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Cortisol and 17-α-hydroxy-progesterone levels in infants with refractory hypotension born at 30 weeks of gestation or less

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Abstract

Refractory hypotension is frequent in very low-birth weight infants, whose hypothalamic-pituitary-adrenal axis has been suggested to be immature. The objective of the present study was to evaluate basal cortisol and 17-α-OH-progesterone in the first 36 h of life in preterm infants with and without refractory hypotension (mean arterial blood pressure below the lower limit for gestational age throughout the study despite aggressive volume expansion and use of vasopressors). Thirty-five infants with ≤30 weeks of gestation and a birth weight ≤1250 g, with no postnatal use of corticosteroid or death in the first 48 h were studied. Mean arterial pressure was measured every 4 h during the first 48 h. Cortisol and 17-α-OH-progesterone were determined at 12 and 36 h and patients were divided into refractory hypotensive (N = 15) and control (N = 20) groups. The groups were not different regarding type of delivery, use of prenatal corticosteroid, requirement of mechanical ventilation, use of vasopressor drugs, morphine, fentanyl, prophylactic indomethacin, and mean sample timing. Although refractory hypotensive newborns were more immature, were smaller, suffered more deaths after 48 h of life and had a higher SNAPPE-2 score, their cortisol and 17-α-OH-progesterone levels were not different from controls at 12 h and at 36 h. The increase of cortisol in newborns with refractory hypotension 36 h after birth was significantly higher than in controls. Despite the fact that refractory hypotensive very low-birth weight neonates were submitted to a very stressful condition, their cortisol and 17-α-OH-progesterone levels were similar to controls.

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Key words
- Hypotension
- Extremely preterm infant
- Adrenal insufficiency
- Cortisol

Introduction

A significant proportion of very low-birth weight (VLBW) infants may present hypotension that is refractory to both volume expanders and vasopressor drugs (1,2). Many of these infants have been shown to respond to the use of corticosteroids, with normalization of arterial blood pressure and a reduction of the dose of vasopressor drugs (3-7).

Although Hanna et al. (8) have reported that very preterm newborn infants have a normal pituitary response to ovine cortico-

tropin-releasing hormone and a normal adrenal response to ACTH, recent studies have shown that those with refractory hypotension have an immature hypothalamic-pituitary-adrenal (HPA) axis and a decreased response to the ACTH test compared to VLBW infants with no refractory hypotension at the end of the first week of life (1,2). Watterberg et al. (9) also suggested that VLBW infants have decreased capacity to synthesize cortisol and present an elevated concentration of cortisol precursors like 17-α-hydroxy-progesterone (17-α-OH-progesterone) after the ACTH stimulation test.

Hypotension is an early manifestation in VLBW infants, usually in the first 48 h of life, and is associated with increased mortality and central nervous system morbidity in preterm infants (10-12). Studies on the HPA axis with corticotropin-releasing hormone or ACTH have conducted on very preterm infants from the 4th to the 15th day of life (1,2,8,9). There is no study evaluating basal serum cortisol levels in VLBW infants with and without refractory hypotension during the critical period for refractory hypotension, i.e., the first 48 h of life. An adrenal response to a stressful situation like severe arterial hypotension resistant to inotropic treatment is expected in VLBW infants with an intact HPA axis (13).

We measured serum cortisol and 17-α-OH-progesterone levels without any HPA axis stimulation test, 12 and 36 h after birth in VLBW infants with and without refractory hypotension.

Material and Methods

The study protocol was approved by the Ethics Committee at Hospital de Clínicas de Porto Alegre, Brazil, and written informed consent was obtained from the infants' parents or guardians.

We conducted a prospective study that included all neonates with a gestational age of ≤30 weeks and a birth weight of ≤1250 g delivered at the obstetric Unit of Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil, and admitted to the neonatal intensive care unit between March 2004 and January 2005. The exclusion criteria were use of corticosteroids in the first 48 h of life, congenital infections, major congenital malformations, and death in the first 48 h of life.

Newborns were followed for the first 48 h of life, starting at 4 h of age, and their mean arterial blood pressure (MABP) was measured every 4 h. Nurses who were blind to the study measured MABP by the oscillometric method with an arm cuff width approximately 45% of the upper arm circumference, during a quiet or sleeping state. An average of three measurements was obtained each time.

At 12 and 36 h, an additional blood sample (0.5 mL) was collected from all newborns for cortisol and 17-α-OH-progesterone measurements. The time of day when the sample was collected does not modify the results obtained because 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 exams. Blood was immediately centrifuged and serum was stored at -80ºC because the hormones were measured in a single assay after the end of the study. Patients were assisted by their neonatologists who were not involved in the study and who were blind to cortisol and 17-α-OH-progesterone levels. The authors were not responsible for the care of the newborns.

Newborns were divided into two groups: refractory hypotensive and control. Refractory hypotensive newborns were those whose MABP remained below the lower limit for gestational age (14,15) throughout the study, with signs of hypoperfusion such as poor capillary return despite the use of aggressive volume expansion (at least 10 mL/kg three times over 30 min) and vasopressor drugs (dopamine up to 20 μg kg^{-1} min^{-1} and/or

dobutamine up to 20 μg kg^{-1} min^{-1}). Controls were newborns with MABP within the normal range for gestational age or hypotensive newborns who responded to the use of volume expansion and/or vasopressor drugs.

Cortisol was measured by ECLIA using the Elecsys 1010/2010/Modular Analytics E170 (Roche Diagnostics GmbH, Mannheim, Germany), and 17-α-OH-progesterone by radioimmunoassay using the ImmunoChem Coated Tube-125/RIA (INC Biomedicals, Inc., Diagnostics Division, Costa Mesa, CA, USA). The lower detection limits were 0.036 μg/dL and 0.1 ng/mL for cortisol and 17-α-OH-progesterone, respectively. Intra-assay and interassay coefficients of variation were 1 and 1.8% for cortisol, and 7.8 and 9.8% for 17-α-OH-progesterone. The person who did all laboratory determinations was blind to the study. All samples were tested in duplicate at the same time.

A sample size of 12 patients in each group was calculated based on an effect size of 1.5, a level of significance of 0.05 and power of 0.9. Since logarithmic transformation was used for comparison of cortisol and 17-α-OH-progesterone, we were able to use the following parametric tests: Student *t*-test and analysis of variance (ANOVA). The chi-square and Fischer exact tests were also used. Spearman correlation was calculated for gestational age, birth weight and cortisol at 12 and 36 h and for gestational age, birth weight and 17-α-OH-progesterone at 12 and 36 h.

Results and Discussion

During the study period, 40 newborns with a gestational age of 30 weeks or less and a birth weight of 1250 g or less were delivered at our institution. Three were excluded: 1 with major multiple congenital malformations and 2 who died within the first 24 h. Two were lost (birth weights 450 and 505 g) because it was impossible to collect blood samples for laboratory analysis. Therefore, a total of 35 patients were analyzed, 15 in the refractory hypotensive group and 20 in the control group. Hypotension was manifested within the first 12 h. MABP was significantly lower in the refractory hypotensive group than in the control group throughout the study period.

Comparison of the two groups is shown in Table 1. There was no difference between groups regarding type of birth, prenatal corticosteroid use, mechanical ventilation requirement, and use of vasopressor drugs, morphine, fentanyl, and prophylactic indomethacin. None of the patients in either group had positive blood and/or cerebrospinal fluid cultures during the study period. The mean sample timings were similar for both groups. Mean gestational age and birth weight were lower in the refractory hypotensive group than in the control group. SNAPPE-2 and

Table 1. Characteristics of the study population.

	Refractory hypotensive (N = 15)	Control (N = 20)
Birth weight (g)[a]	690 ± 176*	1041 ± 164
Gestational age (weeks)[a]	27.02 ± 1.6*	29.4 ± 0.8
Cesarean section[b]	6 (40%)	5 (25%)
Rupture of membranes >18 h[c]	12 (80%)	19 (95%)
Prenatal corticosteroid (number of doses)[a]	1.3 ± 1.7	1.4 ± 1.7
Prenatal corticosteroid (number of patients)[b]	5 (33%)	10 (50%)
Mechanical ventilation[c]	14 (93%)	18 (90%)
Use of dopamine[c]	14 (93%)	18 (90%)
Use of dobutamine[b]	8 (53%)	5 (25%)
Use of morphine[c]	1 (7%)	0 (0%)
Use of fentanyl[c]	4 (27%)	3 (15%)
Prophylactic indomethacin[b]	10 (67%)	10 (50%)
SNAPPE-2[a]	57.9 ± 21.6*	18.4 ± 15.0
First sample timing (h)[a]	13.2 ± 7.4	13.9 ± 6.5
Second sample timing (h)[a]	33.3 ± 9.1	33.6 ± 7.5
Cortisol 12 h (μg/dL)[d]	12.8 ± 15.9+	15.7 ± 29.7
Cortisol 36 h (μg/dL)[d]	25.7 ± 28.9	18.1 ± 28.6
17-α-OH-progesterone 12 h (ng/mL)[d]	32.1 ± 22.9	23.1 ± 14.0
17-α-OH-progesterone 36 h (ng/mL)[d]	49.7 ± 44.3	26.6 ± 17.3
Death after 48 h[c]	10 (67%)*	3 (15%)

Data are reported as means ± SD or as number with percent in parentheses.
*P < 0.05 compared to the control group. [a]Student *t*-test; [b]chi-square test; [c]Fischer exact test; [d]logarithmic transformation and ANOVA. +P < 0.001, cortisol at 12 h compared to 36 h in the refractory hypotensive group. There was no difference in the comparison of cortisol at 12 and 36 h in the control group, and 17-α-OH-progesterone at 12 and 36 h in the refractory hypotensive and control groups.

number of deaths after 48 h of life were significantly higher in the refractory hypotensive group than in the control group. There were no significant differences between groups regarding cortisol at 12 h and 17-α-OH-progesterone levels at 12 and 36 h. There was a trend to higher cortisol levels at 36 h in the refractory hypotensive newborns compared to controls. Cortisol levels at 36 h were significantly higher than at 12 h in the refractory hypotensive group. This difference was not present in the control group, nor was it observed for 17-α-OH-progesterone in either group. The increase of cortisol from 12 to 36 h in the refractory hypotensive group was significantly higher than in the control group.

There were no statistically significant correlations between gestational age or birth weight and cortisol at 12 and 36 h and between gestational age or birth weight and 17-α-OH-progesterone at 12 and 36 h.

It was expected that sick, stressed patients would respond with increased cortisol levels (13) but this was not the case. Cortisol levels were similar in both groups at 12 h of life, even though refractory hypotensive neonates were sicker than controls, as demonstrated by the significantly higher number of deaths after 48 h of life and higher SNAPPE-2 scores.

Early in gestation, maternal cortisol freely crosses an immature placenta. Later in gestation, as placenta metabolism matures, maternal cortisol is oxidized to its inactive metabolite, cortisone. The negative feedback from maternal cortisol decreases, leading to increased fetal synthesis. If the immature newborn lacks the capacity to synthesize cortisol, accumulation of cortisol precursors like 17-α-OH-progesterone occurs (16).

Previous studies have suggested that VLBW infants with refractory hypotension have a decreased capacity to synthesize cortisol (1,2,17). Therefore, we expected to find accumulation of cortisol precursors, especially 17-α-OH-progesterone, in this group; however, there was no difference between groups. This lack of difference may be explained by the fact that we did not perform HPA stimulation tests as done by previous investigators (2,8,9) or by the presence of 17-α-OH-progesterone of maternal origin in the circulation of the newborns studied. Our objective was to study spontaneous cortisol and 17-α-OH-progesterone behavior in the first 36 h of life in refractory hypotensive VLBW infants and compare them to controls.

Studies on cortisol levels in VLBW infants have been performed after the fourth day of life to allow maternal hormones to be metabolized (Ng et al. (2) on day 7, and Jett et al. (18) between day 4 and day 6). However, the critical period for severe hypotension in VLBW infants is the first 48 h (19). Recently Ng et al. (2) published the first blind clinical trial on the use of hydrocortisone for the treatment of refractory hypotension in preterm infants. In the cited study, the median age of commencement of the trial medication (hydrocortisone or placebo) was 11 h of life, suggesting that corticosteroid deficiency might be present already in the first 12 h of life (7). There is no study following cortisol and 17-α-OH-progesterone levels in the first 48 h in refractory hypotensive newborns ≤30 weeks gestation. Our study showed that there was a cortisol increase on the second day of life in refractory hypotensive neonates, probably in response to the high stress situation, but the increase was not sufficient to keep blood pressure at normal levels. Adrenergic receptor down-regulation or nitric oxide synthesis deregulation in refractory hypotensive very preterm infants deserves further investigation.

The present study was an observational uncontrolled study. Management of the patients was the responsibility of the attending neonatologists, who were not involved in the study. Some patients received vasopressor drugs because of poor capillary return

even though they did not present clinical hypotension. However, the prevalence of refractory hypotension found in our population was within the expected values. Ng et al. (7) reported that 30% of infants born after <32 weeks of gestation and with a birth weight of <1500 g had refractory hypotension. Our study was restricted to infants with a birth weight of ≤1250 g and a gestational age of ≤30 weeks, and the prevalence of refractory hypotension was 42%.

Circadian rhythm is not present in the newborn infant, and VLBW infants show little pulsatility in their plasma cortisol over time. Therefore, a single random plasma cortisol level is representative of the plasma cortisol levels over a prolonged period of time (18).

The absence of a significant correlation between gestational age and birth weight and either cortisol at 12 and 36 h of life or 17-α-OH-progesterone at 12 and 36 h of life suggests that there is no relationship between newborn maturity and these hormone levels in neonates born after ≤30 weeks of gestation. Although Scott and Watterberg (17) suggested that there was an inverse relationship between cortisol level and gestational age when they studied newborn infants from 24 to 36 weeks of gestational age, Mesiano and Jaffe (20) suggested that the human fetal adrenal cortex does not produce cortisol *de novo* from cholesterol until about 30 weeks of gestation. After birth, the placental substrate is no longer available, and the VLBW infants will not have the enzymes required for *de novo* cortisol synthesis. This may explain the lack of correlation between maturity and cortisol and 17-α-OH-progesterone in infants with a gestational age of ≤30 weeks. Our study population was situated in a very narrow range of gestational age, around 28 weeks.

Although the standards for the diagnosis of hypotension in VLBW infants in the first 48 h of life are based on invasive measurements, the oscillometric method has a high correlation with direct intra-arterial monitoring in neonates, with a slight overestimation of blood pressure in VLBW infants. There is no correction factor to be used when arterial blood pressure is measured by the oscillometric method. A number of studies have compared the automated oscillometric method with direct intra-arterial blood pressure monitoring in neonates and have found a high correlation between mean blood pressure measurements by these two methods, with correlation coefficients ranging from 0.85 to 0.97 (21). In order to minimize errors in noninvasive MABP measurement it is suggested to choose an appropriate size cuff, to obtain blood pressure during a quiet or sleeping state, and to obtain an average of three measurements (21,22). Our refractory hypotensive patients presented low MABP throughout the study period despite the use of volume expansion and vasopressor drugs. Therefore, there is no doubt about the hypotensive status of these neonates.

Our data showed that cortisol and 17-α-OH-progesterone levels were similar in infants with refractory hypotension born after 30 weeks of gestation or less and with a birth weight of 1250 g or less and controls in the first 36 h of life, despite the fact that refractory hypotensive neonates were submitted to a very stressful condition.

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