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Utility of a precursor-to-product ratio in the evaluation of presumptive positives in newborn screening of congenital adrenal hyperplasia

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Abstract

Title—Utility of a precursor-to-product ratio in the evaluation of presumptive positives in newborn screening of congenital adrenal hyperplasia.

Objective—Screening for Congenital Adrenal hyperplasia (CAH) caused by 21-α hydroxylase deficiency is challenging because factors such as prematurity and stress increase intermediate steroid metabolite levels in newborn infants. The objective of this study was to explore the use of the 17-OHP/11-deoxycortisol ratio as an adjunct measure in the follow-up evaluation of infants with presumptive positive newborn screens for CAH to distinguish between infants with no disorder and those with CAH.

Study Design—This was a retrospective cohort study of infants with presumptive positive newborn screens for CAH. The precursor-to-product ratio of 17-OHP/11-deoxycortisol was compared between infants with no disorder (n=47) and infants with CAH (n=5).

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Results—The CAH infants had higher 17-OHP/11-deoxycortisol ratios than infants with no disorder, 26 (18-58) and 1.05 (0.69-1.46), respectively, $p<0.05$. Among infants with no disorder, higher levels of serum 17-OHP did not reflect higher ratios, indicating sufficient enzyme activity.

Conclusions—The results suggest that a low 17-OHP/11-deoxycortisol ratio represents 21-α hydroxylase sufficiency among presumptive positives in newborn screening of CAH.

Introduction

The newborn screening of congenital adrenal hyperplasia (CAH) in California was implemented in 2005 and is currently mandated in all states. CAH is an autosomal recessive inherited disease and has an incidence of about 1 in 17,000 [1]. CAH caused by 21-α hydroxylase deficiency accounts for about 90% of the diagnosed cases. The salt-wasting form of 21-α hydroxylase deficiency comprises 75% of these cases and is potentially lethal if not treated early[2, 3]. Under normal physiologic conditions, the enzyme 21-α hydroxylase catalyzes the conversion of 17 α-hydroxyprogesterone (17-OHP) to 11-deoxycortisol, and therefore its deficiency in CAH leads to accumulation of 17-OHP. Newborn screening (NBS) programs measure 17-OHP to screen for CAH, however, the frequency of false positive test results remains a problem.

Serum values of adrenal steroids in newborn infants are influenced by many factors such as neonatal stress, assay conditions, maternal steroid treatment, immature adrenal axis, and immature renal functioning[4, 5, 6, 7]. Thus, serum steroid values are not normally distributed, and cut-off values for 17-OHP in premature infants are higher than values for term infants [5, 8]. The variability of steroid values in the newborn is the underlying reason for the large number of false positive results found in newborn screening, which utilizes the method of time-resolved fluoroimmunoassay. Application of age- and weight-based cutoffs, and second-tier screening methods have been unsuccessful in minimizing the number of false positive reports [9]. When an infant screens positive for CAH, the standard of practice is to draw blood for confirmatory measurement of serum 17-OHP by liquid chromatography tandem mass spectrometry (LC-MS/MS). These results are again subject to cut-offs based on gestational age. The abundance of false positives for CAH in newborn screening continues to be a dilemma for the clinician and family because of this need for repeat testing. Repeat testing increases the burden of health care costs and can result in more parental anxiety[2].

The current method of screening examines 17-OHP as a single metabolite within a larger network of interconnecting pathways, and thus does not provide an accurate reflection of enzyme activity. Alternatively, when evaluating the metabolite directly before (17-OHP) and after (11-deoxycortisol) the enzyme block, expressed as a ratio, a more informative depiction of enzyme activity may be observed. When 21-α-hydroxylase is deficient, 17-OHP cannot be converted to its product 11-deoxycortisol and thus 17-OHP rises substantially while 11-deoxycortisol values remain low. Therefore, in patients with CAH, one would expect a high ratio of 17-OHP/11-deoxycortisol. Our group has previously shown that the precursor-to-product ratio of 17 α-hydroxyprogesterone/11-deoxycortisol in newborn dried blood spots accurately reflects 21-α-hydroxylase activity in newborns with

and without CAH [10]. Therefore, there may be potential for using the 17-OHP/11-deoxycortisol ratio as a clinical tool in the evaluation of presumptive positive screens for CAH.

We hypothesize that there is a greater variability of 17-OHP levels compared to the ratio of 17-OHP/11-deoxycortisol, and that the ratio may prove to be useful clinically to reduce the need for repeat confirmatory testing. The objective of this study was to explore whether the 17-OHP/11-deoxycortisol ratio in infants with presumptive positive NBS results may demonstrate a difference between infants with no disorder versus those with CAH. Infants with CAH are expected to have higher ratios, and conversely infants with no disorder are expected to have ratios within a published reference range.

Methods

This is a retrospective cohort study examining presumptive positive cases of CAH, including those with CAH, and those with no disorder. Human Subjects approval was obtained from the Human Subjects Committee at the Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center.

A chart review process identified a total of 63 presumptive positive CAH cases referred to the Regional Newborn Screening Referral Center at Harbor-UCLA Medical Center between October 2010 and June 2013. These included cases from surrounding community hospitals. Data was limited to available information collected by the NBS program and entered into the local database. Subjects were included in the study analysis (n=47) if they met the following criteria: age of screen >24 hours after birth, and availability of serum confirmatory 17-OHP and 11-deoxycortisol measurements drawn at the same time. Subjects were excluded if steroid therapy was initiated prior to confirmatory testing. Maternal data including steroid therapy prior to delivery was not recorded for all cases and therefore was not included in the subject selection process. In addition, the clinical status (i.e. intubated, septic, or healthy, prior transfusions) of each subject during the collection process was not always known and therefore is not addressed in this study.

The primary outcome variables examined were serum 17-OHP, 11-deoxycortisol, and the 17-OHP/11-deoxycortisol ratio. The 17-OHP/11-deoxycortisol ratios were calculated from the reference laboratory values of 17-OHP and 11-deoxycortisol, which were reported in ng/dL. (To determine ratios based on nmol/L, one would multiply each ratio based on the ng/dl results by 1.048.).

Statistical analyses were performed using Excel, SigmaPlot and SYSTAT software. The 17-OHP, 11-deoxycortisol concentrations, and 17-OHP/11-deoxycortisol ratios were examined in frequency distribution graphs. Tests for normality were performed using the Shapiro-Wilk test. Comparisons between groups were made using the two-sided t-test or the Kruskal-Wallis test, with $p<0.05$ considered statistically significant. Normally distributed data is presented as the mean ± the standard error of the mean, while non-parametric data is presented as the median and interquartile ranges (25%–75%). Relationships between the

primary outcome variables and infant characteristics were determined using Spearman's correlations.

Results

The subject characteristics of the no disorder group (n=47) and the positive CAH group (n=5) are displayed in Table 1. Infants with no disorder (n=47) were younger than those with CAH, ($p<0.001$), and correspondingly had lower birth weights ($p< 0.001$). Among the infants with no disorder, males and females did not differ in comparison of hour of screening, NBS 17-OHP, confirmatory 17-OHP, confirmatory 11-deoxycortisol, and the 17-OHP/11-deoxycortisol ratios. In comparison to CAH infants, the infants with no disorder had lower values of NBS 17-OHP ($p < 0.01$) and confirmatory 17-OHP levels ($p <0.05$), but no difference in confirmatory 11-deoxycortisol concentrations. Infants with no disorder exhibited lower 17-OHP/11 deoxycortisol ratios (Figure 1) than infants with CAH ($p < 0.05$), with no overlap between groups

Figure 2 shows the frequency distribution of the 17-OHP concentrations from the newborn screening dried blood test superimposed upon the distribution of the concentrations from serum confirmatory testing. There is generally no overlap in the two sets of values suggesting a rapid maturation of the pituitary adrenal axis in postnatal life between the time points of newborn screening and confirmatory testing.

Figure 3A shows the frequency distribution of 17-OHP serum values from confirmatory tests among the presumptive positive infants, with the corresponding 17-OHP/11-deoxycortisol ratios noted above the bars for the top quartile of 17-OHP. In parallel, Figure 3B, shows the frequency distribution of ratios with the corresponding 17-OHP values noted above the top quartile of ratios. In the highest quartile of 17-OHP (Figure 3A), the corresponding ratios fell below the top quartile values of the ratio (<1.625) except for two values. Thus, an elevated 17-OHP value alone does not correspond to lower enzyme activity based on the precursor-to-product ratios.

Correlations were performed between 17-OHP or 11-deoxycortisol and infant characteristics (Figure 4). 17-OHP negatively correlated with gestational age and birth weight, but positively correlated with hour of screen, which was consistent with findings of previous studies [11]. Similarly, 11-deoxycortisol negatively correlated with gestational age and birth weight, and positively correlated with hour of screen. No other correlations of significance were found in the other data categories, including between NBS 17-OHP and serum confirmatory 17-OHP.

Discussion

The main objective of newborn screening for CAH is to make an early diagnosis in order to prevent or provide timely lifesaving treatment of salt-wasting crisis in 21-α-hydroxylase deficiency [12, 13]. The problem of false positives from NBS frequently occurs, despite efforts in applying age and weight-based cutoffs, and 2nd tier screening. Multiple factors contributing to false positive results include differences in physiology between premature and term infants, maternal conditions and neonatal stress [9, 14]. In this retrospective study, we

demonstrate that the confirmatory 17-OHP/11 deoxycortisol precursor-product ratios differ widely between CAH infants and those with false positive newborn screens. The ratio may be helpful especially in the evaluation of premature infants.

The infants with no disorder tended to be premature in comparison to the term infants with CAH. Our data exhibited corresponding biochemical differences between the two groups (Table 1 and Figure 1), consistent with a delay in maturation of the 21-α-hydroxylase enzyme in prematurity [5, 15]. The 17-OHP/11-deoxycortisol ratio is a measure of 21 α-hydroxylase enzyme activity under steady state[10]. Because of the inability to convert 17-OHP to 11-deoxycortisol in infants with CAH, a disproportionate elevation in 17-OHP with respect to 11-deoxycortisol was expected, despite no difference observed in 11-deoxycortisol between the groups. As a result, the CAH infants demonstrated a concomitant elevation of the 17-OHP/11-deoxycortisol precursor-to-product ratio. In contrast, infants with no disorder demonstrated low ratios, likely reflecting a stabilization, or possibly even a decreasing ratio, as enzyme activity matures.

We have previously suggested establishing cut-off values for the 17-OHP/11-deoxycortisol ratio to be used in the newborn screening of CAH[16]. Reference ranges of steroid precursor-to-product ratios have already been published by Quest Diagnostics, including 17-OHP/11-deoxycortisol ratios in premature (26–28 wk: 0.4–2.4; 34–36 wk: 0.9–4.8) and term infants (0.4-3.1)[17]. All of our subjects with no disorder had ratios below the upper limits, with the highest ratio resulting from a 34-week old infant (ratio 3.6). In our cohort, 28% (13/47) required repeat confirmatory testing due to borderline or persistently elevated confirmatory 17-OHP levels, however their ratios ranged from 0.41 to 1.95. Application of the 17-OHP/11-deoxycortisol ratio could have reduced the need for repeat confirmatory testing in these cases, thus reducing health care costs and relieving parental anxiety. Therefore, we propose that the 17-OHP/11 deoxycortisol has potential for clinical utility during post-NBS confirmatory testing. Whether the ratio could also be useful in second-tier newborn screening would require additional research, as reporting of 11-deoxycortisol in the second-tier metabolite panel has only recently begun.

A limitation of this study was the small sample size based at one Newborn Screening Referral Center for the infant cohort. In addition, limited clinical data for this retrospective analysis did not allow for adjustment of potential perinatal confounding factors that may influence steroidogenesis [4, 5, 6]. In order to establish use of the ratio in ruling out the diagnosis of CAH, a larger-scale study that includes larger sample sizes of CAH infants and infants with false positive tests should be conducted.

Conclusions

The precursor-to-product ratio (17-OHP/11-deoxycortisol) in newborn dried blood spots was previously demonstrated to reflect 21-α-hydroxylase activity [10]. The present study illustrates a wide difference in the serum confirmatory 17-OHP/11-deoxycortisol ratios between infants with and without CAH who tested positive on newborn screening. Further studies on application of the 17-OHP/11-deoxycortisol precursor-to-product ratio in NBS confirmatory

testing are needed. The ratio may be especially helpful in the evaluation of premature infants.

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References

1. CDPH. Califronia Newborn Screening Program. 2015. 4/15/2015 [cited 2015 10/27/2015]webpage]. Available from: https://www.cdph.ca.gov/programs/nbs/Pages/NBSProgrOVforProviders.aspx
2. Speiser PW, Azziz R, Baskin LS, Ghizzoni L, Hensle TW, Merke DP, et al. Congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2010; 95(9):4133–4160. [PubMed: 20823466]
3. Hayashi G, Faure C, Brondi MF, Vallejos C, Soares D, Oliveira É, et al. Weight-adjusted neonatal 17OH-progesterone cutoff levels improve the efficiency of newborn screening for congenital adrenal hyperplasia. Arquivos Brasileiros de Endocrinologia & Metabologia. 2011; 55:632–637. [PubMed: 22218447]
4. Bolt RJ, Van Weissenbruch MM, Popp-Snijders C, Sweep FG, Lafeber HN, Delemarre-van de Waal HA. Maturity of the adrenal cortex in very preterm infants is related to gestational age. Pediatr Res. 2002; 52(3):405–410. [PubMed: 12193676]
5. Nordenstrom A, Wedell A, Hagenfeldt L, Marcus C, Larsson A. Neonatal screening for congenital adrenal hyperplasia: 17-hydroxyprogesterone levels and CYP21 genotypes in preterm infants. Pediatrics. 2001; 108(4):E68. [PubMed: 11581476]
6. Ersch J, Beinder E, Stallmach T, Bucher HU, Torresani T. 17-Hydroxyprogesterone in premature infants as a marker of intrauterine stress. J Perinat Med. 2008; 36(2):157–160. [PubMed: 18211251]
7. Fingerhut R. False positive rate in newborn screening for congenital adrenal hyperplasia (CAH)-ether extraction reveals two distinct reasons for elevated 17alpha-hydroxyprogesterone (17-OHP) values. Steroids. 2009; 74(8):662–665. [PubMed: 19463687]
8. Hingre RV, Gross SJ, Hingre KS, Mayes DM, Richman RA. Adrenal steroidogenesis in very low birth weight preterm infants. J Clin Endocrinol Metab. 1994; 78(2):266–270. [PubMed: 8106610]
9. Sarafoglou K, Banks K, Gaviglio A, Hietala A, McCann M, Thomas W. Comparison of one-tier and two-tier newborn screening metrics for congenital adrenal hyperplasia. Pediatrics. 2012; 130(5):e1261–e1268. [PubMed: 23071209]
10. Hicks RA, Yee JK, Mao CS, Graham S, Kharrazi M, Lorey F, et al. Precursor-to-product ratios reflect biochemical phenotype in congenital adrenal hyperplasia. Metabolomics. 2014; 10(1):123–131. [PubMed: 24489528]
11. van der Kamp HJ, Oudshoorn CG, Elvers BH, van Baarle M, Otten BJ, Wit JM, et al. Cutoff levels of 17-alpha-hydroxyprogesterone in neonatal screening for congenital adrenal hyperplasia should be based on gestational age rather than on birth weight. J Clin Endocrinol Metab. 2005; 90(7): 3904–3907. [PubMed: 15797960]
12. White PC. Neonatal screening for congenital adrenal hyperplasia. Nat Rev Endocrinol. 2009; 5(9): 490–498. [PubMed: 19690561]
13. Trapp CM, Speiser PW, Oberfield SE. Congenital adrenal hyperplasia: an update in children. Current opinion in endocrinology, diabetes, and obesity. 2011; 18(3):166–170.
14. Huynh T, McGown I, Cowley D, Nyunt O, Leong GM, Harris M, et al. The Clinical and Biochemical Spectrum of Congenital Adrenal Hyperplasia Secondary to 21-Hydroxylase Deficiency. The Clinical Biochemist Reviews. 2009; 30(2):75–86. [PubMed: 19565027]
15. Doerr HG, Sippell WG, Versmold HT, Bidlingmaier F, Knorr D. Plasma mineralocorticoids, glucocorticoids, and progestins in premature infants: longitudinal study during the first week of life. Pediatr Res. 1988; 23(5):525–529. [PubMed: 3387174]

16. Hicks RA, Fereira BF, Mao CS, Yee JK, Lee WP. The use of 17-α-hydroxyprogesterone to 11-deoxycortisol ratio in newborn screening of congenital adrenal hyperplasia. J Invest Med. 2012; 60(1):222.

17. Fisher, DA., Salameh, W., Furlanetto, RW. The Quest Diagnostics Manual: Endocrinology. Fourth. San Juan Capistrano: Quest Diagnostics Incorporated; 2007.

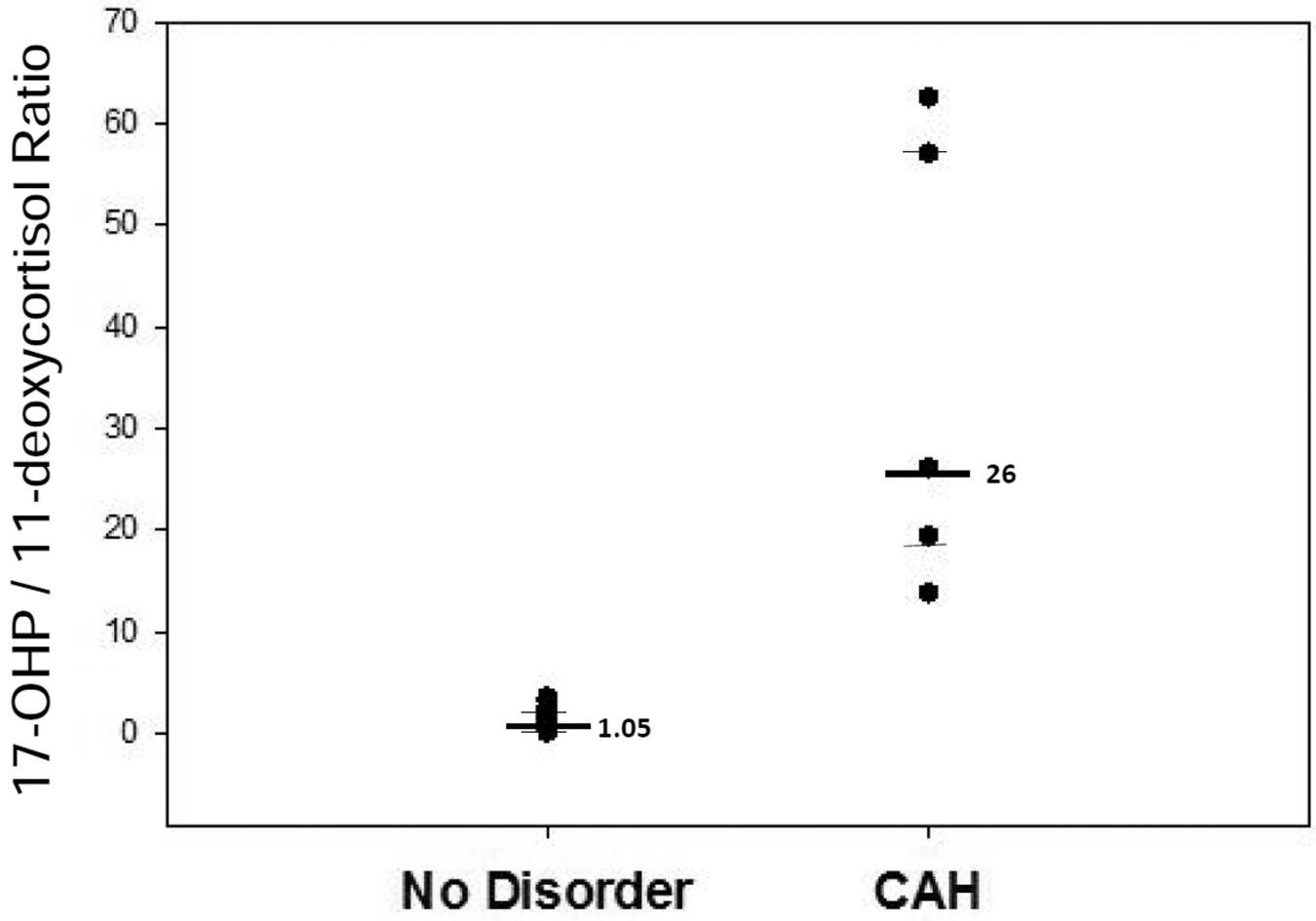

Figure 1. Dot plot of 17-OHP/11-deoxycortisol ratios in infants with congenital adrenal hyperplasia (CAH) (n=5) and with no disorder (n=47)

The medians, and the limits of the 25% to 75% interquartile ranges are represented by dark and thin lines, respectively. Ratios were compared with the Kruskal-Wallis test. Infants with CAH [26 (18 -58)] had higher ratios than infants with no disorder [1.05 (0.69 – 1.46)], $p<0.05$.

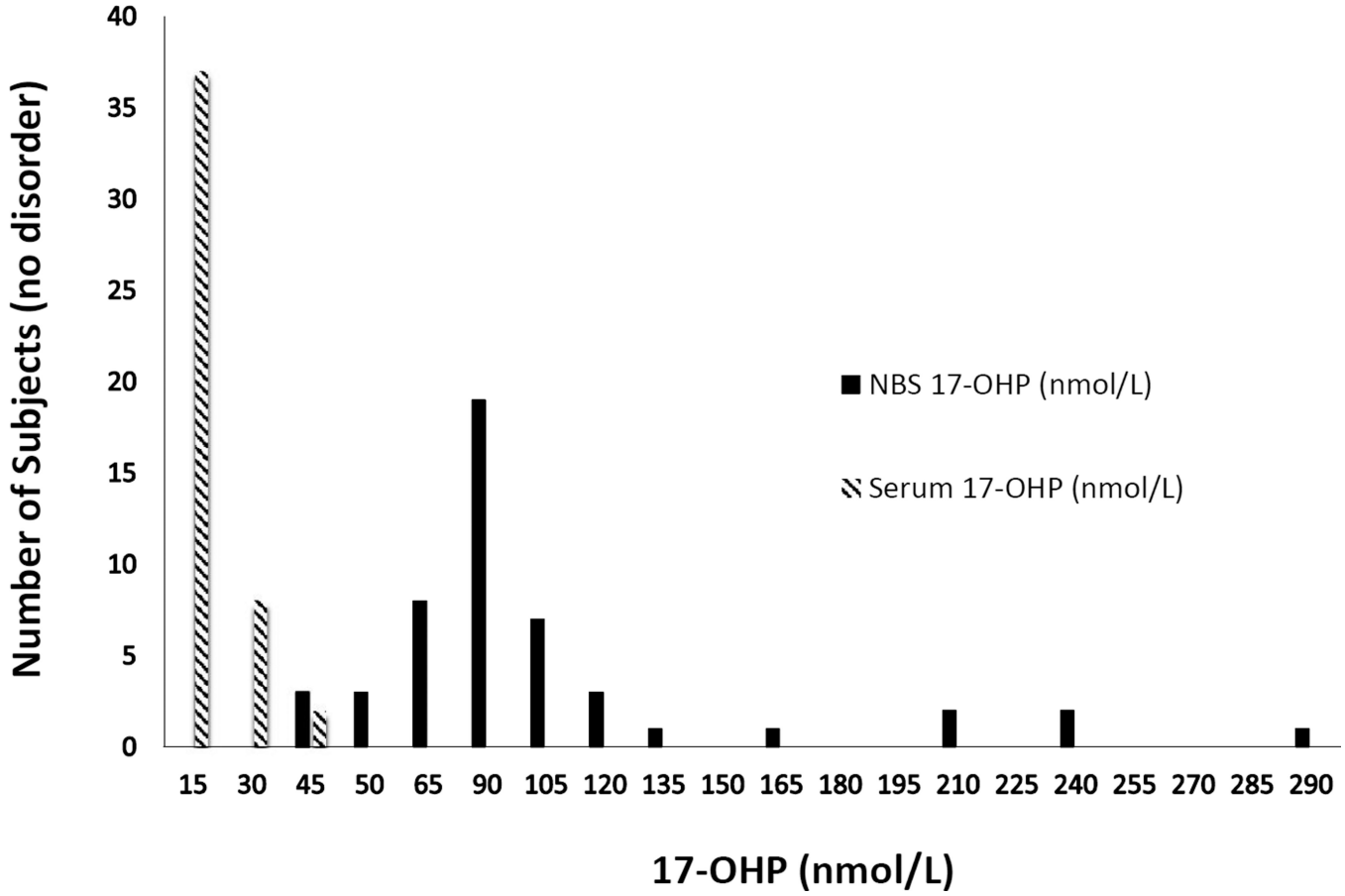

Figure 2. Distribution graph of 17-OHP values from NBS and confirmatory serum testing in infants with no disorders

Data is presented in nmol/L. The solid bars represent NBS 17-OHP values, while the striped bars represent serum confirmatory values.

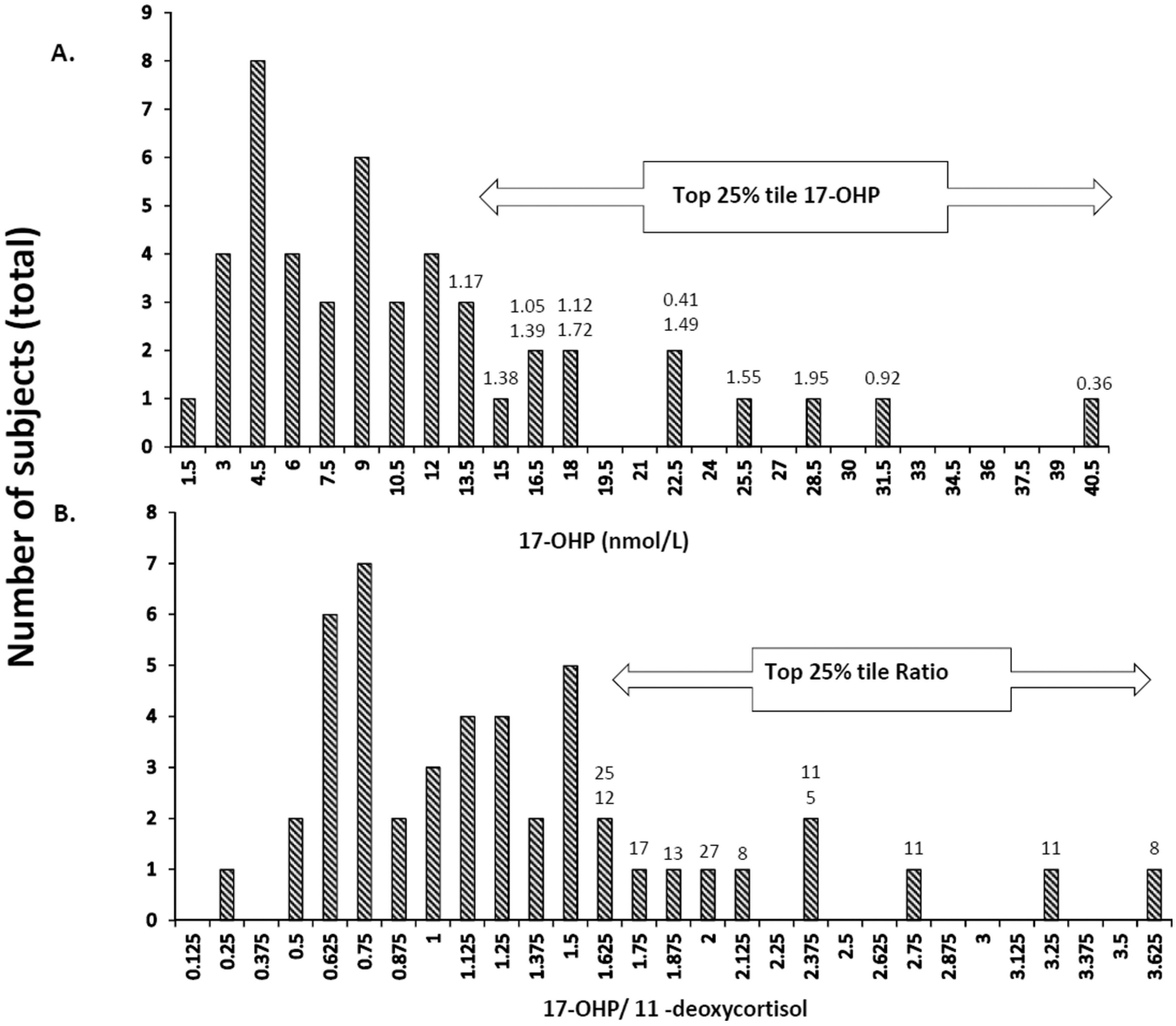

Figure 3. Distribution graphs of confirmatory serum 17-OHP values and 17-OHP/11-deoxycortisol ratios for infants with no disorder (n=47)

(A) Distribution graph of confirmatory 17-OHP values (nmol/L). The numbers above the bars indicate the corresponding 17-OHP/11-deoxycortisol ratios among the top 25% of 17-OHP values. (B) Distribution of 17-OHP/11-deoxycortisol ratios, with the corresponding NBS 17-OHP values (nmol/L) noted above the top 25% of ratios.

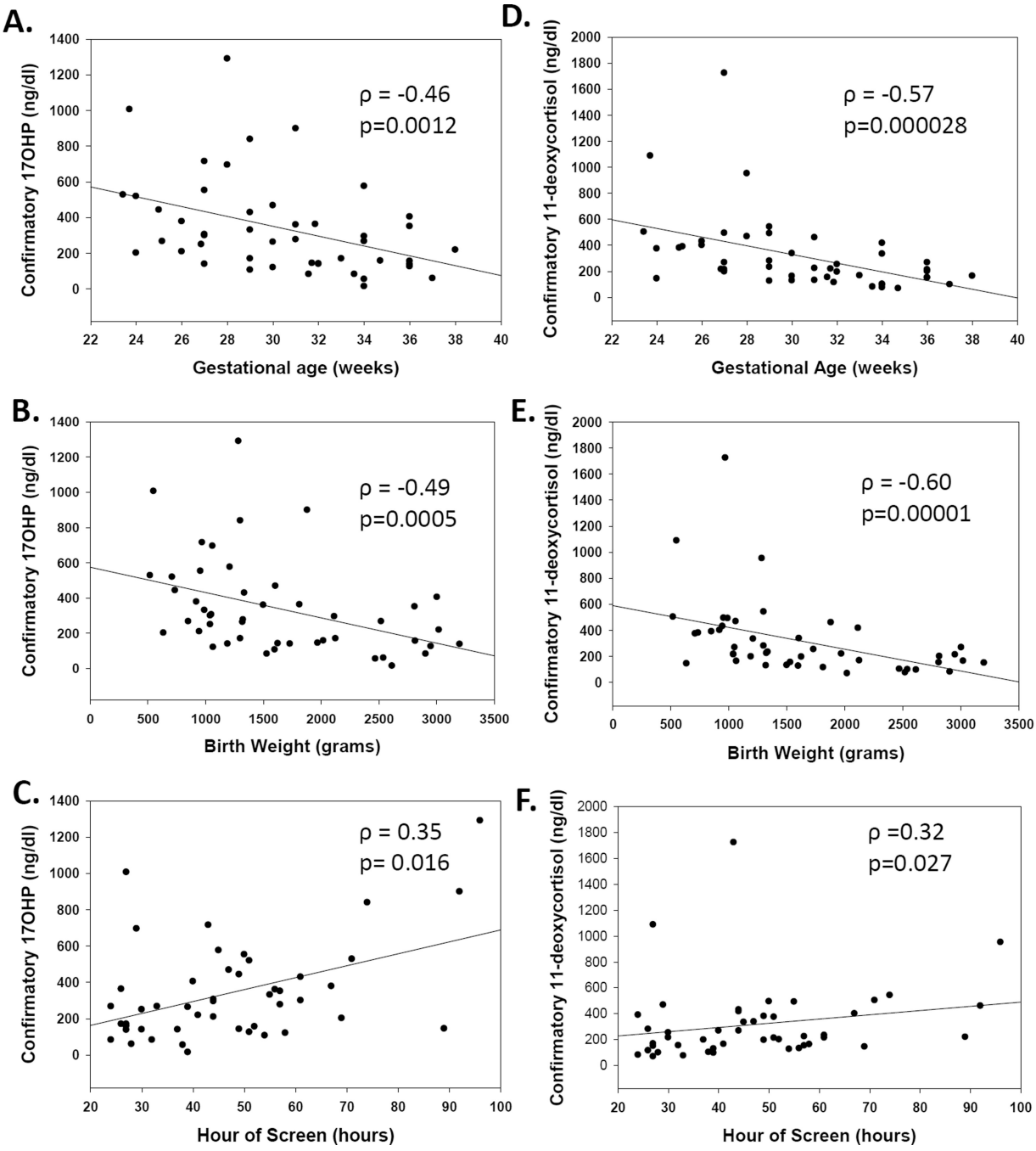

Figure 4. Spearman correlation graphs for 17-OHP and 11-deoxycortisol values vs. infant characteristics

Spearman's rho(ρ) and p values are noted above each graph for the following: (A) gestational age vs. confirmatory serum 17-OHP, (B) birth weight vs confirmatory serum 17-OHP, (C) hour of screen vs. confirmatory serum 17-OHP, (D) gestational age vs confirmatory serum 11-deoxycortisol, (E) birth weight and confirmatory serum 11-deoxycortisol, and (F) hour of screen vs confirmatory serum 11-deoxycortisol.

Table 1

Characteristics of Study Subjects.

	No Disorder (n=47)	CAH (n=5)	P-value
Gender	M: 53%, F: 47%	M:80%, F:20%	NS[1]
Gestational Age (weeks)	30.4 ± 0.6[2]	39.6 ± 0.68	< 0.001
Birth Weight (grams)	1335 (1040 – 2122)	3500 (3395 – 4191)	< 0.001
Hour of Screen (hours)	44 (31 – 57)	24 (17.5 – 30.5)[3]	< 0.01
NBS Dried Blood Spot 17-OHP (nmol/L)	83 (63–101)	593 (399–633)	< 0.01
Confirmatory serum 17-OHP (nmol/L)	8 (4–13)	570 (379–662)	< 0.05
Confirmatory serum 11-deoxycortisol (nmol/L)	6 (4–12)	14 (8–23)	0.26

[1] NS, not significantly different; NBS, newborn screen

[2] Normally distributed data is presented as the mean ± the standard error of the mean. Non-parametric data is presented by the median followed by the 25%–75% interquartile ranges enclosed in parentheses.

[3] n=4 for CAH subjects hour of screen due to inability to obtain one subject's hour of screen from NBS.