

## ORIGINAL ARTICLE

## Severe Thrombocytopenia in Dengue Fever and Vitamin B12 Level

Sandeep Tak<sup>1\*</sup>, Geethu Chachappan<sup>2</sup>, Jagdambe Singh Rathore<sup>2</sup>, Sheshkaran Singh Charan<sup>2</sup>, Ramniwas Bijarniya<sup>2</sup>, Manoj Lakhotia<sup>3</sup>**Abstract**

**Objective:** To document correlation between vitamin B12 deficiency and severity of thrombocytopenia, platelet recovery and duration of hospital stay in dengue fever patients.

**Methods:** This prospective observational study was done in dengue fever patient with severe prolonged thrombocytopenia (<20,000  $\mu$ l and > 2 days duration). Patient with underlying malignancy, hematological disorders, septicemia, or use of any drug which may cause thrombocytopenia, were excluded. Standard statistical methods were used.

**Results:** Total 40 subjects were included in current study. Twenty one were male and mean age was 25 $\pm$ 12 years. Forty percent subjects were having B12 level < 200 pg/L and mean B12 level was 336.9 $\pm$ 362.36 pg/L. SDP requirement was highest in B12<100 pg/L group was (3 $\pm$ 1.41) as compared to other groups. Time required for recovery of platelets to 20000/ $\mu$ l threshold, was also highest in B12<100 pg/L group (5.75 $\pm$ 0.95 days) as compared to other groups. Duration of hospital stay was also highest in B12<100 pg/L group (5.25 $\pm$ 1.25 days) as compared to other groups. There was no relation between B12 levels and other complications of dengue like bleeding, serositis, and shock.

**Conclusion:** Our study suggests that B12 deficiency may be responsible for severe thrombocytopenia; slower platelet count recovery and prolonged hospitalisation in dengue fever patients.

**Introduction**

Dengue is one of the most significant arboviral diseases of humans worldwide. It is predominantly distributed in tropical and subtropical regions that are the natural home for its vector, mosquitoes of the genus *Aedes*.<sup>1</sup> The causative aetiological agent is the Flavivirus genus of family Flaviviridae, otherwise called dengue virus (DENV).<sup>3</sup> Infection with DENV may be subclinical or symptomatic. Dengue fever clinical illness is traditionally classified, in order of increasing severity, as either dengue fever (DF), dengue haemorrhagic fever (DHF) or dengue shock syndrome (DSS). More recently, the WHO proposed a revised classification of clinical infection: dengue; dengue with warning signs; and severe dengue.<sup>1</sup> DF is due to primary infection with any of the serotypes and is typically mild and self-limiting. Recovery from infection is generally complete and confers lifelong

homotypic immunity. DF manifests as a fever for 2–10 days, headache, retroorbital pain, joint and muscle pain with skin rashes.<sup>2</sup> Secondary infection with a other serotype generates cross-reactive antibodies, which increases the potential risk of antibody-dependent enhancement of disease, a form of immunopathology. Hence, recurrent infection is the major risk factor for the serious, often fatal, complications of DHF and the rarer DSS. These are marked by problems of capillary permeability, a decrease in platelet count, disordered blood clotting, severe bleeding, and for DSS alongside systemic shock leading to organ failure.<sup>1,3</sup>

There are no large epidemiological study to assess B<sub>12</sub> level in Indian population but few studies suggest that Vitamin B<sub>12</sub> deficiency is common in Indian populations with prevalence range of 35% to 60%.<sup>8,9</sup> Vitamin B<sub>12</sub> is an important factor required for

erythropoiesis and thrombopoiesis. Approximately 10 % of patients with symptomatic B<sub>12</sub> (cobalamin deficiency) have significant thrombocytopenia.<sup>5</sup> There are case reports associating B<sub>12</sub> deficiency with thrombotic thrombocytopenic purpura (TTP) like picture.<sup>6,7</sup>

However, search on pubmed has failed to reveal any study/case report looking in B<sub>12</sub> level in patients with dengue and severe thrombocytopenia.

**Material and Methods**

We conducted prospective observational study in Department of Medicine at Dr. Sampoorna Nand Medical College, Jodhpur. Admitted patients who were positive for NS1 antigen/IgM/ ELISA for dengue along with platelets counts less than 20000/ $\mu$ l and were showing slow recovery of platelets i.e., persisting below 20000/ $\mu$ l for 2 days or more, were included in the study. Patients with underlying malignancy, hematological disorder, septicemia, or use of any drug which may cause thrombocytopenia, were excluded from study.

Clinical features, hematological and biochemical parameters were noted and vitamin B<sub>12</sub> levels were measured. Single donor platelets (SDP) transfusion was done if patient was actively bleeding or if platelets level were less than 10000/ $\mu$ l. For the analysis purpose patients were divided into four groups according to B12 level were, viz, B12<100 pg/L (n=4), B12 101-200 pg/L (n=12), B12 201-300 pg/L (n=13), and B12>300 pg/L (n=11). Standard statistical methods were used to analyse the data. Patients characteristics were expressed as mean $\pm$  SD for continuous variables and the were compared using Chi-square test.

<sup>1</sup>Professor, <sup>2</sup>Resident, <sup>3</sup>Senior Professor, Department of Medicine, Dr. S.N. Medical College, Jodhpur, Rajasthan;

\*Corresponding Author

Received: 02.08.2017; Accepted: 20.03.2018

**Table 1: Vitamin B12 level and single donor platelets requirement**

B12 group (number)	SDP		ANOVA	P-value
	Mean	Std. deviation		
B12<100 pg/L (4)	3.00	1.414	2.145	.096 Insignificant
B12 101-200 pg/L (12)	1.17	.835		
B12 201-300 pg/L (13)	1.46	1.12		
B12>300 pg/L (11)	1.36	1.286		
Total (40)	1.50	1.198		

**Table 3: Vitamin B12 and hospital stay**

Correlation between	Pearson correlation	P-value	Interpretation
B12 group and SDP	-.190	.239	No correlation
B12 group and number of hospital stay	-.468	.002	Correlation exist
B12 group and platelet recovery	-.644	.000	Correlation exist

## Results

Total forty subjects were included in study. Twenty-one were male and remaining were female subjects. Mean age of the patients were 25±12 year. Twelve patients were non-vegetarian rest all were vegetarian. Forty percent subjects were having B12 level < 200 pg/L and 72% of the patients had B12 level < 300 pg/L and mean B12 level was 336.9±362.36 pg/L. Mean lowest platelets count was 8090±3968/μl (Figure 1). Mean duration of hospital stay was 4.23±1.20 days and mean single donor platelets (SDP) transfusion was 1.5± 1.19 (Figures 2 and 3).

For the analysis purpose patients were divided into four groups according to B12 level were, viz, B12<100 pg/L (n=4), B12 101-200 pg/L (n=12), B12 201-300 pg/L (n=13), and B12>300 pg/L (n=11).

SDP requirement was highest in B12<100 pg/L group was (3±1.41) as compared to other groups (Table 1), but it was not statistically significant (p-value=0.96, ANOVA=2.14). Time required for recovery of platelets to 20000/μl threshold, was also highest in B12<100 pg/L group (5.75±0.95days) as compared to other groups (Table 2 and Figure 4), and it was statistically significant (p-value=0.0, ANOVA=9.07). Duration of hospital stay was also highest in B12<100 pg/L group (5.25±1.25days) as compared to other groups (Table 3), and it was statistically significant (p-value=0.04, ANOVA=2.79) (Table 4).

There was no relation between B12 levels and other complications of dengue like bleeding, serositis, and shock.

## Discussion

Often primary reason for admission in dengue patients is thrombocytopenia and fear factor associated with thrombocytopenia. Duration of admission is also often determined in India by recovery of platelet counts to "safe level" i.e. > 20000/μl. In several studies, there was no direct correlation between severity of thrombocytopenia and dengue complication. In some subset of dengue patients recovery from thrombocytopenia is swift, while in other subset it may take several days. We started this study with presumption that there may be other factors that may be contributing to thrombocytopenia and its slow recovery, during acute hematological/hematopoietic stress. Identifying and correcting contributory factor may probably cut down duration of admission in patients with dengue fever and severe thrombocytopenia. Severe vitamin B12 deficiency may be associated with thrombocytopenia apart from anemia and leukopenia. Vitamin B12 deficiency is common in Indian population; therefore, we planned this pilot study to test the hypothesis that vitamin B12 level may have correlation with prolonged and severe thrombocytopenia in some patients.

In current study requirement for SDP was highest (3.0± 1.41) in a group with vitamin B12 level less than 100pg/L compared to group B12>300 pg/L (1.36±1.2860), although it was not statistically significant. This suggests that severe B12 deficiency may prolong severity of thrombocytopenia, as in current study SDP was transfused only if platelets persisted below 10000/μl.

This was confirmed by another

**Table 2: Vitamin B12 level and platelet recovery time**

B12 group	Platelet Recovery		ANOVA	P-value
	Mean	Std. deviation		
B12<100 pg/L (4)	5.75	.957	9.072	.000 Significant
B12 101-200 pg/L (12)	4.08	1.165		
B12 201-300 pg/L (13)	3.38	0.96		
B12>300 pg/L (11)	3.00	.000		
Total	3.73	1.176		

**Table 4: Vitamin B12 level and duration of hospital stay**

B12 group (number)	Duration of hospital stay		ANOVA	P-value
	Mean	Std. deviation		
B12<100 pg/L (4)	5.25	1.258	2.796	.041 Significant
B12 101-200 pg/L (12)	4.67	1.435		
B12 201-300 pg/L (13)	4.0	1.10		
B12>300 pg/L (11)	3.64	.505		
Total (40)	4.23	1.209		

parameter measured i.e., platelet recovery time (time taken to recover to >20000/μl). This parameter was selected as during in-patient management as the platelets recover beyond 20000/μl and if patient is otherwise fit, she/he is considered for discharge. Platelet recovery time was also maximum in B12<100 pg/L group (5.75±0.95days) as compared to group with B12>300 pg/L (3.0±0days) and it was statistically significant. As it is clear from Table 2 more severe, the B12 deficiency was more prolonged was platelet recovery time.

As discussed earlier, duration of hospital stay is often associated with severity of thrombocytopenia and time it takes to recover to relatively safer level. Similar trend was seen in duration of hospital stay: Hospital stay (in days) was also highest in vitamin B12<100 pg/L group (5.25±1.25days) as compared to group with B12>300 pg/L (3.64±0.5days) and it was statistically significant. As it is obvious from Table 3 as the severity of vitamin B12 deficiency increased so does the hospital stay.

There are no published studies measuring these parameters against vitamin B12 level so we cannot compare with any other study.

Limitation of study: We measured vitamin B12 level only in patients with severe thrombocytopenia as we were testing our hypothesis. Therefore, it had limited number of patients. A larger study is required to confirm these preliminary findings. Best approach would be to measure vitamin B12 level in large cohort of dengue patients and plot it against platelet level. Next logical step should be to see response to injectable B12 supplementation in such patients.

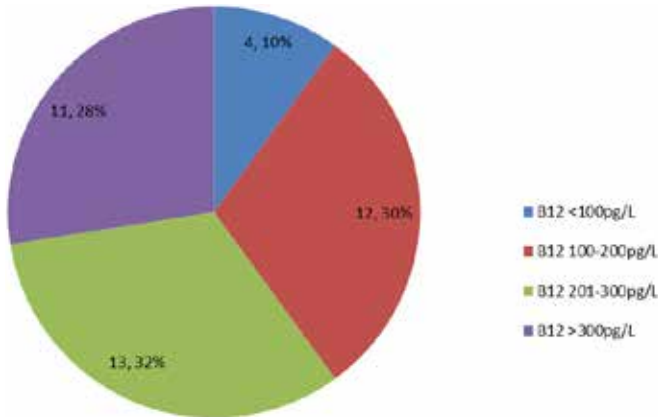


Fig. 1: Vitamin B12 level and severe thrombocytopenia

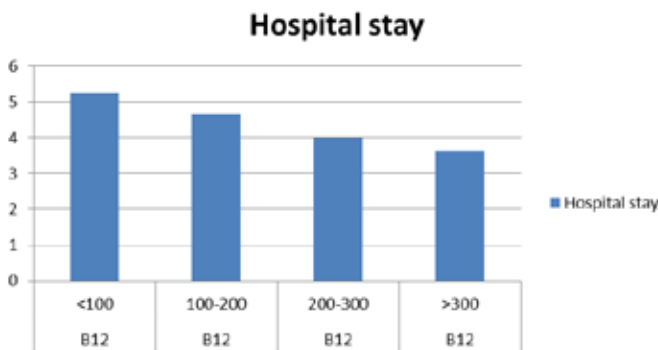


Fig. 3: Vitamin B12 level and duration of hospital stay (in days)

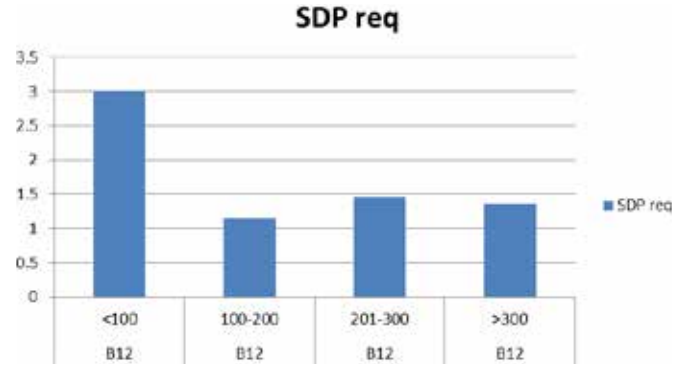


Fig. 2: Vitamin B12 level and single donor platelets requirement

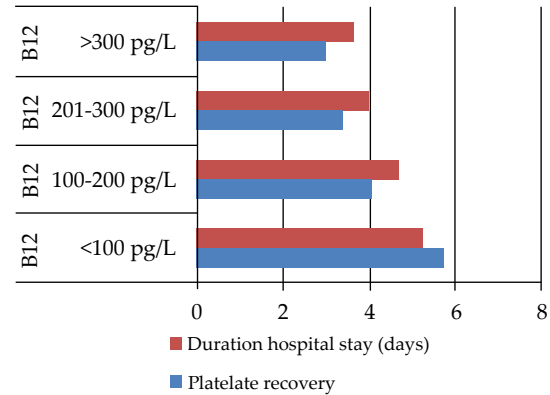


Fig. 4: Vitamin B12 level and duration of hospital stay and platelet recovery time

**Conclusion**

Vitamin B12 deficiency may be a contributing factor to development of severe thrombocytopenia in dengue fever, particularly in Indian population. Severe vitamin B12 deficiency may prolong the hospital stay and increase the requirement of platelets transfusion.

**References**

1. Dengue: Guidelines for diagnosis, treatment, prevention and control. Geneva: World Health Organization 2009; 1-160.

2. Leitmeyer KC, Vaughn DW, Watts DM, Salas R, Villalobos I, de Chacon, et al. Dengue virus structural differences that correlate with pathogenesis. *J Virol* 1999; 73:4738-47.

3. Endy TP. Epidemiology of inapparent and symptomatic acute dengue virus infection: Aprospective study of primary school children in Kamphaeng Phet, Thailand. *Am J Epidemiol* 2002; 156:40-51.

4. Kularatne SA, Gawarammana IB, Kumarasiri PR. Epidemiology, clinical features, laboratory investigations and early diagnosis of dengue fever in adults: a descriptive study in Sri Lanka. *Southeast Asian J Trop Med Public Health* 2005; 36:686-92. PubMed PMID: 16124439.

5. 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; 28:50-6. doi: 10.1111/j.1365-2257.2006.00755.x

6. Chapuis TM, Favrat B, Bodenmann P. Cobalamin deficiency resulting in a rare haematological disorder: a case report. *J Med Case Reports* 2009; 3:80. doi: 10.1186/1752-1947-3-80.

7. Tadakamalla AK, Talluri SK, Besur S. Pseudo-thrombotic thrombocytopenic purpura: A rare presentation of pernicious anemia. *N Am J Med Sci* 2011; 3:472-4. doi: 10.4297/najms.2011.3472.

8. Sivaprasad M, Shalini T, Balakrishna N, Sudarshan M, Lopamudra P, Suryanarayana P, Arlappa N, Ravikumar BP, Radhika MS, Reddy GB. Status of Vitamin B12 and Folate among the Urban Adult Population in South India. *Ann Nutr Metab* 2016; 68:94-102. doi: 10.1159/000442677. PubMed PMID: 26667891.

9. Gupta Bansal P, Singh Toteja G, Bhatia N, Kishore Vikram N, Siddhu A, Kumar Garg A, Kumar Roy A. Deficiencies of Serum Ferritin and Vitamin B12, but not Folate, are Common in Adolescent Girls Residing in a Slum in Delhi. *Int J Vitam Nutr Res* 2015; 85:14-22. doi: 10.1024/0300-9831/a000219. PubMed PMID:2678027.