ORIGINAL ARTICLE

Evaluation of Neonatal Factors on 17 Alpha Hydroxyprogesterone Level in Neonates- An Experience in Tertiary Care Setting

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ABSTRACT

Objective: This study aimed to determine the effect of various neonatal factors on the serum 17 alpha hydroxyprogesterone (17α-OHP) levels in neonates.

Study Design: An observational analytical study.

Place and Duration of Study: This study was conducted at the Department of Chemical Pathology of Shifa International Hospital Islamabad, Pakistan from 29th January 2026 to 30th June 2026.

Materials and Methods: The study was conducted on 132 study participants that included healthy neonates as controls and sick neonates as cases. The sample size was calculated using the WHO sample size calculator. Non-probability consecutive sampling techniques were employed. Clinical data of all neonates were collected using a structured data sheet and their serum 17 alpha hydroxyprogesterone levels were measured using a chemiluminescence-based auto analyzer (Maglumi X3, Snibe Diagnostics, China). Statistical analysis was performed on IBM SPSS Software version 26, with the Mann–Whitney U test applied for comparison of continuous variables to determine statistical significance.

Results: A total of 132 neonates were included in the study, of which 92 (69.7%) were full-term and 40 (30.3%) were preterm. Among them, 44 (33.3%) were sick and 88 (66.7%) were healthy. 17α-OHP levels were significantly higher in preterm compared to term neonates ($p < 0.05$), in sick compared to healthy ($p < 0.05$).

Conclusion: The present study supports existing evidence that several neonatal factors are associated with elevated 17α-OHP levels. Therefore, these neonatal factors should be taken into account when interpreting 17α-OHP levels in the neonates.

Key Words: *17 Alpha Hydroxyprogesterone, Birth Weight, Congenital Adrenal Hyperplasia, Gestational Age.*

Introduction

In the biosynthesis of glucocorticoids and mineralocorticoids in the adrenal glands, a complex cascade of intermediate compounds is involved in the production of cortisol. Among these intermediates, a steroid hormone 17α-OHP, produced in the adrenal cortex, serves as a key precursor in cortisol synthesis. It undergoes hydroxylation by the enzyme 21-hydroxylase, leading to its conversion into 11-deoxycortisol.[1] A genetic defect in the *CYP21A2* gene, encoding the 21-hydroxylase enzyme, results in impaired enzymatic activity, giving rise to a clinical condition known as Congenital Adrenal Hyperplasia (CAH).[2] Although other enzymes in the cortisol biosynthetic pathway may also be involved in causing CAH, 90-95% cases of these cases result from genetic mutation for 21-hydroxylase enzyme only, making 17α-OHP as the sentinel test for biochemical diagnosis of CAH.[2,3] CAH cases are divided into three subgroups based on the severity of *CYP21A2* gene mutation and age at presentation: (a) Salt wasting CAH, in which there is <1% enzyme activity presenting in infancy. (b)Simple virilizing CAH, with 1-2% enzymatic activity presenting in childhood. (c) Non-classical congenital adrenal hyperplasia (NC-CAH) with 20-50% enzymatic activity usually manifested during puberty or later.[4] The classic CAH incidence is around 1: 14,000.[5] The basal serum 17α-OHP concentrations >10 ng/ml (>30 nmol/L) indicate CAH, while lower basal concentrations are usually present in NC-CAH.[6]

Globally, various studies have shown that 17α-OHP levels are not only elevated in CAH but other neonatal factors such as gestational age, birth

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weight, and the presence of illness may significantly raise 17α-OHP concentrations.[7,8,9] Studies done in Korea and Iowa, USA showed that preterm and low-birth-weight infants, in particular, tend to exhibit higher 17α-OHP levels. Similarly, a study conducted in India showed that neonatal stress due to hypoxia, sepsis, or respiratory distress can also elevate 17α-OHP level.[10] An equivocal interpretation of raised 17α-OHP in neonates can lead to unnecessary investigations, parental anxiety, or delayed recognition of true pathology.[11] Given these challenges, there is a clear need to systematically evaluate the impact of neonatal variables on 17α-OHP levels. A better understanding of these associations would help refine interpretation in early life, reduce diagnostic uncertainty, and support more accurate clinical assessment.[12,13] In developing countries such as Pakistan, there is a scarcity of data regarding the impact of these neonatal factors on 17α-OHP levels, primarily due to the absence of a national neonatal screening program. Consequently, the diagnosis of CAH often relies heavily on lab assay of 17α-OHP levels ignoring the complex interplay of other neonatal factors leading to over diagnosis of CAH resulting in unnecessary follow up investigations, parental anxiety, increased health care costs and potential overtreatment.[14] Understanding the influence of neonatal factors, including gender, birth weight, gestational age, and health status on 17α-OHP levels may help improve the accuracy of neonatal screening and the interpretation of results. Apropos, a need was felt to evaluate the effect of these neonatal factors on serum 17α-OHP levels. An observational analytical study was therefore, undertaken to evaluate the effect of these factors on 17α-OHP levels, in order to minimize false positive results and reduce unnecessary parental anxiety.

Materials and Methods

This observational analytical study was conducted in the department of chemical pathology of Shifa International Hospital Islamabad, over a period of 5 months after the ethical approval from the Institutional Review Board and Ethics Committee (IRB & EC) of Shifa International Hospital (IRB# 525-25). The sample size of this study was estimated using WHO sample size calculator that included level of Significance: 5%, power of the test: 80%, population Standard Deviation: 3.67, anticipated Population mean: 1.8.[10] The sample size calculated was 132 that included both healthy and sick neonates within the first week of life delivered at the tertiary care hospital and neonates age greater than 30 days and diagnosed CAH cases were excluded. Out of 132 total subjects, 44 sick neonates were selected as cases and 88 healthy neonates were selected as control subjects, keeping a ratio of 1: 2 for cases and controls respectively.

Cases were sick neonates admitted to the NICU for management of conditions such as respiratory distress syndrome, birth asphyxia, and sepsis, while controls were healthy neonates without any clinical complications and who did not require NICU admission. Each study group was further assigned into one of the two groups; Pre-Term and Term neonates which were classified according to World Health Organization (WHO) guidelines.[15] The Pre-Term as well as Term neonates included both cases and control subjects finally making the distribution of study subjects into four groups; Pre-Term Sick and Term sick and Pre-term healthy and Term Healthy.

A structured data collection form was used to record information regarding neonates that included demographics, maternal gestational status(preterm/term), clinical condition (sick/healthy) and birth weight. Three ml of venous blood was collected in serum separator tubes (Greiner-Bio-One) for estimation of serum 17α-OHP in accordance with standard laboratory protocols. Serum was separated by centrifugation at 4000 rpm for 10 minutes within two hours of collection and stored at -20^{0}Celsius till analysis. Serum specimens were thawed at room temperature prior to analysis and run in batches for determination of 17α-OHP levels by chemiluminescent immunoassay (CLIA) method, using Maglumi X3, fully automated immunoassay analyzer by Snibe Diagnostics, Shenzhen New Industries Biomedical Engineering Co., Ltd., Shenzhen, China. The assay was calibrated using low and high calibrators, with assigned values traceable to Snibe internal reference standard. The assay performance was verified using two levels of controls; 17-a-OHP QC1 and 17-a-OHP QC2.

Data was entered in IBM SPSS Software version 26. The descriptive statistics used for qualitative variables such as preterm/term, sick/healthy in the

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study were frequency and percentages and for quantitative variable such as 17 OHP median and interquartile ranges were used.

Non-parametric test such as Mann Whitney-U test was used to compare 17α-OHP levels among the two gender, birth weight (<2.5kg and >2.5kg), Pre-term and term, sick and healthy. Kruskal Wallis test was used to find the difference between four groups including Preterm Sick, Preterm Healthy, Term Sick and Term Healthy. Additionally, Pearson's correlation analysis was performed to determine association between different variables and 17α-OHP levels.

P value of ≤0.05 was considered statistically significant for all data analysis.

Results

This study included 132 neonates, comprising of 44 sick neonates (33.3%) and 88 healthy neonates (66.7%) (Figure 1). Sick neonates showed higher 17α-OHP levels compared to in healthy neonates ($p < 0.05$). There was no statistically significant difference between the male and female groups ($p = 0.729$). However, neonates with birth weight <2.5 kg had higher 17α-OHP level compared to those with birth weight ≥2.5 kg ($p < 0.001$). Preterm neonates had higher 17α-OHP compared to term neonates ($p < 0.001$). (Table I)

Further subgroup analysis showed that the preterm group included preterm healthy (PH) neonates (n=15 ;11.4%), preterm sick (PS) neonates (n=25; 18.9%), while the term group comprised term healthy (TH) neonates (n=73; 55.3%) and term sick (TS) neonates (n=19; 14.4%). A Kruskal–Walli's test was conducted to compare 17α-OHP levels across these four groups. The mean rank distribution indicated that the PS group had the highest 17α-OHP levels (mean rank = 104.08), followed by the TS group (96.21), while the PH (66.17) and TH (45.97) groups showed comparatively lower levels (Figure 2)

Pearson correlation analysis using log-transformed 17α-OHP levels demonstrated a significant moderate negative correlation with gestational age ($r = -0.465$, $p < 0.001$) and birth weight ($r = -0.443$, $p < 0.001$), indicating that 17α-OHP levels decrease with increasing gestational age and birth weight. Additionally, health status showed a strong positive co-relation with log-transformed 17α-OHP levels ($r = 0.658$, $p < 0.001$), while no significant correlation was observed with newborn gender ($r = -0.017$, $p = 0.848$) (Table II).

Table I: Distribution of 17α-OHP Level by Health Status, Sex, Birth Weight, and Gestational Age (n = 132)

Variable	Category	n (%)	Median 17α-OHP (ng/mL)	IQR (ng/mL)	p-value
Health status	Sick neonates	44 (33.3)	29.23	14.45–47.40	<0.05
	Healthy neonates	88 (66.7)	9.82	6.98–12.25	
Sex	Female	49 (37.1)	12.25	8.27–19.00	0.729
	Male	83 (62.9)	11.04	8.45–17.67	
Birth weight	<2.5 kg	106 (80.3)	22.50	17.25–46.42	<0.001
	≥2.5 kg	26 (19.7)	10.67	7.64–14.60	
Gestational age	Preterm	40 (30.3)	19.12	10.91–42.27	<0.001
	Term	92 (69.7)	10.52	7.45–14.14	

Table II: Association of Log of Serum 17α-OHP Levels and Neonatal Variables Using Pearson Correlation (n = 132)

Variables	Pearson coefficient (r)	p-value
Gestational age group	-0.465	<0.001
Birth weight (BW)	-0.443	<0.001
Sick or Healthy	0.668	<0.001
Gender	-0.017	0.848

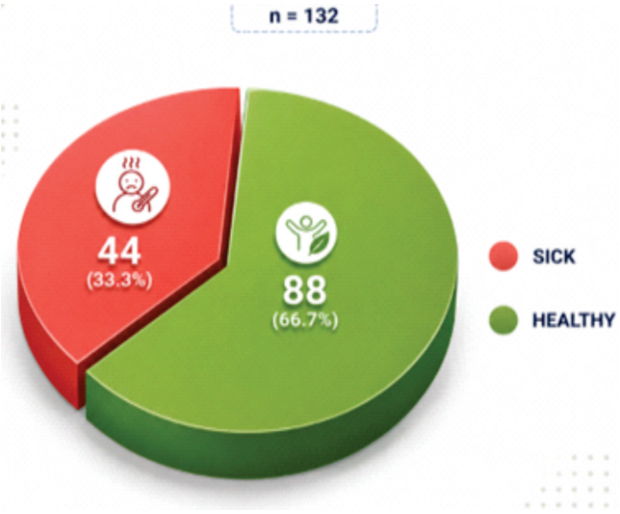

Figure 1: Distribution of Cases and Controls (n=132)

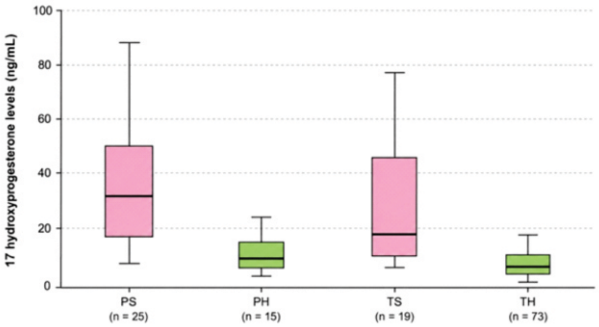

Figure 2: Median and IQR 17 OHP levels Amongst Four Groups: PS, PH, TS, TH.

Discussion

The present study conducted in a tertiary care hospital evaluated the serum 17α-OHP levels in 132 neonates in relation to various neonatal factors. The findings demonstrate that neonatal health status, gestational age which is assigned according to mother, and birth weight significantly influence 17α-OHP concentrations, whereas sex does not appear to have a significant effect.

An important observation from the current study is that serum 17α-OHP levels were significantly higher in sick neonates compared with healthy neonates (median 29.23 ng/mL vs 9.82 ng/mL). Furthermore, the preterm sick subgroup demonstrated the highest levels of 17α-OHP levels with a maximum level of 86.08ng/mL and highest Kruskal-Wallis mean rank (104.08), indicating that the combined effects of prematurity and illness resulted in the higher 17α-OHP compared with other sub-groups. Similar findings have been reported by Vasundhara S. Chennuri, et al., who noted a median value of 34.0 ng/mL of 17α-OHP in sick full-term neonates compared with 8.4 ng/mL in healthy full-term neonate and 58.0 ng/mL in sick preterm infants compared with 20.0 ng/mL in healthy preterm infants, demonstrating that neonatal illnesses such as respiratory distress, sepsis, and perinatal asphyxia can significantly elevate 17α-OHP levels, emphasizing illness as an important confounding factor in neonatal screening.[10] The study conducted by Kum et al. demonstrated that synthesis of steroid hormone in adrenal glands was highly affected by the stress related neonatal conditions such respiratory distress leading to increase in 17α-OHP levels.[8] The large data of a 17α-OHP screening study by Yoon et al., 2023, also identified that preterm sick subgroup neonates have 17α-OHP cutoff values of approximately 35-45 ng/mL. This further strengthens the fact that neonatal health status along with gestational age can act as confounding factors while diagnosing CAH on the basis of serum 17 –OHP levels.[16]

In our study, birth weight and prematurity were also important determinants of serum 17α-OHP concentrations. Neonates weighing less than 2.5kg had median 17α-OHP levels of 22.50 ng/mL which was significantly higher than those weighing ≥2.5kg. This finding is in agreement with a study by de Castro et al. who documented 17α-OHP cutoff at 30.3 ng/mL and 18.1 ng/mL for neonates weighing <2.5kg and ≥2.5kg respectively.[17] Gestational age demonstrated an inverse relation with 17- OHP levels; the 17α-OHP levels were significantly higher in preterm than in term neonates. Our findings are supported by the study by Pode-Shakked et al. who used gestational age specific cutoff values for 17α-OHP: a cutoff of 23.1 ng/mL for preterm infants born at ≤37 weeks of gestation which was and 11.6 ng/mL for infants born > 37 weeks of gestation.[6] Multiple neonatal screening studies on 17α-OHP, have also reported elevated 17α-OHP levels in low-birth-weight infants, emphasizing that birth weight is a stronger predictor in determining 17α-OHP variability.[7,9,12,18] The review paper by Tsuji-Hosokawa et al. done in Japan also highlighted that results of 17α-OHP levels in neonates should be interpreted with caution, particularly in preterm and low-birth-weight infants.[19]

The correlation analyses further strengthened these findings. Serum17α-OHP showed a strong positive correlation with neonatal sickness, and a moderate negative correlation were seen with both gestational age and birth weight, indicating that 17α-OHP concentrations decrease as gestational age and birth weight increases. These findings of the present study are consistent with those reported by Yoon et al. who reported that), who demonstrated a significant negative correlation between 17-hydroxyprogesterone (17α-OHP) concentrations and both gestational age ($r = -0.560$, $p < 0.001$) and birth weight ($r = -0.488$, $p < 0.001$). These findings support the observations that elevated serum 17α-OHP levels in preterm and critically ill neonates reflects physiological immaturity and stress-related adrenal

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activation rather than congenital adrenal hyperplasia.[20]

Our study showed no statistically significant difference between the 17α-OHP levels in male and females. These findings are consistent with the study conducted by Claahsen-Van der Grinten HL et al. which emphasized that 17α-OHP levels in neonates are not affected by gender differences.[2] Speiser PW, et al, Endocrine Society clinical guidelines for congenital adrenal hyperplasia, based on systemic review and expert consensus indicated that gender does not significantly influence serum 17α-OHP levels in neonates.[21]

A retrospective cohort study, done in Canada conducted by Jomaa et al. highlighted that gestational age plays an important role in newborn screening for CAH, as it strongly influences the 17α-OHP concentrations in neonates, with preterm and low birth weight (BW) newborns exhibiting higher 17α-OHP levels that reduce the specificity of CAH screening cut-off value.[12] Similarly, Barra et al. suggested that screening could be made more cost effective by using the 17α-OHP cutoff values adjusted for birth weight, thereby reducing false-positive results.[7] These findings suggested that gestational age and birth weight adjusted interpretation of 17α-OHP, as recommended by the Endocrine Society guidelines can significantly improve diagnostic accuracy and reduce unnecessary recalls.[21] In tertiary care hospitals, the specificity of the test may be further improved by including the health status of neonates, as supported by recent screening strategy improvements and second-tier testing approaches.[18]

The limitations of this study included the absence of repeat 17α-OHP measurements, multivariable regression analysis and biochemical or genetic confirmation of CAH in neonates with elevated 17α-OHP levels. Additionally, the study was conducted at a single center with a small sample size. To validate these findings further studies involving multicentric populations and follow-up protocols are needed.

Conclusion

In conclusion, the present study supports existing evidence that neonatal illness, prematurity, and low birth weight significantly influence 17α-OHP levels. Therefore, these neonatal factors should be taken into account when interpreting 17α-OHP levels in the neonates.

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CONFLICT OF INTEREST

Authors declared no conflicts of Interest.

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DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.