

Salivary and serum cortisol levels in newborn infants

María V. Forclaz, M.D.,^a Eduardo Moratto, M.D.,^b Alicia Pennisi, M.D.,^c Siloína Falco, M.D.,^c Graciela Olsen, M.D.,^c Patricia Rodríguez, M.D.,^b Regina Papazian, M.D.,^a and Ignacio Bergadá, M.D.^d

ABSTRACT

Introduction. Given that serum cortisol level interpretation in newborn infants (NBIs) is hard, the objective of this study was to correlate baseline salivary and serum cortisol levels and to describe salivary cortisol levels in the first month of life.

Population and Methods. Descriptive, prospective, longitudinal, and correlational study. Term NBIs were selected from the Division of Neonatology of Hospital Nacional Profesor Alejandro Posadas in 2014. Cortisol was measured in saliva specimens while cortisol, cortisol-binding globulin, and albumin were measured in blood specimens. A linear correlation was performed to relate serum and salivary cortisol levels; Friedman test was conducted to compare cortisol levels during the first month of life, and the difference was used to analyze the performance of values equal to or lower than the first quartile.

Results. Fifty-five NBIs were studied. Serum cortisol: 7.65 (1.0-18.1 $\mu\text{g/dL}$); salivary cortisol: 35.88 (5.52-107.64 nmol/L); cortisol-binding globulin: 22.07 (16.5-33.0 $\mu\text{g/mL}$), expressed as median and range. The correlation coefficient between serum and salivary cortisol was 0.54, $P = 0.001$. Cortisol performance during the first month of life showed no statistically significant differences, and the difference between the second and the first specimen of values equal to or lower than the first quartile increased in 10 out of 12 patients.

Conclusion. The measurement of cortisol in saliva reflects serum cortisol levels in normal NBIs. Some patients had low levels of cortisol at 36 hours of life and showed a trend towards a spontaneous increase during the first month of life.

Key words: newborn infant, salivary cortisol, serum cortisol.

<http://dx.doi.org/10.5546/aap.2017.eng.262>

- a. Division of Pediatric Endocrinology, Department of Pediatrics, Hospital Nacional Prof. Dr. A. Posadas, Buenos Aires, Argentina.
- b. Endocrinology Lab, Laboratory Department, Hospital Nacional Prof. Dr. A. Posadas, Buenos Aires, Argentina.
- c. Division of Neonatology, Hospital Nacional Prof. Dr. A. Posadas, Buenos Aires, Argentina.
- d. Department of Endocrinology, Hospital de Niños Dr. Ricardo Gutiérrez, Buenos Aires, Argentina.

E-mail address:

María V. Forclaz, M.D.:
mforclaz@intramed.net

Funding:

CBG level determination funded by Bodiagnóstico S.A.

Conflict of interest:

None.

Received: 4-7-2016

Accepted: 12-22-2016

INTRODUCTION

Activation of the hypothalamic-pituitary-adrenal axis with cortisol release is critical to maintain stress-induced homeostasis.¹ In the fetus, over 80% of the adrenal cortex consists of the fetal zone that predominantly synthesizes dehydroepiandrosterone-sulfate (DHEA-S)¹ and low cortisol levels, which account for 3- β -ol-dehydrogenase deficiency.¹⁻³ After birth, the fetal cortex progressively disappears and a concomitant increase in the activity of the enzyme 3- β -ol-dehydrogenase is observed.

Serum cortisol levels in healthy NBIs are low, within a range similar to that observed in those with hormone deficiency. This renders the diagnosis among children with low clinical suspicion of adrenal insufficiency difficult, limiting its diagnostic value and leading to hormone replacement therapy with an uncertain diagnosis. However, it has been described that free hormone fraction level tends to be similar to that observed among adults, apparently secondary to a low serum cortisol-binding globulin (CBG) concentration.^{4,5} Cortisol in saliva is a reliable marker of the hypothalamic-pituitary-adrenal axis activity; it correlates with plasma cortisol and reflects plasma free cortisol levels.⁶ Saliva specimens are relatively easy to collect with a non-invasive technique^{6,7} and they can be successfully collected in newborn infants by using different stimulating agents like citric acid^{4,7,8} or lemon juice.

In newborn infants, the correlation between blood and salivary levels has been shown in specimens processed by immunoradiometric assays.⁹ Cortisol measurement in saliva enabled to monitor circadian rhythm variations

To cite: Forclaz MV, Moratto E, Pennisi A, et al. Salivary and serum cortisol levels in newborn infants. *Arch Argent Pediatr* 2017;115(3):262-266.

in adrenal activity in newborns and infants^{4,10} and to study the impact of stress on newborn infants in Neonatal Intensive Care Units and in close contact with the mother.^{6,11}

OBJECTIVE

1. To correlate salivary and serum cortisol baseline levels.
2. To determine salivary cortisol levels on serial specimens collected during the first month of life from healthy term NBIs.

MATERIAL AND METHODS

Design: prospective, descriptive, longitudinal, and correlational study.

This study was approved by the Ethics Committee of Hospital Nacional Profesor Alejandro Posadas. An informed consent was obtained from one of the NBI's parents or legal guardian.

Population

The sample size was estimated based on previous studies. In NBIs, in order to estimate the median serum morning cortisol level with a 95% confidence interval, considering an error of 3 nmol/L as acceptable, and estimating a 72 nmol/L⁹ variance and a loss of 15% because of different reasons, at least 36 NBIs should be re-enrolled in the study.

Healthy NBIs born in Hospital Nacional Profesor Alejandro Posadas, in working days from March 1st to 15th, 2014 were selected. Children born to healthy mothers with a normal pregnancy, born by vaginal or abdominal delivery, with an Apgar score ≥ 7 at 1 minute, documented oral feeding, and a normal physical examination were included. Newborns with congenital malformations, born to mothers who had received corticosteroid treatment and those who had not fasted at least for an hour before specimen collection were excluded. Gestational age (GA) was based on the date of the mother's last menstrual period (LMP); if LMP was uncertain, GA was estimated by an early ultrasound and/or physical examination (Capurro) assessed by 2 qualified professionals. A term newborn was defined as an infant born between ≥ 37 and < 42 completed weeks of gestation; NBI of appropriate weight was defined as a NBI with a birth weight (BW) between the 10th and 90th percentile for the GA; low weight NBI referred to a NBI with a BW below the 10th percentile for the GA; and high weight NBI, if the

BW was above the 90th percentile for the GA.¹²

Relevant population demographic outcome measures were: NBI sex, birth weight (g), length (cm), GA (weeks).

Specimens

Saliva (100 μ L) and peripheral blood (1 mL) specimens were collected, the latter by venipuncture, between 7:00 and 8:00 AM on business days, after 36 hours of life, coinciding with the specimen requested for the neonatal screening. Cortisol was measured in saliva specimens while total cortisol, CBG, and albumin were determined in peripheral blood specimens. Saliva specimens were collected immediately before blood withdrawal, with 1 or more hours of fasting. Salivary secretion was induced by placing 2 drops of 5% citric acid on the tongue, 2 minutes before sample collection.^{4,7,8} To avoid interferences, the stimulating agent was administered to all the infants instead of using it only when specimen collection was difficult.⁸ Gentle examination of the oral cavity was performed to minimize the risk of contamination with milk that could have interfered with the assay.¹³ Saliva specimens were obtained by careful aspiration of the floor of the mouth with soft plastic Pasteur pipettes and were placed in plastic tubes which were kept frozen at -20 °C until their processing.⁴ Peripheral blood specimens were placed in dry tubes, they were centrifuged and kept frozen at -20 °C until their processing.

Additional saliva specimens were collected from those infants who were seen at the office within the first 30 days of life, from 9:00 to 11:00 AM.

Serum and salivary cortisol determinations were performed using automated competitive chemiluminescence immunoassays in an ACCESS analyzer (Beckman-Coulter),¹⁴ without previous collection,^{4,15} with a limit of detection of 0.4 μ g/dL and 11.0 nmol/L, respectively and an inter- and intra-assay coefficient of variation of 6.0% and 2.14%, respectively. CBG concentration was determined by means of an immunoradiometric assay (Diasource) with a limit of detection of 6.5 mg/L and an intra- and inter-assay coefficient of variation of 8.6% and 10.8%, respectively.

Serum cortisol values were expressed in μ g/dL; salivary cortisol levels, in nmol/L and μ g/dL; and CBG values, in mg/L.

Conversion factors: nmol/L \times 0.036 = μ g/d; μ g/dL \times 27.6 = nmol/L.

Statistical analysis

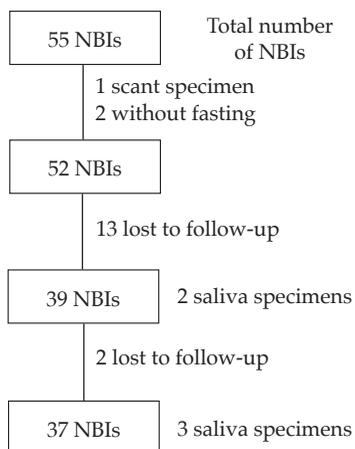
Continuous data were expressed as mean and median with their respective dispersions (standard deviation [SD]/range) whereas categorical data were described as absolute numbers and measures of frequency (percentage). The correlation between serum and salivary cortisol was evaluated with the linear correlation test. Differences were considered significant if the *p* value was < 0.05. The Mann-Whitney-Wilcoxon test was used to compare serum and salivary cortisol performance based on the approach to birthing. The Friedman test was conducted to compare the 3 non-consecutive levels of morning salivary cortisol during the first month of life. The performance of cortisol in patients' saliva with values equal to or lower than the first quartile in the first specimen was analyzed in terms of the difference of salivary cortisol values between the second and the first specimens.

TABLE 1. Characteristics of the population under study

Clinical outcome measures (n= 52)	Median and range n (%)
Female/male	26 / 26 (50%)
Gestational age (weeks)	39.00 (37.0-41.6)
Birth weight (g)	3210 (2450-4420)
Length (cm)	50.0 (45.0-52.0)
Age (days)	1.9 (1.5-3.1)
Serum cortisol (µg/dL)	7.65 (1.0-18.1)
Salivary cortisol (nmol/L)	35.88 (5.52-107.64)
CBG (µg/mL) (n= 48)	22.07 (16.5-33.0)
Albúmin (g/dL) (n= 41)	4.10 (3.5-5.2)

CBG: cortisol-binding globulin.

FIGURE 1. Flowchart of newborn infants



NBIs: newborn infants.

RESULTS

Fifty-five term newborn infants were examined; parents were asked to return for follow-up and saliva specimen collection during the first month of life, as observed in Figure 1. Clinical characteristics and results are described in Table 1.

Linear regression results among serum and salivary cortisol levels (*r*= 0.54, *P*= 0.001) are presented in Figure 2.

Out of the 52 patients with sufficient specimens, 39 (75%) could be evaluated more than once during the first month of life. The comparison of non-consecutive measurements of cortisol in morning saliva during the first month of life is shown in Figure 3.

FIGURE 2. Correlation between serum and salivary cortisol levels in 52 baseline specimens in term newborn infants

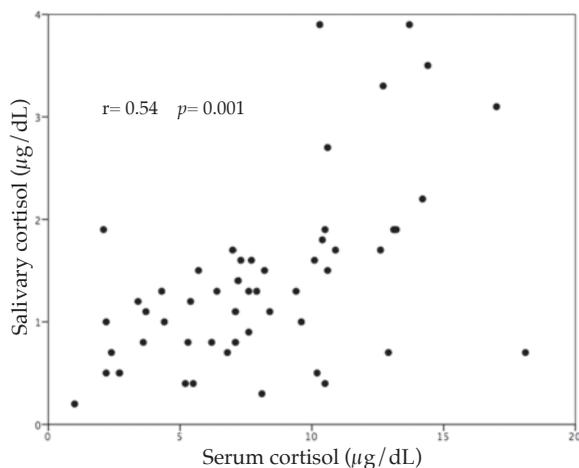
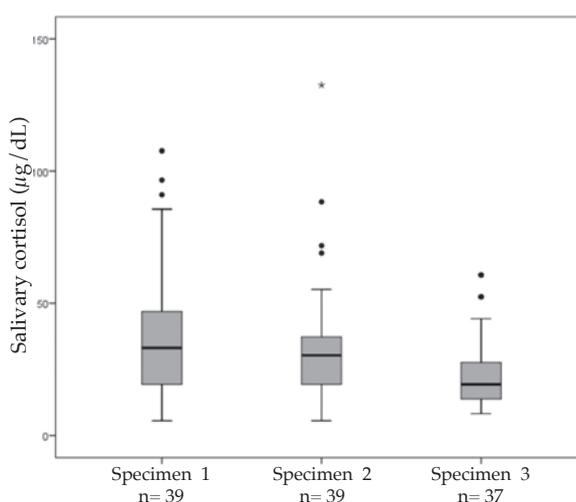


FIGURE 3. Salivary cortisol levels during the first month of life



In specimen 1 ($n=39$), obtained at a median age of 1.9 days of life (range: 1.5-3.1), the median salivary cortisol was 33.12 nmol/L (range: 5.52-107.6); in specimen 2 ($n=39$), obtained at a median age of 5 days of life (range: 3-20 nmol/L), the median salivary cortisol was 30.3 nmol/L (range: 5.52-132.48); and in specimen 3 ($n=37$), obtained at a median age of 16 days of life (range: 7-30), the median salivary cortisol was 19.3 nmol/L (range: 8.28-60.7). Salivary cortisol values along the first month of life showed no statistically significant differences ($P=0.99$).

In 12 out of the 16 patients with values of salivary cortisol equal to or lower than the first quartile (22.0 nmol/L), a second specimen was obtained (Figure 4). The difference described an upward trend in 10 patients, a decreasing trend in 1, and it remained without changes in 1.

Median serum CBG was 22.07 $\mu\text{g}/\text{mL}$ (range: 16.5-33.0).

DISCUSSION

In this prospective study on term NBIs, a correlation was found between serum and salivary cortisol levels. Such correlation was 0.54, which is consistent with what has been published by Maas et al. who, in a systematic review of the literature, reported that it varied between 0.44 and 0.83.¹⁶ Based on the results, it is inferred that low serum cortisol concentrations would match low salivary cortisol concentrations. This implies that, under physiological conditions,

there would be a rate of healthy NBIs that would have "physiological hypocortisolemia," which may correlate with low salivary cortisol levels. These findings –which are different from those published in the literature– are associated with CBG concentrations comparable to that of adults,^{8,16,17} which would confirm that some NBIs have a low cortisol secretion at this stage of life.⁶⁻⁸ Hadjian et al. showed, by means of balance of dialysis and an adsorbent technique, a lower capacity in serum proteins to bind cortisol in term NBIs.⁷ However, Scott et al., in a sample of 120 preterm NBIs, obtained low cortisol values that could not account for CBG deficit since CBG values determined in 61 NBIs ($16 \pm 5 \text{ mg}/\text{L}$) were only slightly below the normal adult range (19-45 mg/L).¹⁷

It is likely that low serum cortisol levels in some of these infants correspond to a phenomenon of immaturity or to different disappearance times in the adrenal fetal zone associated with low adrenocorticotrophic hormone (ACTH) and 3- β -ol-dehydrogenase levels, as described.¹⁻³ It has been postulated that the human placenta synthesizes large quantities of corticotropin-releasing hormone (CRH), which is released by maternal and fetal circulation resulting in the highest CRH concentrations in life. After birth, there is an abrupt decrease in circulating CRH. Based on the above, the hypotheses would be that, in the NBI, the hypothalamus has not reached sufficient maturity to release CRH or that the pituitary gland, having been exposed to large amounts of CRH, would be temporarily refractory to the low concentrations produced by the hypothalamus in the NBI.³

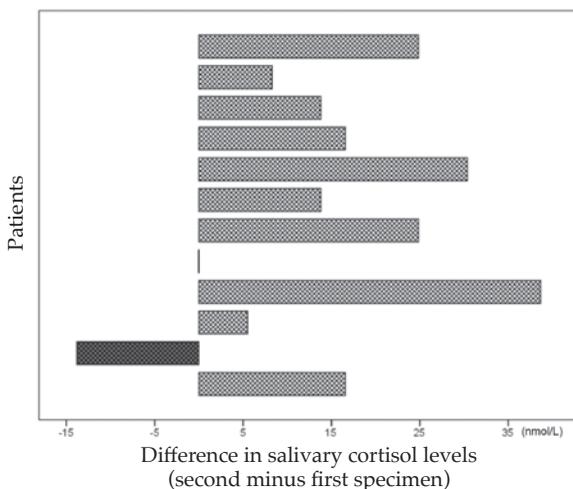
Median serum cortisol was 7.65 $\mu\text{g}/\text{mL}$ (range: 1-18.1) in specimens obtained from 7:00 to 8:00 AM with, at least, 1 hour of fasting, similar to what has been reported.⁴

In a pilot sample of NBIs between 2 and 7 days of life, fed on demand, it has been observed that serum cortisol concentrations obtained between 10:00 and 11:00 AM were $3.5 \pm 2.3 \mu\text{g}/\text{dL}$ (mean \pm SD) whereas the median was 2.7 $\mu\text{g}/\text{dL}$ (range: 0.7-11.5 $\mu\text{g}/\text{dL}$).¹⁸

Median salivary cortisol levels were similar to those reported with a higher dispersion.^{4,11} This variability can be a limiting factor for the definite clinical diagnosis of adrenal insufficiency.

It is worth pointing out that the use of citric acid was useful to obtain saliva specimens in adequate quantities to process in most of the infants without adverse events and, by using it in all NBIs, any interference was prevented.

FIGURE 4. Performance of salivary cortisol in 12 newborn infants born with levels equal to or lower than the first quartile in the first specimen (each bar corresponds to a patient)



In the longitudinal sampling of salivary cortisol during the first three weeks of life, more than 70% of the study population was evaluated; no significant changes were seen regarding the specimen collected on the second day of life. However, the difference of salivary cortisol values between the second and the first specimens of patients who were within or below the first quartile showed an upward trend in 10 out of 12 patients.

A limitation of this study was that the fasting time was reported by the mother without having been checked by a witness. Specimens were obtained only during business days because of staff availability. Another limitation was that neither ACTH nor CRH could be measured, which may account for the presence of inadvertent biases. The wide ranges in the results of salivary cortisol determinations reflect the high inter-individual variability, as reported.⁹

Salivary cortisol determinations could be a tool for the diagnosis of adrenal insufficiency in these infants, although further studies are necessary to confirm these findings. There is a rate of normal NBIs whose serum and salivary cortisol levels are low, which renders the diagnosis of adrenal insufficiency difficult if only this measurement is taken into account.

CONCLUSION

The measurement of cortisol in saliva reflects serum cortisol levels in normal NBIs. Low levels of cortisol observed at 36 hours of life in normal NBIs showed a trend towards a spontaneous increase during the first month of life. ■

Acknowledgments

We would like to thank Fernando Ríos, M.D., for his outstanding collaboration in the statistical analysis, Silvana Varela, B.S., for her contribution, and Horacio Repetto, M.D., for his collaboration and dedication to editing this article.

REFERENCES

1. Fernandez E, Watterberg K. Relative adrenal insufficiency in the preterm and term infant. *J Perinatol* 2009; 29(Suppl 2):S44-9.
2. Voutilainen R. Endocrine pathology of the premature infant-adrenal function [abstract]. *Horm Res* 2009;72(Suppl 3):11.
3. Fernandez E, Montman R, Watterberg K. ACTH and Cortisol response to critical illness in term and late preterm newborns. *J Perinatol* 2008;28(12):797-802.
4. Calixto C, Martinez F, Jorge S, Moreira A, et al. Correlation between plasma and salivary cortisol levels in preterm infants. *J Pediatr* 2002;140(1):116-8.
5. Francis S, Walker R, Riad-Fahmy D, Hughes D, et al. Assessment of adrenocortical activity in term newborn infants using salivary cortisol determinations. *J Pediatr* 1987;111(1):129-33.
6. Hadjian A, Chedin M, Cochet C, Chambaz E. Cortisol binding to proteins in plasma in the human neonate and infant. *Pediatr Res* 1975;9:40-5.
7. Neu M, Laudenslager M, Robinson J. Coregulation in Salivary Cortisol during maternal holding of premature infants. *Biol Res Nurs* 2009;10(3):226-40.
8. Talge N, Donzella B, Kryzer E, Gierens A, et al. It's Not That Bad: Error Introduced by Oral Stimulants in Salivary Cortisol Research. *Dev Psychobiol* 2005;47(4):369-76.
9. Gröschl M, Rauh M, Dörr H. Circadian Rhythm of salivary cortisol, 17 α -Hydroxyprogesterone, and Progesterone in healthy Children. *Clin Chem* 2003;49(10):1688-91.
10. de Weerth C, Zijl C, Buitelaar J. Development of cortisol circadian rhythm in infancy. *Early Hum Dev* 2003;73(1-2): 39-52.
11. Cabral D, Antonini S, Custódio R, Martinelli C Jr, et al. Measurement of Salivary Cortisol as a marker of stress in newborns in a Neonatal Intensive Care Unit. *Horm Res Paediatr* 2013;79(6):373-8.
12. Lejarraga H, Diaz Ballvé, Guerra A. Estudio antropométrico de 1.401 recién nacidos sanos del área urbana de Buenos Aires. *Rev Hosp Niños B Aires* 1976;18:9-21.
13. Magnano CL, Diamond EJ, Gardner JM. Use of salivary cortisol measurements in young infant: a note of caution. *Child Dev* 1989;60(5):1099-101.
14. Van Aken M, Romijn J, Miltenburg J, Lentjes E. Automated measurement of Salivary Cortisol. *Clin Chem* 2003; 49(8): 1408-9.
15. Flück CE. Assessing the function of the human adrenal cortex. In Ranke M, Mullis P-E, eds. *Diagnostics of endocrine function in children and adolescents*. 4th ed. Basel: Karger; 2011. Pages 350-78.
16. Maas C, Ringwald C, Weber K, Engel C, et al. Relationship of Salivary and Plasma Cortisol levels in Preterm Infants: Results of a prospective observational study and systematic review of the literature. *Neonatology* 2014;105(4):312-8.
17. Scott S, Watterberg K. Effect of gestational age, postnatal age and illness on plasma cortisol concentrations in premature infants. *Pediatr Res* 1995;37(1):112-6.
18. Forclaz V, Moratto E, Falco S, Romero E, et al. Growth Hormone (GH), Insulin (I) and Cortisol (F) values in relation to glycaemia (G) in term neonates [abstract]. *J Pediatric Endocrinol Metab* 2006;19(Suppl 3):1071.