A Correlation of the Platelet Count with D-Dimer Levels as an Indicator for Component Therapy in Children with Dengue Hemorrhagic Fever

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Abstract Dengue Fever (DF) may evolve into two life threatening forms—Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS). DHF is associated with increased vascular permeability and plasma leakage causing thrombocytopenia and loss of clotting factors into the third space and may result in bleeding initially due to thrombocytopenia and later due to disseminated intravascular coagulation (DIC), often as a terminal event. Prompt recognition and treatment of minor bleeds in DF children with incipient DIC with component therapy may be associated with improved survival while failure to do so is usually catastrophic. A sensitive marker for early DIC is the presence of D-dimer (DD) in the blood. To determine the correlation between the severity of thrombocytopenia and early DIC in children with DHF. The impact of additional factors like age and shock will also be evaluated. Case control prospective study of 60 DHF sero-positive children (1–15 years) with thrombocytopenia. After clinical evaluation they were divided into two equal groups based on the degree of thrombocytopenia (more than/less

than 30,000/mm³). PT/APTT and DD levels were estimated in all children of both groups and statistical correlation was done. There was no significant difference in the DD levels between the two groups. However, children in either group, presenting with clinical features of shock and thrombocytopenia had significantly higher DD levels. Empirical component therapy in children with DHF based purely on their low platelet counts may not be justified. However, in DHF children with thrombocytopenia and features of shock, aggressive component therapy may prevent subsequent bleeding and may be justified.

Keywords Dengue fever · Dengue hemorrhagic fever · D-dimer · Thrombocytopenia · Disseminated intravascular coagulation

Introduction

Dengue Fever (DF) is a mosquito borne arbo—viral infection caused by a virus of the flaviviridae family. Of global importance, the World Health Organization (WHO) has estimated that 2.5 billion people (roughly 40 % of the world's population) currently, are at risk of the disease with an estimated 50–100 million new cases every year [1]. The virus is transmitted by the non-nocturnal *Aedes aegypti* mosquito, which dwells in urban areas and bites early in the morning or late at night. The mosquito, once infected, is capable of transmitting this infection for life and infected humans are the only definitive hosts of this virus.

The two life threatening forms of dengue infection, also called severe DF, are DHF and DSS. The hallmark of progression to severe dengue is increased vascular permeability and consequent plasma leakage. If left untreated, plasma leakage may become severe enough to

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cause circulatory compromise and shock as well as coagulation abnormalities due to loss of platelets and clotting factors into the third space. This generally does not cause major bleeding but when associated with shock, hypoxia or acidosis often leads to DIC and multiple organ failure.

It is well known that treatment of severe dengue is mainly supportive and not specific [2]. Of late, however, component transfusions (PRBC, Platelets and FFP) are being used indiscriminately, with a view to ‘prevent’ the occasionally occurring subsequent life threatening bleeding due to DIC. However in some children with DHF, a combination of thrombocytopenia associated with prolongation of both PT and APTT is almost uniquely suggestive of early or incipient DIC which is then invariably noted to be associated with an increase in fibrin degradation products (FDP) and D dimer (DD) levels [3, 4] and a higher risk of subsequent severe bleeding. Early recognition and prompt therapy of minor bleeds with components in these patients may then be lifesaving.

Therefore, this study was undertaken to assess if children with DHF and significant thrombocytopenia were at increased risk of developing DIC and to assess the contribution, if any, of other co morbid conditions like age and the presence of shock leading to this increase.

Objective

- To compare the D-dimer levels in DHF children with and without severe thrombocytopenia (above and below platelet counts of $30,000/\text{mm}^3$ [5]).
- To assess if children with DHF and shock had higher levels of DD than children with DHF without shock.
- To assess if age is a factor while determining the need for component transfusion in children with DHF.

Methodology

Type of Study

Case control approach and prospective study of DHF after due informed consent.

Selection of Patients

Children between the age group of 1–15 years who were seropositive for DF (NS 1 and/or IgM positive) with thrombocytopenia and presenting to a teaching hospital in South India, were selected for the study. The history and clinical features were recorded with particular attention to the presence of the clinical features of shock which included (a) BP less than 3rd centile for the age, sex

and stature of the child (b) Delayed Capillary Filling Time (CFT) > 2 s and (c) cold, clammy extremities. Children with other concomitant infections or who had been treated earlier for DF or those with thrombocytopenia due to any other cause or those with pre-existing bleeding or coagulation disorders and liver disease were excluded from the study.

The subjects were then divided into two age and sex matched groups. Group I with severe thrombocytopenia (platelet count $<30,000$ cells/ mm^3) and group II with platelet counts of $>30,000$ cells/ mm^3 at admission. The PT, APTT and D dimer levels were estimated for all children in both groups and compared statistically. The contribution of other factors such as age and the presence of shock was also evaluated statistically.

Sample Size

As published literature indicates that 25 % of patients with DF are likely to develop DHF and with an absolute precision on ± 9 and alpha error = 0.1, it was estimated that a minimum of 60 cases needed to be studied

Sample Collection

Blood samples from sero-positive cases that fulfilled the inclusion criteria were collected on admission and the serum stored at -80°C

Sample Analysis

The samples were subjected to a quantitative ELISA test using an ELISA strip reader with 8 wells which is the current gold standard technique for the detection of DD [6]. All samples were analyzed within 4 weeks of collection.

Statistical Analysis

All qualitative parameters were expressed as a proportion with 95 % confidence interval level ($p \leq 0.05$). Clinical parameters and data such as age, duration of dengue fever are summarized in terms of median and SD as the data was not normally distributed [7]. The parameters, compared in the two groups, included the platelet count and DD levels. The Chi square test of significance using the SPSS version 20 software was employed.

Results

Group 1 included children with platelet count $<30,000/\text{mm}^3$ (range 10,600–30,000/ mm^3) and Group 2 had children with platelet count $>30,000/\text{mm}^3$ (range 30,510–78,000/ mm^3).

Both groups were age and sex matched as depicted in Table 1.

Comparison of the PT, APTT and DD values in between groups I and II and in different age groups are given below (Table 2)

All children in Group I (platelet counts $<30,000/\text{mm}^3$) had altered PT/APTT or both, while only 6 out of 30 children in group II showed similar findings (p value 0.00001). The median of the DD values in Group 1 was 5.50. The median of the DD values in Group 2 was 4.15. The p value was found to be insignificant (p value 0.804) indicating thereby that the DD levels were not significantly different in different age groups.

A total of 24 children had clinical evidence of shock of which 14 were from group I and 10 from group II (Table 3). All children in group I had altered PT/APTT or both while only two from group II had similar findings (p value 0.00004). Median and peak DD levels in group 1 (4.0 and 40.1) was also found to be more than 25 % higher than in group 2 (3.01 and 22.8 respectively) but this was found to be statistically incomparable due to paucity of numbers. Further, when comparing the PT and APTT values alone in children of either group (i.e. irrespective of the degree of thrombocytopenia) with and without shock, we found that there was no statistically significant difference between the two groups (p value 0.389). However, a comparison of the DD values in children who presented with and without clinical features of shock irrespective of the degree of thrombocytopenia revealed that children in shock had much higher DD levels than children without thereby indicating an increased risk of incipient DIC in the former (p value 0.012) (Table 3).

An age-wise evaluation of DD values in children who presented with features of shock was also done to assess whether age could influence the need for component therapy (Table 4). The median value of DD levels in children with shock in the age group 1–7 years was 7.25 while in

the age group 8–14 years was 6.4. On comparison, this was found to be NOT significant (p value = 0.591).

Discussion

Dengue fever ranks as the most important mosquito-borne viral disease in the world at present. An estimated 2.5 billion people live in over 100 endemic countries where dengue viruses are transmitted. Up to 50 million infections occur annually with 22,000 deaths mainly amongst children [1]

Severe DF has two life threatening forms which include the *Dengue Hemorrhagic Fever (DHF)* and *Dengue Shock Syndrome (DSS)* the former culminating in uncontrolled bleeding and the latter in irreversible shock [8]. The illness is characterized by the occurrence of a 'Cytokine Tsunami' whose genesis, not fully understood as yet, is attributed to the release of cytokines, IL—6, free radicals and histamine in sequence which causes a sudden increase in vascular permeability leading to the development of a leak syndrome 4–6 days after the onset of fever [8]. This leads to the movement of body fluids and its components including platelets and clotting factors into the extravascular spaces and to the loss of intravascular volume. Persistent hypotension characterizes DSS while decrease in platelet count, elevated prothrombin Time (PT) and Activated partial thromboplastin time (APTT) identifies DHF [9, 10]. In severe and persistent cases, there is also an associated early increase in Fibrin Degradation Products (FDP) and DD, which indicates the possibility of incipient DIC, which can be fatal if recognized late. A recent study in Chennai has shown that six out of nine patients who died of severe DF and shock also developed features of DIC [11, 12]. Plasma leakage therefore contributes to both bleeding and shock in severe DF [13, 14] either of which can have significant consequences.

DIC, by itself can cause an increase in vascular permeability and plasma leakage and in the setting of DF can potentiate devastating effects. If recognized and treated early, incipient DIC is reversible. The D-dimer (DD) is a specific and sensitive marker of incipient DIC and can help identify early cases [1]. In a study done by Setrkraising et al. [13], 41 patients with sero- positive DF were studied to evaluate the usefulness of DD (measured by the ELISA test) as an indicator of dengue severity. Twelve of these cases had DF while 29 had DHF. DD was found to be positive in 13 % of the DF patients and in 87 % of patients with DHF (p value <0.01). Further the sensitivity and specificity of DD in predicting DHF was found to be 90 and 67 % respectively. This study also showed positive correlation between severe dengue infection and DD levels [13]. Serum fibrinogen level, another marker for incipient

Table 1 Demographic characteristics of subjects under study

Age (in years)	Frequency			
	Group I		Group II	
	Male	Female	Male	Female
1–3	2	0	2	0
4–5	1	2	1	2
6–7	2	0	2	0
8–9	0	0	0	0
10–11	2	5	2	5
12–13	6	6	6	6
14–15	4	0	4	0
Total	17	13	17	13

Table 2 PT/APTT and median DD values in both groups and distributed age-wise

	Group 1 (n = 30) (plt < 30,000/mm ³)	Group 2 (n = 30) (plt count > 30,000/mm ³)	<i>p</i> value
Altered PT/APTT	30	6	0.00001
Median DD value (range)	5.50 3.5–11.25	4.15 1.325–10.65	0.804
Median DD value age group 1–7 years	9.0	4.0	0.146
Median DD value age group 8–15 years	5.0	5.0	0.162

Table 3 PT, APTT and D-dimer levels in children with and without shock

	Group I with shock (n = 14)	Group II with shock (n = 10)	<i>p</i> value
Altered PT/APTT or both	14	2	0.00004
Median DD value range	4.0 (0.5–40.3)	3.01 (0.5–22.1)	0.536
Peak DD values	40.3	22.1	
	Children with shock Group I—14; Group II—10 (n = 24)	Children without shock Group I—16; Group II—20 (n = 36)	<i>p</i>
Altered PT/APTT or both	16	20	0.389
Median DD value range	6.4 (3.0–14.25)	4.0 (1.8–5.3)	0.012

Table 4 Age-wise distribution of DD levels in children presenting with shock

	Children with shock (age 1–7 years)	Children with shock (age 8–14 years)	<i>p</i> value
Median DD value	7.25	6.4	0.591

DIC has not been used in our study since elevated levels of D dimer and soluble fibrin are equally sensitive parameters for the diagnosis of DIC, and a normal level of D dimer has high negative predictive value [15]. Additionally, serum fibrinogen levels may be falsely decreased by ongoing third space loss due to the ‘plasma leak’ that occurs in this illness.

It is well known that current therapy for dengue is mainly supportive and includes fluid to maintain intra vascular volume and integrity and component therapy (PRBC, platelets and colloids) in case of fall in hematocrit or with bleeding [2]. Clinical bleeding hardly ever manifests with platelet counts of more than 30,000 cells/mm³ and platelet transfusion is usually initiated in clinical settings (i.e. with bleeding) and counts much lower than this figure. However nowadays, platelet and other component transfusions are demanded by parents and instituted indiscriminately with a view to allay anxiety and prevent subsequent bleeding. DHF children with thrombocytopenia

and altered coagulation parameters who have raised levels of DD in the plasma may justifiably be given early and aggressive component transfusions for even minimal bleeding which may occur subsequently. In others, it may be considered inappropriate and in fact dangerous. It may subject the child to the various well known risks and complications of transfusion [16].

Therefore, we wanted to determine whether thrombocytopenia in children with DHF was a reflection of imminent DIC. In a study conducted by Makroo et al. [17], it was found that bleeding occurred significantly more often in patients with a platelet count less than 30,000/mm³. Accordingly, we studied the DD levels in two groups of sex and age matched children who on admission had platelet counts of above and below the platelet count of 30,000/mm³.

While we found that all children in Group I with a platelet count of less than 30,000/mm³ had altered PT/APTT or both, only six children in group II had similar

findings ($p < 0.05$). However, on further analysis we found that there was no statistical difference in DD values between the two groups indicating thereby that thrombocytopenia of less than $30,000/\text{mm}^3$ alone did not indicate incipient DIC. Therefore, component therapy in these children will not be justified. In a study done in Thailand [13], it was seen that DD levels were significantly increased in children with DHF (with associated thrombocytopenia) in comparison to those with only DF. However, in that study there was no correlation of the platelet counts with DD levels.

Twenty-four children presented with shock of whom 14 were from group I and ten from group III. In these, alterations in PT/APTT or both were seen in 14 and 2 respectively ($p < 0.05$). Further, elevation of median and peak DD values was found to be more in the former though statistical correlations could not be done due to paucity of numbers but as either parameter was more than 25 % greater than its counterpart in the other group, it could suggest that DHF children in shock with significant thrombocytopenia were at higher risk for developing incipient DIC than those without. When we further compared the DD levels in children with DHF, irrespective of the degree of thrombocytopenia, who presented with and without clinical features of shock, we found that DD levels were significantly elevated in the children with shock (p value < 0.05). In contrast, prolongation of PT/APTT alone occurred in only 16 of the 24 children in this group as compared to 20 out of 36 in those without shock ($p > 0.05$) indicating that the PT and APTT, while correlating well with the platelet count in DF children without shock, had no such correlation in DF children with shock indicating that significant thrombocytopenia may be a better marker of altered coagulation in these children than PT and APTT alone. This further suggests that early component therapy in seriously ill children with shock, with platelet counts of $< 30,000/\text{mm}^3$ and irrespective of the PT/APTT values, may minimize subsequent bleeding and may be justified.

Further, the same group of children presenting with shock were then analyzed in order to determine whether a relationship existed between elevated DD levels and the age. In a study conducted by Toulon, it was found that normal DD levels were found to be elevated especially in the first 6 months of life. Data suggests that most coagulation test results are highly dependent on age, mainly during the first year of life, and age specific reference ranges needed to be used to maintain accuracy [18]. We wanted to see if there was any significant relationship between the DD levels in different age groups in our study. It was found that statistically, there was no significant relationship between the DD levels in different age groups suggesting that the approach to treatment need not be different in different age groups.

The diagnosis of DIC involves a large number of sophisticated tests in the setting of a disease. Most of the tests are not easily available to clinicians working in non tertiary hospitals. While the ISTH system of scoring is an excellent method of diagnosing overt and incipient DIC, it again requires the use of expensive tests to reach the diagnosis [19]. One of the aims of this paper was to use the platelet count (a cheap and easily available test) as a marker to identify incipient DIC in children with DHF and hence initiate, possibly life -saving component therapy early. We have found that while it is not useful for this purpose in DHF children without shock, it correlated with the presence of incipient DIC in DHF children with shock. This would be of immense benefit in situations where health care facilities are limited, especially in resource poor settings. A clinical diagnosis of shock and an estimation of the universally available platelet count may be all that is required by the treating physician to either arrange for components early in the course of the disease or to refer the patient to a center where facilities to treat this complication exist, thereby saving lives.

While other studies and standard 'practice' have suggested a correlation between significant thrombocytopenia and the development of subsequent life threatening bleeds in children with DHF, to the best of our knowledge, this is the first study in literature which indicates that severe thrombocytopenia may indeed be associated with an increased risk of DIC in children with DHF in shock.

Conclusion

The DD levels in the children with *severe thrombocytopenia* (platelet counts $< 30,000/\text{mm}^3$) and *without* (platelet counts $> 30,000/\text{mm}^3$) are not significantly different. Therefore, empirical component therapy in these children based purely on their platelet count may not be justified. However, in DHF children with *severe thrombocytopenia and clinical features of shock*, median and peak DD levels were found to be elevated and *early component therapy* in these children with minor bleeds may be justified with the ultimate aim of minimizing or aborting subsequent catastrophic bleeding.

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Compliance with Ethical Standards

Conflict of interest The authors report no conflict of interest in this paper.

Human and Animal Rights All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964

Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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