demographic profile of patient cohort reveals a predominant representation of younger individuals (59.7%) affected by dengue. This distribution underscores the susceptibility of younger populations to dengue fever, aligning with the known epidemiological patterns of the disease [19]. Furthermore, gender distribution shows a higher prevalence among males, accounting for 68.3% of the patients, consistent with existing literature documenting a slightly increased risk of dengue fever among male [7]. Among the patients, the majority (76.3%) presented with no premorbid conditions, while a small proportion had pre-existing medical conditions such as diabetes mellitus (12.2%), hypertension (4.3%) and chronic obstructive pulmonary disease (COPD) (2.2%). 5% of patients have other premorbid like Interstitial lung disease or hypothyroidism etc. Interestingly the drop in platelets was more severe in patients with comorbid conditions and that relationship was significant. The diagnosis of dengue fever was predominantly confirmed by NS1 antigen testing (89.9%), highlighting the reliability of this diagnostic modality in identifying acute dengue infections. Additionally, a noteworthy proportion of patients (6.5%) were diagnosed based on IgM serology, with a small subset (3.6%) diagnosed based on typical clinical symptoms supported by the blood CP report characteristic of bi-cytopenia. This underscores the importance of utilizing multiple diagnostic approaches to ensure accurate and timely diagnosis, particularly in resource-constrained settings where access to laboratory testing may be limited [4]. Evaluation of platelet counts revealed varying degrees of thrombocytopenia severity among patients, but the incidence of severe bleeding manifestations was relatively low, with only a small percentage of patients experiencing petechial hemorrhage (10.1%) or more severe bleeding such as melena or upper gastrointestinal bleeding 2.9% (4 patients). Out of 139 patients, only 17 (12.2 %) went into DHF (dengue hemorrhagic fever). Importantly, no cases of intracranial hemorrhage were reported, highlighting the overall manageable nature of bleeding complications in current patient cohort. In present study, the average duration of hospital stay was five days, with the majority of patients experiencing a favourable clinical course. However, one unfortunate fatality occurred due to dengue myocarditis with associated arrhythmia, emphasizing the importance of vigilant monitoring for cardiac complications in dengue patients [20]. Greater severity of thrombocytopenia was significantly associated with longer hospital stays. Similar findings are supported by various studies done in India [15, 16]. In summary, we studied the relationship of underlying B12 and folate deficiency with the severity of thrombocytopenia in dengue patients which turned out to be non-significant. The findings emphasize the necessity for further investigation into the mechanisms driving thrombocytopenia in dengue fever and suggest the potential for developing tailored therapeutic interventions to improve patient outcomes in this prevalent disease.

CONCLUSIONS

It was concluded that the severity of thrombocytopenia is not associated with underlying serum B12 deficiency and the relationship is not significant.

Authors Contribution

Conceptualization: UB
Methodology: UB, AS, AA, BR, RA
Formal analysis: AA, BR, RA, TZ
Writing review and editing: TZ

All authors have read and agreed to the published version of the manuscript

Conflicts of Interest

All the authors declare no conflict of interest.

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