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Term neonates with infection and shock display high cortisol precursors despite low levels of normal cortisol

Abdelmoneim Khashana ^{1,2}, Marja Ojaniemi ^{1,3}, Markku Leskinen ³, Timo Saarela ³ and Mikko Hallman ^{1,3}

1. PEDEGO Research Center, and Medical Research Center Oulu, University of Oulu, Oulu, Finland

2. Department of Paediatrics and Neonatology, Suez Canal University Hospital, Ismailia, Egypt

3. Department of Children and Adolescents, Oulu University Hospital, Oulu, Finland

Short title

Cortisol precursors in neonates with infection

Correspondence

A. Khashana. Ismailia, Egypt. E-mail.; Abdelmoneim_khashana@hotmail.com

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Circulatory collapse, cortisol, dehydroepiandrosterone, newborn infant, sepsis

Abbreviations

DHEA, dehydroepiandrosterone; HPA axis, hypothalamic-pituitary-adrenal axis; PPAR α , peroxisome proliferator-activated receptor α ; 3 β -HSD, 3-beta-hydroxysteroid dehydrogenase; NICU, neonatal intensive care unit

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ABSTRACT

Aim: Neonatal therapy-resistant septic shock is a common problem in middle and low-income countries. We investigated whether newborn infants with infection and therapy-resistant hypotension showed evidence of abnormal levels of cortisol or cortisol precursors.

Methods: A total of 60 term or near term neonates with evidence of infection were enrolled after informed consent. Of these, 30 had an infection and refractory shock and 30 had an infection without shock. There were no detectable differences between the groups in the length of gestation, birth weight or gender distribution. Serum was obtained during days four and 14 after birth. Cortisol and cortisol precursor concentrations were analysed using liquid chromatography-tandem mass spectrometry.

Results: The cortisol concentrations were low considering the expected responses to stress and they did not differ between the groups. The infants with infection and shock had higher serum dehydroepiandrosterone (DHEA) levels than those without shock ($319.0 \pm 110.3 \mu\text{g/dl}$, versus $22.3 \pm 18.3 \mu\text{g/dl}$; $p < 0.0001$) and they also had higher 17-hydroxy-pregnenolone, pregnenolone and progesterone concentrations. There were no detectable differences in the levels of 17-hydroxy-progesterone, 11-deoxy-cortisol, cortisol or cortisone.

Conclusion: Septic newborn infants with therapy-resistant hypotension had very high DHEA levels, suggesting that 3-beta-hydroxysteroid dehydrogenase activity limited the rate of cortisol synthesis.

Key notes

- This study explored whether deficient endocrine stress responses in newborn infants influenced the risk of therapy-resistant shock during infection.

- We found that when infections were complicated by shock, blood cortisol was within the normal range but cortisol precursors, particularly dehydroepiandrosterone, were much increased.
- According to these findings there was limited cortisol synthesis in term infants affected by neonatal septic shock, supporting the rationale of corticosteroid therapy.

INTRODUCTION

Circulatory collapse is a complication of neonatal infection. Despite treatment with intravenous fluids and vasopressors, adequate blood flow to vital organs is affected and life-threatening neonatal complications are common (1). Clinical findings observed in neonates with infection, such as hypotension and decreased urine output, are similar to the symptoms of adrenal insufficiency. The immaturity of the hypothalamic-pituitary-adrenal axis (HPA axis) in the neonatal period can lead to an inadequate response to stressful conditions, which may contribute to circulatory collapse. Volume supplementation and inotropes are the treatments of choice. However, a significant proportion of newborn infants who suffer from septic shock with refractory hypotension may respond favourably to corticosteroids (1).

Cortisol in the early days of life in preterm infants can be affected by an intrinsic transient defect in synthesis because of the immaturity of the HPA axis (2,3). Later in infancy, immaturity of the HPA axis limits the ability to increase cortisol production in response to stressful conditions (4) and adrenal insufficiency may be prevalent in critically ill children with systemic inflammatory response syndrome and vasopressor dependent shock (5).

Adrenal insufficiency is diagnosed when baseline cortisol levels are less than 10 microgram/dl (6) and a single random cortisol measurement reflects the adrenal status of the newborn infant to some extent (7). Relative adrenal insufficiency is present when the cortisol response to stress is inadequate (8) and serious adrenal insufficiency may present with life-

threatening cardiovascular collapse. Relative adrenal insufficiency is an important clinical entity in critically ill patients with significant associated morbidity (9,10). Critically ill newborn infants have frequently been reported to have low cortisol as the expected increase in response to stress (11,12). Many neuroendocrine factors participate in the regulation of HPA axis stress response and may account for the difficulty of the diagnosis and for the conflicting results of corticosteroid replacement therapy in septic shock (13,14,15).

According to our hypothesis, neonates with infection and therapy resistant hypotension may have a defect in the metabolic pathways of cortisol synthesis, which results in the accumulation of cortisol precursors in their blood. In this study, we first aimed to clarify whether the cortisol levels were abnormally low in infants with therapy resistant septic shock and secondly, whether these neonates showed evidence of a limitation in the synthesis of cortisol. We compared the blood levels of both cortisol and its precursor steroids in neonates with infection and therapy-resistant shock with those who had infection without shock.

PATIENT AND METHODS

Study population and ethics

This was a prospective, case-control study performed in the newborn ward of the Department of Paediatrics, Suez Canal University, Egypt. Ethical approval for the study was obtained from the research ethics committee of the Faculty of Medicine, Suez Canal University, and from the research committee of the Faculty of Medicine, Oulu University, Finland. Informed consent was obtained from the parents.

Neonates of 34 to 42 weeks of gestation who were aged from four to 14 days and were suffering from neonatal infections were included in the study, which was carried out from January 2012 to June 2014. We assumed that by the fourth day of life, the direct influence of

the maternal compartment had disappeared and by the 14th day of life the function of the adrenal gland may have stabilised (16).

All neonates with signs and symptoms of infection, including diminished spontaneous activity, less vigorous sucking, anorexia, apnea, bradycardia and temperature instability, laboratory evidence of infection, were divided into groups A and B.

Group A neonates had infection and manifestations of circulatory collapse, including refractory hypotension and oliguria. Refractory hypotension was defined as a mean blood pressure < 10% percentile for age not responding to high dose inotropes, including dopamine 15 µg/kg per minute or dobutamine 10 µg/kg per minute (1,29). Systolic, diastolic and mean blood pressure values were recorded by digital intra-arterial or external blood pressure using a M747 monitor (Meditec, London, UK). The blood pressure values that are presented in this study represent the average of three consecutive blood pressure recordings. Oliguria was defined as a urine output of <1 ml/kg/h, measured at four-hour intervals (1).

Group B neonates had manifestations of infection without circulatory collapse. During the 48 hours before blood sampling, the following were used as exclusion criteria: major surgery in the preceding week or major stress induced by medical procedures (16,17), a congenital anomaly (1), postnatal corticosteroid treatment before blood sampling (1) and a maternal history of endocrine disorders (1). A detailed history was taken and full clinical examinations were performed on each patient.

Blood sampling and laboratory studies

Blood specimen of two millilitres was withdrawn from the venous line and serum was separated and frozen at -20°C. The time of the day when the sample was collected was not expected to modify the results obtained due to the lack of circadian rhythm in neonates. An

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evaluation for sepsis was carried out by analysing complete blood picture and the levels of C-reactive protein. In the differential leukocyte count, the abnormal number of band cells and the elevated ratio of immature to total polymorphonuclear leukocytes (> 0.20) were considered diagnostic for infection. Blood cultures were also recovered.

Baseline serum cortisol concentrations and several of its precursors - pregnenolone, 17-OH-pregnenolone, dehydroepiandrosterone (DHEA), progesterone, 17-OH-progesterone, 11-deoxy-cortisol and cortisone - were determined using liquid chromatography-tandem mass spectrometry (18). All the laboratory analyses were performed by staff blinded to the clinical study and were performed in duplicate at the end of the clinical study.

Statistics

Clinical data were collected prospectively. The means and standard deviations were determined for continuous variables, frequencies were determined for nominal and ordinal variables and an unpaired, two-tailed t-test was performed. A value of $p < 0.05$ was considered to be statistically significant.

RESULTS

Patient characteristics

A total of 60 newborn infants with neonatal infections were analysed during the study: 30 in group A and 30 in group B. There were no significant differences in the demographic characteristics of the two groups.

With regard to the microorganisms identified in the blood cultures, we found that 67% of the isolates were Gram negative organisms while 31% were Gram positive organisms and 2% of the isolates were *Candida*. *Klebsiella pneumoniae* was the most common pathogen,

accounting for (24%) of the total isolates, followed by *Pseudomonas* (19.9%) and *Staphylococcus aureus* (15.4%).

The near term and term patients with evidence of infection and shock were given intravenous infusions of fluids and vasopressors. Hypotension was defined as a mean blood pressure < 10% percentile for age. The patients that had low blood pressure despite a high dose of dopamine (15 µg/kg/min) or dobutamine (10 µg/kg/min) within one hour after the start of the infusion, were eligible to join study group A, while newborn infants admitted with suspected sepsis without refractory hypotension during the same period were considered eligible to join group B, to permit recruitment of matched controls.

Table 1 shows the demographic characteristics of group A and group B. There were no significant differences between the groups in gestational age at birth, postnatal age, gender distribution or birth weight. The male to female ratio was 1.5 to 1.0 in group A and 1.6 to 1.0 in group B.

Levels of adrenal steroids

The concentrations of cortisol, cortisone and cortisol precursors were measured, as indicated. The levels of cortisol were clearly low in all neonates considering their critical illness. In contrast, most of the cortisol precursor hormones were at higher levels than expected, but there was a marked individual variation between the newborn infants. When we compared the vasopressor resistant and normotensive newborns, the cortisol concentrations did not differ significantly between the groups (Table 2). As the enzyme activity of 3β-hydroxysteroid dehydrogenase (3β-HSD) may be impaired in infected neonates with circulatory collapse, the precursor hormones that are substrates for 3β-HSD were compared between the groups. Post 3β-HSD steroid hormones, namely 17-OH-progesterone and progesterone, were also examined. Interestingly, there were highly significant differences between the groups regarding the level of DHEA ($p < 0.0001$), 17

hydroxy-pregnenolone ($p < 0.0001$) and pregnenolone ($p < 0.05$), suggesting a blockade in hormone synthesis in group A with circulatory collapse (Table 2). There was also a significant difference between the groups regarding the level of progesterone, suggesting a blockade in hormone synthesis in group A with circulatory collapse ($p < 0.05$). No significant differences between the groups were detected with regard to 17 hydroxy-progesterone, 11 deoxy-cortisol, cortisone and cortisol ($p > 0.05$) (Table 2).

In group A, no significant correlation was found between the weight of the neonates and any of the hormones. In addition, there were no significant correlations between the cortisol level and any of the other hormones using parametric correlation.

DISCUSSION

Understanding the pathophysiology of adrenal insufficiency in infected neonates is important for improving treatment strategies. Therefore, in this study, the levels of cortisol and its precursors in a total of 60 term and near term newborn patients with infection were analysed. We found that 30 of the neonates demonstrated evidence of circulatory collapse, despite the use of vasopressors, and the control group of 30 infants with similar birth weight and gestation at birth had signs and laboratory findings of infection without refractory hypotension. The cortisol concentrations in the study population were similar to healthy infants beyond the first week of life (19-21), which was rather unexpected since critically ill patients have been found to have higher cortisol concentrations than healthy individuals (22). In addition, serum cortisol concentrations did not differ between the groups. However, we found that the newborn infants diagnosed with treatment resistant hypotension had a significantly higher accumulation of cortisol precursors prior to the action of 3-beta-hydroxysteroid dehydrogenase (3β -HSD) activity.

In our study, cortisol concentrations were not increased in critically ill neonates with infection and refractory hypotension. Thus, circulatory collapse in this group of patients may have been a result of relative adrenal insufficiency under excess stress from the infection, which partly explains the severe symptoms. In the group A patients with shock, the high DHEA levels may have influenced the function of the glucocorticoid receptor, potentially suppressing the vasopressor response. Similar results have been demonstrated (23). This phenomenon may be transient, whereby infants suffering from circulatory collapse during infection may have a partial metabolic block that manifests during infection or in the first few weeks of life, suggesting a delay in the maturation of cortisol production. We cannot exclude the possibility that the infants with refractory hypotension had a genetic predisposition of poor adrenal responsiveness during inflammatory stress. These alternatives still need to be studied in the future.

Previous studies have suggested that preterm infants with refractory hypotension had a decreased capacity to synthesise cortisol, which is in agreement with our findings. Cortisol levels in neonate infants were measured after the fourth day of life to allow maternal hormones to be metabolised (24,25). The critical period for severe hypotension in critically ill newborn infants has been reported to be evident during the first 48 hours after the unresponsiveness to vasopressors was noted (26). That is why we carried out the blood sampling within one hour after the unresponsiveness to vasopressors was noted. Our study showed that there was no increase in cortisol levels in refractory hypotensive neonates, in response to the high stress situation, and that the endogenous cortisol levels were not sufficient to keep the blood pressure at normal levels. Adrenergic receptor down-regulation or excessive nitric oxide synthesis in refractory hypotensive newborn infants deserves further investigation.

In our study, we found that there was a highly significant difference between the levels of DHEA between A and B groups and this reached ten times the normal level in patients with circulatory collapse. DHEA is a precursor of adrenal steroids and is a peroxisome proliferator that induces genes for the classical peroxisomal and microsomal enzymes associated with this stress response. These effects are mediated through activation of peroxisome proliferator-activated receptor α (PPAR α). DHEA has been shown to decrease the level of 11 β -hydroxysteroid dehydrogenase 1 in a dose-dependent pattern (27). Regulation of 11 β -hydroxysteroid dehydrogenase 1 expression is important because the enzyme is believed to amplify local glucocorticoid signalling and suppression of it has been shown to decrease the metabolism of cortisone to cortisol (27,28). However, in the present study the cortisol to cortisone ratio was unaffected. This was in accord with our proposal that when newborn infants have septic shock, there is a defect in the synthesis of the cortisol precursors in the adrenal cortex. Adrenal steroid precursor concentrations have also been shown to be abnormal in preterm infants (1). In addition, infants born before 32 weeks of gestation have been found to be prone to develop catecholamine-resistant hypotension, with low cortisol concentrations that were apparently due to the decreased adrenal stimulation HPA axis, rather than an impaired adrenal response to stimulation (30).

CONCLUSION

In this study, serum cortisol concentrations did not differ between the two groups of infected neonates with and without circulatory collapse. However, we found that newborn infants diagnosed with septic shock with refractory hypotension had a significantly higher accumulation of cortisol precursors prior to the action of 3 β -HSD activity. This suggested a limitation in its activity.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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Table 1: Demographic characteristics of group A and group B subjects

Variable	group A	group B
Gestational age (weeks)	38.2±1.8	38.4±1.7
Birth weight (grams)	2861±201	2950±181
Age (days)	4.8±0.7	5.0±0.6
Sex (male/female)	19/11	17/13

Table 2: Cortisol and its precursor's levels in group A and group B infants

Variable (µg/dl)	Group A Mean±SD	Group B Mean±SD	p - value
Pregnenolone	55.35±46.8	36.9±14.7	0.047
17-hydroxy-pregnenolone	149.3±24.0	29.6±11.3	< 0.0001
Dehydroepiandrosterone	319.0±110.3	22.3±18.3	< 0.0001
Progesterone	1.5±0.4	1.2±0.4	0.0052
17-hydroxy-progesterone	4.7±1.5	4.4±2.3	0.552
11-deoxy-cortisol	16.4±5.9	15.0±6.1	0.370
Cortisone	7.2±1.9	7.1±3.2	0.884
Cortisol	13.5±4.3	13.3±3.5	0.844
Cortisol/Cortisone ratio	1.7±0.2	1.9±0.1	0.650