Association of abnormal coagulation tests with dengue virus infection and their significance as early predictors of fluid leakage and bleeding

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Abstract
Introduction: Dengue virus infection is endemic in Sri Lanka and is a major health concern. Fluid leakage and bleeding are important features in the spectrum of symptomatic dengue. Several laboratory tests have been evaluated for their association with dengue in order to find a predictor of complications.

Objective: To find the association of blood coagulation tests with dengue virus infection and their significance as early predictors of leaking and bleeding.

Method: A prospective study was carried out in ward 8, Lady Ridgeway Hospital on 384 patients admitted with a non-specific febrile illness without clinical features of fluid leakage or bleeding. They were followed up clinically to detect features of leaking and bleeding and with serial coagulation tests [Activated partial thromboplastin time (APTT) and international normalized ratio (INR)] and liver enzymes. Data analysis was done to detect associations between dengue and these tests.

Results: Eighty three patients with dengue haemorrhagic fever (DHF) and 75 patients with dengue fever (DF) were identified. Twelve DHF patients and 1 DF patient had bleeding manifestations. A statistically significant association was seen between abnormal coagulation results and DHF/bleeding when compared with DF. Ninety five

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percent had a raised APTT and 12% had a raised INR *before* leaking. One hundred percent had raised APTT and 46% had raised INR *before* bleeding. APTT had a *good sensitivity and specificity as a predictor* while INR was low in sensitivity and high in specificity. Liver transaminases also showed a positive correlation but lacked specificity.

Conclusion
In febrile patients with dengue virus infection raised APTT and INR are significantly associated with leaking and bleeding and so are the liver enzymes alanine transaminase (ALT) and aspartate transaminase (AST). Out of the laboratory parameters studied day 4 and day 5 APTT is the best predictor of leaking and bleeding. INR and liver transaminases could be used as supportive predictors.

Introduction
Dengue virus infection is a major health concern in Sri Lanka. It was one of the most prevalent severe infections among in-ward paediatric patients in many hospitals at the time of the study. According to reports from the Sri Lanka Epidemiology Unit, the number of suspected dengue cases reported in 2013 was 32,063. Peak incidence occurred during the months of July and August when this study was carried out.

The spectrum of symptomatic dengue virus infection spreads from an undifferentiated fever and dengue fever (DF) to dengue haemorrhagic fever (DHF) with shock and expanded dengue syndrome[1]. The hallmark of DHF is plasma leakage. This may lead to shock. Both DF and DHF could have bleeding manifestations. Many factors play a part in the pathogenesis of bleeding including coagulopathy which results in derangement of activated partial thromboplastin time (APTT) and the international normalised ratio (INR).

Many studies have been conducted in various Asian countries to find a predictor of clinical outcome in

dengue virus infection[2]. There have been several studies showing a correlation between abnormal coagulation studies and the severity of infection[2-5].

Objectives

1. To study the association between blood coagulation test (APTT and INR) abnormalities and dengue virus infection

2. To assess the possibility of using an abnormal APTT and/or INR as an early predictor of leaking and bleeding.

Method
A prospective study was conducted from April to October 2013 in ward 8, Lady Ridgeway Hospital. Patients admitted during this period with a non-specific febrile illness but not showing clinical evidence of leaking or bleeding at the time of admission were recruited to the study. Patients with a past medical history of a bleeding disorder or those on anti-coagulant medication were excluded. Informed written consent was obtained from the mother/father or guardian of each patient. Ethical clearance for the study was obtained from the Ethical Review Committee of the Lady Ridgeway Hospital for Children, Colombo.

Blood sampling was done on completion of 72 hours (early day 4) since the onset of fever. In patients who presented after 72 hours, sampling was done on admission. Samples were obtained for full blood count, APTT, prothrombin time (PT) with INR, alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Patients who continued to have non-specific symptoms or clear evidence of dengue infection were followed up with serial blood tests. Particular care was taken in obtaining samples for APTT and PT/INR to ensure a proper sample suitable for credible analysis was taken. 1.8 ml of blood was taken into a container with 0.2 ml of sodium citrate and was analysed within 4 hours in the Haematology Department of the Lady Ridgeway Hospital. For this study APTT was considered abnormal if it was more than 33.6s (i.e. >20% higher than the control of 28s) and INR was considered abnormal if >1.2. Patients were followed up during their stay in the hospital for clinical progression and for evidence of leaking and bleeding. To ensure reliability of clinical evidence only examinations performed by a Paediatric Registrar, Senior Registrar or Consultant Paediatrician, were accepted. On day 7 of illness blood samples were analysed for dengue serology (IgG and IgM). In patients already discharged this was done as an outpatient procedure.

Data analysis was carried out with Microsoft Excel using appropriate statistical tests. Correlation scores were calculated to determine correlations between variables as appropriate and Chi-square tests were carried out to calculate sensitivity and specificity values.

Results
During the study period 384 patients were recruited of whom 219 were boys and 165 were girls. Ages ranged from 7 months to 12 years and 6 months with a mean age of 6 years. There was no statistically significant difference between different age groups and sex in the development of leaking or bleeding.

Ten patients were removed from the study either because they were taken against medical advice or did not visit for serology tests. Out of 374 who completed the study, 158 were serologically confirmed as having a current dengue virus infection. Of the 158, eighty three (52.5%) patients had fluid leakage (DHF) whilst 75 (47.5%) patients had no evidence of leakage (DF). Two hundred and sixteen patients did not have a current dengue viral infection (non-DF/DHF). These included 7 patients with urinary tract infection, 23 patients with respiratory tract infections and 186 patients with viral fever. Therefore, the total number of patients who did not have evidence of fluid leakage was 291. Table 1 shows patients with primary and secondary dengue infection.

Table 1: *Primary and secondary infection*

Infection	Primary No. (%)	Secondary No. (%)
DF (n=75)	45 (60.0)	30 (40.0)
DHF (n=83)	12 (14.5)	71 (85.5)

Out of the 83 DHF patients 7 (8.43%) patients went into shock while 12 (14.46%) patients had bleeding. Out of the 75 DF patients 1 (1.33%) patient had bleeding. Therefore the total number of dengue virus infected patients who had bleeding were 13. All patients with these complications (shock and /or bleeding) had secondary infection. Of the 83 patients who leaked, 82 patients had an abnormal APTT at some stage during the course of their illness. Only one patient had a normal APTT. Table 2 shows the number of patients in different patient groups who had abnormal test result at some stage during their illness. As liver enzymes, ALT and AST are routinely done in patients with suspected dengue, the association of these liver enzymes with leaking and bleeding was also analysed. An ALT of >40 u/l and an AST >60 u/l were considered as abnormal.

Table 2: *Coagulation and liver enzyme abnormalities in different patient groups*

Patient group	Patients with abnormal APTT No. (%)	Patients with abnormal INR No. (%)	Patients with abnormal ALT No. (%)	Patients with abnormal AST No. (%)
DHF (n=83)	82 (98.8)	12 (14.4)	72 (86.7)	83 (100.0)
DF (n=75)	24 (32.0)	00 (0.0)	51 (68.0)	72 (96.0)
Dengue with bleeding (n=13)	13 (100.0)	08 (61.5)	13 (100.0)	13 (100.0)
Other (n=216)	05 (02.3)	06 (02.8)	61 (28.2)	119 (55.1)

If a test was to be used as a predictor it is important that abnormal results are seen prior to the clinical onset of complications. Table 3 shows the number of patients who showed abnormal results prior to the onset of leaking or bleeding.

Table 3. *Number of patients who had abnormal results prior to onset of leaking and bleeding*

Patient group	APTT No. (%)	INR No. (%)	ALT No. (%)	AST No. (%)
DHF (n=83)	79 (95.2)	10 (12.1)	54 (65.1)	70 (84.3)
Dengue with bleeding (n=13)	13 (100.0)	06 (46.2)	13 (100.0)	13 (100.0)

The mean values for day 4 and day 5 APTT and INR showed a significant difference ($p<0.05$) in patients with leaking and bleeding when compared with patients with DF and Non-DF/DHF as shown in Table 4. The mean INR in patients with leaking and bleeding was less than 1.2, but differences of the means when compared with DF and Non-DF/DHF patients was significant ($p<0.05$). The t test was used to calculate the significance between the different means for APTT and INR in the different groups having leaking, bleeding and dengue fever. Correlation analysis was done between day 4 (and day 5) coagulation test result abnormalities and subsequent positivity for leaking/bleeding as shown in Table 5

Table 4. *Mean APTT and INR on day 4 and 5 in different patient groups.*

	DHF	Dengue with bleeding	DF	Non-DF/DHF
APTT	40.5 seconds	42.3 seconds	31.8 seconds	29.8 seconds
INR	1.02	1.12	0.94	0.97

Table 5. *Pearson correlation scores and p values for day 4 and day 5 coagulation tests*

Patient group	Day 4 APTT Pearson score	p Value	Day 4 INR Pearson score	p Value	Day 5 APTT Pearson score	p Value	Day 5 INR Pearson score	p Value
DHF (leaking)	0.654	< 0.0001	0.138	0.009	0.828	< 0.0001	0.205	< 0.0001
Dengue with bleeding	0.297	< 0.0001	0.311	< 0.0001	0.261	< 0.0001	0.373	< 0.0001
DF	0.001	0.981	0.092	0.084	0.089	0.108	0.074	0.183

Finally Chi-square analysis were carried out on day 4 and 5 coagulation test results to find the best predictor of leakage and bleeding by assessing sensitivities and specificities at the selected cut-offs of 33.6s for APTT and 1.2 for INR. These results are shown in Table 6.

The parameters used for correlation were, a patient being positive/negative for an abnormal APTT (or INR) and the same patient being positive/negative for leaking (or bleeding).

Table 6: *Sensitivities and specificities for day 4 and 5 coagulation abnormalities to predict leaking and bleeding*

		Sensitivity	Specificity
Day 4 APTT >33.6s	Leaking	72.1%	92.7%
	Bleeding	84.6%	80.6%
Day 4 INR >1.2	Leaking	7.6%	97.8%
	Bleeding	30.8%	97.6%
Day 5 APTT >33.6s	Leaking	94.00%	93.4%
	Bleeding	92.3%	74.8%
Day 5 INR >1.2	Leaking	9.6%	99.6%
	Bleeding	46.2%	99.1%

Chi-square test done on liver transaminases using a cut-off of 40 u/l for ALT and 60 u/l for AST showed the following sensitivity and specificity. These results are shown in Table 7.

Table 7: *Sensitivities and specificities for day 4 and 5 liver enzyme abnormalities to predict leaking and bleeding*

		Sensitivity	Specificity
Day 4 ALT >40 u/l	Leaking	67.1%	70.8%
	Bleeding	100%	65%
Day 4 AST >60 u/l	Leaking	88.6%	40.1%
	Bleeding	100%	35%
Day 5 ALT >40 u/l	Leaking	79.5%	60.6%
	Bleeding	100%	52.9%
Day 5 AST >60 u/l	Leaking	100%	30.5%
	Bleeding	100%	28.6%

Discussion

In our study 52.5% of patients with confirmed dengue viral infection ended up with fluid leakage (DHF) and 8.4% of them went into shock and 8.2% of dengue patients developed bleeding. This highlights the importance of finding an early predictor for leaking and bleeding. A reliable test which can be done on day 4 (or 5) to predict subsequent development of leaking or bleeding is of utmost importance in dengue endemic countries such as Sri Lanka to anticipate and treat patients and to manage resources.

In Taiwan, Huang et al in 2001 showed an association of raised APTT values in patients with DHF and shock[3]. In 2003, Liu et al states that 97.5% of patients with DHF had prolonged APTT values when compared with those having DF (68.8%)[5]. They found that prothrombin time did not show this positive correlation. However, abnormalities in the liver enzyme AST showed a significant correlation with DHF when compared with DF[5]. In 2010 Chuansumrit et al, from a study done in Thailand, found a positive correlation between abnormal coagulation studies and dengue shock[4]. In Indonesia, Budastra et al concluded that APTT can be used as a predictor of bleeding manifestations in DHF[2].

Our study showed that a prolonged APTT and a raised INR was significantly associated (p<0.01) with DHF when compared with DF and Non-DF/DHF patients. However, although a raised INR showed a significant association it was raised only in 14.4% of 83 DHF patients, limiting the value of using it as a predictor. In contrast APTT was raised in 98.8% of patients with DHF making it a potentially good tool to use as a predictor. There was also a significant association of these test abnormalities with bleeding when compared with DF patients who did not bleed and Non-DF/DHF patients. Here again an abnormal APTT showed a better association than INR (100% vs. 61.5%). Pearson correlation tests done on day 4 and day 5 APTT and INR also confirmed this positive correlation with DHF and with bleeding but not with DF. Although the main objectives of the research was to study the association of coagulation tests with dengue, as liver enzymes are routinely done, we also analyzed the association of AST and ALT. Although both AST and ALT show a significant association (p<0.01) with DHF and bleeding they also show significant association (p<0.01) with DF as well, again limiting the value of using them as early predictors.

To use any test result as an early predictor of leaking or bleeding it must show an abnormal result before the onset of clinical features. Ninety five percent of patients who subsequently developed leaking had an abnormal APTT prior to the onset of clinical features. This was higher than any other laboratory marker that we studied. One hundred percent of patients who

subsequently had bleeding had abnormal APTT, ALT and AST levels prior to clinical features making them potentially useful markers for bleeding.

To decide on the best predictor we analyzed sensitivities and specificities with Chi-squares using above cut-offs and this showed clearly that INR lacked adequate sensitivity (but has high specificity) and that both ALT and AST lacked adequate specificity to predict leaking and bleeding. The best predictor was APTT for both leaking and bleeding and day 5 values showed higher levels of sensitivity and specificity than day 4. But as some patients start leaking or bleeding before day 5 it is important to do day 4 APTT as well as day 5 APTT and if it is >33.6 it is a very good predictor of leaking and/or bleeding. INR, AST and ALT could also be used as supportive predictors bearing in mind their different sensitivity and specificity patterns.

Conclusions
In febrile patients with dengue viral infection raised APTT and INR are significantly associated with leaking and bleeding and so are the liver enzymes ALT and AST. Out of the laboratory parameters studied day 4 and day 5 APTT is the best predictor of leaking and bleeding. INR and liver transaminases could be used as supportive predictors.

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