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Original Article

Biochemistry Section

Cord Blood Levels of Insulin, Cortisol and HOMA2-IR in Very Preterm, Late Preterm and Term Newborns

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ABSTRACT

Introduction: Alteration in the glucose homeostasis is still the major cause of morbidity and mortality in the newborns. Intrauterine undernutrition plays an important role in causing adult insulin resistance and diabetes but the exact cause is still unknown.

Aim: To estimate the plasma glucose, serum insulin and cortisol levels at birth in newborns at different gestational age.

Materials and Methods: The present cross-sectional study conducted from December 2014 to June 2015 included 58 newborns enrolled as per the inclusion criteria and further categorized into Group I (very preterm; n=19; gestational age < 32 weeks), Group II (late preterm; n=20; gestational age between 32-37 weeks) and Group III (full term; n=19; gestational age >37 weeks) newborns. Venous Cord Blood (VCB) was collected and plasma glucose was analysed by GOD-POD (Glucose Oxidase-Peroxidase) method in auto analyser whereas serum insulin and cortisol were analysed by ELISA (Enzyme Linked Immunosorbent Assay). HOMA2-IR (Homeostatic Model Assessment) calculator was used to assess insulin resistance. All parametric data was expressed as mean±SD and analysed using ANOVA with Tukey's as the Post-Hoc test. Correlation analysis was done using Pearson's correlation co-efficient with scatter plot as the graphical representation.

Results: Significantly increased insulin and HOMA2-IR levels were found in group I (13.7±4.7μIU/mL and 1.6±0.58 respectively) when compared to group II (8.3±2.9μIU/mL and 0.93±0.2 respectively) and group III (8.3±2.1μIU/mL and 1.03±0.26 respectively). A positive correlation between cortisol levels and gestational age (r = 0.6, n = 58, $p < 0.001$) and a negative correlation between insulin and gestational age (r = -0.654, n = 58, $p < 0.001$) was observed in the study population.

Conclusion: Increased levels of insulin and HOMA2-IR as seen in the very preterm newborns signify the predisposition of these newborns to development of diabetes in later stages of life. The inverse association of cortisol and insulin with gestational age suggests that cortisol could also be responsible for impaired β cell function and insulin sensitivity.

Keywords: Insulin resistance, Neonates, Postnatal life

INTRODUCTION

The intrauterine environment and early postnatal life are now well recognized as imperative determinants of the risk of disease in later life. Low birth weight, an indicator of poor intrauterine condition, has constantly been related with a variety of adult-onset diseases, including type 2 diabetes mellitus, hypertension, dyslipidemia, coronary artery disease and cerebrovascular diseases [1].

Thrifty phenotype hypothesis acclaims that type 2 diabetes in an adult is the outcome of the nutritionally thrifty gene which develops during fetal programming in utero. This thrift results in impaired growth of the β cells of the pancreas. Insulin requirement is lesser in an individual in the nutritionally deprived state. However, a sudden move to good or over-nutritional state triggers the dormant phase of β cell function to release insulin in impulsive manner which leads to hyperinsulinemia and later diabetes in them [2]. 'Fetal insulin hypothesis' proposes the relationship between inherited insulin resistance and altered growth mediated by insulin [3]. Hill et al., postulated that in utero alteration in the levels of hormones like insulin and cortisol that are involved in glucose homeostasis increases the risk of developing insulin resistance and obesity in the later stages of life [4].

At delivery, newborn passes a transitional phase from a mother-dependent to an independent environment where continuous transplacental supply of nutrients from the mother to the fetus stops abruptly. At this time, there is fluctuation in the concentration of insulin and cortisol, for adaptation to the external environment [5]. An adrenocortical hormone, cortisol, is known for stress-responsive action and its concentration is used as a stress marker at the time of delivery in cord blood. It modulates a large number of physiological actions involved in metabolic, inflammatory, cardiovascular and behavioral processes. The molecular mechanisms and the physiological effects of cortisol have been broadly studied. However, the involvement of cord blood cortisol action in the etiology of diabetes and insulin resistance has not yet been studied in preterm and term newborns. Recent mounting clinical evidence and animal studies have attracted growing interest in the role of cortisol action in obesity and insulin resistance [6].

Limited studies have been reported on the glucose, insulin, cortisol and insulin resistance levels in cord blood of newborns at different gestational age. As in mid gestation both insulin and cortisol are present in the fetus blood for maintaining the glucose homeostasis. Hence, this study was planned with an objective to determine the glucose, insulin, cortisol and HOMA2-IR levels at birth in newborns at different gestational age.

MATERIALS AND METHODS

This was a cross-sectional study comprising of 99 newborns from the constituent Medical College Hospitals, conducted between December 2014 and June 2015. The study was approved by Institutional Ethics Committee and an informed consent was obtained from mothers. The study population comprised of residents of southern India from in and around the city of Mangalore, Karnataka, India.

Samples were collected (in six months duration) from 99 normal vaginal deliveries, of which 39 were preterm and 60 were term delivery. Fifth minute Apgar score >8 for term and preterm newborns only were included in the study. Pregnant women with a history of any infectious disease or obstetric complications

like gestational diabetes, hypertension, kidney disease, thyroid disease, polycystic ovarian disease etc. were excluded from the study. So, during recruitment for the study only 58 newborns were included in the study as per selection criteria.

All 58 selected newborns were further divided into three groups according to their gestational age. Group I (n=19) < 32 weeks (or < 224 days) called as very preterm (VP); Group II (n=20) 32–37 weeks (224-259 days) as late preterm (LP); and Group III (n=19) > 37 weeks (>259 days) as full term (FT) [7]. Venous cord blood (VCB) was collected under aseptic conditions from the umbilical cords of all 58 newborns, born through normal vaginal deliveries. Blood collected after delivery, but prior to the expulsion of placenta. A 3ml VCB was drawn from umbilical cord into each plain and fluoride vacutainer. Plasma glucose was analysed within 4 hours of collection. Serum was separated by centrifugation at 3000 rpm for 10 minutes and was stored at -20ºC for a maximum period of one month as per the manufacturer's stability and storage instructions for the ELISA kits [8,9]. All data regarding mother and newborn were collected from case sheet.

Biochemical Estimation and Analysis

Plasma glucose estimation was done by glucose oxidase – peroxidase (GOD-POD) method using Agappe diagnostic kits, Ernakulam, Kerala with an inter and intra assay CV of less than 5% in Roche Hitachi P800 auto-analyser (Roche Diagnostics GmbH, Mannheim). Commercially available ELISA kits were used to estimate insulin (DRG, Germany; inter and intra assay CV <3%) and cortisol (CalBiotech, USA; inter and intra assay CV <6%) based on sandwich principle in ELx 800 ELISA reader by BIO TEK® Instruments, Inc. Insulin resistance was calculated using HOMA2-IR (Homeostatic Model Assessment) calculator v2.2.2 [10].

STATISTICAL ANALYSIS

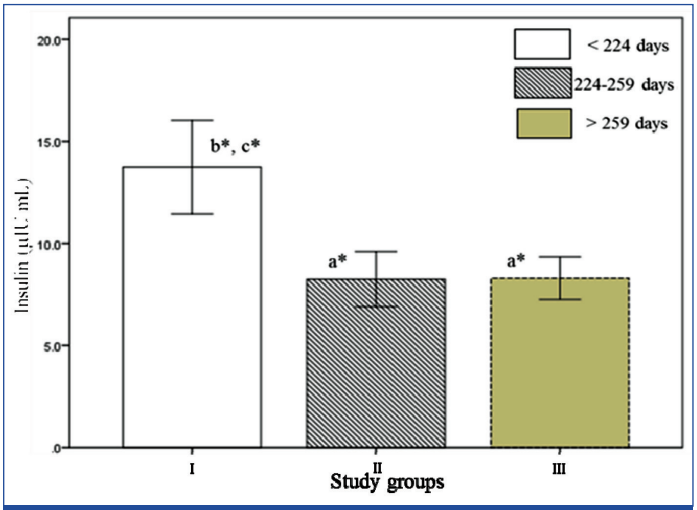
Data was analysed using IBM SPSS version 20.0 (SPSS, Chicago, IL, USA). The parametric data were presented as Mean ± SD. Intergroup mean comparison of parametric data was done using one way ANOVA with Tukey's as the Post-Hoc test. Correlation analysis was done using Pearson's correlation coefficient with scatter plot as the graphical representation. The p-value less than 0.05 was considered statistically significant.

RESULTS

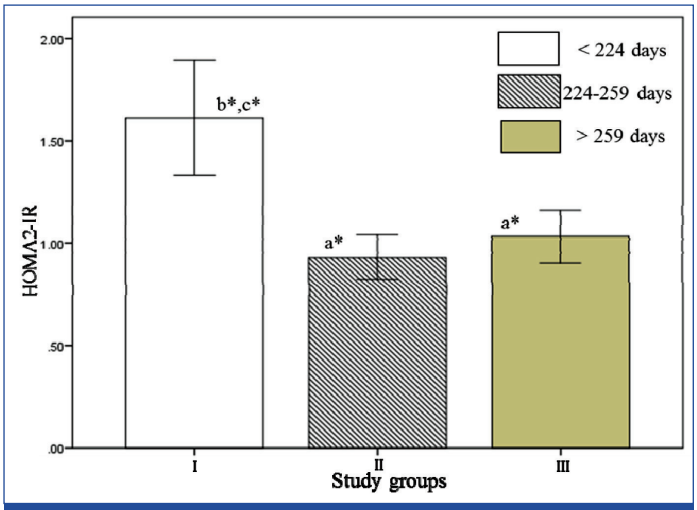
Intergroup mean comparison of anthropometry and biochemical parameters among the three groups is depicted in [Table/Fig-1]. Among the anthropometry parameters, birth weight and length of the newborns were found to be significantly different in all three groups [Table/Fig-1]. Mean levels of insulin and HOMA2-IR were found significantly increased in group I (13.7 mg/dL and 1.6, respectively) when compared to group II (8.3 mg/dL and 0.93, respectively) and group III (8.3 mg/dL and 1.03 respectively) [Table/Fig-1-3] respectively. Serum mean cortisol level was highest in group III (126.1 ng/mL) as compared to group II and I (122.7 and 81 ng/mL respectively) but significant difference was found between group I and II and between I and III whereas no significant difference was seen between group II and III [Table/Fig-1,4] respectively. Correlation analysis revealed a significant positive correlation between cortisol levels and gestational age (r = 0.6, n = 58) [Table/Fig-5] and a significant negative correlation was found between insulin and gestational age (r = -0.654, n = 58) [Table/Fig-6].

Variables	Group I (n=19)	Group II (n=20)	Group III (n=19)
Birth weight (kg)	2.2±0.2^{b*,c*}	2.6±0.23^{a*,c*}	3±0.33^{a*,b*}
Length (cms)	44.6±2.2^{b,c*}	46.6±0.8^{a,c}	48.6±1.8^{a*,b}
Head circumference(cm)	32.1±1.4^{c}	32.9±1.05	33.4±1.9^{a}
Chest circumference(cm)	30.8±0.5^{b,c*}	31.5±1.3^{a}	35.6±0.7^{a*,b*}
Mid arm circumference (cm)	8.1±0.15^{c}	8.3±0.31^{c*}	9.6±0.59^{a*,b*}
Glucose (mg/dL)	72.1±21	69.4±11.7	80.4±12
Insulin (µIU/mL)	13.7±4.7^{b*,c*}	8.3±2.9^{a*}	8.3±2.1^{a*}
Cortisol (ng/mL)	81±32.3^{b*,c*}	122.7±27.7^{a*}	126.1±33^{a*}
HOMA2-IR	1.6±0.58^{b*,c*}	0.93±0.2^{a*}	1.03±0.26^{a*}

[Table/Fig-1]: Anthropometric and biochemical variables in three groups. Data is expressed as mean ± standard deviation, p<0.05 is denoted when a is Vs Group I, b is Vs Group II, c is Vs Group III, * is used when p<0.001 (ANOVA).



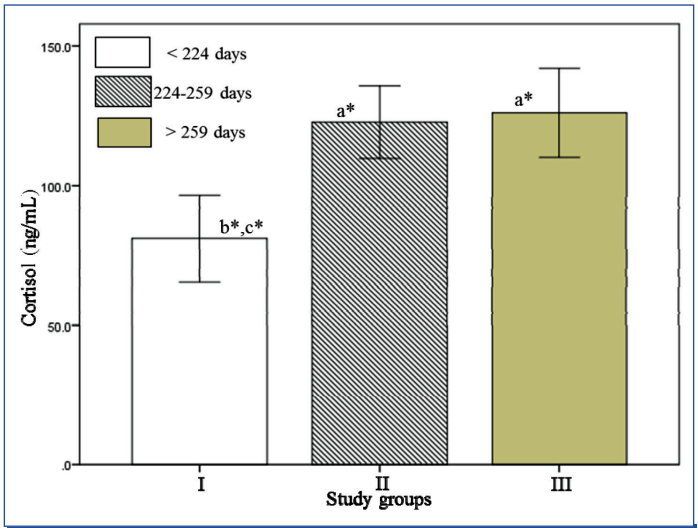
[Table/Fig-2]: Error bar showing comparison of Insulin in different groups. a*(p<0.001) comparison with group I, b*(p<0.001) with group II and c*(p<0.001) with group III. *p*-Values by ANOVA followed by Post Hoc Tukey's test.



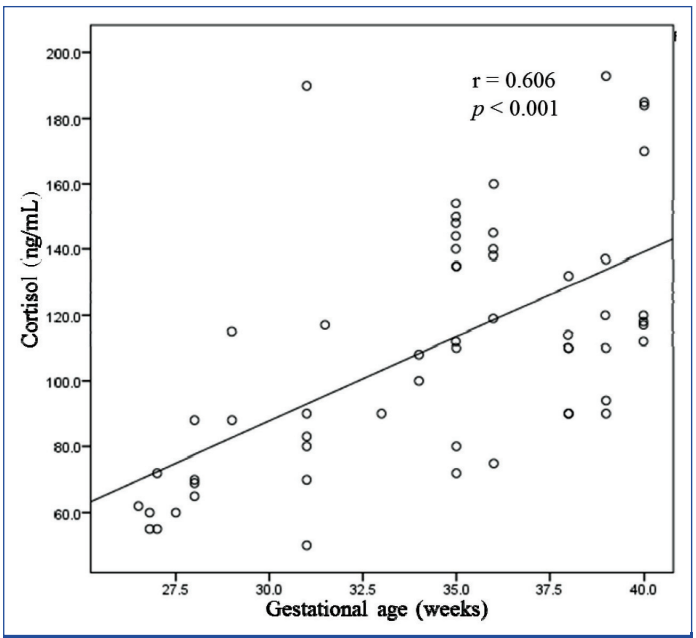
[Table/Fig-3]: Error bar showing comparison of HOMA2-IR in different groups. a*(p<0.001) comparison with group I, b*(p<0.001) with group II and c*(p<0.001) with group III. *p*- Values by ANOVA followed by Post Hoc Tukey's test.

DISCUSSION

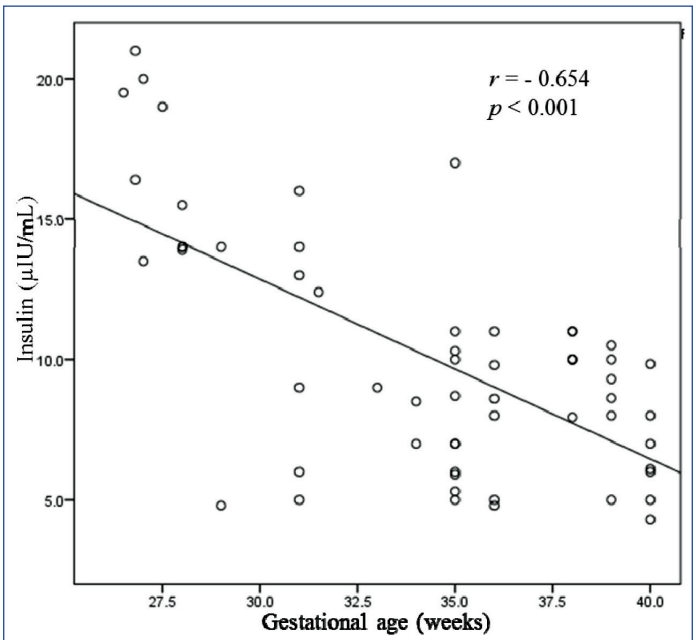
The present study reports significantly higher insulin and HOMA2-IR levels in cord blood of VP newborns as compared to LP and FT newborns. No significant difference was found between LP and FT. The increased insulin levels in VP newborns can be attributed to the anabolic role of insulin and its action on fetal growth and development [11]. Because of immature development of insulin signal transduction pathways in VP newborns, the insulin requirement increases for its anabolic action thus enhancing the likelihood of hyperinsulinemia and development of insulin resistance in them. These changes lead to the down-regulation of insulin-dependent glucose transporter isoform 4 (GLUT4) expression as observed in preterm animal models [11,12]. This mechanism explains the existence of high insulin resistant state in VP newborns as observed in the present study. High insulin levels might improve with growth later on via an increase in the receptor



[Table/Fig-4]: Error bar showing comparison of cortisol in different groups. a*(p<0.001) comparison with group I, b*(p<0.001) with group II and c*(p<0.001) with group III. *p*- Values by ANOVA followed by Post Hoc Tukey's test.



[Table/Fig-5]: Scatter plot between cord blood cortisol and gestational age in total study Population.



[Table/Fig-6]: Scatter plot between cord blood insulin and gestational age in total study Population.

number and maturity thus increasing the peripheral sensitivity to the hormone [5].

Bagnoli et al., study reported more fat deposition and percentage weight gain in VP newborns after few days of birth because of higher insulin levels in them [5,13]. There have been lots of mechanistic frameworks in the past decade to explain the biological basis of the association observed between birth weight and health outcomes in the adult life. Hales and Barker's thrifty phenotype hypothesis suggested that a poor fetal environment results in the development of an adaptive response which improves the progress of crucial body organs to the detriment of others. This results in an alteration in the postnatal metabolic pathways to support survival under poor nutritional status. These alterations in metabolic pathways become detrimental only when abundant postnatal nutrition is present [14].

Seldom studies have reported the levels of cortisol in cord blood of preterm and term newborns. In corroboration with the findings of Srivastava et al., this study found significantly lower cortisol levels in VP than the LP and FT newborns [15]. Cortisol has its major regulatory action in terminal maturation of the fetus and also helps the fetus for neonatal adaptation at birth. Its levels remain low till 30 weeks of gestation followed by a progressive rise approximately reaching 200μg/mL near term [16-18]. The significant correlation found between cord blood cortisol levels and the gestational age in the present study is indicative of an increase in the cortisol levels with the advancement of gestational age [19].

Glucocorticoids in newborns are primarily derived from the maternal circulation through the fetoplacental compartment. Majority of the glucocorticoid circulating in the fetal compartment is cortisone while that in the maternal circulation is cortisol. In the early period of pregnancy, 80-85% of cortisol from the maternal circulation is converted to cortisone in the placenta before entering the fetal circulation. In the later half of pregnancy, there is increased conversion of cortisone to cortisol in placenta by the action of enzyme 11 β-hydroxy steroid oxidoreductase; activity of which increases with the increase in gestational age, hence lower cortisol levels are seen in preterm newborns [15].

In this study, an inverse correlation was noted between insulin and cortisol with gestational age indicating an association of cortisol with a decrease in insulin sensitivity in children and adolescents. This can be attributed to a decrease in β cell function and impaired insulin secretion in response to a glucose stimulus [20]. Thus, cortisol can be hypothesized as an essential factor in intrauterine programming, as postulated by several other studies [20,21]. Fasting cortisol levels were higher in adults who were born with lower birth weight and were associated with features of metabolic syndrome such as insulin resistance, hypertension and type 2 diabetes [21]. Cortisol was shown to be responsible for various grades of β cell dysfunction thus contributing to impairment of insulin sensitivity [22]. Children and young adults with history of very low birth-weight and preterm delivery have shown to exhibit higher HOMA2-IR indices as compared with those delivered at term [1,23,24]. This explains the association of prematurity with the development of insulin resistance in the later stages of life in very preterm newborns. These findings point towards a causal relationship between birth-weight and development of abnormal glucose homeostasis in the adulthood; thus postulating a U-shaped association with both lower and higher birth weight leading to an increased risk of metabolic diseases like diabetes.

CONCLUSION

In conclusion, this study describes the trend of insulin, cortisol and HOMA2-IR levels in cord blood of newborns with different gestational ages. Higher insulin and HOMA2-IR levels in very preterm newborns as compared to late preterm and full term newborns could be responsible for development of metabolic

diseases like diabetes and its complications in adult life. This increase in insulin and HOMA2-IR levels might suggest that the defects contributing to insulin resistance in the pathophysiology of diabetes might have initiated in the intrauterine phase itself. The inverse association of cortisol and insulin suggests that cortisol could also be responsible for impaired β cell function and insulin sensitivity. However, further prospective studies are required in larger population to ascertain the cause-effect relationship and validate the fetal hypothesis in relation to development of metabolic diseases like diabetes in later stages of life.

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