Journal of Clinical Virology 50 (2011) 8–12

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

High plasma mid-regional pro-adrenomedullin levels in children with severe dengue virus infections

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ARTICLE INFO

Article history:
Received 12 July 2010
Received in revised form
13 September 2010
Accepted 14 September 2010

Keywords:
Dengue hemorrhagic fever/dengue shock syndrome
Biomarkers
Adrenomedullin
Mid-regional pro-adrenomedullin
Vasopressin
Copeptin

ABSTRACT

Background: Dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) is characterized by hemorrhage, plasma leakage and shock. Adrenomedullin and vasopressin are vaso-active hormones that mediate endothelial permeability, vascular tone and water balance and may therefore play a role during DHF/DSS. Adrenomedullin reduces endothelial permeability and has vasodilatory properties, while vasopressin is a potent vasoconstrictor with anti-diuretic effects.
Objectives: To determine mid-regional pro-adrenomedullin (MR-proADM) and copeptin, which are reliable and stable markers for adrenomedullin and vasopressin response, respectively, and relate their plasma concentrations to outcome and markers of plasma leakage in Indonesian children with DHF and DSS.
Study design: In this observational cohort study Indonesian children with DHF/DSS were enrolled. On study days 0 and 2, plasma MR-proADM and copeptin concentrations as well as parameters of plasma leakage were determined. Plasma MR-proADM and copeptin concentrations were compared to values of healthy controls.
Results: MR-proADM was increased in both DHF ($n = 43$) and DSS ($n = 28$) vs. controls ($n = 17$), with median (IQR) values of 0.47 (0.40–0.68), 0.56 (0.44–1.00) vs. 0.22 (0.19–0.29) nmol/L, respectively. Additionally, MR-proADM correlated with signs of increased vascular leakage such as low albumin and increased pleural effusion. Copeptin concentrations showed no significant changes as compared to controls.
Conclusions: MR-proADM concentrations are elevated in children with DHF and DSS and correlate with the severity of plasma leakage, in contrast to copeptin concentrations. We speculate that adrenomedullin has a functional role in limiting endothelial hyperpermeability during DHF/DSS. Finally, MR-proADM may be a candidate biomarker to predict development of DHF/DSS.

1. Background

The past decades have seen a remarkable increase in the incidence and global expansion of dengue virus infections. Dengue virus infection usually manifests as a non-severe febrile illness, called dengue fever (DF). However, each year 250,000–500,000 of the estimated 100 million patients with dengue virus infection develop more severe disease: dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS).[1] A central phenomenon of DHF/DSS is a transient dysfunction of the endothelial barrier, which results in plasma leakage, shock and hemorrhage. The pathogenesis of this vascular hyperpermeability is still largely unknown, although the excessive release of pro-inflammatory cytokines and vaso-active mediators is generally assumed a central process.[2]

Adrenomedullin and vasopressin are vaso-active hormones that exert specific hemodynamic effects and have received attention in recent years as biomarkers for sepsis. Adrenomedullin is predominantly released by the endothelium, acts as a potent vasodilator and has natriuretic effects.[3] Other properties of adrenomedullin include a reduction in endothelial permeability,[4,5] bactericidal effects[6] and down-regulation of pro-inflammatory cytokines.[7] Administration of exogenous adrenomedullin in animal models of sepsis results in reduced mortality.[5] Vasopressin (also known as the antidiuretic

Abbreviations: DF, dengue fever; DHF, dengue hemorrhagic fever; DSS, dengue shock syndrome; MR-proADM, mid-regional pro-adrenomedullin; IQR, interquartile range; WHO, world health organization.

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doi:10.1016/j.jcv.2010.09.008

hormone) is released from the hypothalamus and has potent antidiuretic and vasoconstrictive effects.[8] The hemodynamic effects of adrenomedullin and vasopressin are thus partly opposite.

Recently, assays have become available to determine circulating mid-regional pro-adrenomedullin (MR-proADM) and copeptin concentrations.[9,10] These peptides are co-synthesized with adrenomedullin and vasopressin, respectively, and have the advantage of a longer half-life, lack of bioactivity and lack of protein binding, which makes them more suitable for daily practice.[11,12] Patients with septic shock have elevated plasma concentrations of both MR-proADM and copeptin, which correlate with prognosis.[13,14]

Vasopressin and adrenomedullin may play a pathogenic role in DHF/DSS through maintaining vascular tone and via regulating vascular endothelial integrity. Moreover, copeptin and MR-proADM may be valuable biomarkers to predict those patients at risk for developing DHF/DSS. No data are yet available on copeptin and MR-proADM during dengue virus infection.

2. Objectives

To determine plasma MR-proADM and copeptin concentrations in a cohort of children with DHF or DSS and relate their plasma concentrations to outcome and markers of plasma leakage.

3. Study design

This study was designed as an observational cohort study and was conducted in the Dr. Kariadi University Hospital in Semarang, Indonesia. Children, aged below 15 years, admitted to the paediatric ward or intensive care unit with a clinical diagnosis of suspected DHF or DSS were included from July 2005 until May 2006; 17 healthy children, age 6–14 years, were included as controls. Demographic, clinical and laboratory data were collected using a standardized data collection form. A chest X-ray was performed with the patient lying in right lateral decubitus position to detect pleural effusion and a tourniquet test was performed to detect bleeding tendency. In children with pleural effusion, the pleural effusion index (PEI) was calculated. The PEI was defined as 100 times the maximum width of the (right or left) pleural effusion, divided by the maximum width of the hemi-thorax on that side.

Children were classified as having suspected DHF or DSS according to WHO criteria.[15] In summary, suspected DHF was defined as presence of fever, a hemorrhagic tendency, thrombocytopenia ($<100 \times 10^9$/L), evidence of pleural effusion and/or a >20% rise or drop in hematocrit after volume replacement therapy. DSS was defined as DHF with evidence of circulatory failure. All patients had a positive dengue specific IgM, determined by ELISA. Blood was collected in EDTA and citrate blood tubes on day 0 (day of admission), day 2 and the day of discharge.

3.1. Laboratory procedures

EDTA blood was centrifuged at 15 °C for 20 min at $1600 \times g$ and plasma was stored at −80 °C until further analysis. MR-proADM and copeptin were measured in 50 μl of plasma by a Time-Resolved Amplified Cryptate Emission (TRACE) technology assay,[16] using kits designed for automated sandwich immunofluorescent assay of MR-proADM and copeptin, respectively (KRYPTOR; BRAHMS AG). The KRYPTOR MR-proADM and copeptin assays have a detection range of 0.05–100 nmol/L and 4.8–1200 pmol/L, respectively.

A full blood count was performed daily by a standard hematology analyzer. Serum total protein and albumin concentrations were measured by Biuret and Bromocresol–Green method respectively, as described before.[17,18] Presence of dengue specific IgM and IgG antibodies was determined by capture and indirect ELISA (Focus Technologies, Cypress, Calif., USA).

Table 1
Patient characteristics and baseline data.

Characteristic	DHF (DHF I and II) $n = 43$	DSS (DHF III and IV) $n = 28$
Male sex; *n* (%)	15 (35)	9 (32)
Age; years	8 (6–9)	7 (6–9)
Body weight; kg	20 (18–28)	22 (17–30)
Body height; cm	120 (111–133)	120 (112–137)
Body temperature; °C	37.6 (37.0–38.4)	38.0 (37.1–38.5)
Duration fever until admission; days	4.0 (3.0–5.0)	4.0 (4.0–5.0)
Pulse rate; rate per minute	100 (90–110)	NA
Systolic blood pressure; mmHg	100 (100–110)	NA
Diastolic blood pressure; mmHg	70 (60–70)	NA
Mean arterial pressure; mmHg	80 (73–85)	NA
Respiratory rate; rate per minute	28 (24–29)	28 (24–30)
Tourniquet test positive; *n* (%)	29 (67)	14 (50)
Petechiae; *n* (%)	13 (30)	7 (25)
Epistaxis; *n* (%)	6 (14)	0 (0)
Gum bleeding; *n* (%)	1 (2)	0 (0)
Hematemesis; *n* (%)	2 (5)	1 (4)
Melena; *n* (%)	0 (0)	1 (4)
Hemoglobin; g/dL	13.1 (12.4–14.0)	13.3 (11.9–14.2)
Hematocrite; %	38.2 (36.4–41.7)	39.8 (35.6–43.3)
Platelet count; $\times 10^9$/L	63 (40–85)	38 (26–67)
White blood cell count; $\times 10^3$ cells/mL	4.4 (3.1–6.3)	5.0 (3.0–9.5)
Albumin serum; g/dL,	3.5 (2.9–3.8)	2.8 (2.5–3.3)**
Total protein serum; g/dL	5.9 (4.9–6.5)	4.8 (3.8–5.7)**
Pleural effusion index at day 0	14 (0–22)	18 (12–29)*

Data represent medians with interquartile ranges or numbers with percentages. No data are given for pulse rate and blood pressure in the DSS group as all children received immediate intravenous fluid therapy at the time of presentation. DHF, dengue hemorrhagic fever; DSS, dengue shock syndrome; NA, not applicable; *n* (%), absolute numbers (percentage) within DHF or DSS group.

* $p < 0.05$; calculated by Mann–Whitney *U* test.

** $p < 0.01$; calculated by Mann–Whitney *U* test.

3.2. Statistical analyses

Data are expressed as medians with corresponding interquartile range (IQR) unless stated otherwise. Levels of MR-proADM and copeptin below the detection limit were assigned a value equal to the lower detection limit of the assay. Frequency comparison of categorical data was done by chi-square test. Mann–Whitney *U* test was used for statistical evaluation of continuous variables between 2 independent groups; Kruskal–Wallis test with Dunn's multiple comparison test was used for evaluation between 3 or more groups. Wilcoxon matched pairs test was used to evaluate changes in variables in time within groups. Relationships between continuous variables were examined by Spearman correlation analysis. A *p*-value of <0.05 indicated a significant difference. Statistical analyses were performed with SPSS version 16.0.

4. Results

4.1. Clinical characteristics

Seventy-one children with severe dengue virus infection were included in this study. According to WHO criteria, 43 (61%) were categorized as suspected DHF and 28 (39%) as suspected DSS. Patient characteristics and relevant baseline data are shown in Table 1. Children with DHF/DSS were generally young with a median age of 8 and 7 years for the DHF and DSS group, respectively. There were no significant differences in demographic characteris-

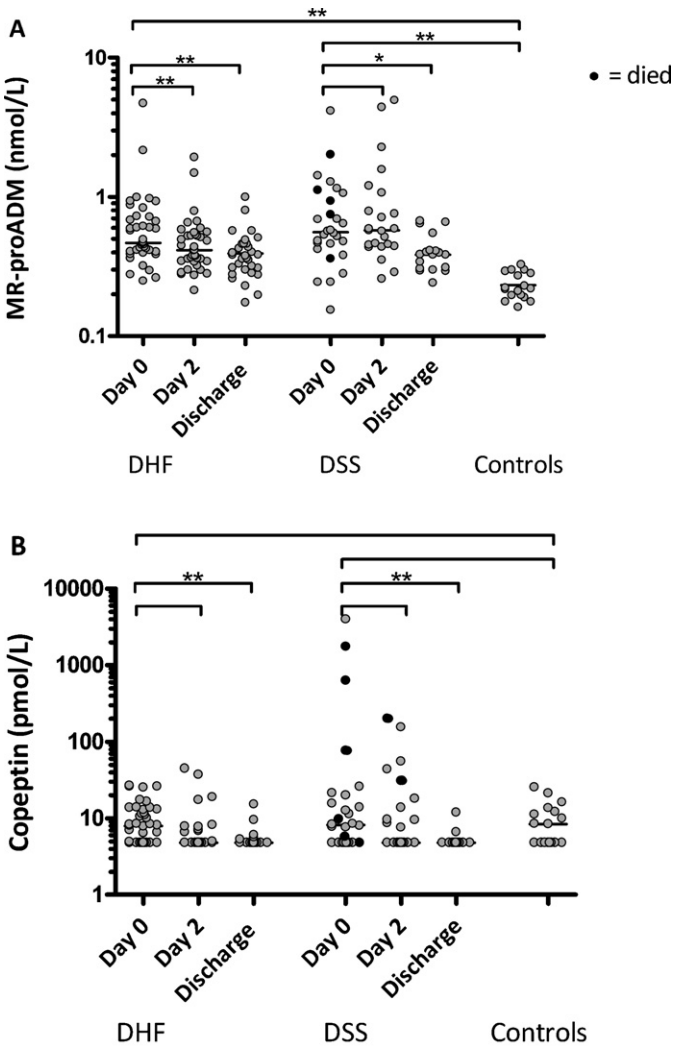

Fig. 1. Plasma mid-regional pro-adrenomedullin and copeptin concentrations in Indonesian children with DHF/DSS and in healthy controls. Plasma mid-regional pro-adrenomedullin (MR-proADM) (A) and copeptin (B) concentrations in Indonesian children with DHF and DSS vs. healthy controls. Data are depicted at day 0 (day of study enrollment), day 2, and the day of discharge. The lines represent the median values. *p* values are determined by Mann–Whitney *U* test for non-paired data and Kruskal–Wallis test for paired data. *Represents a *p* value < 0.05; **p value < 0.01.

tics between both groups. Except for a significantly lower platelet count in the DSS group, there were also no significant differences in hematological values. As expected, parameters for plasma leakage were consistent with more severe plasma leakage in the DSS group; children with DSS had a significantly higher PEI values and lower concentrations of serum albumin and serum protein. The median duration of hospital admission was 4 days (IQR 3–5). Six patients, all with DSS grade IV, died in hospital despite treatment. Treatment included intravenous fluid replacement ($n = 65/71$), fresh frozen plasma ($n = 11/71$), fresh plasma transfusion ($n = 1/71$), platelet transfusion ($n = 6/71$) and dopamine ($n = 7/71$).

4.2. MR-proADM and copeptin

Plasma MR-proADM concentrations were significantly higher in the DHF and DSS groups at enrollment than in the healthy controls with median (IQR) values of 0.47 nmol/L (0.39–0.68) and 0.56 nmol/L (0.44–1.00) vs. 0.22 nmol/L (0.19–0.29), respectively (Fig. 1A). While MR-proADM concentrations in the DHF group had decreased by day 2 to 0.41 nmol/L (0.34–0.54 nmol/L), MR-proADM concentrations remained elevated with 0.57 nmol/L (0.44–1.07 nmol/L) in the DSS group. All 6 patients who died of DSS had MR-proADM concentrations that were around or above the highest concentration found in the healthy controls.

Copeptin concentrations were not significantly higher in either of the patient groups as compared to the healthy controls on any day (Fig. 1B). Copeptin concentrations in the control group showed a larger variation and a broader overlap with the DHF and DSS concentrations than MR-proADM concentrations. Of the 6 patients that died, 3 patients had concentrations above the maximum concentration of the control group and 3 patients had copeptin concentrations within the range of the control group. Of the 4 highest copeptin concentrations on admission, 3 patients died.

4.3. MR-proADM is associated with increased plasma leakage

In both groups, protein and plasma leakage was already present at enrollment, indicated by the low serum concentrations of albumin and total protein (normal minimum values of 3.3 and 6.0 g/dL respectively) and the presence of pleural effusion (Table 1). The pleural effusion index increased from enrollment to day 2 in both groups, suggesting ongoing plasma leakage (Fig. 2A).

There was an inverse correlation of MR-proADM with serum albumin concentrations at enrollment (Spearman $r = -0.45$, $p = 0.0001$) (Fig. 2B). MR-proADM concentrations were positively associated with the pleural effusion index at day 2 (Spearman $r = 0.51$, $p = 0.0001$) (Fig. 2C). In contrast, there was no significant correlation of copeptin concentrations with either PEI or albumin concentrations (data not shown). There was also no significant correlation of blood pressure, which was corrected by intravenous fluids whenever necessary, with either MR-proADM or copeptin concentrations (data not shown).

5. Discussion

In the present study, we show that DHF/DSS is associated with elevations of plasma concentrations of MR-proADM. MR-proADM concentrations reflect the production of the vaso-active hormone adrenomedullin, which is considered to play an important role in the initiation of the early hyperdynamic response of septic shock.[19] Various studies have indeed reported elevated MR-proADM in patients with septic shock.[13,14] Among its diverse properties, the ability of adrenomedullin to reduce endothelial permeability is of particular interest in DHF/DSS, because this condition is characterized by a transient dysfunction in the endothelial cell barrier resulting in plasma leakage in predominantly the pleural and peritoneal cavities. Disintegration of the endothelial cell barrier may also contribute to the bleeding complications of DHF/DSS. Thus, although the vasodilatory effects of adrenomedullin may potentially have detrimental effects in DSS, our findings of elevated MR-proADM concentrations being associated with low serum albumin concentrations and with an increased pleural effusion index, may support the hypothesis that an increased adrenomedullin response is beneficial during DHF/DSS by counter-regulating the endothelial vascular hyperpermeability. MR-proADM levels gradually decreased over time but were not normalized at the time of discharge from the hospital. It should be noted however that improvement of the clinical condition together with elevation of platelets numbers are reason for discharge, which occurred after an average of 4 days in hospital. No follow-up samples were available after discharge.

Copeptin, a marker for vasopressin concentrations, has shown to be elevated during sepsis and septic shock as well.[20] We found no significant differences in copeptin concentrations between DHF and DSS patients and healthy controls. Notably, as has been

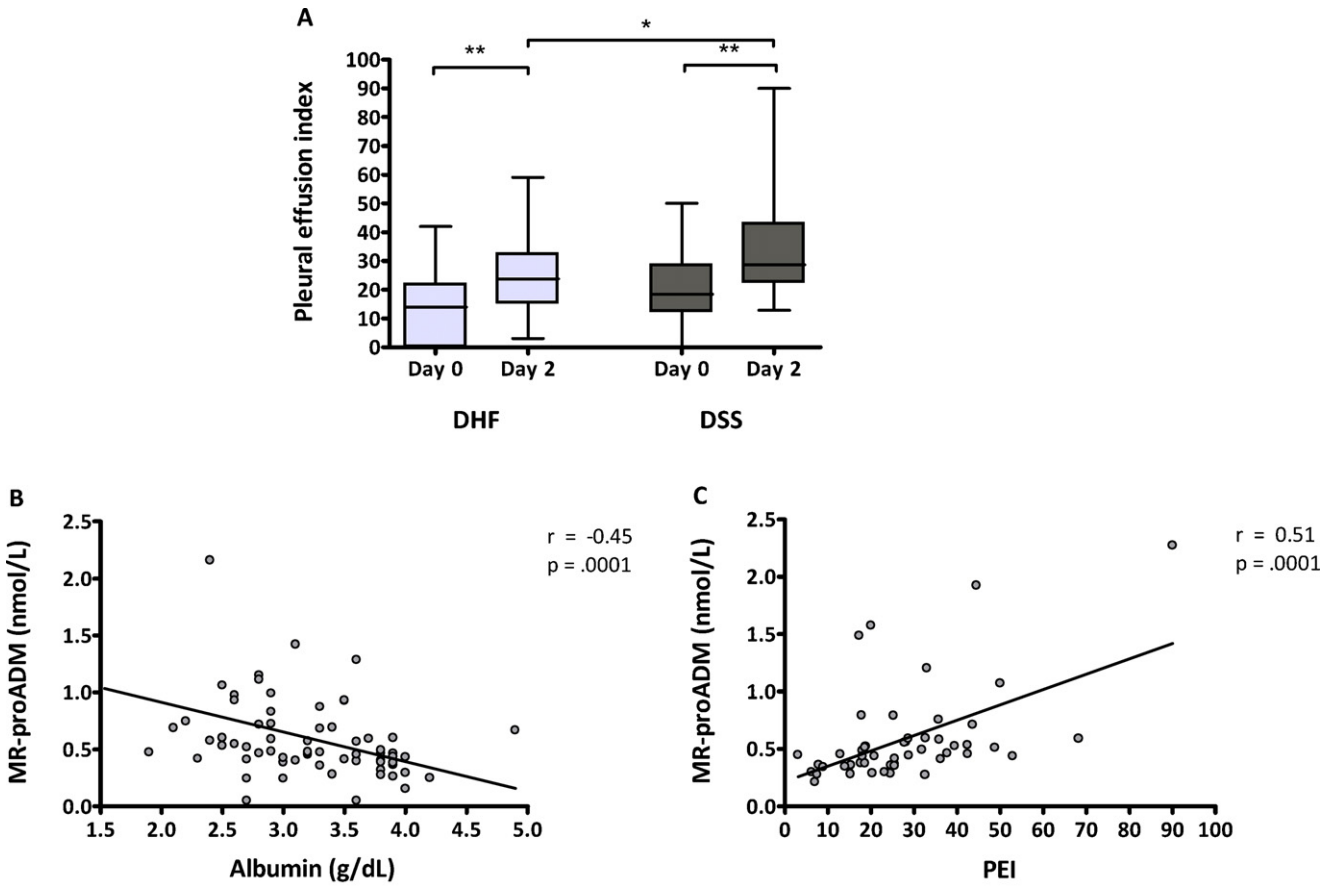

Fig. 2. Correlation of parameters plasma leakage parameters with mid-regional pro-adrenomedullin concentrations in Indonesian children with DHF/DSS. (A) Pleural effusion index (PEI) in Indonesian children with DHF and DSS on day 0 (day of study enrollment) and day 2 respectively. *p* values are determined by Mann–Whitney *U* test for non-paired data and Kruskal–Wallis test for paired data. *p* value < 0.05 is considered significant. *Represents a *p* value < 0.05; **p value < 0.01. Correlation between plasma mid-regional pro-adrenomedullin (MR-proADM) and serum albumin concentrations (B) on the day 0 ($n = 69$, 2 outliers are not represented in the figure). Correlation between MR-proADM and the PEI (C) at day 2 ($n = 52$, 1 outlier is not represented in the figure). Correlations are determined by Spearman correlation analysis (r).

observed for other stress-hormones like cortisol, the vasopressin response during human septic shock occurs in a biphasic manner, with initially high vasopressin concentrations, followed by a relative vasopressin deficiency. This phenomenon may also explain the rather large variation in copeptin concentrations in our patients. In addition, being a major regulator of water balance, factors such as water intake may also have a strong impact on vasopressin secretion. The observed variation in copeptin concentrations in the healthy controls may be attributed to such factors as well.

There is an urgent need for biomarkers in dengue virus infection to select those patients that are at risk for developing DHF/DSS. Presently, such biomarkers are lacking and this results in many more admissions of patients with dengue virus infection than the eventual number of DHF/DSS cases warrant, thereby seriously overloading the health care facilities in resource poor endemic countries. MR-proADM might be a potential biomarker to stratify patients with dengue infection for the risk of DHF/DSS. Prospective studies evaluating the predictive value of MR-proADM are needed, which should also include patients with uncomplicated dengue.

In conclusion, MR-proADM concentrations are elevated in children with DHF and especially with DSS. We hypothesize that adrenomedullin has a functional role in DHF/DSS by limiting endothelial hyperpermeability.

Funding

None.

Conflict of interest statement

None.

Ethical approval

The Study Research Ethics Committee of the Faculty of Medicine Diponegoro University in Semarang, Indonesia, approved all legal, ethical and laboratory aspects of the study. Written informed consent was obtained from parents or legal guardians of the patients and healthy controls.

Acknowledgements

We thank all the children and their parents/caretakers for participating in this study.

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