The Relationship of Salivary and Cord Blood Cortisol in Preterm Infants

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ABSTRACT

Recent studies reveal that salivary cortisol measurements accurately reflect blood cortisol levels in older children and adults; yet the relationship between the two values in premature infants has not been established. This study explores the use of salivary cortisol as an accurate measure of adrenal steroid concentrations in premature infants to provide a reliable and less invasive tool for investigating hormonal stress response. Premature infants (n=51) were recruited, with saliva and blood collected immediately after birth, and cortisol levels measured by radioimmunoassay. A linear relationship emerged between cord plasma and salivary cortisol values in the 102 paired samples [(salivary cortisol)=0.546 + 0.192*(plasma cortisol), r=0.481 and P=.0003). Findings demonstrate salivary and plasma cortisol levels as correlated in premature infants. This information will be useful in future studies that assess use of salivary cortisol to evaluate neonatal stress axis function.

KEY WORDS

saliva, cortisol, preterm, stress

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INTRODUCTION

In current medical practice, premature infants are frequently exposed to potent steroid drugs like betamethasone and dexamethasone, with considerable beneficial effects on premature lung problems and low blood pressure. The mechanism by which steroid exposure can cause problematic hypothalamic-pituitary-adrenal (HPA) axis function is not yet completely understood. Although most premature infants recover normal HPA axis function within one to two months after completing a prolonged steroid course, a few continue to have abnormal function[1]. Data on long-term effects of exposure to glucocorticoids in infants are limited. A plethora of evidence supports the hypothesis that events in fetal life permanently alter the structure or function of an individual, programming later adult disease[2]. In animal studies, steroid treatment during neuro-ontogeny leads to abnormal brain development[3-5], raising concern about possible long-term effects of steroid treatment.

With cortisol seen as a major indicator of altered physiological states in response to stressful stimulation, most human studies of it face numerous problems associated with venipuncture for blood sampling. Cortisol level in saliva has recently become a valuable alternative to blood analysis for assessing adrenocortical function in humans. Saliva is much more convenient to collect than blood or urine, and concentration of corticosteroid in saliva generally

reflects that of the free fraction of corticosteroid in blood plasma[6-7]. According to the free hormone concept, unbound cortisol reaches the target tissue and elicits glucocorticoid effects[8-9]. Correlation of saliva to free cortisol in plasma does not depend on saliva flow rate[6,10,11] and changes in salivary cortisol concentration with systemic cortisol change occur in minutes[12]. Significant doubts as to reliability and validity of cortisol levels measured in saliva compared to cortisol concentration in the blood still exist. Although more than a dozen papers indicate high correlation between the two values[13], and additional studies compare salivary cortisol with serum/plasma cortisol concentrations in different populations[14-16], data on the relationship between them in premature infants are limited. In newborns, the affinity constant for cortisol binding globulin (CBG) is reportedly similar to that in adults, but concentrations of this protein are low, such that binding globulin capacity of CBG in neonates is approximately half of that in adults. This could explain salivary cortisol concentrations in newborn infants higher and more variable than those in adults[17].

There is a need for a reliable and less invasive tool for investigations of HPA axis activity in premature infants. Thus, correlation between salivary and serum cortisol in premature infants looms as a possible alternative. This study explores use of salivary cortisol as an accurate measure of adrenal steroid production in premature infants. Results can be used in future study relying on salivary cortisol measurements for evaluation of neonatal stress axis function.

SUBJECTS AND METHODS

Neonates ≤ 37 weeks gestation (n=51) were selected from patients admitted to the nursery of China Medical University Hospital. Only those with actual or potential respiratory complications requiring oral nasopharyngeal suctioning as part of their routine initial stabilization were included. Infants with multiple congenital anomalies, congenital adrenal disorder and those judged too clinically unstable to accommodate a cotton swab in their oral cavity for 30-60 sec were excluded. Approval from the Institutional Review Board of China Medical University Hospital and written informed parental consent was obtained. Saliva was collected immediately after oral nasopharyngeal suctioning during routine initial stabilization. A modified sampling device (Salivette, Sarstedt) was placed in the oral cavity, adjacent to buccal mucosa within 15 min of delivery. Salivette was modified by cutting it in half transversely and attaching a swab to a smooth 2 mm diameter stick from a culture swab. Dry Salivette stimulates salivary flow so that sufficient material can be collected within 30-60 sec. Salivettes were centrifuged for 5 min (5,000 rpm) to isolate saliva. Blood (0.5-1 cc) was aspirated from the umbilical cord or placental vessels after cord clamping and delivery of the placenta. Samples were frozen for later batch analysis of plasma and salivary cortisol concentrations by radio immunoassay. Patient data, including birth weight, length, head circumference, gender, Apgar scores at 1 and 5 min, gestational age, delivery route (spontaneous vaginal, vacuum-assisted vaginal, cesarean section), maternal age, and maternal antenatal steroid treatment, were collected. Body mass index was calculated as weight (kg)/[length (m)]^2.

STATISTICAL ANALYSIS

Continuous variables were reported as mean ± standard deviation (SD) and categorical variables as percentages. Intergroup differences in means or medians were calculated via t-test or Mann-Whitney test when appropriate. Pearson coefficients determined any linear relationship between cord plasma cortisol and salivary cortisol. Stepwise linear regressions plotted association between salivary cortisol and umbilical cord plasma cortisol, gestation age, birth weight, length, body mass index, and gestational age. All p values were those of two-sided tests; statistical significance was set at $p < 0.05$. Analyses used SAS version 9.1 (SAS Institute Inc, Cary, NC).

TABLE 1
The Pearson correlation coefficients between umbilical cord plasma cortisol, salivary cortisol, birth weight, birth length, body mass index (BMI) and gestation age.

	umbilical cord plasma cortisol		**salivary cortisol**	
	r	p-value	r	p-value
umbilical cord plasma cortisol	1.000		0.481	0.0003*
birth weight	0.085	0.5509	0.200	0.1586
birth length	0.136	0.3411	0.352	0.0112*
BMI	0.015	0.9167	-0.057	0.6935
gestation age	0.235	0.0975	0.236	0.0953

*p<0.05

RESULTS

The 51 newborns included 21 males and 30 females; gestational age ranged from 28 to 37 weeks (mean ± SD, 34.2 ±2.2 weeks), birth weights 1050 g to 4200 g (2164 ± 598 g). Eleven women were given antenatal steroid and followed recommendations for their use by the National Institutes of Health[18]. One body weight was 4200 g in a 37-week infant of an insulin-dependent mother. Mean (±SD) salivary and plasma cortisol concentrations were 1.36±1.05 μg/dl and 4.21±2.63 μg/dl, respectively. Cord plasma cortisol levels were <5 μg/dl in 39/51; only one was >8 μg/dl, in a 34-week infant of a mother with chronic hypertension and superimposed preeclampsia, and whose plasma and salivary cortisol levels were 20.2 and 3.41 μg/dl, respectively.

Table 1 depicts a linear relationship between cord plasma and salivary cortisol values [salivary cortisol (μg/dl) = 0.546 + 0.192*(plasma cortisol (μg/dl), r = 0.481 and P =.0003]. By stepwise regression considering plasma cortisol, birth weight, birth length, gestation and Apgar scores, the only measured variables which contributed significantly to variation in salivary cortisol were plasma cortisol and birth length. There was no difference in either plasma or salivary cortisol concentration between males and females. Mean salivary cortisol, not plasma cortisol, was lower in infants whose mothers were treated with antenatal steroids (0.78±0.47 μg/dl, n=11) than in those of mothers not treated (1.51±1.11 μg/dl, n=40, p<0.05, t-test or Mann-Whitney). No difference appeared in mean umbilical plasma or salivary cortisol concentration among infants delivered by spontaneous vaginal (n=11) *vs.* vacuum assisted vaginal (n=3) *vs.* caesarean section (n=20). There was no relationship between birth weight, length, body mass index, or gestational age and umbilical cord plasma cortisol, nor between birth weight or body mass index and salivary cortisol. A modest direct linear relationship arose between birth length and salivary cortisol (p=0.0112, r=0.352); the relation between gestational age and salivary cortisol was nearly significant (p=0.054.

DISCUSSION

Increasing use of salivary cortisol as a biomarker of stress has facilitated research of HPA function. This method requires only saliva collection and thus is minimally intrusive, easy for a caregiver or family member to accomplish, and most importantly, capable of yielding valid and reliable detection of cortisol. Correlation between serum and salivary cortisol levels is well-established in adults and children; however, few investigations target salivary cortisol in preterm infants[19]. Feasibility of using salivary cortisol for gauging corticosteroid in smaller or premature infants is not yet well evaluated. This study shows salivary cortisol concentration reflecting plasma cortisol concentration in premature infants at birth. Saliva specimen collection by “Salivette” is practical in premature infants of 28 weeks gestation and 1 kg birth weight.

Range of salivary cortisol concentrations at birth found by this study is similar to those reported by others in preterm infants[19]. In

addition, there is no significant rise in either plasma or salivary cortisol concentration at birth with advancing gestation. A prior study reported umbilical plasma cortisol level rising abruptly at term and higher after spontaneous delivery than after cesarean section[20]. Absence of these trends in our data reflects the exclusion of term infants and commencement of sample collection immediately after birth to avoid the increased cortisol levels after delivery.

Infants in this study must be hemodynamically stable and not under respiratory distress to avoid adrenal suppression. Mean salivary cortisol was lower when mothers were treated with antenatal steroid than in mothers not treated ($p<0.05$). Questions arise as to whether exposure to corticosteroids may have an impact on the HPA axis in preterm infants. A previous study reported suppression of maternal adrenal function in women receiving at least two weekly courses of prenatal betamethasone, as proven by both decreased basal and post-stimulatory maternal serum cortisol concentrations[21,22]. While betamethasone crosses the placenta, this causes suppression of the fetal pituitary-adrenal axis[23]. Multiple doses of corticosteroids may suppress the adrenocorticotrophic hormone, with subsequent suppression of adrenal function and cortisol production, which may ultimately adversely affect infant health.

In conclusion, results indicate it is possible to collect saliva from extremely low birth weight infants, thus providing a technique that is easy, noninvasive and can be completed in less than 5 min. Salivary and serum cortisol levels in premature infants are correlated. This information will prove useful in future study relying on salivary cortisol measurement for evaluation of neonatal stress axis function.

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