Original Article

Association between Circulating Cortisol and ACTH and Severity of Dengue Infection in Adult Patients

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Objective: To study the kinetics of endocrine changes in adult patients with dengue hemorrhagic fever (DHF) and dengue fever (DF) and association to disease severity.

Materials and Methods: This hospital-based observational clinical study involved 48 adult patients, 32 with DHF and 16 with DF, who presented with clinical features and positive serological testing for dengue infection on the day of diagnosis (D1). Serial circulating ACTH and cortisol concentrations were determined on D1, day of defervescence (Ddef), day 1 of convalescence (DC1 or 24 h after Ddef), day 2 of convalescence (DC2 or 48 h after Ddef), at 2-week follow-up (F1), and 2-month follow-up (F2).

Results: The median cortisol concentration in the DHF group was higher on D1 than that at F2. This was not found in patients with DF who had no difference of median cortisol concentration at D1 and F2. The median ACTH concentrations in both DHF and DF groups were low on D1 and trended toward recovery at F2. There was association between circulating ACTH and cortisol concentrations at D1 in patients with the severity of DHF ($r = 0.309$, $p = 0.042$) but not of DF.

Conclusion: The ACTH and cortisol responses were associated with severity of dengue infection and recovery after 2 months.

Keywords: ACTH, Cortisol, Dengue fever, Dengue hemorrhagic fever

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Dengue is an important infectious disease in tropical areas which affects both children and adult patients. The infection exhibits a wide spectrum of clinical presentations and physiologic especially endocrine responses. The kinetics of endocrine changes in adult patients with dengue hemorrhagic fever (DHF) and dengue fever (DF) may be associated with disease severity, but such information in adults is limited. In a study of cortisol sufficiency in Burmese children, patients with DHF who developed shock during the acute and convalescent phases demonstrated the highest mean cortisol level during the acute stage; patients with DHF but without shock and patients with DF demonstrated the next highest mean cortisol levels, respectively[(1)]. In another study of Thai children with either DHF or DF, the serum cortisol concentration trended toward insufficiency during the febrile period in patients with DF and the shock period in patients with DHF[(2)].

In a stress-free healthy human, cortisol is secreted from the adrenal cortex in a diurnal pattern. Cortisol release is controlled by adrenocorticotropic hormone (ACTH, also known as corticotropin), which is produced by the pituitary. ACTH is in turn under the influence of hypothalamic corticotropin-releasing hormone[(3)]. Cortisol itself exerts negative-feedback control on both hormones. More than 90% of circulating cortisol is bound to proteins (predominantly corticosteroid-binding globulin but also albumin). Only the free hormone, however, is biologically active[(4)].

The cortisol concentration usually rises in the early phase of critical illness, proportional to the severity of stress, in response to increased release of corticotropin-releasing hormone and ACTH. This occurs either directly or via resistance to or inhibition of the negative-feedback mechanism exerted by cortisol[(5)]. Such endocrine changes have been widely studied in many critical illnesses; however, they have infrequently been studied in adult patients with dengue virus infection. Moreover, knowledge regarding the mechanism underlying the severity of dengue infection in relation to ACTH and cortisol is limited. Thus, the objective of this study was to evaluate the changes in the circulating ACTH

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and cortisol concentrations at different time points in adult patients with either DHF or DF. In addition, the relationship among circulating cortisol, ACTH, and the severity of dengue infection in both patient groups was evaluated.

Material and Methods

Patients, blood collection and hormonal determination

This hospital-based longitudinal clinical study was performed at the outpatient division of Faculty of Medicine Vajira Hospital, Navamindradhiraj University and Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand from August 2012 to December 2014. The study was approved by the ethics committees of both institutions (Faculty of Medicine Vajira Hospital, No. 015/12; Faculty of Tropical Medicine, TMEC 13-041).

Inclusion criteria were patients who aged ≥15 years with body temperature of ≥38°C and had history of fever within 72 hours, flushed face, and laboratory-confirmed dengue based on positive serological testing for dengue infection by rapid tests for NS1 antigen. The exclusion criteria were a history of fever for >72 h, focal source of infection (e.g., otitis media, pneumonia, meningitis), coryza, chronic illness (including anemia), or hemodynamic unstable evidenced from vital signs.

Dengue infection was defined according to the World Health Organization (WHO) 1997 classification criteria[6]. In brief, the patient with dengue fever (DF) must have acute febrile illness and two or more of the following symptoms: headache, retro-orbital pain, myalgia, arthralgia, rash, and hemorrhagic manifestations. Dengue hemorrhagic fever (DHF) was defined as fever with hemorrhagic tendencies (evidenced by at least one of the following: petechiae, ecchymoses, or purpura; bleeding from the mucosa, gastrointestinal tract, injection sites, or other locations; hematemesis or melena; or thrombocytopenia as shown by a platelet count of ≤100,000 platelets/mm^3) and evidence of plasma leakage.

The patients with a diagnosis of dengue infection by NS1 antigen who gave consent to participate in the study signed informed consent. Blood sample for laboratory testing were collected for the status of viremia by Reverse Transcription Polymerase Chain Reaction (RT-PCR), immunoglobulin M (Standard Diagnostics Inc., Yongin, Gyeonggi, Korea), cortisol and ACTH levels on the first day of enrollment (D1). The serum cortisol and plasma ACTH concentrations were determined with a two-site chemiluminescent immunometric assay (IMMULITE®1000; Siemens, Munich, Germany) at Vajira Hospital in Bangkok, Thailand. The detection limits of cortisol and ACTH were 0.2 µg/dl and 1.0 pg/ml, respectively.

All patients were followed-up five times after D1: the day of defervescence (Ddef, 1 day after the fever disappeared), the first day of convalescence (DC1, 24 h after Ddef), the second day of convalescence (DC2, 48 h after Ddef), the 2-week follow-up (F1), and the 2-month follow-up (F2). The patients' clinical features and laboratory parameters including complete blood count, cortisol and ACTH levels were measured during each visit.

Statistical analysis

Data were analyzed using SPSS software version 18.0 (SPSS Inc., Chicago, IL). The circulating cortisol and ACTH concentrations are presented as median and interquartile range. Significant differences in the cortisol and ACTH concentrations between two independent subject groups at different time points of blood collection were compared with the Mann-Whitney U test. The patients' age, sex, and serotype were also evaluated. Correlations between the circulating cortisol and ACTH concentrations were analyzed using Spearman's rank correlation test. A *p*-value of <0.05 was considered statistically significant.

Results

Forty-eight adult patients with either DHF (n = 32) or DF (n = 16) who presented with clinical features and positive serological testing for dengue infection on the day of enrollment (D1) were included in the present study. Demographic data and clinical and laboratory parameters of all 48 patients with dengue virus infection are shown in Table 1. No significant differences in these parameters were found between patients with DHF and DF.

The circulating cortisol and ACTH concentrations at the time of enrollment were compared between 32 patients with DHF and 16 with DF. Low ACTH but high cortisol concentrations were found in patients with DHF upon enrollment (D1). Patients with DHF had higher cortisol concentrations (median, 16.33 µg/dl; IQR, 5.85 to 27.30 µg/dl than patients with DF (median, 13.15 µg/dl; range, 3.05 to 27.09 µg/dl), but the difference was not statistically significant ($p = 0.314$) (Figure 1A). In contrast, patients with DHF had significantly lower ACTH concentrations (median, 1.84 pg/ml; range, <1.00 to 44.60 pg/ml) than patients with DF (median, 4.30 pg/ml; range, <1.00 to 41.41 pg/ml) ($p = 0.030$) (Figure 1B).

To evaluate the kinetic pattern of cortisol and ACTH, the circulating cortisol and ACTH concentrations were measured at D1, Ddef, DC1, DC2, F1, and F2. Decreased circulating ACTH at D1 but recovery after 2 months (F2) in both DHF and DF were demonstrated. The median ACTH concentration in both the DHF and DF groups was low at D1 (1.840 and 6.000 pg/ml, respectively) and tended to increase after Ddef, DC1, DC2, F1, and F2 (DHF: 7.130, 10.780, 10.065, 17.270, and 21.085 pg/ml, respectively; DF: 12.300, 9.695, 12.300, 24.650, 14.730, and 24.300 pg/ml, respectively) (Figure 2B). These findings indicated recovery after cure. The circulating ACTH concentration in patients with DHF at Ddef was lower than that in patients with DF, but not significantly ($p = 0.530$).

In contrast to ACTH, high circulating cortisol during fever was found in DHF. The median cortisol concentration in patients with DHF at D1 was higher than that at F2 (16.33 vs. 9.33 µg/dl, respectively), whereas that in patients with DF was similar between D1 and F2 (13.15 vs. 14.70 µg/dl, respectively) (Figure 2A). The median

Table 1. Demographic data and clinical and laboratory parameters according to clinical categories of 48 patients with dengue virus infection

Characteristics	DHF (n = 32)	DF (n = 16)
Age, years	21.5 (15 to 64)	19 (15 to 45)
Sex (male/female)	13/19	10/6
Sex ratio	1.00/1.46	1.67/1.00
Serotype identification		
Serotype 1	2 (6.25)	4 (25.00)
Serotype 2	6 (18.75)	3 (18.75)
Serotype 3	15 (46.88)	7 (43.75)
Serotype 4	9 (28.13)	2 (12.50)
Hemoglobin, g/dl	13.75 (6.6 to 16.4)	14 (10.9 to 15.8)
Hematocrit %	41.6 (20.5 to 49.4)	41.4 (34.6 to 47)
White blood cells, 10^3/ml	3.2 (1.5 to 6.6)	2.75 (1.2 to 7.10)
Atypical lymphocytes, %	3 (0 to 32)	0.5 (0 to 25)
Band neutrophils, %	4.5 (0 to 33)	4 (0 to 15)
Red blood cells, 10^6/ml	5.08 (2.9 to 6.33)	4.98 (4.14 to 6.88)
Platelets, 10^3/ml	82 (16 to 178)	85.5 (15 to 229)
High fever at time of enrollment (°C)	37.8 (37.5 to 40.6)	38 (37.6 to 39.2)
Duration of high fever before treatment (days)	4 (2 to 7)	4 (1 to 6)
Duration of high fever during treatment (days)	2 (1 to 4)	2 (1 to 5)

Data are presented as median (range) or n (%) unless otherwise indicated.
DHF = dengue hemorrhagic fever, DF = dengue fever, were defined according to World Health Organization criteria, DHF = dengue hemorrhagic fever, DF = dengue fever

cortisol concentration at D1 was higher in patients with DHF than DF, but not significantly (p = 0.314), and the median cortisol concentrations were similar at Ddef (DHF: 13.60 µg/dl, DF: 14.28 µg/dl) and DC2 (DHF: 13.50 µg/dl, DF: 14.06 µg/dl). These findings indicate that a more severe dengue viral infection will result in a stronger bodily response in terms of cortisol secretion, especially in the early stage of infection.

The correlation of circulating cortisol and ACTH at different time points were assessed (Table 2). The relationship between the circulating ACTH and cortisol concentrations was found. The ACTH concentration in patients with DHF and DF was positively correlated with cortisol production at D1, DC1, F1, and F2, whereas the ACTH concentration was negatively correlated with cortisol at Ddef and DC2. These findings indicate that the cortisol and ACTH axis is associated with dengue virus infection.

To evaluate the correlation of fever with the ACTH and cortisol concentrations, the body temperature of patients with either DHF or DF who presented with high fever at the time of enrollment (D1) was assessed. The ACTH concentration in patients with DHF tended to decrease with high fever, but not significantly (p = 0.403, r = -0.055). In contrast, the ACTH concentration tended to increase with high fever in patients with DF; again, however, the relationship was not significant (p = 0.373, r = -0.073). Patients with DHF showed no change in the cortisol concentration with high fever (p = 0.073, r = 0.446), whereas the cortisol concentration in patients with DF tended to increase with high fever (p = 0.373, r = 0.446).

Discussion

The design of the present study allowed us to measure the cortisol and ACTH concentrations early in the course of dengue viral infection and to evaluate the kinetics of these responses in individual patients during the course of their illness. The most notable finding of this study was the higher cortisol concentration in patients with DHF. This indicated a more severe infectious process than in patients with DF, with more pronounced ACTH suppression via negative feedback by the hypothalamic-pituitary-adrenal axis. This was statistically significant on day 1 of clinical dengue infection. This finding supported the patho-physiologic response of body to stress or infectious state, particularly in patients with more severe disease, by secretion of cortisol followed by negative feedback to suppress ACTH secretion. The higher cortisol concentrations found in patients with DHF in the present study were consistent with the findings of studies by Myo-Khin et al from Myanmar[1] and Joob and Wiwanitkit from Thailand[7]. These data suggested that serum cortisol might serve as a biomarker for severe dengue infection. During the course of the study, the cortisol concentration in patients with DF remained stable until follow-up at week 8 while the ACTH concentration gradually increased. In patients with DHF, however, the cortisol concentration gradually decreased as the ACTH concentration steadily increased. The authors also found a positive correlation between the circulating ACTH and cortisol concentrations with statistical significance in patients with DHF and DF at D1, DC1, F1, and F2, whereas the ACTH concentration in these patients was negatively correlated with the cortisol

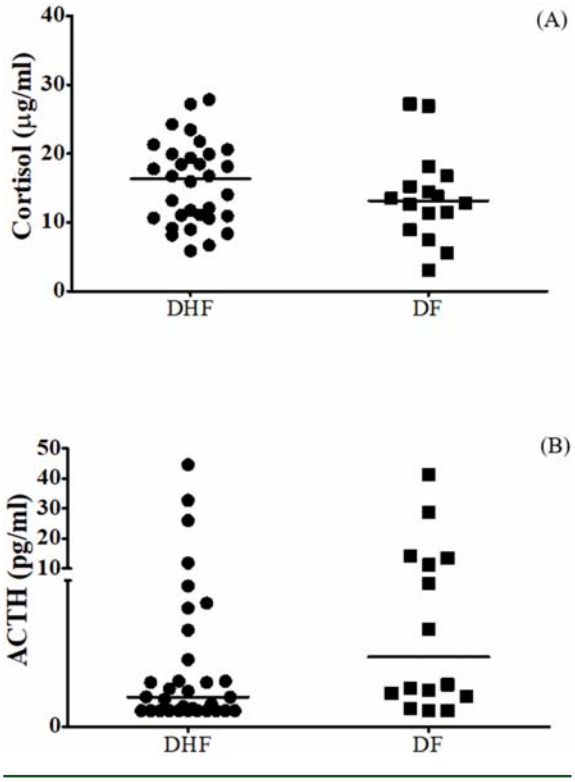

Figure 1. Distribution of (A) circulating cortisol and (B) ACTH in adult patients with DHF (n = 32) and DF (n = 16). Each dot represents one patient. The bar demonstrates the median in each group. ACTH = adrenocorticotropic hormone; DHF = dengue hemorrhagic fever; DF = dengue fever.

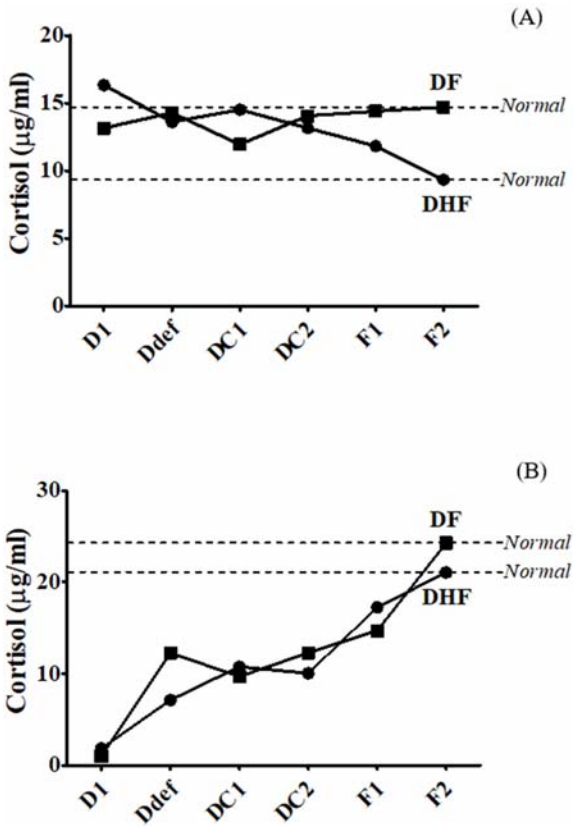

Figure 2. Circulating (A) cortisol and (B) ACTH in adult patients with DHF (n = 32) and DF (n = 16) at different time points. Each dot represents the median cortisol or ACTH concentration at each time point. The dashed line indicates the normal value after 2 months of follow-up (F2). ACTH = adrenocorticotropic hormone; DHF = dengue hemorrhagic fever; DF = dengue fever; D1 = day of enrollment; Ddef = day of defervescence; DC1 = first day of convalescence; DC2, second day of convalescence; F1 = 2-week follow-up; F2 = 2-month follow-up.

concentration at Ddef and DC2. This indicated that the ACTH and cortisol axis was associated with the active stage of dengue virus infection and that complete recovery occurred after resolution of the infection. These findings support a role of the endocrine response in the severity of dengue illness. The present study has observed the kinetics of these responses in adult patients with DF and DHF. During the period of clinical risk and potential for capillary leakage and intravascular volume depletion, the body would try to maintain hemodynamic stability and cortisol secretion would be part of this stress response. Stress in the human body affects in modulation of the autonomic nervous system, neuroendocrine axis, and metabolic and immune systems. When stress rapidly diminishes, these systems come into energetic equilibrium with the pre-stress state. Infectious processes cause stress and transiently disturb the endocrine system until the infectious source has been eliminated[8], as shown in the present study.

The hypothalamic-pituitary-adrenal axis is the main neuroendocrine structure involved in modulating the adaptive response to different stressors. It works through interconnection of the sympathoadrenal and neurohypophyseal systems, which in turn are responsible for catecholamine secretion, cytokine activation, and vasopressin release[9].

The proper cortisol concentrations in patients with DF and DHF are of great interest. Cortisol plays a role in suppressing the release of interferon-gamma; granulocyte-monocyte colony-stimulating factor; interleukins 1, 2, 3, and 6; tumor necrosis factor-alpha; inflammatory mediators such as eicosanoids, bradykinin, serotonin, and histamine; and tissue-destroying enzymes such as collagenase and elastase. The response of the pituitary and adrenal glands may have

Table 2. Correlation between circulating cortisol and ACTH in patients with either DHF or DF at different time points

Correlation between cortisol and ACTH	Spearman's rank test			
	DHF (n = 32)		DF (n = 16)	
	p	r	p	r
D1	0.038	0.318	0.017	0.551
Ddef	0.002	0.499	0.057	-0.503
DC1	0.005	0.462	0.045	0.469
DC2	0.047	0.384	0.247	-0.209
F1	0.010	0.431	0.004	0.653
F2	0.279	0.304	0.094	0.957

Data are analyzes by Spearman's rank test
DHF = dengue hemorrhagic fever; DF = dengue fever; ACTH = adrenocorticotropic hormone; DHF = dengue hemorrhagic fever; DF = dengue fever; D1 = day of enrollment; Ddef = day of defervescence; DC1 = first day of convalescence; DC2 = second day of convalescence; F1 = 2-week follow-up; F2 = 2-month follow-up

evolved to suppress and modulate inflammatory responses to infectious processes[10]. Corticosteroids are not a recommended treatment in the WHO dengue guidelines. The main criticism of this recommendation is that it is based on poor-quality evidence; thus, the interest in corticosteroids for treating dengue has continued. High-quality evidence regarding the effects of corticosteroids in patients with dengue, either for prevention of complications or treatment of established shock, is lacking. The observed kinetic change of ACTH and cortisol in the present study demonstrated the proper cortisol concentrations in dengue viral infection (both DF and DHF). This is a supporting evidence that there is no benefit of steroid treatment for DF and DHF; however, this is not proof of dengue shock syndrome because no patients in this series developed signs anywhere along the spectrum of dengue shock syndrome.

Nevertheless, the beneficial effects of corticosteroids in patients with severe shock cannot be overlooked, given the high mortality associated with the most severe forms of dengue. There is an obvious demand for a sufficiently powered randomized controlled trial of high-dose corticosteroids in the treatment of patients with severe dengue shock syndrome. Nickels and Moore showed that increases in the cortisol concentration are not related to increases in body temperature[11].

Conclusion

This is the first report on the endocrine dynamics related to disease severity in adult patients with DHF and DF. The hypothalamic-pituitary-adrenal axis is affected differently depending on the severity of dengue infection. The cortisol and ACTH axis is associated with the severity of dengue infection and recovery after 2 months of infection. Moreover, cortisol and ACTH might serve as important parameters of the illness status in patients with dengue virus infection. However, the clinical implications of these findings with respect to endocrine changes require further study.

What is already known on this topic?

Dengue has a wide spectrum of clinical presentations and physiology especially endocrine responses. In Burmese children with DHF who developed shock during the acute and convalescent phases demonstrated the highest mean cortisol level during the acute stage; patients with DHF but without shock and patients with DF demonstrated the next highest mean cortisol levels, respectively. In another study of Thai children with either DHF or DF, the serum cortisol concentration trended toward insufficiency during the febrile period in patients with DF and the shock period in patients with DHF.

What this study adds?

The cortisol and ACTH concentration is associated with the severity of dengue infection in adults and recovery after 2 months of infection. They might serve as important parameters of the illness status in adult patients with dengue virus infection.

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Potential conflicts of interest

The authors declare no conflict of interest.

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