INTRODUCTION

Dengue is a debilitating vector-borne disease with a 30-fold rise in disease incidence over the past five decades. According to the World Health Organization (WHO), dengue is a major global threat [1]. More than 70% of the dengue disease burden is in South-East Asia and the Western Pacific. Urbanization, rapid movement of people and goods, favourable climatic conditions and lack of trained staff have all contributed to the global increase of dengue. An estimated 500,000 deaths due to dengue fever [3].

Dengue fever is one of the important tropical diseases of public health significance caused by flavivirus. It is a major cause of morbidity and mortality worldwide. Identification of factors associated with severity of dengue can improve the prognosis of the disease. This study tried to assess the factors associated with severity of dengue.

METHODS

A record based study was conducted in a tertiary care hospital setting in southern India. A total of 550 case files were reviewed to ascertain demographic, clinical and laboratory parameters among confirmed cases of dengue. The severity of dengue was categorized using WHO 2009 classification.

RESULTS

Of 550 records reviewed, 449 (81.6%) were classified as non-severe dengue and 101 (18.4%) as severe dengue. Factors associated with severe dengue on univariate analysis were: gender, backache, skin rash, nausea and vomiting, abdominal distension, haemorrhage, breathlessness, oliguria, haematocrit, lymphopenia, high AST or ALT levels, and raised serum protein levels. ALT > 63 IU/L or oliguria (OR = 1.77, 95%; CI = 1.01-3.1) and hypoproteinemia (OR = 5.57, 95%; CI = 2.82-10.98) were found to have significant association with the development of severe dengue.

Conclusion: This study indicates that when dengue patients present with bleeding episodes, ascites, oliguria, raised ALT and low serum protein levels, clinicians should be alert to the appearance of severe complications. Early identification of these factors will help clinicians to recognize the severity of dengue illness and enable them to implement appropriate interventions.

Keywords: Dengue infection, Flavivirus, Plasma leakage, Severe dengue.
exposure variables (clinical, demographic and laboratory) with severe dengue. Independent sample t-test (Mann-Whitney U test) was used to compare mean (median) of continuous exposure variables (clinical, demographic and laboratory) with severe dengue. Logistic regression analysis was used to find factors associated with severe dengue. All variables with p<0.02 on univariate analysis were considered for logistic regression analysis. Backward Wald elimination procedure was used to select significant variables. A p value of <0.05 was considered as statistically significant.

RESULTS
A total of 550 patient's medical records were reviewed. Out of which 405 (73.6%) were males and 145 (26.4%) were females. The male to female ratio was 2.8:1. The most affected age group was 21-30 years followed by 31-40 years. The mean age of the patients was 32.41 (SD=11.64) years. There was no statistically significant difference observed in mean age between the two groups. Majority of the patients were working in agriculture setting (114/550) 20.7%.

Clinical characteristics in patients with dengue fever are described in Table 1. Fever was the most consistent complaint 547 (99.5%) followed by headache 263 (47.8%), myalgia 241 (43.8%), vomiting 227 (41.3%) and pain abdomen 165 (30%).HAemorrhagemannifested in 79 (14.4%) cases in the form of: petechiae 35 (6.4%); melena 18 (3.3%); hematemesis 8 (1.5%); gum bleed 13 (2.4%); epistaxis 4 (0.7%); haematuria 9 (1.6%); and menorrhagia 7 (4.8%). Some patients presented with more than one haemorrhagic manifestation.

Twenty-nine (5.3%) patients had shock as the predominant complication followed by AKI (4.2%). Acute hepatitis with aminotransferase levels increased to at least 10 times their normal values was observed in 16 (2.9%) cases.All patients with acute respiratory distress syndrome (ARDS)required ventilator support. Of these, eight patients improved and were successfully weaned off the ventilator.

Rapid decline in WBC and platelet counts were frequently noted in the early phase of the disease. Large number of patients had a platelet count of less than 50,000/mm³ (Table 2). Further, the prevalence of bleeding episodes among patients with platelet count <50,000/mm³ was significantly higher (p=0.001) among SD group (22/60) as compared to NSD group (22/60) as compared to NSD group (29/225). Thirte in transaminase levels was commonly observed and in most cases, there was a greater elevation in AST than ALT levels. The salient laboratory findings are summarized in Table 3.

Antipyretics (oral paracetamol) were used along with intravenous fluids when required. Platelet transfusion was done in 103 (18.7%) patients with low platelet counts and bleeding during hospitalization. Packed red blood cells were transfused in 12 cases with anaemia and bleeding episodes. The median duration of hospitalization was 5 days (IQR, 4-6 days) in mostcases. Fifty-four (9.8%) patients required admission to intensive care units (ICU). The mortality was observed in 7 (1.3%) and all fatal cases were due to shock, ARDS and multi-organ failure.

The results of multivariate logistic regression analysis for factors associated with severe dengue are presented in Table 4. From univariate analysis of clinical, demographic and laboratory parameters, we found that gender, backache, skin rash, nausea and vomiting, abdominal distension, haemorrhage, breathlessness, oliguria, hepatomegaly, splenomegaly, ascites, leukopenia, hypoproteinemia and high ALT showed significant association with severe dengue. The corresponding unadjusted Odd's ratio ranges from 0.4 to 7.93. After taking into consideration all these variables for binary logistic regression analysis, skin rash, haemorrhagic episodes, oliguria, low serum protein, high ALT and ascites were found to be associated with severe disease. The corresponding adjusted Odd's ratio ranges from 0.42 to 11.75. The risk of severe dengue was more among patients who had haemorrhage (OR=11.75, 95%; CI=6.38-21.62), oliguria (OR=4.01, 95%; CI=1.32-12.15), ascites (OR=2.68, 95%; CI=1.19-6.01), hypoproteinemia (OR=5.57, 95%; CI=2.82-10.98) and high ALT (OR=1.77, 95%; CI=1.01-3.1).

DISCUSSION
The existing global distribution of the risk of dengue virus infection and its complications are now becoming prevalent and severe. Additionally, the changing epidemiology and rapid urbanization in developing countries such as India has enormously increased the prevalence of life threatening diseases like dengue [8]. Frequent outbreaks of dengue fever have been reported over the last few decades with large number of patients presenting with atypical features such as pancreatitis, myositis, serositis, myocarditis, hepatitis, acalculous cholecystitis, central nervous system involvement, and even death [9,10]. Given the wide range of clinical manifestations of dengue fever, one of the challenges to the treating clinician is to be able to identify which patients are likely to develop severe dengue. Early recognition of factors associated with the severity of dengue will help identify the patients with severe infection.

Table 1: Demographic and clinical profile of dengue infection

| Patient characteristics   | Total (n=550) | Dengue with or without warning signs (n=449) | SD (n=101) | p  |
|---------------------------|--------------|----------------------------------------------|------------|----|
| Male                      | 405 (73.6)   | 342 (76.2)                                   | 63 (62.4)  | 0.004* |
| Female                    | 145 (26.4)   | 107 (23.8)                                   | 38 (37.6)  |    |
| Age [years]*              | 32.41±11.64  | 31.96±11.51                                  | 34.39±12.04| 0.058|
| Fever                     | 547 (99.5)   | 447 (99.6)                                   | 100 (99.0) | 0.502|
| Headache                  | 263 (47.8)   | 223 (49.7)                                   | 40 (39.6)  | 0.067|
| Myalgia                   | 241 (43.8)   | 198 (44.1)                                   | 43 (42.6)  | 0.780|
| Arthralgia                | 98 (17.8)    | 81 (18.0)                                    | 17 (16.8)  | 0.774|
| Backache                  | 35 (6.4)     | 32 (7.1)                                     | 3 (3.0)    | 0.122|
| Pain abdomen              | 165 (30.0)   | 133 (29.6)                                   | 32 (31.7)  | 0.683|
| Abdominal distension      | 50 (5.5)     | 18 (4.0)                                     | 12 (11.9)  | <0.001*|
| Nausea and vomiting       | 227 (41.3)   | 177 (39.4)                                   | 50 (49.5)  | 0.063|
| Hemorrhage                | 79 (14.4)    | 37 (8.2)                                     | 42 (41.6)  | <0.001*|
| Breathlessness            | 13 (2.4)     | 6 (1.3)                                      | 7 (6.9)    | 0.001*|
| Oliguria                  | 19 (3.5)     | 8 (1.8)                                      | 11 (10.9)  | <0.001*|
| Conjunctival congestion   | 94 (17.1)    | 79 (17.6)                                   | 15 (14.9)  | 0.508|
| Skin rash                 | 170 (30.9)   | 145 (32.3)                                   | 25 (24.8)  | 0.138|
| Hepatomegaly              | 62 (11.3)    | 42 (9.4)                                     | 20 (19.8)  | 0.003*|
| Splenomegaly              | 32 (5.8)     | 23 (5.1)                                     | 9 (8.9)    | 0.142|
| Pleural effusion          | 13 (2.4)     | 8 (1.8)                                      | 5 (5.3)    | 0.038*|
| Ascites                   | 44 (8)       | 27 (6.0)                                     | 17 (16.8)  | <0.001*|
| SBP (mmHg)*               | 119±14       | 120±12                                      | 115±19     | 0.001*|
| DBP (mmHg)*               | 77±12        | 78±9                                         | 72±21      | <0.001*|

*Data are expressed as mean±SD. SD: Standard deviation, SBP: Systolic blood pressure, DBP: Diastolic blood pressure. *p<0.05. SD: Severe dengue
Although no difference in the gender distribution of dengue cases exist, several studies have reported varying results. In the majority of reports of dengue outbreaks in India, males outnumbered females [1,1,2]. This gender difference in dengue cases may be due to increased mobility of the male population in the society and better access to health-care [13]. However, our study did not find gender as a risk factor for dengue infection severity. Dengue virus infections affect human populations of all age groups worldwide. In some parts of the world, dengue is mainly a pediatric health problem[1]. In this study, majority of cases were seen in the 21-30 years’ age group which supports the observations made by some authors who reported that dengue infection in India is predominantly a disease of young adults [14,15].

The classical symptoms of dengue infections such as fever, headache, and pain abdomen with vomiting were observed in majority of cases. Although bleeding in the form of petechiae and melena was seen in many patients, none of our patients had severe haemorrhage. Bleeding in severe dengue infection may be related to impaired platelet function with moderate to severe thrombocytopenia and coagulopathy, with activation of the coagulation system and fibrinolysis [16]. Fluid accumulation due to plasma leakage including ascites and pleural effusion was noted in 8% of the coagulation system and fibrinolysis [16]. Fluid accumulation due to plasma leakage including ascites and pleural effusion was noted in 8% of patients. Although bleeding in the form of petechiae and melena was seen in many patients, none of our patients had severe haemorrhage. Bleeding in severe dengue infection may be related to impaired platelet function with moderate to severe thrombocytopenia and coagulopathy, with activation of the coagulation system and fibrinolysis [16]. Fluid accumulation due to plasma leakage including ascites and pleural effusion was noted in 8% and 2.4% of patients on ultrasound scan of abdomen while acalculous cholecystitis was noted in only 5 cases.

Clinical evidence includes hepatomegaly, with liver involvement being more frequent in the severe form of disease [19]. Hepatomegaly was found to be a strong risk factor for severe dengue in many studies in the past [20,21], however, our study did not find hepatomegaly as a risk for dengue infection severity. Though splenomegaly is not a common feature in dengue fever it was noted in 5.8% of patients. In the current study, we did not find statistically significant association of splenomegaly with severe illness. In a recently published report from Pakistan, it was observed that significant number of patients with severe dengue had splenomegaly [22]. The reason for spleen enlargement in severe dengue infection may be due to replication of dengue virus in spleen, resulting in splenomegaly [23]. These clinical findings emphasize the fact that presence of hepatomegaly and ascites in addition to with or without splenomegaly in a patient with dengue infection should prompt a clinician to suspect severe illness and manage severity early in the course of the illness.
Asignificant number of patients (5.3%) had shock in the present series. Leo et al., conducted a 5-year retrospective study in Singapore on confirmed adult dengue fatalities and found that deaths among dengue cases were due to shock (100%) and organ failure (85.7%) [24]. Prolonged shock in severe dengue is most often complicated by metabolic acidosis, multi-organ impairment and severe bleeding which carries a poor prognosis [16].

Hepatic dysfunction is a well-recognized feature of dengue infection, often demonstrated by hepatomegaly and mild-to-moderate increases in transaminase levels although jaundice and acute hepatitis are uncommon. The liver dysfunction in dengue infection can be a result of the direct effect of the virus on liver cells or the dysregulated host immune response against the virus [19,25]. The present study showed AST−ALT which is consistent with the observations made by Kuo et al. in an evaluation of 270 dengue patients, observed abnormal levels of AST and ALT in 9.3% and 82.2% respectively. They reported that elevation of AST levels was usually greater than ALT [26]. This may be due to the release of AST during myocyte damage in dengue infection. However, on regression analysis, we noticed that raised ALT was independently associated with severe disease. ALT is primarily associated with hepatocytes, with minimal activity in kidney and skeletal muscle. It is possible that raised ALT in severe dengue may reflect both liver and renal dysfunction.

The sinister symptom that points to renal dysfunction such as oliguria was observed in 19 (3.5%) cases with AKI (n=23, 4.2%). It was noted to be associated with severe illness and in majority it was self-limited. The proposed mechanisms include increased plasma leakage and loss of fluid from the intravascular compartment leading to shock which may lead to reduced renal perfusion and acute tubular necrosis [27].

We observed that serum protein levels were associated with severe dengue, whereas haematocrit, platelet count, leucocyte count and serum albumin were not. In addition to hypoproteinaemia, we found that raised ALT levels were associated with severe form of disease. This is in contrast to observations made by Jayaratn et al., stated that raised AST levels were associated with severe dengue and have better predictive value in predicting severe disease [28]. Ours findings indicate that in addition to evaluating haemoconcentration and decreased platelet counts, the presence of low serum protein and raised ALT levels may be taken as a marker for predicting severe disease.

Our study had some limitations such as only hospitalized patients with dengue fever were included in this study and secondly, few studies are available pertaining to factors associated with severe dengue based on the 2009 WHO classification. In the 1997 WHO classification, DHF and DSS comprise the severe form of the disease. Due to differences in the variables included in each classification, comparing the results of the present study with the 1997 WHO classification may not be much justifiable.

CONCLUSION
Based on the results, we conclude that bleeding episodes, ascites, oliguria, raised ALT and low serum protein levels were associated with severe illness. When dengue patients present with these features, the clinician should be aware of the possibility of severe complications. Early identification of factors associated with dengue severity is important for the clinicians in order to implement appropriate interventions for better prognosis.

AUTHOR CONTRIBUTIONS
MSP, CUK, SN and GT planned the study. MSP and CUK designed the study protocol. MSP compiled the data. MSP, CUK, VK and VG analysed and interpreted the relevant data. CUK and MSP drafted the manuscript. CUK, SN and GT critically revised the manuscript for intellectual content. All authors read and approved the final manuscript. MSP, CUK, SD and VG are guarantors of the paper.

CONFLICT OF INTEREST:
None declared.

REFERENCES
1. WHO. Dengue: Guidelines for diagnosis, treatment, prevention and control, 1st ed. Geneva, World Health Organization; 2009.
2. WHO. Dengue and severe dengue Fact sheet. World Health Organization, 2017. Available: from: http://www.who.int/mediacentre/ factsheets/fs117/en/. [Last accessed on 2017 Sep 06].
3. WHO. Global Health Estimates 2015: Deaths by Cause, Age, Sex, by Country and by Region, 2000-2015. Geneva, World Health Organization; 2016.
4. Anung KL, Thanachartwet V, Desakorn V, Chamnancharun S, Sahassananda D, Chierakul W, et al. Factors associated with severe clinical manifestation of dengue among adults in Thailand. Southeast Asian J Trop Med Pub Health 2013;44(4):562-12.
5. Thomas L, Broustey Y, Najiloullah F, Hocchedez P, Hatchuel Y, Moravie V, et al. Predictors of severe manifestations in a cohort of adult dengue patients. J Clin Virol 2010;48(2):96-9.
6. Malavige GN, Velathamani P, VG, Wijewickramena ES, Fernando S, Jayaratne SD, Aaskov J, et al. Patterns of disease among adults hospitalized with dengue infections. QJM 2006;99(5):299-305.
7. Thanachartwet V, Oër-Areemitr N, Chamnancharun S, Sahassananda D, Jittmittrapah A, Suwannakudt P, et al. Identification of clinical factors associated with severe dengue among Thai adults: A prospective study. BMC Infect Dis 2015;15:420.
8. Gupta N, Srivastava S, Jain A, Chaturvedi UC. Dengue in India. Indian J Med Res 2012;136(3):373-90.
9. Goyal V, Gill GS, Singh J, Singh GP, Singh V, Singh S, et al. Clinical spectrums of dengue fever in a tertiary care centre with particular references to atypical presentation in the 2011 outbreak at Bathinda, Punjab, India. Int J Pharm Sci Res 2013;5:Suppl 4:363-67.
10. Nayak J, Behera S, Swain SK, Panda SR. A study of multiorgan dysfunction in patients with dengue and its clinico-hematological correlation with severity. Asian J Pharm Clin Res 2017;10(2):218-21.
11. Singh NP, Jhumr B, Agarwal SK, Gaitha M, Dewan R, Daga MK, et al. The 2003 outbreak of dengue fever in Delhi, India. Southeast Asian J Trop Med Public Health 2005;36(5):1174-8.
12. Sinha N, Gupta N, Jhumr B, Gulati S, Kulkarni AV. The 2006 dengue outbreak in Delhi, India. J Commun Dis 2008;40(4):243-8.
13. Chakravarti A, Arora R, Luxemburger C. Fifty years of dengue in India. Trans R Soc Trop Med Hyg 2012;106(5):273-82.
14. Gupta E, Dar L, Narang P, Srivastava VK, Broor S. Serodiagnosis of dengue during an outbreak at a tertiary care hospital in Delhi. J Med Res 2005;121(1):36-8.
15. Gupta E, Dar L, Kapoor G, Broor S. The changing epidemiology of dengue in Delhi, India. Virol J 2006;3:92.
16. Yacoub S, Farrar J. Dengue. In: Farrar J, Hotez PJ, Junghanss T, Kang G, Laloo D, White NJ, editors. Manson’s Tropical Diseases. 23rd ed. Elsevier; 2014. p. 162-170.
17. Setiawan MW, Samsi TK, Wulur H, Sugianto D, Pool TN. Dengue haemorrhagic fever: Ultrasound as an aid to predict the severity of the disease. Pediatr Radiol 1996;26(1):1-4.
18. Quiroz MR, Mendez GF, Ovando RK. Clinical utility of ultrasound in the identification of dengue haemorrhagic fever. Rev Med Inst Med Seguro Soc 2006;44(3):243-8.
19. Seneviratne SL, Malavige GN, de Silva HJ. Pathogenesis of liver involvement during dengue viral infections. Trans R Soc Trop Med Hyg 2006;100(7):608-14.
20. Falconar AK, Romero-Vivas CM. Simple prognostic criteria can definitively identify patients who develop severe versus non-severe dengue disease, or have other febrile illnesses. J Clin Med Res 2012;4(1):33-44.
21. Pham TB, Nguyen TH, Vu JQ, Nguyen TL, Malvy D. Predictive factors of dengue shock syndrome at the children hospital no.1, Ho-Chi-Minh City, Vietnam. Bull Soc Pathol Exot 2007;100(1):43-7.
22. Raza FA, Rehman Su, Khalid R, Ahmad J, Ashraf S, Iqbal M, et al. Demographic and clinico-epidemiological features of dengue fever in Faisalabad, Pakistan. PLOS One 2014;9(3):89868.
23. Gulati S, Maheshwari A. Atypical manifestations of dengue. Trop Med Int Health 2007;12(9):1087-95.
24. Leo YS, Thein TL, Fisher DA, Low JG, Oh HM, Narayanan RL, et al. Confirmed adult dengue deaths in Singapore: 5-year multi-center retrospective study. BMC Infect Dis 2011;11:123.
25. Trung DT, Thao LT, Hien TT, Hung NT, Vinh NN, Hien PT, et al. Liver
involvement associated with dengue infection in adults in Vietnam. Am J Trop Med Hyg 2010;83(4):774-80.

26. Kuo CH, Tai DI, Chang-chien CS, Lan CK, Chiou SS, Liaw YF. Liver biochemical tests and dengue fever. Am J Trop Med Hyg 1992;47(3):265-70.

27. Lima EQ, Noqueira ML. Viral hemorrhagic fever-induced acute kidney injury. Semin Nephrol 2008;28(4):409-15.

28. Jayaratne SD, Atukorale V, Gomes L, Chang T, Wijeysinghe T, Fernando S, et al. Evaluation of the WHO revised criteria for classification of clinical disease severity in acute adult dengue infection. BMC Res Notes 2012;5:645.