ORIGINAL ARTICLE

Determination of Variation in Neonatal Serum 17 Hydroxyprogesterone Levels in Relation with Gestational Age & Low Birth Weight

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ABSTRACT

Objective: To determine the variation in neonatal Serum 17 – hydroxyprogesterone levels in newborns in accordance with the birth weight and gestational age.

Study Design: It was an analytical cross-sectional study.

Place and Duration of Study: The study was carried out at Armed Forces Institute of Pathology Rawalpindi. The duration of study was 6 months i.e., 17th Nov, 2021 – 17th May, 2022 after the approval from Institutional Review Board (FC-CHP21-12/Read-IRB/22/845).

Materials and Methods: A sum of 210 individuals were included by convenient non-probability sampling technique and divided into 3 groups. Group I included 70 neonates which were delivered at full term (38 – 40 weeks of gestation) with birth weight of 2500 – 4000 g as healthy controls. Group II included 70 neonates delivered at 32 - 37 weeks of gestation with birth weight of 1500 – 2500 g. Group III included 70 neonates delivered at 28 – 32 weeks of gestation with less than 1500 g weight at the time of birth.

Results: The gender-wise distribution of patients, was with males accounting for 65% and females for 35%. The mean value of Serum 17 hydroxyprogesterone (17 – OHP) exhibited a significant increase in Group III (118+8.05 nmol/l), followed by Group II (58+15.66 nmol/l), while Group I had normal levels (21+8.08 U/l) as shown in Figure II. After conducting a one-way ANOVA, post-hoc Tukey analysis was performed to compare three study groups. The Tukey HSD q statistics revealed significant differences between Group I and Group II (72.86), Group I and Group III (180.8), and Group II and Group III (107.95), all with a p-value of < 0.01. Significance in terms of statistics was determined by considering a Tukey HSD p-value below 0.05. Data was analyzed on SPSS version 23.

Conclusion: Neonatal Serum 17 – hydroxyprogesterone levels vary in accordance with the birth weight and gestational age. False-positive newborn-screening rates are disproportionately increased in prematurity and low birth weight. The optimal cut of levels adjusted to birth weight and gestational age of Serum 17 – hydroxyprogesterone should be established for screening of patients of congenital adrenal hyperplasia.

Key Words: *Birth Weight (BW), Congenital Adrenal Hyperplasia (CAH), 17 hydroxyprogesterone (17-OHP), Gestational Age (GA).*

Introduction

Congenital Adrenal Hyperplasia (CAH) is a very complex disease. In this disease there is impaired biosynthesis of cortisol and aldosterone resulting in increased production of 17-hydroxyprogesterone (17-OHP). CAH can be broadly divided into two major forms classical CAH and non-classical CAH. Classical CAH is a more severe form of disease and usually presented in neonates with markedly increased levels of (17-OHP) and ambiguous genitalia. While non-classical CAH is mostly presented later in life with moderate rise of (17-OHP). Screening of CAH is very complex due to variability in nature of serum (17-OHP) after birth. Adjusted Serum 17-hydroxyprogesterone levels according to gestational age and birth weight are used for screening of CAH and can be fatal if undiagnosed. (17-OHP) are erroneously elevated in prematurity and low birth weight.[1] Several countries are conducting the screening process of the neonates which have greater concentration of 17-hydroxyprogesterone for deficiency of classic (severe) 21-hydroxylase enzyme (the most routine kind CAH); However, co-

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syntropin stimulation testing in order to affirm the establishment or diagnosis non-classic (mild) subtypes.[1,6] The projected global incidence of classic CAH is around 1:14,000.[6] The scale of clinical presentations varies from forms with neonatal symptoms, i.e. Simple Virilizing (SV) and Salt Wasting (SW) forms, to non-classical forms which may not become obvious until adulthood. [3,14]

However, CAH screening is plagued by significant issues. Sensitivity of the diagnosis for Simple Virilizing SV-CAH is not optimal when screening is limited to one sample taken immediately after birth due to cost restraints. However, getting a 2nd sample can resolve this problem, it might increase the detection of non-classical CAH too, that isn't the optimal aim of CAH screening.[4] Rest of the problems come from the low specificity of the methods used for the screening of immunologically based 17-hydroxyprogesterone (17-OHP). Cross-reactivity, particularly with steroid sulphates, and illness-related stress causes an increase in the recall rate, more commonly in cases of infants that are born prematurely. The chances of little increased newborn 17-OHP levels because of heterozygosity for CYP21 mutations could potentially intensify this problem in newborns [5-7]. The main objective of the study was to determine the variation in neonatal Serum 17 – hydroxyprogesterone levels in newborns in accordance with the birth weight and gestational age. Increased false positive results of Serum 17-hydroxyprogesterone are associated with prematurity and low birth weight. No local study has been found after extensive literature review hence there is utmost need to establish adjusted gestational age and birthweight cut - off levels.

Materials and Methods

An analytical, cross-sectional research was carried out at Armed Forces Institute of Pathology Rawalpindi. The duration of the study was 6 months. An overall 210 neonates were included by using non-probability convenient sampling technique and divided into three groups on basis of birth weight and gestational age. The study started after the approval of Ethical Review Committee i.e., 17 Nov 2021 – 17 May 2022 (FC-CHP21-12/Read-IRB/22/845). Group I included 70 neonates delivered at full term 38 - 40 weeks with normal birth weight 2500 – 4000 g. Group II included 70 neonates delivered at 32 – 37 weeks of gestation period with less weight at the time of birth ranging from 1500 to 2500 grams. Group III included 70 neonates delivered at 28 – 32 weeks of gestation with very low birth weight that's less than 1500 g. The terminologies like little before term, long before term, lower weight at the time of birth and seriously less birth weight is as per classification of World Health Association (WHO).[25]

We have excluded neonates delivered with maternal steroidal history and other endocrine disorders. As they could interfere with our results misleading the diagnosis.

All eligible participants were informed of the objectives of this study. For participation in research groups and venipuncture, all parents of study participants were provided with written consent. The voluntary nature of participation in this study was highlighted. During a standard interview, sociodemographic variables and the background characteristics of CAH were also emphasized. In addition, each participant received a standard, comprehensive medical examination, which included collecting blood. Each participant's venous blood was taken in quantity of 5 ml while making use of a disposable vacutainer equipment (Plain tube). Within half an hour, serum or plasma was separated and then analyzed accordingly. Serum 17 – OHP was analyzed on Snibe Maglumi immunoassay analyzer using chemiluminescence technique.

Data was parametric therefore One-way ANOVA and post-hoc Tukey analysis was used to compare the intergroup mean. Variance and Tukey HSD q statistics were also calculated among groups. p value of < 0.01 was believed to be statistically substantial.

Results

The gender-wise distribution of patients as depicted in Figure 1, was with males accounting for 65% and females for 35%. The mean value of Serum 17 hydroxyprogesterone (17 – OHP) exhibited a significant increase in Group III (118+8.05 nmol/l), followed by Group II (58+15.66 nmol/l), while Group I displayed normal levels (21+8.08 U/l) as shown in Figure 2. After conducting a One-way ANOVA, post-hoc Tukey analysis was performed to compare the different groups. The Tukey HSD q statistics revealed significant differences between Group I and Group II (72.86), Group I and Group III (180.8), and Group II and Group III (107.95), all with a p-value of < 0.01.

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Significance in terms of statistics was determined by considering a Tukey HSD p-value below 0.05. Data was analyzed on SPSS version 23.

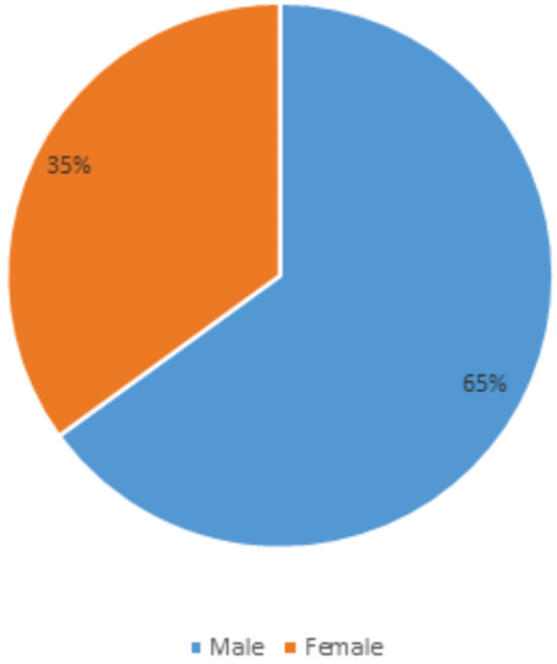

Figure 1: Gender Wise Distribution of Patients

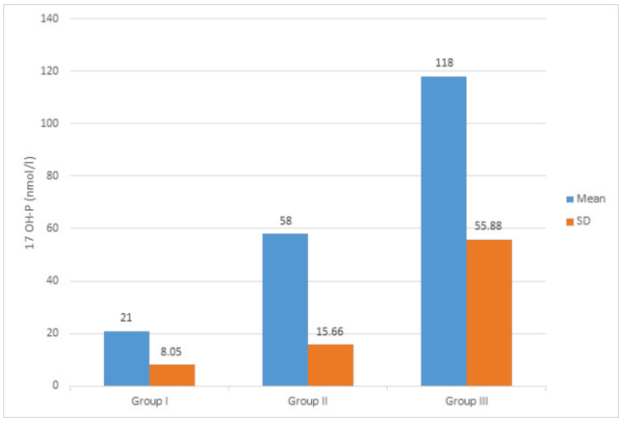

Figure 2: Comparison of Mean & SD of 17-OHP among Groups (N = 210)

Table : I Results of Post hoc Analysis among Groups (N = 210)

Groups	Comparison	Tukey HSD q Statistics	Tukey HSD p-Value
Group I	Group II	72.86	< 0.001*
Group I	Group III	180.8	< 0.001 *
Group II	Group III	107.95	< 0.001 *

**p* value < 0.05 considered as Statistically Significant

Discussion

CAH is a collection of multiple genetic disorders of autosomal recessive pattern resulted by transformations in specific genes encoding for enzymes in cortisol biosynthesis pathways: 21-hydroxylase (21OH), 11-hydroxylase (11OH), 17-hydroxylase (17OH; also named as 17,20-lyase), 3-hydroxysteroid dehydrogenase type 2 (3HSD2), steroidogenic acute regulatory protein (StAR), P450 (POR). CAH manifests with a spectrum of biochemical and clinical phenotypes, with or without changes in glucocorticoid, production of sex steroid and mineralocorticoid. Due to these disorder, there are some direct and indirect effects on steroidogenic pathways and the rarity of these conditions, congenital adrenal hyperplasia remains among the most difficult endocrine disorders to diagnose, treat, and manage. Continued advances in the field of genetics, metabolomics, and treatment strategies result in improvement of patient outcomes by enhancing our understanding of these complex diseases.[9,15,18]

High financial and psychological expenses are related to false-positive CAH screening results. The laboratory charges associated with a false result of positive screening, including copies of internal repeats and following samples, and the separate sample processing, are approximately ten times the price of a standard sample of screening. When we include the charges associated with optional clinical follow-ups, the total becomes considerably high. Therefore, schemes that are meant for the considerable physiological variation in the values of newborn 17-OHP have been implemented, such as the adjustment of the cut-off values of 17-OHP in relation to Gestational Age (GA) and Birth Weight (BW).[10]

In this study, it is evident that there are significant differences in serum 17-OHP levels between infants who are premature and those with low birth weight. This variation results in suspiciously elevated levels of serum 17-OHP, which ultimately leads to false positive results for coronary artery disease. The mean serum 17-OHP concentration ranges from 21 nmol/l in the control group to 118 nmol/l in Group III, which consists of neonates born between 28 and 32 weeks of gestation and weighing less than 1500 g. The following studies support our findings as well.

Danny Joma et al screened newborns for CAH, both with and without a reported Gestational Age. Screening based on GA was performed on infants with a reported Gestational Age; elsewise, screening

based on Birth Weight was performed. Using this method, 2,588 infants (0.37%) positively tested, including newborns with congenital heart disease. The re-screening of the population was done, but infants having unreported Gestational Age were screened again using GA-adjusted cutoff levels after a thorough history was taken. A sum of 2,562 infants (0.36%) tested positive, where all the true positive cases had corrected identification. 26 cases were identified as false positive, and these cases were eliminated. It increased the positive predictive value (PPV) of the test from 1.31 percent to 1.33 percent. This variation was caused by a 24% elevation in the Positive Predictive Value of newborn screening for unreported Gestational Age.[1]

Miranda et al. subdivided the neonates based on their birth weight and determined that after applying stringent cut-off levels, body weight was adjusted accordingly. This results in a reduction of false positive results to 0.73 percent and a growth in the positive predictive value (PPV) for neonates born with an extremely low birth weight, i.e., below 2000 grams.[10]

Atsumi et al study's focuses on the primary cause of cross reactivity in immunoassay, which leads to an increase in false positives in screening preterm neonates for CAH. In preterm infants, 17-hydroxypregnenolone sulphate and 15-hydroxylated compounds are elevated. Immunoassay screening for CAH identified 653 individuals with positive results; 38 cases were confirmed, resulting in a PPV of 5.8% (38/653). Alternatively, the PPV for LC-MS/MS screening was 40% (6/15). In response, the Ministry of Health added LC-MS/MS as a second-layer CAH screening test with BW and GA adjusted levels having 99.95 % specificity with minimal risk of missing CAH. [11]

Conclusion

Neonatal Serum 17 – hydroxyprogesterone levels vary in accordance with the birth weight and gestational age. False-positive newborn-screening rates are disproportionately increased in prematurity and low birth weight. The optimal cut of levels adjusted to birth weight and gestational age of Serum 17 – hydroxyprogesterone should be established for screening of patients of congenital adrenal hyperplasia.

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CONFLICT OF INTEREST

Authors declared no conflicts of Interest.

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Authors have declared no specific grant for this research from any funding agency in public, commercial or nonprofit sector.

DATA SHARING STATMENT

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