Tropical Biomedicine 38(3): 396-402 (2021)
https://doi.org/10.47665/tb.38.3.076

ORIGINAL ARTICLE

Total serum cortisol level is low in children with severe dengue shock syndrome

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ARTICLE HISTORY

Received: 26 March 2021
Revised: 6 July 2021
Accepted: 6 July 2021
Published: 30 September 2021

ABSTRACT

Dengue shock syndrome (DSS) is a potentially critical and life-threatening concern, especially in children of tropical countries. The serum cortisol levels in severe DSS or later stages of DSS are limited references. We prospectively studied an association between of serum cortisol as well as interleukin levels and the severity of DSS in pediatric patients. A prospective cross-sectional study of 35 consecutive DSS cases (3 months to 16 years old) admitted to our institution from July 1, 2019, to June 30, 2020 was conducted. Serum cortisol, IL-6, and IL-10 were measured at T0 (shock recognition) and T12 (12 hours after T0); their values were presented as median and interquartile ranges (25%–75%). Severe DSS included patients with impalpable pulses or systolic blood pressure < 70 mmHg, recurrent shock, and prolonged shock. In contrast, non-severe DSS presented circulatory failure without any features of severe DSS. A total of 8 (22.8%) severe DSS patients expressed the cortisol (T0) significantly lower compared to the non-severe DSS group (7.3 µg/dl versus 14.3 µg/dl, p=0.008). In severe DSS, there was a minimal change in cortisol levels between T0 and T12 (7.3 µg/dl and 4.7 µg/dl p>0.05), whereas the decrease is significant in their counterparts (14.3 µg/dl to 5.6 µg/dl, p<0.005). Additionally, there were moderate correlations between IL-6 (T0), IL-10 (T0), IL-10 (T12) and total fluid requirement (Spearman's rho = 0.47, 0.4, and 0.36, respectively; p<0.05). Our study demonstrated that adrenal dysfunction was present in patients with severe and non-severe DSS, as noted by cortisol level at T12. In addition, IL-6 and IL-10 levels are correlated with the total fluid requirement, which is a marker of DSS severity. Further studies could reveal how adrenal dysfunction in pediatric patients with DSS can affect outcomes and the potential roles of interleukin levels in fluid management strategy.

Keywords: Serum cortisol; adrenal insufficiency; interleukin; dengue shock syndrome.

INTRODUCTION

Dengue hemorrhagic fever (DHF) has remained a grievous public health problem in tropical countries for more than two centuries (Guzman *et al*., 2010; Bhatt *et al*., 2013). Dengue shock syndrome (DSS) occurs in 15% of dengue cases and can lead to multiorgan dysfunction and death (Anders *et al*., 2011). In patients with DSS and multiple organ dysfunction, coagulation disorder is a common consequence resulting in hemorrhage (Wills *et al*., 2002; WHO, 2009). In addition to prolonged shock, bleeding by coagulation disorders and an increase in cytokine levels may contribute to adrenal insufficiency in children with a critical illness such as sepsis (Pizarro *et al*., 2005; Hebbar *et al*., 2011; Annane *et al*., 2017). Elevated cortisol level has been reported in several forms of severe dengue infection (early phase of DSS vs DHF without shock, and DHF vs dengue fever) (Myo *et al*., 1995; Joob & Wiwanitkit, 2014). However, data on adrenal response and interleukin (IL) levels in children with varying severity of DSS are limited. In this study, we prospectively enrolled children with DSS and measured cortisol, IL-6, IL-10 levels at shock recognition and 12 hours later. It is presented that the cortisol level was lower, while the IL-6 and IL-10 were higher in almost severe forms of DSS compared to their counterparts. Moreover, this study also assessed the association between cortisol and interleukin levels with the total fluid requirement, an indirect marker of DSS severity (Nguyen *et al*., 2019). The results may be a contribution to research aiming hemodynamic stable and improving outcomes.

MATERIALS AND METHODS

Study population

Consecutive patients between 3 months and 16 years of age admitted to our institution from July 1, 2019, to June 30, 2020, with DSS were prospectively recruited. DSS diagnosis criteria used in our study were based on WHO 2009 guideline. All patients presented with clinical characteristics of dengue infection (fever, hemorrhagic manifestation, and thrombocytopenia), positive dengue NS1 antigen (Humasis Co., Ltd., South Korea) or dengue IgM (NovaLisa™, Novatec Germany), and circulatory failure (WHO, 2009; Vietnam MoH, 2019). Circulatory failure was determined by rapid and weak pulse, cold and clammy skin and restlessness, and pulse pressure ≤ 20 mmHg or hypotension (blood pressure < 80 mmHg in children < 5 years old and < 90 mmHg in children ≥ 5 years of age) (WHO, 2009; Vietnam MoH, 2019). In addition, blood samples were collected for cortisol, IL-6, IL-10 measurement at two time-points, at shock recognition (T0) and 12 hours after T0 (T12), to assess the changes in adrenal function over time and in response to fluid resuscitation.

Severe DSS was defined as a shock with no detectable pulse or blood pressure or systolic blood pressure <70 mmHg (WHO, 1997). Recurrent DSS cases had recurred shock after at least 2 hours of recovery from the first episode of shock. Prolonged DSS is shock-resistant to fluid therapy (≥ 60 ml/kg) and/or shock persisted ≥ 6 hours (Vietnam MoH, 2019). In this study, severe DSS refers to all categories, as mentioned earlier.

All underlying health conditions (asthma, bronchopulmonary dysplasia, congenital heart disease, immunodeficiency) or received corticosteroids within the previous four weeks were excluded from the study. The study was approved by the Institutional Scientific and Ethical Committee, and written informed consent was obtained from parents or legal guardians of all patients.

Clinical data

Demographic and clinical data were recorded on a standardized questionnaire. All patients recruited in the study were closely monitored and managed following the Vietnam Ministry of Health (MoH) guidelines (Vietnam MoH, 2019). Obesity was defined as BMI ≥ 95 percentile for age and sex. Liver function and coagulation tests (INR, PTT, and fibrinogen) were measured at baseline and repeated 6 hours later or as clinically indicated. Hepatic damage was defined when patients had AST or ALT levels ≥ 120 UI/l (WHO, 2009). Coagulation disorder was defined when patient had PT > 1.2 times of control, aPTT > 1.2 times of control, or fibrinogen < 1 g/l (Kathleen *et al*., 2019). Arterial blood gas was performed when there was tachypnea and/or retractions or SpO_2 < 90% on room air. Respiratory distress was defined as tachypnea, retractions, hypoxemia (SpO_2 < 90% and/or PaO_2 < 60 mmHg on room air), or $PaCO_2$ > 50 mmHg (Hammer, 2013).

Cortisol and interleukins measurement

Whole blood samples were taken based on standard procedures in collection tubes containing anticoagulant EDTA and were soon centrifuged to obtain plasma fractions. Plasma samples were stored at -20°C and analyzed in a set of 10-15 samples. Total serum cortisol was quantitative using chemiluminescent immunometric assay (Elecsys® Cortisol II, Roche Diagnostics, Germany) via Cobas e 602 machine (Roche Diagnostics, Germany). The threshold of detection is 0.054 µg/dl. Critical illness-related corticosteroid insufficiency, or adrenal insufficiency (A.I.), was defined as total random serum cortisol <10µg/dl in the setting of shock, according to the consensus of the Society of Critical Care Medicine and European Society of Intensive Care Medicine (Annane *et al*., 2017). IL-6 was measured using the Cobas e 411 machines (Elecsys® IL-6, Roche Diagnostics, Germany) at the minimum 1.5 pg/ml concentration, and IL-6 was increased when the concentration >7 pg/ml. Similarly, the IL-10 threshold is 1.5 pg/ml (using IMMULITE 1000, Siemens Healthineers, Germany), and the increasing concentration is more than 9.1 pg/ml.

Statistical analysis

Variates were presented as numbers and percentages, median with interquartile range (IQR, 25%–75%), or mean ± standard deviation. Categorical variables were compared using the χ^2 test or Fisher's exact test, and continuous variables were compared using the Student t-test, Mann-Whitney *U* test or Kruskal Wallis test. Normality of continuous variables was assessed by frequency distribution (histogram) and Shapiro-Wilk test. Individual changes in cortisol and interleukin levels at T0 and T12 were evaluated using paired T-test or Wilcoxon signed-rank test. In addition, the association between cortisol and interleukin levels and the total fluid requirement was assessed by Spearman's rank-order correlation test. All analyses were conducted using the Statistical Package for Social Sciences version 20.0 (SPSS, Inc., Chicago, IL, USA). A two-sided $p<0.05$ was considered statistically significant.

RESULTS

Patient characteristics

From July 1, 2019, to June 30, 2020, a total of 35 consecutive patients were prospectively enrolled in the study at a mean age of 6.9 ± 3.8 years. Thus, 27/35 (77.1%) patients with non-severe DSS and 8/35 (22.9%) patients with severe DSS, including one with prolonged DSS and one with recurrent DSS.

Overall, 21/35 (60%) patients had hepatic damage. A total of 27/35 (77.1%) patients had coagulation disorders and 14/35 (40%) patients with respiratory distress. All 14 patients with respiratory distress received oxygen, 11/14 (78.5%) needed nasal continuous positive airway pressure therapy and one required mechanical ventilation. Patients received a total mean fluid volume of 164 ± 44 ml/kg, infused average 31.3 ± 7.9 hours. Of the clinical characteristics, only respiratory distress was statistically more common in patients with severe DSS than non-severe DSS (Table 1).

Cortisol and interleukin levels

The main purpose is to determine the difference between cortisol and interleukin levels between patients with severe DSS and non-severe DSS. The cortisol level at T0 was significantly lower in the severe DSS group than the other group (median 7.3 µg/ml versus 14.3 µg/ml, $p<0.05$) (Figure 1 & Table 2). In all 35 patients, there was no significant correlation between cortisol levels and IL-6 or IL-10 levels at either T0 or T12 (Figure 4 & Table 2). Cortisol and IL-10 levels decreased, whereas IL-6 increased from T0 to T12 in both severe and non-severe DSS. However, only cortisol and IL-10 in the non-severe group and IL-6 in the severe group were statistically significant changes (Table 2 & Figure 2). Additionally, IL-6 (T0), IL-10 (T0), and IL-10 (T12) showed a moderate correlation with total fluid requirement (rho = 0.47, 0.4, and 0.36 with $p < 0.005$, $p = 0.018$, and $p = 0.033$, respectively; Figure 3).

Table 1. Baseline characteristics between non-severe DSS and severe DSS group

	Non-severe DSS (n=27)	Severe DSS (n=8)	P
Age (years), median (IQR)	5 (4, 7)	6 (5, 8)	0.77*
Male, n (%)	14 (51.8)	3 (37.5)	0.70†
Obesity, n (%)	4 (14.8)	2 (25)	0.60†
Hematocrit at T0, %	47.4 ± 4.7	50.6 ± 2.6	0.08***
Platelet count (x 1000/µL), median (IQR)	21 (10.2, 31)	23 (18, 25)	0.90*
Lactate (mmol/L), median (IQR)	3 (2.5, 4)	3 (2.5, 3.4)	0.69*
AST (IU/L), median (IQR)	156 (97., 284)	198 (131, 309)	0.91*
ALT (IU/L), median (IQR)	62 (39, 169)	89 (23, 127.2)	0.85*
INR, median (IQR)	1.1 (1, 1.4)	1.3 (1.1, 1.5)	0.13*
aPTT (seconds), median (IQR)	43.7 (38.8, 61.2)	61.5 (41.8, 120)	0.05*
Fibrinogen (g/L), median (IQR)	1.1 (0.7, 2.1)	0.8 (0.7, 1.6)	0.62*
Respiratory distress, n (%)	8 (29.6%)	6 (75%)	**0.04**†
Hepatic damage, n (%)	16 (59.2%)	5 (62.5%)	1.00†
Coagulation disorder, n (%)	20 (74.1%)	7 (87.5%)	0.65†
Total fluid volume (ml/kg)	173.6 ± 38	188.8± 68.7	0.41‡
Fluid infusion time (hours)	33.8 ± 7.5	37.1 ± 7.1	0.11‡
Total colloid fluid volume (ml/kg)	101.2 ± 44	135.4 ± 31.8	0.15‡

ALT: alanine aminotransferase, aPTT: activated partial thromboplastin time, AST: aspartate aminotransferase, INR: International Normalized Ratio, IQR: interquartile range (25% and 75%).
* Mann – Whitney U test; † Fisher's exact test; ‡ T-test.

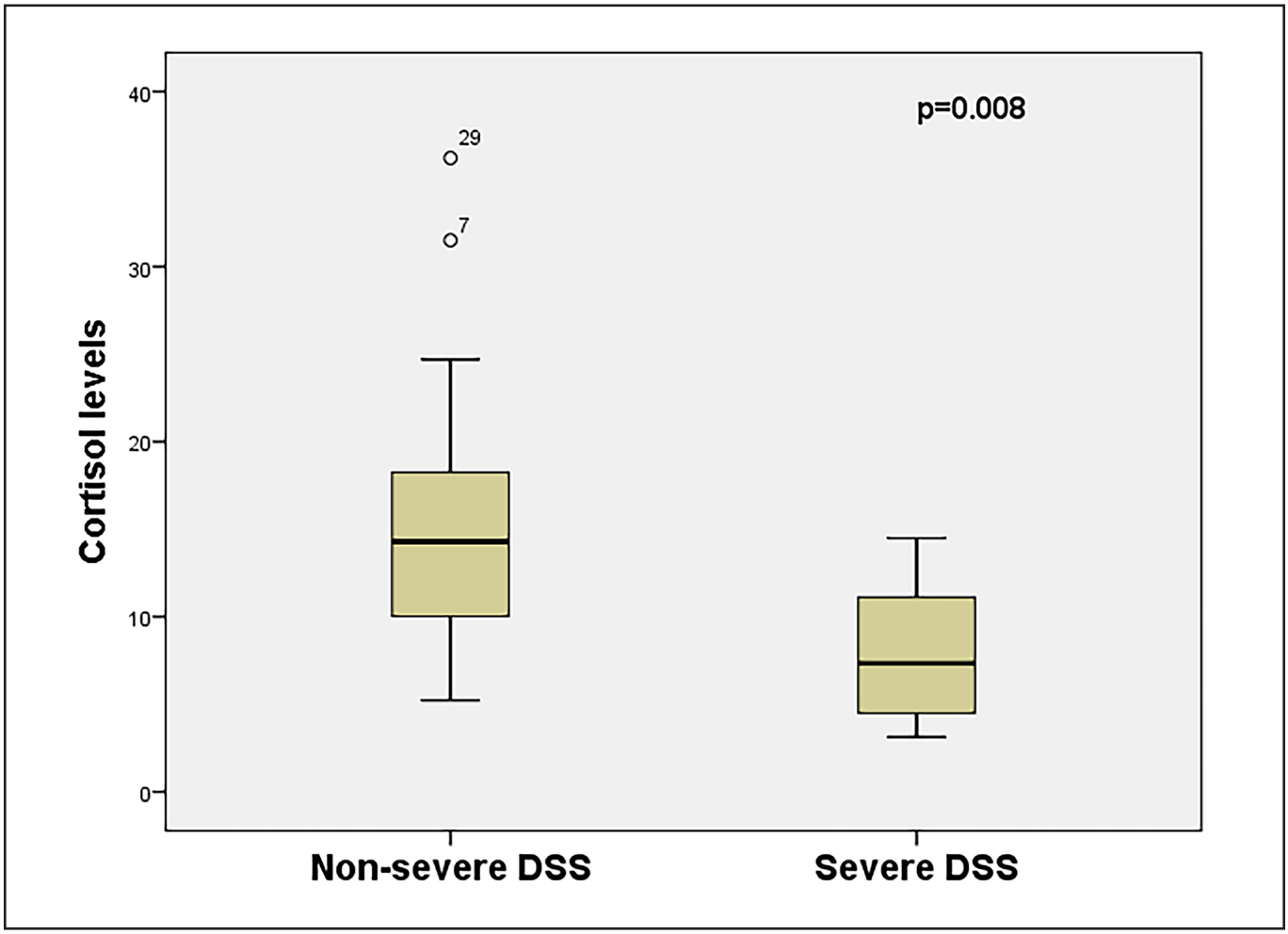

Figure 1. Cortisol level at presentation (T0) between non-severe DSS and severe DSS
Illustration of the difference in cortisol levels between severe DSS and non-severe DSS group. Box plot charts represent median and 25^{th} – 75^{th} percentiles. Mann – Whitney U test was performed to compare the median of each group.

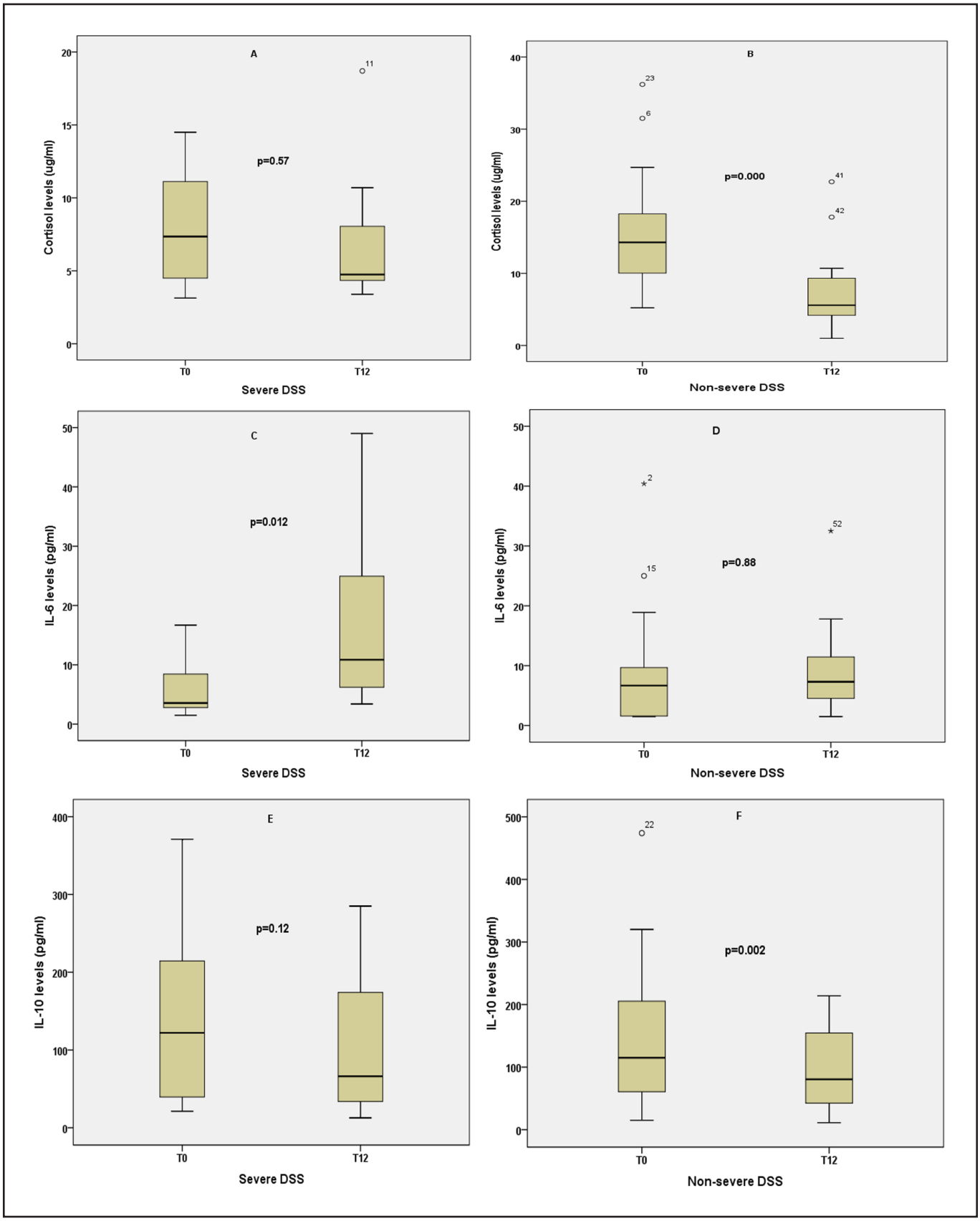

Figure 2. Changes in cortisol, IL-6, IL-10 from T0 to T12 in severe DSS and non-severe DSS groups. Shown are changes in cortisol levels (A & B), IL-6 levels (C & D), IL-10 levels (E & F) from T0 to T12 in severe DSS and non-severe DSS groups, respectively. Data are shown as the box plot (the top of the box represents the 75th percentile, the bottom of the box represents the 25th percentile and the line in the middle indicates the 50th percentile). The statistical method performed was Wilcoxon signed-rank test.

DISCUSSION

Dengue shock syndrome due to plasma leakage is the most severe form of dengue infection in children, accounting for about 15% of all cases (Anders *et al*., 2011). Unfortunately, despite clear and updated treatment guidelines, the pathophysiology of DHF and DSS has not been fully understood, and the management of pediatric patients with DSS remains a challenge in tropical countries.

Interestingly, the elevated cortisol levels at T0 in the non-severe DSS group eventually dropped to levels similar to those in the severe DSS group both at T0 and T12. This observation suggested there was likely a typical pattern of adrenal response in the early phase of DSS. Still, adrenal dysfunction existed in both groups of severe and non-severe DSS. It remains to be investigated if more severe and/or prolonged hypoperfusion of the adrenal gland or other factors affected the adrenal function in DSS. Previously, cortisol level has was demonstrated higher in DHF than in non-dengue controls, and there was no AI in DHF cases (Myo *et al*., 1995). In addition, higher cortisol level was observed in DHF compared to dengue fever (Joob & Wiwanitkit, 2014). These observations are likely similar to the pattern observed in other critical illnesses such as trauma, sepsis, acute respiratory distress syndrome, etc., in which hypercortisolemia was positively related to the severity of the condition (Widmer *et al*., 2005; Mesotten *et al*., 2008). The deficiency of cortisol levels has also been reported and associated with increased mortality from critical illness (Rothwell & Lawler, 1995). In this study, we hypothesized that the adrenal function in pediatric patients with severe and non-severe DSS might be dysregulated, which resulted in an inappropriate adrenal response potentially affecting patient outcomes. Corticosteroid therapy has been shown to be effective in some patients with severe DSS (Bandara & Herath, 2018). Further studies with larger sample sizes are likely needed to understand how adrenal dysfunction in pediatric patients with DSS can affect outcomes.

Many studies showed that cytokines play a major role in the pathogenesis of DHF, reflecting an imbalance between Th1 and Th2 cells. The early Th1 response was characterized by the production of IFN-γ, IL-2 for viral clearance, characterized by dengue fever. The response of Th2 produced cytokines IL-4, IL-5, IL-6, IL-10, and IL-13 which were accountable for inflammatory responses, vascular endothelial cells insult, and changes in homeostasis leading to plasma loss, which was the hallmark of DHF and DSS (Chaturvedi *et al*., 2000). Elevated IL-6, IL-10 levels were considered to be markers of DHF severity in various previous studies and associated with vascular leakage, bleeding, and other complications presence (Juffrie *et al*., 2001; Iani *et al*., 2016; Singla *et al*., 2016). In our study, there were no significant differences in the IL-6 and IL-10 levels between the severe and non-severe DSS at both T0 and T12. In addition, from T0 to T12, both severe and non-severe DSS showed an increase in IL-6 and a decrease in IL-10. However, the increase in IL-6 and decrease in IL-10 were only significant in the severe DSS and non-severe DSS, respectively (Table 2 & Figure 2).

Furthermore, our study results demonstrated that IL-6 levels at T0, IL-10 levels at both T0 and T12 were positively correlated with the total fluid requirement, which is considered as a marker of DSS severity in clinical practice. Fluid replacement is the cornerstone of DSS treatment. A strict fluid replacement strategy is strongly recommended as too much fluid administration may increase patient morbidity and mortality. Our study results may serve as a proof that IL-6 and IL-10 levels may be studied as markers

Table 2. Cortisol, IL-6, and IL-10 levels at T0 and T12 between two groups of DSS

		Non-severe DSS (n=27)	Severe DSS (n=8)	P
Cortisol (µg/ml)	T0	14.3 (9.4, 18.3)	7.3 (4.2, 12)	**0.008***
	T12	5.6 (4.1, 9.4)	4.7 (4.3, 9.4)	0.91*
IL-6 (pg/ml)	T0	6.7 (1.5, 10.4)	3.5 (2.6, 8.6)	0.94*
	T12	6.9 (4.5, 11.8)	10.9 (5.7, 42.1)	0.13*
IL-10 (pg/ml)	T0	115 (52.3, 209)	122 (32.6, 241.7)	0.91*
	T12	80.6 (37.4, 167)	66.2 (31.3, 176)	0.83*

All data were presented as median and interquartile ranges (25% and 75%).
* Mann – Whitney U test was performed.

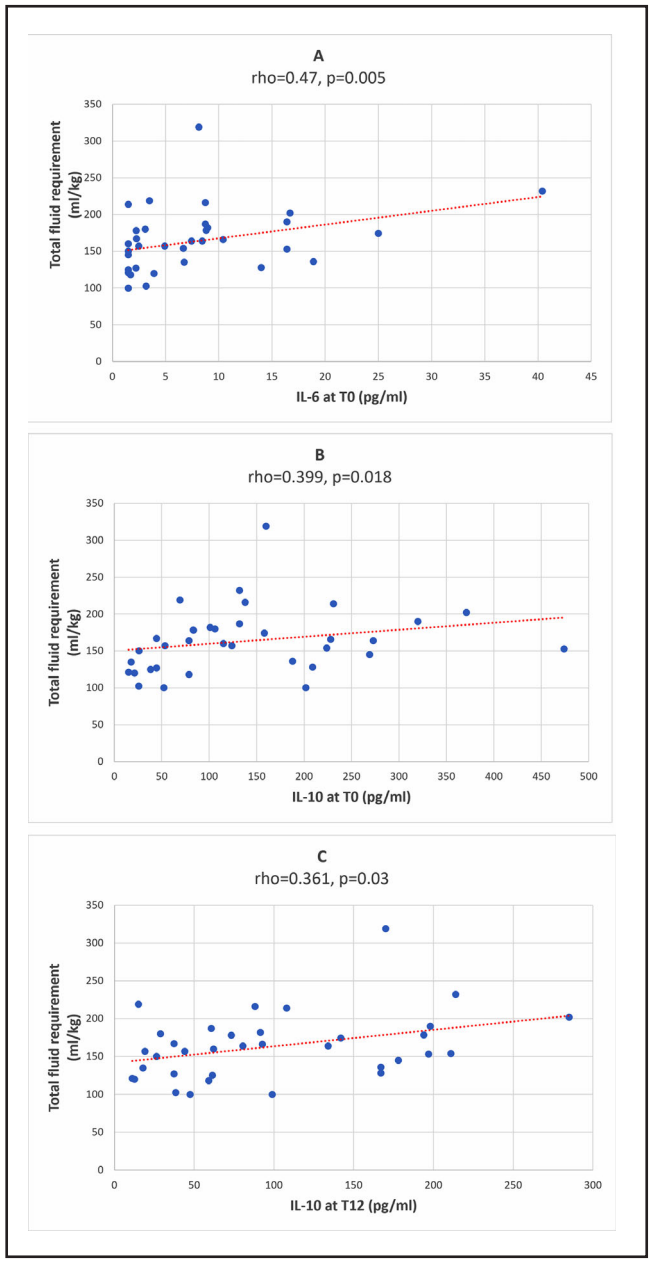

Figure 3. Correlations between total fluid requirement and IL-6 (T0), IL-10 (T0), and IL-10 (T12). Scatter plots show the correlations of total fluid requirement with IL-6 levels at T0 (panel A), IL-10 levels at T0 (panel B), and IL-10 levels at T12 (panel C). The red dotted lines indicate regression lines. Spearman rank correlation test was used to measure the degree of association.

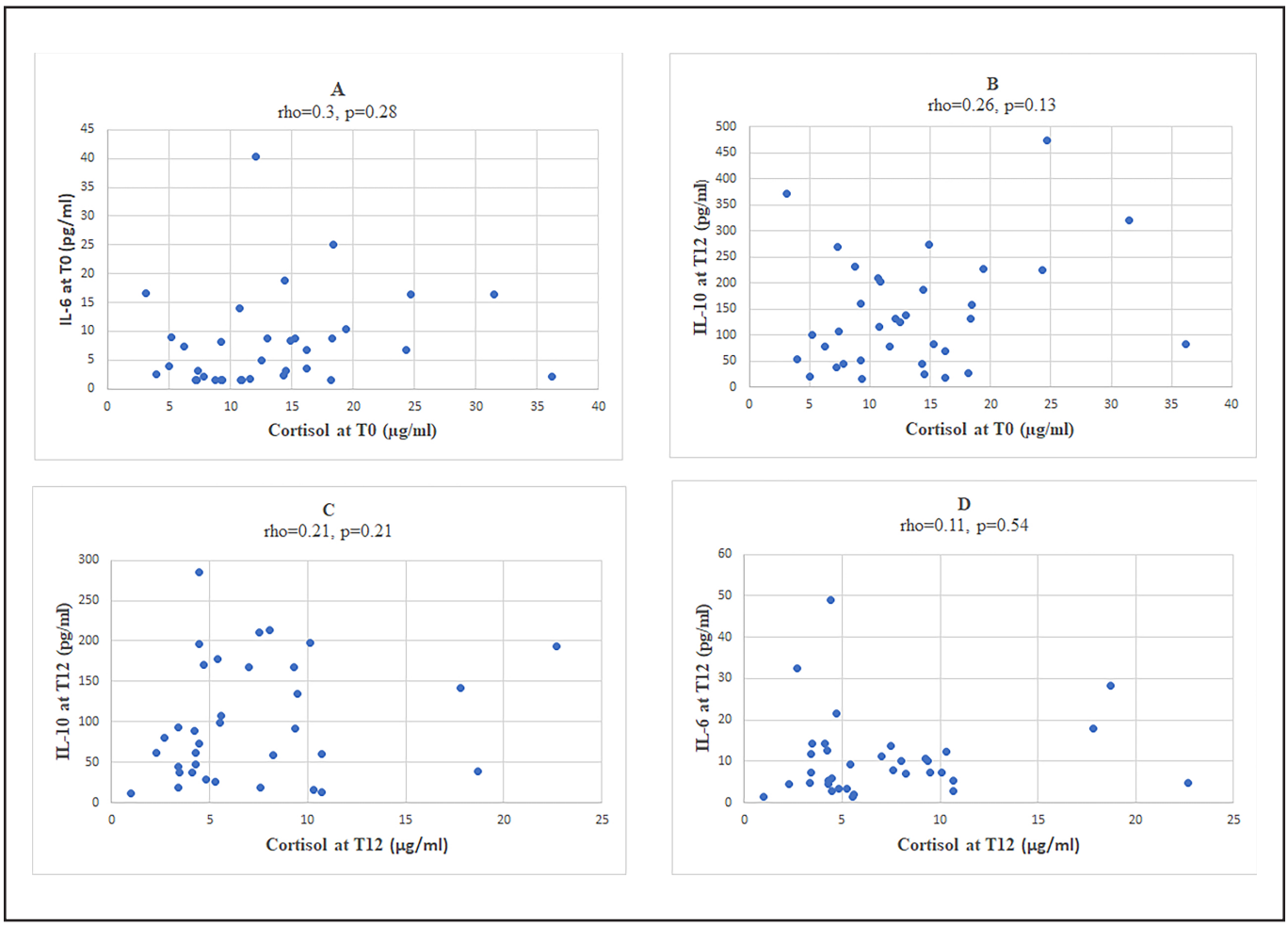

Figure 4. Spearman correlation between cortisol and IL-6, IL-10 at T0 and T12.

of plasma leakage, which can potentially guide fluid replacement therapy in DSS.

As for the relationship between cortisol and interleukin in infectious diseases, Marieke den Brinker *et al*. studied 69 pediatric patients with meningococcal sepsis and reported a positive correlation between cortisol and IL-6 (r = 0.45), with cortisol/ACTH ratio decreasing by 19% for every doubling of IL-6 (den Brinker *et al*., 2005). However, to the best of our knowledge, no studies had examined both cortisol and IL-6, IL-10 levels in patients with dengue infection, especially in patients with DSS. In our study, cortisol levels did not correlate with IL-6 or IL-10 levels in the samples collected both at T0 and T12, although a decrease in cortisol and increase in IL-6 levels were observed in all DSS patients.

Our prospective study in a tertiary pediatric center was limited by its small sample size and a limited number of pediatric patients with severe DSS. In addition, investigation of the adrenal gland using imaging studies in patients with low cortisol levels was not performed in our study, limiting our ability to understand the underlying pathologies of the adrenal gland in these patients. Furthermore, the categorization of DSS into severe DSS and non-severe DSS could be vague, considering the features of "severe DSS" in this study could be secondary to late recognition. Finally, blood sampling was limited to a very tight period (12 hours) which may not give the complete picture of the cortisol and interleukins kinetics in response to the severity or progression of DSS.

In conclusion, our study demonstrated that patients with severe DSS had lower cortisol levels when compared to non-severe DSS patients at shock recognition. However, the inappropriate adrenal response was noted in both severe and non-severe DSS groups as indicated in cortisol levels at the 12-hour mark. In addition, IL-6 and IL-10 levels are correlated with a total fluid requirement, which is a marker of DSS severity. Thus, further studies may help reveal how adrenal dysfunction in pediatric patients with DSS can affect outcomes and the potential roles of interleukin levels in fluid management strategy.

ACKNOWLEDGMENT

The authors appreciate all patients and their families who participated in this research and many healthcare professionals who helped make this project possible.

Conflict of interest

The authors report no conflict of interest.

REFERENCES

Anders, K.L., Nguyet, N.M., Chau, N.V.V., Hung, N.T., Thuy, T.T., Lien, L.B., Farrar, J., Wills, B., Hien, T.T. & Simmons, C.P. (2011). Epidemiological factors associated with dengue shock syndrome and mortality in hospitalized dengue patients in Ho Chi Minh City, Vietnam. *The American Journal of Tropical Medicine and Hygiene* **84**: 127-134. https://doi.org/10.4269/ajtmh.2011.10-0476

Annane, D., Pastores, S.M., Rochwerg, B., Arlt, W., Balk, R.A., Beishuizen, A., Briegel, J., Carcillo, J., Christ-Crain, M., Cooper, M.S. et al. (2017). Guidelines for the diagnosis and management of Critical Illness-Related Corticosteroid Insufficiency (CIRCI) in critically ill patients (Part I): Society of Critical Care Medicine (SCCM) and European Society of Intensive Care Medicine (ESICM) 2017. *Critical Care Medicine* **45**: 2078-2088. https://doi.org/10.1097/ccm.0000000000002737

Bandara, S.M.R. & Herath, H. (2018). Effectiveness of corticosteroid in the treatment of dengue – A systemic review. *Heliyon* **4**(9): e00816. https://doi.org/10.1016/j.heliyon.2018.e00816

Bhatt, S., Gething, P.W., Brady, O.J., Messina, J.P., Farlow, A.W., Moyes, C.L., Drake, J.M., Brownstein, J.S., Hoen, A.G., Sankoh, O. et al. (2013). The global distribution and burden of dengue. *Nature* **496**: 504-507. https://doi.org/10.1038/nature12060

Chaturvedi, U.C., Agarwal, R., Elbishbishi, E.A. & Mustafa, A.S. (2000). Cytokine cascade in dengue hemorrhagic fever: implications for pathogenesis. *FEMS Immunology and Medical Microbiol*ogy **28**: 183-188. https://doi.org/10.1111/j.1574-695X.2000.tb01474.x

den Brinker, M., Joosten, K.F.M., Liem, O., de Jong, F.H., Hop, W.C.J., Hazelzet, J.A., van Dijk, M. & Hokken-Koelega, A.C.S. (2005). Adrenal insufficiency in meningococcal sepsis: Bioavailable cortisol levels and impact of interleukin-6 levels and intubation with etomidate on adrenal function and mortality. *The Journal of Clinical Endocrinology & Metabolism* **90**: 5110-5117. https://doi.org/10.1210/jc.2005-1107

Guzman, M.G., Halstead, S.B., Artsob, H., Buchy, P., Farrar, J., Gubler, D.J., Hunsperger, E., Kroeger, A., Margolis, H.S., Martínez, E., Nathan, M.B. et al. (2010). Dengue: a continuing global threat. *Nature Reviews Microbiology* **8**: S7-16. https://doi.org/10.1038/nrmicro2460

Hammer, J. (2013). Acute respiratory failure in children. *Paediatric Respiratory Reviews* **14**: 64-69. https://doi.org/10.1016/j.prrv.2013.02.001

Hebbar, K.B., Stockwell, J.A., Leong, T. & Fortenberry, J.D. (2011). Incidence of adrenal insufficiency and impact of corticosteroid supplementation in critically ill children with systemic inflammatory syndrome and vasopressor-dependent shock*. *Critical Care Medicine* **39**: 1145-1150. https://doi.org/10.1097/CCM.0b013e31820eb4e4

Iani, F.C., Caldas, S., Duarte, M.M., Cury, A.L., Cecílio, A.B., Costa, P.A., Antonelli, L.R. & Gollob, K.J. (2016). Dengue patients with early hemorrhagic manifestations lose coordinate expression of the anti-inflammatory cytokine IL-10 with the inflammatory cytokines IL-6 and IL-8. *American Journal of Tropical Medicine and Hygiene* **95**: 193-200. https://doi.org/10.4269/ajtmh.15-0537

Joob, B. & Wiwanitkit, V. (2014). Serum cortisol in dengue and dengue hemorrhagic fever: Is there any clinical implication? *Asian Pacific Journal of Tropical Disease* **4**: 313-314. https://doi.org/10.1016/S2222-1808(14)60579-X

Juffrie, M., Meer, G.M., Hack, C.E., Haasnoot, K., Sutaryo, Veerman, A.J. & Thijs, L.G. (2001). Inflammatory mediators in dengue virus infection in children: interleukin-6 and its relation to C-reactive protein and secretory phospholipase A2. *American Journal of Tropical Medicine and Hygiene* **65**: 70-75. https://doi.org/10.4269/ajtmh.2001.65.70

Kathleen, P., Timothy, P. & Theresa, P. (2019). Mosby's Diagnostic and Laboratory Test Reference. Missouri: Elsevier, pp. 255.

Mesotten, D., Vanhorebeek, I. & Van den Berghe, G. (2008). The altered adrenal axis and treatment with glucocorticoids during critical illness. *Nature Clinical Practice Endocrinology and Metabolism* **4**: 496-505. https://doi.org/10.1038/ncpendmet0921

Myo, K., Soe, T., Thein Thein, M., Than Nu, S., Tin Tin, S. & Muya, T. (1995). Serum cortisol levels in children with dengue haemorrhagic fever. *Journal of Tropical Pediatrics* **41**: 295-297. https://doi.org/10.1093/tropej/41.5.295

Nguyen, P.N.T., Trang, P.T.K. & Tuan, T.D. (2019). Respiratory distress associated with dengue hemorrhagic fever on paediatric patients: Learning from a provincial hospital in Southern Vietnam. *Archives of Pharmacy Practice* **10**: 92-97.

Pizarro, C.F., Troster, E.J., Damiani, D. & Carcillo, J.A. (2005). Absolute and relative adrenal insufficiency in children with septic shock. *Critical Care Medicine* **33**: 855-859. https://doi.org/10.1097/01.ccm.0000159854.23324.84

Rothwell, P.M. & Lawler, P.G. (1995). Prediction of outcome in intensive care patients using endocrine parameters. *Critical Care Medicine* **23**: 78-83. https://doi.org/10.1097/00003246-199501000-00015

Singla, M., Kar, M., Sethi, T., Kabra, S.K., Lodha, R., Chandele, A. & Medigeshi, G.R. (2016). Immune response to dengue virus infection in pediatric patients in New Delhi, India. Association of viremia, inflammatory mediators and monocytes with disease severity. *PLoS Neglected Tropical Diseases* **10**: e0004497. https://doi.org/10.1371/journal.pntd.0004497

Vietnam MoH (Vietnam Ministry of Health). (2019). Dengue Hemorrhagic Fever diagnosis and treatment guideline. Hanoi.

Widmer, I.E., Puder, J.J., König, C., Pargger, H., Zerkowski, H.R., Girard, J. & Müller, B. (2005). Cortisol response in relation to the severity of stress and illness. *The Journal of Clinical Endocrinology and Metabolism* **90**: 4579-4586. https://doi.org/10.1210/jc.2005-0354

Wills, B.A., Oragui, E.E., Stephens, A.C., Daramola, O.A., Dung, N.M., Loan, H.T., Chau, N.V., Chambers, M., Stepniewska, K., Farrar, J.J. et al. (2002). Coagulation abnormalities in dengue hemorrhagic fever: Serial investigations in 167 Vietnamese children with dengue shock syndrome. *Clinical Infectious Diseases* **35**: 277-285. https://doi.org/10.1086/341410

WHO (1997). Dengue haemorrhagic fever: diagnosis, treatment, prevention and control. pp. 12-23.

WHO (2009). Dengue: guidelines for diagnosis, treatment, prevention and control. pp. 3-132.