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Original Article

Biochemistry Section

Comparison of Biochemical Parameters in Cord Blood versus Venous Blood among Premature and Term Neonates: A Cross-sectional Study

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ABSTRACT

Introduction: Premature and low birth weight neonates are more prone to develop various disorders. These neonates undergo various investigations to confirm diagnosis. Cord blood samples are obtained without any intervention on the neonate, making them a useful source for analysing biomarkers and predicting disorders in premature and low birth weight neonates.

Aim: To compare the biochemical parameters among premature neonates (low birth weight) versus matured term neonates (normal birth weight) in cord blood and venous samples.

Materials and Methods: This analytical cross-sectional study was conducted at SRM Medical College Hospital and Research Centre in Kattankulathur, Kancheepuram, Tamil Nadu, India from July 2022 to September 2022. The study included two groups: Group 1 consisted of premature and low birth weight neonates, and Group 2 consisted of normal birth weight term neonates, with 53 participants in each group. Biochemical parameters like creatinine, sodium, potassium, calcium, magnesium, phosphorus, High-Density Lipoprotein Cholesterol (HDL-C), Low-Density Lipoprotein Cholesterol (LDL-C), Albumin, Alkaline Phosphatase (ALP), Gamma-Glutamyl Transferase (GGT), iron, and Creatine Kinase (CK) were analysed in cord blood and venous samples of these groups. Student's t-test was used to compare between groups, and Pearson's correlation was used to assess the association of biochemical parameters in cord blood and venous blood samples.

Results: The mean age of the mothers and gestational age in group 1 were 24±4 years and 33±2.3 weeks, respectively. The mean weight of the neonates was 2135±241 grams. When cord blood samples were compared between the study groups, sodium, phosphorus and albumin were found to be significantly decreased and LDL-C was significantly increased in group 1, whereas GGT was significantly decreased in group 2 for venous blood. When cord blood samples were compared between the study groups, magnesium (r-value=0.2) was found to be significantly decreased in group 1.

Conclusion: It was observed that biochemical parameters were significantly correlated, except for sodium, HDL-C, LDL-C, and GGT, between cord blood and venous blood samples. Hence, cord blood samples collected through non invasive methods can be used for sample analysis in neonates.

Keywords: Lipid profile, Low birth weight, Neonatal mortality, Newborns

INTRODUCTION

Baby born before 37 weeks of pregnancy is considered as preterm, and a Low-Birth-Weight infant (LBW) is a baby with a birth weight of less than 2500 grams. The foetus matures during the last week of gestation and attains full maturity. Globally, prematurity accounts for a neonatal mortality rate of 10%, or nearly 500,000 deaths annually [1]. In India, the incidence of preterm births is 14.5%, and LBW contributes to about 33% of newborns [2]. Perinatal mortality and subsequent neurological disorders and respiratory sequels are consequences of preterm birth [3]. Some neonates may develop cerebral palsy and intellectual disability, while ongoing investigations have revealed mild cognitive, adjustment, and behavioural problems [4,5].

LBW is also associated with kidney diseases [6-8]. Before the last few weeks of pregnancy, the lungs, pancreas, and kidneys rapidly develop essential functional cell structures in humans, and preterm birth may impact their final development [9]. Premature birth has been linked to impaired nephron development in the postnatal period [10,11]. The number of nephrons and gestational age are substantially associated. As a result, preterm birth reduces the number of nephrons, which might impact blood pressure and long-term renal function [12,13]. Birth weight plays an important role in mineral status, which can lead to neuronal and bone diseases [14,15]. Supportive studies have also revealed the influence of birth weight on lung diseases [16].

In term and preterm neonates, venous blood is used to assess various biochemical parameters; however, it is an invasive procedure. Cord blood samples, obtained without intervention on the neonate, can be considered for evaluating biomarkers of various disorders in neonates. In India, only a few supportive studies have been conducted on premature neonates [17,18]. Hence, the present study aimed to analyse and compare the biochemical parameters in cord blood and venous blood samples of both premature neonates and LBW neonates, compared to term neonates with normal birth weight, at a tertiary care centre in Southern India.

MATERIALS AND METHODS

This analytical cross-sectional study was conducted at SRM Medical College Hospital and Research Centre, Kattankulathur, Kancheepuram, Tamil Nadu, India, from July 2022 to September 2022. The study was initiated after obtaining approval from the Institutional Ethical Committee (IEC) (Ethics clearance number: 8390/IEC/2022). After obtaining written consent in both the local language and English from one of the parents, the study included participants from both groups.

Inclusion criteria: Premature neonates (born before 37 weeks of pregnancy) and neonates with LBW (birth weight of less than 2500 grams) were included in group 1. Term neonates with normal birth weight neonates were included in group 2. Neonates with APGAR

scores of 9 at one minute and 10 at five minutes were included in both Group 1 and Group 2.

Exclusion criteria: Newborns with critical illnesses, congenital abnormalities, serious systemic diseases present at birth, and mothers with co-morbidities were excluded from the study.

Sample size: The sample size was calculated to be 53 in each group, based on the advice of the statistician using data from the National Family Health Survey-4 conducted by the Indian Institute of Population Sciences [19]. The sample size was calculated using the Cochran formula:

$n=z^2 * p * (1-p)/d^2$

$n=(1.96)^2 * 16.4 * 83.6/(10)^2$

$=52.7$

where:

z-Confidence level (95%)

p-Proportion of the population

d-Absolute precision

Data collection: The study participants were selected based on the inclusion and exclusion criteria. Cord blood and venous blood samples (3-5 mL) were collected from premature neonates and transported to the Department of Biochemistry, Central Laboratory. Umbilical cord samples were collected following the methodology described by Baer VL et al., [18]. Samples collected from mature term neonates as part of routine analyses were used in this study. Precautions were taken during sample transportation to avoid haemolysis. The collected samples were allowed to clot for 20-30 minutes, and standard preanalytical procedures were followed. Serum from cord blood and venous blood samples was separated after centrifugation at 3500 rpm for 15 minutes and estimated in Auto-analyser Beckman-Coulter AU480 Auto-analyser for biochemical parameters, following the respective methods [Table/Fig-1] [20-26].

Parameter (normal range)	Method	Cut-off range
Creatinine	Jaffe's Kinetic	(Males: 0.7-1.3 mg/dL; Females: 0.4-1.0 mg/dL) [20]
Sodium	Ion selective electrode	(135-145 mmol/L) [21]
Potassium	Ion selective electrode	(3.6-5.0 mmol/L) [21]
Calcium	Arsenazo	(8.8-10.7 mg/dL) [21]
Magnesium	Xylidyl blue	(1.46-2.68 mg/dL) [21]
Phosphorus	UV Molybdate	(3.4-4.5 mg/dL) [21]
High density lipoprotein-Cholesterol (HDL-C)	Direct antibody inhibition	(>40 mg/dL) [22]
Low density lipoprotein-Cholesterol (LDL-C)	Direct antibody inhibition	(<130 mg/dL) [22]
Albumin	BCG	(4-5.03 g/dL) [23]
Alkaline Phosphatase (ALP)	PNP AMP KINETIC	(80-290 IU/L) [24]
Gamma Glutamyl Transferase (GGT)	UV kinetic/IFCC	(GGT) (5-30 IU/L) [24]
Iron	TPTZ binding	(35-145 µg/dL) [25]
Creatine Kinase (CK)	NAC Action-IFCC	(10-400 IU/L) [26]

[Table/Fig-1]: Biochemical parameters and methods of estimation [20-26].

STATISTICAL ANALYSIS

The data were analysed using Statistical Package for Social Sciences (SPSS) (version 28.0) statistical software. The means and standard deviations of the parameters were compared between cases and controls using Student's t-test. Pearson's correlation was used to assess the association of biochemical parameters between cord blood and venous blood samples.

RESULTS

The mean age and gestational age of mothers included in group 1 were 24±4 years and 33±2.3 weeks, respectively. The mean weight of the neonates in group 1 was 2135±241 grams. In group 2, the mean age of the mothers was 25±2 years, and the gestational age was 38±1.1 weeks. The mean weight of the neonates in group 2 was 2879±122 grams. On comparing the biochemical parameters between groups in cord blood samples, it was found that creatinine, potassium, CK, and magnesium were lower in premature neonates. There was also a significant decrease in sodium and phosphorus in premature neonates compared to mature term neonates. The levels of calcium, HDL-C, ALP, iron, and GGT were slightly elevated in premature neonates, and there was a statistically significant alteration in LDL-C and albumin levels among premature neonates compared to mature term neonates [Table/Fig-2].

Similarly, when analysing the biochemical parameters between groups in venous blood samples, it was found that sodium, magnesium, phosphorus, LDL-C, and albumin were lower in premature neonates. The levels of potassium, calcium, HDL-C, ALP, CK, and iron were slightly elevated in premature neonates, and there was a statistically significant increase in GGT levels among premature neonates compared to mature term neonates [Table/Fig-2].

In the present study, the comparison of biochemical parameters showed that there was no significant difference found between cord blood and venous blood among LBW and preterm neonates (group 1), except for magnesium levels, which were significantly higher in venous blood. However, there was no significant difference found between cord blood and venous blood parameters in group 2 [Table/Fig-3].

When correlating the biochemical parameters between cord blood and venous blood samples among premature and mature term neonates, parameters like sodium, HDL-C, LDL-C, and GGT showed a negative correlation in premature neonates, and sodium, calcium, and GGT showed a negative correlation in mature term neonates. Other analysed biochemical parameters showed a positive correlation between cord blood and venous blood samples [Tables/Fig-4,5].

DISCUSSION

The ALP, creatinine, and potassium in the current study did not show any difference between the groups. When comparing cord blood samples between the groups, sodium was found to be significantly reduced in concentration among premature neonates. The concentrations of other electrolytes in the serum did not differ significantly in a previous study [27]. Hyponatraemia and hypocalcaemia occurred in an average of 30.9% and 28.4% of infants with perinatal asphyxia in the first 72 hours of life, as reported in a study [28]. Creatinine values did not vary between the groups in the current study. However, a previous study found

Parameters	Cord blood		p-value	Venous blood		p-value
	Group 1 (n=53)	Group 2 (n=53)		Group 1 (n=53)	Group 2 (n=53)	
Creatinine (mg/dL)	0.57±0.15	0.6±0.1	0.09	0.8±0.2	0.9±0.2	0.9
Sodium (mmol/L)	115.4±16.3	123.3±8.7	0.002*	119.5±17.7	123.0±18.1	0.05
Potassium (mmol/L)	19.3±8.4	19.4±8.4	0.9	22.7±12.9	20±13.8	0.3
Calcium (mg/dL)	9.9±0.9	9.8±1.8	0.7	9.5±0.8	9.3±1.1	0.2
Magnesium (mg/dL)	2.6±0.7	2.7±0.7	0.4	2.9±0.6	3.3±2.7	0.2

Phosphorus (mg/dL)	8.9±4.4	12.3±7.4	0.005*	8.4±3	9.2±4.3	0.2
HDL-C (mg/dL)	28.5±8.4	27.8±5.8	0.6	31.2±10.2	28.6±7.2	0.1
LDL-C (mg/dL)	47.8±22.6	39.4±12.3	0.02*	77.1±25	80.1±28.2	0.5
Albumin (g/dL)	3.5±0.5	3.7±0.4	0.002*	4.0±0.5	4.1±0.9	0.3
ALP (IU/L)	185±127.8	148.6±56.2	0.06	169.2±50.2	155.1±52.8	0.1
GGT (IU/L)	162.3±106.2	159.5±84.2	0.8	180±141	132.7±80.9	0.03*
Iron (µg/dL)	152.5±48.4	144.7±47.9	0.4	107.6±51.1	90.6±44.5	0.07
CK (IU/L)	425.4±491.5	442.4±237.5	0.8	410±291.8	393.8±270.4	0.7

[Table/Fig-2]: Comparison of cord blood in group 1 vs group 2 and venous blood in group 1 versus group 2.

Parameters	Group 1		p-value	Group 2		p-value
	Cord blood	Venous blood		Cord blood	Venous blood	
Creatinine (mg/dL)	0.57±0.15	0.8±0.2	0.5	0.6±0.1	0.8±0.2	0.8
Sodium (mmol/L)	115.4±16.3	119.5±17.7	0.6	123.3±8.7	123.0±18.1	0.8
Potassium (mmol/L)	19.3±8.4	22.7±12.9	0.9	19.4±8.4	20±13.8	0.4
Calcium (mg/dL)	9.9±0.9	9.5±0.8	0.3	9.8±1.8	9.3±1.1	0.2
Magnesium (mg/dL)	2.6±0.7	2.9±0.6	0.04*	2.7±0.7	3.3±2.7	0.8
Phosphorus (mg/dL)	8.9±4.4	8.4±3	0.7	12.3±7.4	9.2±4.3	0.9
HDL-C (mg/dL)	28.5±8.4	31.2±10.2	0.2	27.8±5.8	28.6±7.2	0.5
LDL-C (mg/dL)	47.8±22.6	77.1±25	0.1	39.4±12.3	80.1±28.2	0.7
Albumin (g/dL)	3.5±0.5	4.0±0.5	0.4	3.7±0.4	4.1±0.9	0.7
ALP (IU/L)	185±127.8	169.2±50.2	0.8	148.6±56.2	155.1±52.8	0.7
GGT (IU/L)	162.3±106.2	180±141	0.2	159.5±84.2	132.7±80.9	0.5
Iron (µg/dL)	152.5±48.4	107.6±51.1	0.9	144.7±47.9	90.6±44.5	0.6
CK (IU/L)	425.4±491.5	410±291.8	0.8	442.4±237.5	393.8±270.4	0.2

[Table/Fig-3]: Comparison of cord blood vs venous blood in group 1 and cord blood vs venous blood in group 2.

Parameters	r-value	p-value
Creatinine (mg/dL)	0.07	0.5
Sodium (mmol/L)	-0.07	0.6
Potassium (mmol/L)	0.01	0.9
Calcium (mg/dL)	0.1	0.3
Magnesium (mg/dL)	0.2	0.04*
Phosphorus (mg/dL)	0.03	0.7
HDL-C (mg/dL)	-0.1	0.2
LDL-C (mg/dL)	-0.2	0.1
Albumin (g/dL)	0.1	0.4
ALP (IU/L)	0.03	0.8
GGT (IU/L)	-0.1	0.2
Iron (µg/dL)	0.008	0.9
CK (IU/L)	0.01	0.8

[Table/Fig-4]: Correlation between cord and venous samples among group 1 (Premature and low birth weight neonates).
*p<0.05 is statistically significant

Parameters	r-value	p-values
Creatinine (mg/dL)	0.02	0.8
Sodium (mmol/L)	-0.2	0.8
Potassium (mmol/L)	0.1	0.4
Calcium (mg/dL)	-0.1	0.2
Magnesium (mg/dL)	0.02	0.8
Phosphorus (mg/dL)	0.2	0.9
HDL-C (mg/dL)	0.08	0.5
LDL-C (mg/dL)	0.03	0.7
Albumin (g/dL)	0.04	0.7
ALP (IU/L)	0.05	0.7
GGT (IU/L)	-0.08	0.5
Iron (µg/dL)	0.06	0.6
CK (IU/L)	-0.1	0.2

[Table/Fig-5]: Correlation between cord and venous samples among group 2 (full term and normal birth weight neonates).

that creatinine concentration was significantly increased among premature neonates compared to term infants [29]. According to a previous study, premature and near-term newborns have higher LDL cholesterol levels than term neonates [30].

This finding was in concordance with the present study, which showed increased LDL-C levels among group 1 (47.8±22.6 mg/dL) compared to group 2 (39.4±12.3 mg/dL), with a p-value of 0.02. A previous study showed that premature newborns had a significant decrease in HDL levels compared to term and near-term neonates, while term and near-term neonates did not show any significance [31].

In a study by Kumar AJ et al., it was found that LDL-C and HDL-C levels were higher in preterm newborns compared to term neonates, and this difference was statistically significant (p-value=0.001). However, there was no statistically significant difference in HDL levels [32]. LBW newborns had higher levels of HDL and LDL, although these differences were not statistically significant. Therefore, there was a tendency towards a poorer lipid profile in Indian preterm and near-term infants [33]. In contrast to the above studies, HDL-C did not show any difference between the groups in the current study.

Previous studies indicated that preterm newborns had significantly higher serum levels of ALP, while the levels of total protein were not significantly different [34]. In present study, albumin was found to be decreased among preterm and LBW neonates (3.5±0.5 mg/dL) compared to term and normal weight neonates (3.7±0.4 mg/dL), with a significance of p-value=0.002. Preterm newborns were also associated with increased ALP activity and decreased calcium and phosphorus levels. Serum sodium and potassium were also found to be altered [35]. In the present study, among the liver function parameters, apart from albumin, GGT was found to

be increased among group 1 (180±141 mg/dL) compared to group 2 (132.7±80.9 mg/dL), which was statistically significant (p-value=0.03).

Serum calcium is considered an undependable parameter because neonates can preserve normal calcium levels despite bone calcium deprivation. Additionally, calcium concentrations can be influenced by other conditions like hypophosphatemia [36]. Hypophosphatemia is an early marker of impaired mineral metabolism and occurs between 7 and 14 days of age [37]. A serum phosphate concentration less than 5.6 mg/dL was associated with a mean gestational age of 30.3 weeks and a mean birth weight of 1490 grams, as reported in a study [38]. In the current study, phosphorus concentration was lower among group 1 (8.9±4.4 mg/dL) compared to group 2 (12.3±7.4 mg/dL), with a significance of p-value=0.005.

Over 30% of late preterm newborns at six weeks of age had iron deficiency anaemia, according to a research study [39]. However, iron levels did not vary significantly between the study groups in the present study. CK levels were found to increase significantly with gestational age, as reported in a study [40].

When correlating the biochemical parameters between cord blood and venous blood samples among premature infants, parameters like sodium, HDL-C, LDL-C, and GGT showed a negative correlation, while other analysed biochemical parameters were positively correlated with venous blood.

Limitation(s)

The mode of delivery (normal or lower segment casearean section) was not taken into account during sample collection.

CONCLUSION(S)

The study concludes that, when comparing the analysed biochemical parameters between preterm and full-term neonates, there were significant differences in cord blood sodium, phosphorus, LDL-C, and albumin. In venous blood samples, only GGT showed a statistically significant difference between the groups. It was observed that most analysed biochemical parameters were positively correlated, except for sodium, HDL-C, LDL-C, and GGT. Hence, cord blood samples collected non invasively can be used for biochemical analyses in neonates.

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Authors contribution: YY-sample collection and coordination between the Neonatal Intensive Care Unit (NICU), labour ward, and Clinical Laboratory of the Department of Biochemistry. MV-Initiated the study, guided the student, and served as a scientific advisor. GPB-collected data and helped define the study population. VMV-critically reviewed the study proposal and served as a scientific advisor.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Jun 16, 2023
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