Cortisol precursors in neonates with vasopressor resistant hypotension in relationship to demographic characteristics

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Abstract

***Objective*:** To correlate between cortisol precursors in neonates with vasopressor resistant hypotension and demographic characteristics.

***Methods*:** We investigated 48 neonates with vasopressor-resistant hypotension. Gestation at birth ranged from 34 to 42 weeks and postnatal age from four to 14 days. Cortisol and precursor steroids were measured soon after the onset of volume expansion and inotropes for treatment of shock. Their concentrations were determined using liquid chromatography-mass spectrometry.

***Results*:** In neonates with vasopressor resistant hypotension, the serum levels of cortisol were within normal non-stress range. There was a strong negative linear association between postnatal age and dehydroepiandrosterone level ($r = -0.50$, $p < 0.01$), which decreased with neonatal age. In addition, there was a significant positive association between gestational age at birth and 17–hydroxy–pregnenolone ($r = 0.33$, $p = 0.02$). No further significant associations were evident between the neonatal weight, duration of gestation or gender, and of the levels of cortisol or the other steroids ($p > 0.05$). The cause of therapy-resistant hypotension did not appear to influence the steroid levels.

***Conclusions*:** cortisol stress response is absent in these severely ill late preterm and term infants. This may be due to inhibition of the distal pathway of cortisol synthesis.

Keywords; Newborn, late preterm, critical illness, refractory hypotension, shock, adrenal dysfunction

Introduction

Vasopressor resistant hypotension is described as a mean blood pressure less than ten per percentile for age, not reacting positively to the maximum dose of inotropes (dobutamine 10 µg/kg/min or dopamine 15 µg/kg/min). The efficacy of prophylactic hydrocortisone supplementation for the prevention of hypotension in low birth weight infants has been shown [1–4].

The immaturity of hypothalamic pituitary adrenal axis in neonatal period directs to an insufficient recovery from stressful diseases, which may contribute to the etiology of the circulatory failure. Circulatory collapse is a common complication of the life-threatening diseases in neonates as a result of numerous conditions due to hypovolemic shock, diminished cardiac function or peripheral vasodilatation. In spite of restoration of intravascular volume and vasopressor therapy, circulatory collapse continues to be an important cause of poor perfusion of the vital organs [2, 5].

Persistent hypotension associated with poor organ perfusion is a serious problem in preterm neonates and it is linked to significant adverse effects including death, intra-ventricular hemorrhage, and abnormal neuro-development [6]. Watterberg and colleagues pointed out that premature neonates developing chronic lung disease had low serum cortisol values shortly after birth and concluded that immature neonates have a reduced capability to produce cortisol, which may lead to adrenal inadequacy in face of critical sickness [7]. In addition, Watterberg et al. also observed that very preterm infants who had a serious disease a reduced response to adrenal stimulus persisted through the first month of life [8].

Martins and Procianoy located that cortisol and 17–hydroxy–pregnanolone intensities were low in neonates with intractable hypotension born after thirty weeks of gestation [9] and Scott and Watterberg proposed that there was an inverse association between cortisol level and gestational age in tiny neonates [10].

In the present study in neonates with vasopressor resistant hypotension, we aimed to associate the serum levels of cortisol and its precursor steroids with the demographic characteristics including gestational and postnatal age, birth weight and gender. This approach identified some interesting associations between steroid metabolism and perinatal development in present population.

Research design and methods

Altogether, the study included 48 infants with vasopressor resistant hypotension and critical neonatal disease for cortisol metabolism. All infants were non-responders to plasma expanders and to inotropes. Serum concentrations of steroids were analysed soon after the onset of volume expansion and inotrope treatment for shock. The patients were recruited from August 2010 to October 2014.

It was estimated that we would require enrolling 48 newborn infants by using the value of standard normal distribution for type I error probability which is equal 1.96 for 95 % significance and the value of standard normal distribution for the desired statistical power of 90 % and equal 1.28.

Our team included critically ill newborn infants with vasopressor resistant hypotension which is defined as a lack of response to intravenous fluid bolus (10–20 ml/kg) and a high dose of inotropes (maximal dopamine 15 μg/kg per minute or dobutamine 10 μg/kg per minute). Inclusion criteria were: neonates with serious disease with gestational age ranging from 34 weeks to 42 weeks. Gestational age was determined on the basis of the last menstrual period and if necessary by examination of the newborn as described by Ballard et al. [11]. The postnatal age ranged from day four to fourteen. We assumed that before day four the metabolism of the mother and the placenta may interfere with the chemistry of the newborn. On the other hand, two weeks after the near-term or term birth, the maturity of the adrenal gland may become evident [12].

Systolic, diastolic and mean BP values were recorded by digital BP monitoring. The BP values which were presented in this report represent the average of three BP recordings.

The following exclusion criteria were considered before obtaining the blood specimens: surfactant administration within less than 24 hours before the blood sampling, and stress-inducing medical procedures such as ultrasound scans, and X-ray or endotracheal intubation within four hours before blood sampling. The exclusion criteria were as follows: serious anomalies (congenital heart diseases, central nervous system malformation and renal anomalies), postnatal corticosteroid therapy before blood sampling, and maternal history of endocrine diseases such as diabetes mellitus, thyroid or adrenal problems. These diseases and events may interfere with cortisol metabolism in neonates [2, 12, 13].

Blood cell-counts, arterial blood gases, serum electrolytes, blood sugar and serum albumin were analyzed. Septic work up included complete blood counts and C-reactive protein.

Once circulatory collapse occurred with intractable hypotension not reacting to high dose inotropes (dopamine ≥ 15 μg/kg per minute or dobutamine ≥ 10 per minute) [2, 3] within 1 hour after start of the infusion without improvement, 2 ml of blood was obtained. Time of sampling was recorded.

The time of day when the sample was collected did not modify the results obtained because of the circadian rhythm is not present in newborn infants. No venous puncture was performed only for the purposes of the study. Rather, blood samples were collected at the time of routine laboratory tests.

Serum cortisol and its precursors (pregnenolone, 17–hydroxy–pregnenolone, dehydroepiandrosterone, progesterone, 17–hydroxy–progesterone, 11–deoxy–cortisol and cortisone concentrations) were quantitated using liquid chromatography-mass spectrometry (LC–MS). Besides the immunoassays, LC–MS has the potential to find its place in the clinical laboratory medicine for quantification of steroid hormones. A prerequisite for the application of

a new analytical procedure in clinical diagnostics is standardization to dismiss analytical intra- and inter laboratory variability and inaccuracy [14]. The laboratory technician was blinded to the study. All samples were tested in duplicate.

Ethical approval of the study was obtained and an informed consent was obtained from the parent.

Results

There was a strong negative association between age and dehydroepiandrosterone levels in sick neonates with vasopressor resistant hypotension. During days four to 14, the serum concentrations of dehydroepiandrosterone decreased significantly (Table 1).

There was a significant positive correlation between the gestational age and 17–hydroxy–pregnenolone. 17–hydroxy–pregnenolone levels were higher in critically ill neonates with higher gestational age than those with lower gestational age (Table 2).

The following correlations between the neonatal birth weight (BW) and the intensities of cortisol and its precursor hormones in vasopressor resistant hypotension were obtained: r = – 0.17 (p = 0.26) between BW and pregnenolone; r = 0.26 (p = 0.08), between BW and 17–hydroxy–pregnenolone; r = 0.03 (p = 0.86), between BW and dehydroepiandrosterone; r = – 0.11 (p = 0.44), between BW and progesterone; r = – 0.17 (p = 0.25) between BW and 17–hydroxy–progesterone; r = – 0.04 (p = 0.78), between BW and 11–deoxy–cortisol; r = – 0.01 (p = 0.94), between BW and cortisone; and r = – 0.06 (p = 0.70) between BW and cortisol.

There was no detectable correlation between the weights of neonates with refractory hypotension and any of the cortisol or its precursor concentrations. Also, there were no statistically significant differences between cortisol and its precursors intensities between the near term and term neonates with vasopressor resistant hypotension ($p > 0.05$). It means that vasopressor resistant hypotension may take place in both term and near-term neonates during serious illnesses (Table 3).

No significant differences in cortisol (12.3 ± 4.3, 11.8 ± 4.0; $p = 0.709$) and its precursor concentrations were observed in vasopressor resistant hypotension as compared between neonates with BW <2500 g and with BW ≥2500 g.

During vasopressor resistant hypotension, there were no significant differences male and female infants in the serum concentrations of cortisol (males: 13.1 ± 4.0; females 11.9 ± 4.9; $p = 0.360$) or its precursors. Male to female ratio was 1.4:1 in this study.

The estimated causes of neonatal vasopressor resistant hypotension were as follows: respiratory distress syndrome, 34 cases (70.8%); neonatal hypoxic ischemic encephalopathy, one case (2.1%); septicemia, five cases (10.4%); congenital pneumonia, three cases (6.3%); severe brain hemorrhage, one case (2.1%); miscellaneous, four cases (8.3%).

Discussion

In the present analysis, a cohort of 48 full-term and late preterm neonates with intractable hypotension were submitted for quantitative analysis of serum cortisol and seven other steroids that serve as precursors of cortisol. The concentrations of cortisol were within the normal limits. However, considering a severe stress the cortisol concentrations in a number of cases were very low. This is consistent with the findings of Watterberg who found that very preterm infants developing chronic lung disease had low serum cortisol levels [7, 8]. Earlier reports have suggested that low birth weight infants with refractory hypotension have a decreased capacity to synthesize cortisol [10, 15, 16]. We found a strong negative association between the postnatal age and dehydroepiandrosterone levels in ill neonates with vasopressor resistant hypotension. We propose that in late preterm and term neonates, enzymes that influence dehydroepiandrosterone metabolism undergo a development, possibly involving enzymes hydrolyzing dehydroepiandosterone.

Although the hypotension during the first 48 hours of life is defined by invasive measurements, the oscillometric measurements correlate with direct intra-arterial monitoring in neonates with correlation coefficients of 0.85-0.97 [17]. Selecting a fitting size cuff, measuring pressure during quiet sleep, and recording a mean of three measurements further improve the accuracy [17, 18]. Our hypotensive patients presented with consistently low mean arterial blood pressures throughout the study period despite volume expansion and vasopressor medications. Therefore, there is no uncertainty about the hypotensive status of these neonates. Refractory hypotension is common in neonatal intensive care units and it may present with poor capillary return even

though they did not present clinical hypotension. However, our cases of refractory hypotension were within the expected values as reported by Ng and colleagues [19].

Circadian rhythm of adrenal gland is poorly developed in newborn infants. Consequently, a single random plasma cortisol intensity assessment is demonstrative of the plasma cortisol levels over a lengthy period of time [20]. It was predictable that sick, stressed patients would reply with amplified cortisol levels [21]. Cortisol levels were similar in diseased and non-diseased neonates at 12 h of life, even though refractory hypotensive neonates were sicker than the controls, as proven by the significantly higher number of deaths after 2 days of life [9].

We reported cortisol and steroid levels first after the fourth day of life to permit catabolism of maternal/placental hormones in infants [19, 20]. However, the two first days of life constitute the most critical period for severe hypotension in very low birth weight infants [22]. Ng et al 2004 performed a randomized trial using hydrocortisone for treatment of refractory hypotension in preterm infants. The median age of the onset of medication was 11 h of birth [15, 16]. Martins and Procianoy [9] found an increase in cortisol levels after preterm birth, but this was associated with failure to increase blood pressure. Adrenergic receptor down-regulation or excessive nitric oxide synthesis in refractory hypotension deserves further investigation. In present study we found no association between cortisol levels and postnatal age. Additional studies are required on the development of hypothalamus-pituitary and adrenal-cortex hormones in preterm and term newborn [23]. It is problematic to identify corticosteroid deficiency in patients with critical illnesses. A low threshold for testing of the hypothalamic-pituitary-adrenal axis and recommendation of corticosteroid replacement treatment in acutely ill patients has been proposed [24].

Maternal cortisol is oxidized in placenta to its inactive metabolite, cortisone that enters the fetal compartment. Some cortisone may be converted to cortisol in fetal tissues. In newborn with inadequate synthesis of cortisol, cortisol precursors, such as 17–hydroxy–progesterone accumulate [25]. In present study we identified a significant positive association the gestational age and the accumulation of 17–hydroxy–pregnenolone in sick infants. 17–hydroxy–pregnenolone level increased more in neonates with higher gestational age than in those with lower gestational age. This could be the result of the combination of the developmental stimulation of hypothalamic adrenal axis and an acquired metabolic block in distal pathway of cortisol synthesis.

According to Martins and Procianoy there were no correlations between GA or BW and cortisol or 17-OH-progesterone at 12 and 36 h of life in very preterm infants with refractory hypotension [9]. On the other hand, Scott and Watterberg [10] showed an inverse relationship between cortisol level and gestational age when they examined the early neonatal (<7 day-old) infants from 27 to 36 weeks of gestational age with mild to moderate stress. In contrast, Mesiano and Jaffe [26] proposed that the human fetal adrenal cortex does not synthetize cortisol *de novo* from cholesterol until the age of thirty weeks of gestation. The variation in the results may reflect the differences in environment and population characteristics.

Our study population of refractory hypotension ranged from 34 to 42 weeks of gestation at birth and the postnatal age ranged from four to 14 days. In these severely ill infants we failed to observe any association between serum cortisol and the duration of pregnancy or the postnatal age. We detected no gender differences in serum cortisol or in serum levels of the other steroids. In contrast, we found a significant positive association between the duration of pregnancy and postnatal 17-hydroxy-pregnenolone and a strong negative association between postnatal age and

dehydroepiandrosterone. We propose that these findings may be the result of the prenatal and postnatal maturation of steroid metabolism, respectively.

We observed low levels of the major endogenous glucocorticoid (cortisol) despite persistent vasopressor resistant hypotension and high cortisol precursor levels [cf. 27]. This suggests a metabolic block in the distal pathway of cortisol synthesis. The exact causes and biochemical features of this postulated life-threatening defect remain unknown. There is inadequate information on the indications and basis of steroid therapy in newborn infants with low blood pressure [28]. We propose that a therapeutic trial of hydrocortisone is indicated, as it may improve the outcome of present patient population and it may enable the evaluation of the recovery of cortisol synthesis.

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Declaration of interest

The authors have no conflict of interest to declare.

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Table 1. Correlations between the postnatal age and the concentrations of cortisol and its precursors in vasopressor resistant hypotension

Variable	*Pearson correlation coefficient*	*P Value*
Pregnenolone	-0.05	0.76
17-hydroxy-pregnenolone	-0.06	0.67
dehydroepiandrosterone	-0.50	0.00
Progesterone	0.24	0.10
17-hydroxy-progesterone	0.03	0.85
11-deoxy-cortisol	0.03	0.83
Cortisone	0.06	0.68
Cortisol	-0.13	0.36

Correlation is highly significant at the 0.01 level (2-tailed)

Table 2. Correlations between the gestational age at birth and the concentrations of cortisol and its precursors in vasopressor resistant hypotension after birth

Variable	*Pearson correlation coefficient*	*P Value*
Pregnenolone	-0.14	0.34
17-hydroxy-pregnenolone	0.33	0.02
dehydroepiandrosterone	0.13	0.39
Progesterone	-0.03	0.83
17-hydroxy-progesterone	-0.19	0.19
11-deoxy-cortisol	0.07	0.65
Cortisone	-0.03	0.82
Cortisol	-0.12	0.42

Correlation is significant at the 0.05 level (2-tailed)

Table 3. The mean (SD) differences in cortisol and its precursor levels between in late preterm and full-term neonates in vasopressor resistant hypotension

Variable (µg/ dL)	*34 to 36 weeks*	*Equal or More than 37 weeks*	*P Value*
Pregnenolone	63.0 (60.9)	42.5 (33.7)	0.054
17-hydroxy-pregnenolone	120.4(42.9)	144.8 (88.3)	0.088
dehydroepiandrosterone	284.9 (127.4)	341.7 (178.6)	0.076
Progesterone	1.7 (0.4)	1.5 (0.6)	0.057
17-hydroxy-progesterone	4.5 (1.4)	4.3 (1.2)	0.454
11-deoxy-cortisol	17.1 (6.8)	16.5 (4.7)	0.613
Cortisone	7.4 (2.3)	6.9 (1.4)	0.201
Cortisol	12.4 (4.6)	11.5 (4.3)	0.324