INTRODUCTION

Dengue is a mosquito-borne viral disease that has rapidly spread in all regions of WHO in recent years. The incidence of dengue has grown dramatically around the world in recent decades. A vast majority of cases are asymptomatic or mild and self-managed, and hence the actual numbers of dengue cases are under-reported. Many cases are also misdiagnosed as other febrile illnesses. The virus is transmitted to humans through the bites of infected female mosquitoes, primarily the Aedes aegypti mosquito. It is caused by a virus of the Flaviviridae family and there are four distinct serotypes of the virus that cause dengue (DENV-1, DENV-2, DENV-3 and DENV-4). It causes a wide spectrum of disease. This can range from sub clinical disease (people may not know they are even infected) to severe flu-like symptoms in those infected. Although less common, some people develop severe dengue, which can be associated with severe bleeding, organ impairment and/or plasma leakage. Severe dengue has a higher risk of death when not managed appropriately. Dengue fever is a severe flu-like infection that involves individuals of all age groups (infants, children, adolescents, and adults). Transmission among human beings occurs by the mosquito Aedes aegypti and chiefly occurs during the rainy season. The proposed etiologies for dengue virus infection are Viral replication, primarily in macrophages, direct skin infection by the virus, immunological and chemical-mediated mechanism induced by host–viral interaction. Thrombocytopenia may be related to alterations in megakaryocytopoiesis, manifested by...
infection of human haematopoietic cells and compromised progenitor cell growth. This may cause platelet dysfunction, damage, or depletion, leading to significant hemorrhages. In 2009, the new World Health Organization (WHO) dengue case classification – dengue/severe dengue (D/SD) – was introduced, replacing the 1997 WHO dengue case classification: dengue fever/dengue haemorrhagic fever/dengue shock syndrome (DF/DHF/DSS). Expanded Dengue syndrome is a term announced by the WHO in 2011 to cover the uncommon expressions of dengue involving severe damage to the liver, kidneys, bone marrow, heart, or brain.

Dengue symptoms is characterized by a biphasic, high-grade fever lasting for 3 days to 1 week. Other symptoms are retrobulbar pain, rashes, myalgia and painful joint, metallic taste, appetite loss, diarrhea, vomiting, and abdomen pain. Dengue is also known as break-bone fever because of the associated myalgia and pain in joints. Flushing, a characteristic feature is commonly observed on the face, neck, and chest. The most common hemorrhagic manifestation are epistaxis, bleeding gums, skin hemorrhages and gastrointestinal hemorrhages.

Vitamin B12 deficiency causes anemia, leukopenia and thrombocytopenia. Approximately 10 percent of patients with symptomatic B12 (cobalamin) deficiency have significant thrombocytopenia. Patients with vitamin B12 deficiency infected with dengue can develop severe thrombocytopenia with bleeding manifestations. There are no sufficient studies associating severe dengue viral infection with Vitamin B12 deficiency. So, this study has been taken.

**Inclusion criteria**
1) Patients with aged more than 18 years.
2) Confirmed cases of dengue fever with NS 1 Ag positive and Ig M antibody positive
3) Patients giving consent for the study

**Exclusion criteria**
1) Patient with sepsis
2) Underlying malignancy, on chemotherapy, autoimmune disorder,
3) Hematological disorder, drugs causing thrombocytopenia

For the study purpose, we defined the case definition as below

**Case definition**
- Dengue fever positive = NS 1 antigen positive, Ig M antibody positive
- Thrombocytopenia – Platelet less than 1.5 lac
  - Mild thrombocytopenia – 1 to 1.5 lac
  - Moderate thrombocytopenia – 50,000 to 1 lac
  - Severe thrombocytopenia – less than 50,000
- Vitamin B12 deficiency – less than 190pg/ml
- Normal Vitamin B12 – 190pg/ml to 950pg/ml

**Statistical analysis**
Data was entered into Microsoft excel data sheet and was analyzed using SPSS 22 version software. Categorical data was represented in the form of Frequencies and proportions. Chi-square test was used as test of significance for qualitative data. Continuous data was represented as mean and standard deviation. Independent t test was used as test of significance to identify the mean difference between two quantitative variables.

**RESULTS**

**Profile of subjects with Dengue fever in the study**
Mean age of subjects was 39.20 ± 13.77 years. Majority of subjects were in the age group 31 to 40 years (26%), 50% were males and females respectively.

In the study 78% were NS1 +ve and 22% were IgM +ve. Most common clinical feature was fever in 70%, 48% had bleeding manifestations. Most common bleeding manifestation was bleeding gums 28% and 16% had Polyserositis (Table 1).

\[ \chi^2 = 31.1, df = 3, p < 0.001^* \]
In the study, among subjects with no thrombocytopenia and mild thrombocytopenia, 100% had normal Vitamin B12 levels. Among those with Moderate thrombocytopenia, 62.5% had Vitamin B12 Deficiency and 37.5% had normal Vitamin B12 levels and among those with severe thrombocytopenia, 94.4% had Vitamin B12 deficiency and 5.6% had normal Vitamin B12 levels. There was significant association between platelet count and Vitamin B12 levels (Table 2).

In our study there was significant positive correlation between Vitamin B12 and Platelet count that is with decrease in platelet count there was decrease in Vitamin B12 levels and vice versa (Figure 1).

In our study there was significant negative correlation between Vitamin B12 and Platelet transfusion that is with decrease in B12 levels there was increase in need of platelet transfusion and vice versa (Figure 3).

In the study among subjects with Vitamin B12 deficiency mean platelet count was 43037.0 ± 27732.9 and among subjects with normal Vitamin B12 was 164434.8 ± 92198.8. There was significant difference in mean platelet count among those with and without B12 deficiency (Table 3).

In our study there was significant difference in mean Vitamin B12 levels with respect to Severity of Thrombocytopenia. Mean Vitamin B12 was significantly lower among those with Severe Thrombocytopenia and highest among those with No Thrombocytopenia.

Similarly, there was significant difference in mean Platelet transfusion and duration of hospital stay with respect to Severity of Platelet count (Table 4).

**DISCUSSION**

Vitamin B12 acts as a co-factor during synthesis phase of the cells in bone marrow. So, Vitamin B12 deficiency causes thrombocytopenia. Serum Vitamin B12 levels are not frequently tested in patients being treated for thrombocytopenia secondary to spectrum of dengue viral infection.

In our study out of 50 patients, 78% (39) patients had dengue NS1 antigen +ve and 22% (11) patients had dengue IgM +ve. Most common clinical feature was fever followed...
Bleeding manifestations were noted in 21% of patients in the study conducted by Laul et al.19 In our study, 16% of the patient had polyserositis. A study conducted by Senthamarai et al., showed 21% of the patient had polyserositis.19 A study done by Tak et al., showed 32.5% patients had Vitamin B12 deficiency in dengue patients.20 In our study, 87.5% patients had Vitamin B12 deficiency less than 190pg/dl, which was statistically significant.

In our study among dengue patients with normal platelet count and mild thrombocytopenia, had normal Vitamin B12 levels. Among those with moderate thrombocytopenia, 62.5% patients had Vitamin B12 deficiency and 37.5% patients had normal Vitamin B12 levels and among those with severe thrombocytopenia, 94.4% had Vitamin B12 deficiency and 5.6% had normal Vitamin B12 levels. Mean Vitamin B12 was significantly lower among those with severe thrombocytopenia. Similarly there was significant difference in mean Platelet transfusion and duration of hospital stay with respect to Severity of Platelet count. Patients with decrease in B12 levels there was increase in need of platelet transfusion and increased duration of hospital and vice versa.

<table>
<thead>
<tr>
<th>Platelet Count</th>
<th>Vitamin B12 pg/ml</th>
<th>Platelet Transfusion RDP</th>
<th>Hospital Stay</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Thrombocytopenia</td>
<td>373.2 ± 133.9</td>
<td>3.1 ± 3.9</td>
<td>3 ± 2.8</td>
</tr>
<tr>
<td>Mild Thrombocytopenia</td>
<td>329.0 ± 110.4</td>
<td>3.2 ± 2.2</td>
<td>3 ± 2.4</td>
</tr>
<tr>
<td>Moderate</td>
<td>174.8 ± 91.3</td>
<td>4.7 ± 1.0</td>
<td>6.0 ± 2.9</td>
</tr>
<tr>
<td>Severe</td>
<td>107.2 ± 108.4</td>
<td>6.1 ± 2.4</td>
<td>9.3 ± 2.6</td>
</tr>
</tbody>
</table>

P value:
- Vitamin B12 pg/ml: <0.001*
- Platelet Transfusion RDP: 0.045*
- Hospital Stay: 0.001*

Figure 3: Scatter Plot showing Negative correlation between Vitamin B12 and Platelet Transfusion

by bleeding manifestations. A study done by Neeraja et al., showed fever was the most common presenting complaint.17 Most common bleeding manifestation was bleeding gums. In our study, 48% patients had bleeding manifestations.
CONCLUSION

Dengue fever patients with Vitamin B12 deficiency had moderate to severe thrombocytopenia and more bleeding manifestations. Those patients required more platelet transfusion and increased duration of hospital stay. So serum Vitamin B12 levels can be tested in dengue patients with severe thrombocytopenia. Supplementation of Vitamin B12 in those patients may improve the platelet count, may decrease the bleeding manifestations and platelet transfusion. It may also reduce the duration of hospital stay. Large number of studies may require for confirming it.

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