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A Study of Cord Blood Lipid Profile in Preterm and Term Neonates

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Abstract
Background: According to foetal origin hypothesis, atherosclerosis originates during foetal period. Studies have shown an enhanced rate of coronary heart disease among men and women whose birth weights were at a lower end of normal range.
Objectives: 1) to compare lipid profile of umbilical cord blood of preterm and term neonates. 2) To compare lipid profile between male and female preterm neonates. 3) To determine Atherogenic Index of Plasma (AIP) and compare the obtained values between preterm and term neonates.
Methodology: A case-control study was conducted in OBG Department, Cheluvamba Hospital, from June 2014 to July 2014. All the preterm deliveries reported during this period were included in the study. Gender matched term babies were selected as controls. The proforma included socio-demographic profile, obstetric history and cord blood lipid profile among preterm and term neonates.
Results: The mean gestational age in term and preterm neonates was 39.16 ± 1.01 weeks and 33.13 ± 3.28 weeks and birth weight was 3.13 ± 0.45 kg and 2.04 ± 0.55 kg respectively. Among term and preterm neonates the mean value of serum cholesterol, serum LDL and and Atherogenic Index were more among preterm neonates except serum Triglycerides, serum HDL, serum VLDL and the difference was not statistically significant. Among male and female preterm neonates the mean value of serum cholesterol, serum triglyceride, serum HDL, serum LDL and serum VLDL were more among female preterm neonates except Atherogenic Index and the difference was not statistically significant except HDL.
Conclusion: Altered cord blood lipid profile values were found in preterm neonates in comparison with term neonates except HDL. Atherogenic Index of Plasma was more in preterm neonates in comparison with term neonates.

Keywords: Term and Preterm infants, Lipid Profile, Atherogenic Index.

1. Introduction
Cardiovascular diseases (CVDs) are the largest single contributor to global mortality and will continue to dominate mortality trends in the future [1]. It is a worldwide disease with 7.2 million deaths and 12.2% of total death. Coronary artery disease (CAD) is assuming a serious dimension in developing countries. It is expected to be the single most important cause of death in India by the year 2015. There is a considerable increase in the prevalence of CVD in urban area in India in last decade [2]. Atherosclerosis is considered as a major cause of CVDs; it is a process that begins in early life and progresses silently for decades. Recent studies have shown that, low birth weight and preterm births are linked to a higher prevalence of CVDs [3].
In adults, increased low density lipoprotein cholesterol (LDL-C) and reduced high density lipoprotein cholesterol (HDL-C) levels are associated with atherosclerotic lesion, with its prodromal stages formed early in life. Children with high level LDL-C at birth might be more liable to high lipoprotein serum levels as they reach adulthood [4].
According to foetal origin hypothesis, atherosclerosis originates during foetal period. Studies have shown an enhanced rate of coronary heart disease among men and women whose birth weights were at a lower end of normal range. Barker *et al.* demonstrated that low birth weight is associated with preterm births and correlated with increased prevalence of CVDs. Recent experiments in animals and human studies have shown that the risk factors for CVD are the influence of the intrauterine environmental like poor nutrition that affects

Development during a critical period of life, may permanently change structure and physiology of organ and tissue [5]. The arterial hypertension of adults causes changes in plasma levels of fibrinogen, dyslipidemia, impaired glucose tolerance or type 2 diabetes which start during foetal life, thus the overall nature of the progress of the atherosclerosis is age dependent, which begins in childhood and progresses with advancing age [6, 7].

A North American study has shown that, every child over the age of 3 years old has some degree of aortic fatty streaks [8].The cord blood cholesterol level in infants is lower than the adults [10]. Small for gestational age (SGA) infants have higher levels of triglyceride, rich VLDL and intermediate LDL, in comparison with the AGA (appropriate for gestational age) infants. These findings suggest a link between higher triglyceride, rich VLDL subclasses in SGA infants and future coronary artery disease [9]. Determination of various lipid fractions in the paediatric age group has increased awareness about the origins of the atherosclerosis in early life [10].With this background, an attempt is made to compare the cord blood lipid profile in preterm and term neonates.

2. Objectives

1. To compare lipid profile of umbilical cord blood of preterm and term neonates.
2. To compare lipid profile between male and female preterm neonates.
3. To determine Atherogenic Index of Plasma (AIP) and compare the obtained values between preterm and term neonates.

3. Material and Methods Study Setting

A case-control study was conducted in Obstetrics and Gynaecology Department, Cheluvamba Hospital, which is a part of Mysore Medical College and Research Institute, Mysore, Karnataka, from June 2014 to July 2014. All the preterm deliveries reported during this period were included in the study. Gender matched term babies were selected as controls. Non probability convenient sampling technique was used to obtain the samples. Written consent from the mother was taken before conducting the study.

Method of data collection

A pretested semi-structured questionnaire was used to collect the data. The proforma included socio-demographic profile, obstetric history and cord blood lipid profile among preterm and term neonates.

Inclusion criteria:

1. All newborns who are delivered in Cheluvamba Hospital includes both booked and unbooked cases.
2. All newborns with gestational age less than 37 weeks were included under preterm and all others as term neonates.

Exclusion criteria:

Neonates with

1. Congenital malformations
2. Neonates borne to mother with maternal illness like Diabetes mellitus (DM) including Insulin dependent diabetes mellitus (IDDM) & gestational diabetes, Tuberculosis, Asthma, Pregnancy induced hypertension.
3. Family history of coronary heart disease / hypercholesterolemia
4. Any maternal medication, except iron & vitamin supplements
5. Neonates with perinatal problems like Hypoglycaemia, pathological jaundice
6. Drug abuse in mother and antenatal medications
7. Instrumental delivery including extraction.

Specimen collection and Analysis

After delivery of the placenta and immediately after cord clamping, 5 ml of umbilical venous blood was obtained from the placental end of the umbilical cord under aseptic precautions. It was then allowed to stand for few minutes. Serum was separated from the clotted blood after centrifuging at 3000 rpm for 30 minutes. From the serum, total cholesterol (TC), total triglyceride (Tg), high density lipoprotein (HDL-C), low density lipoprotein (LDL-C) and very low density lipoprotein (VLDL-C) were calculated as follows:

1. Measurement of Serum Cholesterol: It was measured by using Cholesterol DES (Dynamic Extended Stability), CHOD – PAP Method.

Principle: Cholesterol esterase hydrolyses cholesterol esters in the specimen into free cholesterol and fatty acid. In the second reaction, cholesterol oxidase converts cholesterol to cholest-4-en-3-one and hydrogen peroxide. In presence of peroxidase, hydrogen peroxide oxidatively couples with 4-aminoantipyrine and phenol to produce red quinine imine dye which has absorbance maximum at 510 nm (505-530 nm). The intensity of red colour is proportional to the total cholesterol in the specimen [11].

2. Measurement of HDL Cholesterol: It was measured by using HDL – Cholesterol DES (Dynamic Extended Stability) method.

Principle: Phosphotungstate Mg^{2+} precipitates chylomicrons, low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) fractions. After centrifugation, high Density Lipoprotein (HDL) fraction remains unaffected in supernatant. Cholesterol content of HDL fraction is assayed using ready to use reagent supplied with cholesterol kit (11).

1. Measurement of Serum Triglyceride: It was measured by using TG – DES (Dynamic Extended Stability) method.

Principle: Glycerol released from hydrolysis of serum triglycerides by lipoprotein lipase of the kit is converted by glycerol kinase to glycerol 3-phosphate, which is oxidized by glycerol phosphate oxidase to dihydroxy acetone phosphate and H_2O_2. In presence of peroxidase, H_2O_2 oxidizes phenolic chromogen to a red coloured compound. The intensity of the colour was measured on autoanalyzer (11).

1. Measurement of LDL Chlesterol: It was measured by direct method using a commercially available kit.

1. Estimation of VLDL Cholesterol:

VLDL Cholesterol is calculated using Friedewaldformula [12]

VLDL Cholesterol in mg% = Serum Triglyceride / 5

1. Atherogenic Index of Plasma will be calculated from the obtained HDL-C and TGS values.

Calculation of AIP (Atherogenic Index of Plasma)

Atherogenic index of plasma (AIP) is a logarithmically transformed ratio of molar concentrations of triglycerides to HDL-cholesterol. Atherogenic index of plasma (AIP) is calculated as log (TG/HDL-C).

Statistical Tools

The data was entered in Excel and analysed using EPi-Info Software. Descriptive statistics like mean and standard deviation was applied. Student t test were done to find the correlation between term and preterm neonates and male & females preterm neonates, p- value ≤ 0.05 at 95% confidence interval was considered as significant.

Institutional ethical clearance certificate was obtained before starting the study.

5) Results

This study was carried out at Cheluvamba Hospital which is a part of Mysore Medical College and Research Institute (MMC & RI), Mysore, Karnataka. The salient feature of the present study was the comparison of the cord blood lipid profile and the Atherogenic Index values among preterm and term neonates.

Table 1: Gender Distribution among Preterm & Term Neonates.

Gestation	Term Neonates		Preterm Neonates	
Gender	Male	Female	Male	Female
Frequency	18	12	18	12
Percentage (%)	60	40	60	40

The groups of term and preterm neonates each (n =30) contained 18 males (60%) and 12 females (40%). Thus the present study shows male predominance in preterm as well as term births.

Table 2: Gestational Age and Birth Weight among Preterm and Term Neonates

	Term		Preterm		P value
	Mean	SD	Mean	SD	
Gestational Age(Wks)	39.6	1.01	33.13	0.45	<0.05
Birth Weight	3.13	3.28	2.04	0.55	<0.05

The mean gestational age of distribution in term and preterm neonates was found to be 39.16 ± 1.01 weeks and 33.13 ± 3.28 weeks respectively. The mean value of birth weight in term and preterm neonates was found to be 3.13 ± 0.45 kg and 2.04 ± 0.55 kg respectively.

Table 3: Cord Blood Lipid Profile among Preterm and Term Neonates.

	Term		Preterm		p value
	Mean	SD	Mean	SD	
Total Cholesterol	64.76	26.66	70.4	34.98	0.48
Triglycerides	47.73	16.64	46.06	25.4	0.76
HDL	27.26	8.51	25.46	9.35	0.43
LDL	29.6	12.04	30.96	11.2	0.65
VLDL	9.56	3.32	9.21	5.08	0.75

The mean value of serum Total Cholesterol in term and preterm neonates was found to be 64.76 ± 26.55 and 70.40 ± 34.98 respectively. A higher value of total cholesterol was observed in preterm neonates as compared to term neonates and the difference was not statistically significant.

The mean value of serum Triglycerides in term and preterm neonates was found to be 47.73 ± 16.64 and 46.06 ± 25.40 respectively. A lower value of serum triglyceride was observed in preterm neonates as compared to term neonates, however the difference was not statistically significant.

The mean value of serum high density lipoprotein cholesterol (HDL) in term and preterm neonates was found to be 27.26 ± 8.51 and 25.46 ± 9.35 respectively. A lower value of serum high density lipoprotein cholesterol was observed in preterm neonates as compared to term neonates but, the difference was not statistically significant.

The mean value of serum low density lipoprotein cholesterol (LDL) in term and preterm neonates was found to be 29.60 ± 12.04and 30.96 ± 11.20 respectively. A higher value of low density lipoprotein cholesterol was observed in preterm neonates as compared to term neonates. Even here, the difference was not statistically significant.

The mean value of serum very low density lipoprotein cholesterol (VLDL) in term and preterm neonates was found to be 9.56 ± 3.32 and 9.21 ± 5.08 respectively. A lower value of VLDL Cholesterol was observed in preterm neonates as compared to term neonates and the difference was not statistically significant.

Table 4: Atherogenic Index of Plasma in Preterm and Term Neonates.

	Term		Preterm		P value
	Mean	SD	Mean	SD	
AIP	0.232	0.19	0.238	0.34	0.93

The mean value of Atherogenic Index of Plasma (AIP) in term and preterm neonates was found to be 0.232 ± 0.19 and 0.238 ± 0.34 respectively. A higher value of AIP was observed in preterm neonates as compared to term neonates and the difference was not statistically significant.

Table 5: Comparison of Gestational Age, Birth Weight Lipid Profile and Aip between Preterm Male and Female Neonates.

	Males		Females		P value
	Mean	SD	Mean	SD	
Gestational Age	32.39	3.94	34.25	1.48	0.13
Birth weight	1.91	0.6	2.25	0.43	0.11
Total Cholesterol	67.72	37.25	74.42	32.47	0.61
Triglycerides	44.22	23.93	48.83	28.32	0.63
LDL	28.61	10.57	34.5	11.64	0.13
HDL	22.39	8.59	30.08	8.82	0.02
VLDL	8.84	4.78	9.77	5.66	0.63
AIP	0.267	0.38	0.19	0.29	0.59

The mean value of gestational age of male and female preterm neonates was found to be 32.39±3.94 and 34.25±1.48 respectively.

The mean value of birth weight of male and female preterm neonates was found to be 1.91±0.6 and 2.25±0.43 respectively.

The mean value of serum total cholesterol of male and female preterm neonates was found to be 67.72±37.25 and 74.42±32.47 respectively. Higher values of serum total cholesterol observed in preterm female as compared to preterm male neonates and the difference was not statistically significant.

The mean value of serum triglyceride of male and female preterm neonates was found to be 44.22±23.93 and 48.83±28.32 respectively. Higher values of serum triglycerides observed in preterm female as compared to

preterm male neonates and the difference was not statistically significant.

The mean value of serum high density lipoprotein (HDL) of male and female preterm neonates was found to be 22.39±8.59 and 30.08±8.82 respectively. A significantly higher values of HDL Cholesterol observed in preterm female as compared to preterm male neonates and the difference was found to be statistically significant.

The mean value of serum low density lipoprotein (LDL) of male and female preterm neonates was found to be 28.61±10.57 and 34.5±11.64 respectively. Higher values of LDL Cholesterol observed in preterm female as compared to preterm male neonates and the difference was not statistically significant.

The mean value of serum very low density lipoprotein (VLDL) of male and female preterm neonates was found to be 8.44±4.78 and 9.77±5.66 respectively. Higher values of VLDL Cholesterol observed in preterm female as compared to preterm male neonates and the difference was not statistically significant.

The mean value of Atherogenic Index of Plasma (AIP) of male and female preterm neonates was found to be 0.26±0.38 and 0.19±0.29 respectively. Slightly lower value of AIP observed in preterm female as compared to preterm male neonates and the difference was not statistically significant.

Gestational age, Birth weight and Lipid profile were found to be higher in female preterm than male preterm neonates. Of these the difference in the HDL values among male and female preterm neonates shows statistical significance (p – value = 0.02). The AIP value is found to be higher in male preterm neonates than in female preterm neonates.

6) Discussion

Numerous studies have been conducted comparing the cord blood lipid profile in preterm and term neonates. However, very few studies have been done addressing the AIP values in preterm and term neonates. Most of the studies done till date have shown that high cholesterol levels is more likely to be observed in preterm and SGA infants. Early prevention and close follow up would help to prevent the complications of atherosclerosis in these patients. Young age can be viewed as an opportunity to begin preventive interventions to change risk factors for cardiovascular diseases. Increasing awareness about origin of atherosclerosis in early life has renewed interest in determination of various lipid profile in cord blood.

Gestational Age and Birth Weight

The study shows that gestational age and birth weight in preterm neonates were lower than those in term neonates. This difference is found to be statistically significant. Kumar *et al.* [13]. Showed that TG levels are higher in low birth weight new borns and concluded that cholesterol levels were not affected by birth weight. Koklu *et al.* [14]. Showed that TG, TC, LDL and VLDL levels in macrosomic neonates are clearly higher than those of normal birth weight neonates. Donega *et al.* [15]. Showed that the levels of TC, LDL and HDL in preterm newborns are higher than those in full term newborns, but TG levels in preterm newborns are lower than those in term newborns. They also found that birth weight was not related to umbilical cord lipid levels. Ajay Kumar *et al.* (13).in their study demonstrated that cord blood cholesterol levels are not influenced by birth weight and gestation, but levels of cord blood triglyceride and free fatty acids are affected by birth weight and gestation. In this study, most of the lipid parameters (TC, TG, LDL and VLDL) were found to be high whereas HDL levels were found to be low in preterm neonates compared to term neonates.

Serum Total Cholesterol

The results demonstrated that the serum TC levels in the preterm neonates were higher than term neonates though it was not statistically significant, which is in agreement with Diaz M *et al*. (2004) [16]. Similarly, the finding also mimics with Ginsburg BE *et al.* [17]. and the results obtained from the study by Avinash N. Jadhao, Arun K. Tadas and Swati A. Tadas (2014) (11). They found that the TC concentration in cord blood was higher in preterm infants than term infants.

From this study, it was evident that the mean value of serum TC was higher in preterm than term neonates. It has been reported that the plasma depletion of cholesterol that occurs at term is due to a decrease in HDL-C and LDL-C levels. LDL-C plasma levels are low in term neonates, most likely due to its rapid uptake and metabolism by the foetal adrenal as precursor or substrate for steroid hormone biosynthesis, postulated by Parker Jr *et al.* [18]. They found that TC and LDL-C levels in foetal plasma declined progressively from 33 to 42 weeks of gestation. At 41 to 42 weeks of gestation, the foetal plasma concentrations of TC and LDL-C were significantly lower than those at 33 to 34 weeks of gestation. It has also been demonstrated that Lecithin- Cholesterol Acyl Transferase (LCAT) activity was lower in preterm neonates than term neonates. So, fall in HDL-C may be associated with an increase in the LCAT activity during intrauterine life of foetus.

The lack of statistical significance may be explained due to the small sample size of this study.

Serum LDL – Cholesterol

It is observed that LDL-C in preterm neonates was higher than those in term neonates, though it was not statistically significant. This finding of the study is in agreement with previous reports. As we have already discussed, the cause of the fall in plasma LDL-C concentration is explained by the increase of its uptake by the foetal adrenal gland for steroid hormone production during foetal development as postulated by Parker Jr *et al* (18).

Serum HDL – Cholesterol

It was found that HDL – C in preterm neonates was lower than those of term neonates. The difference was not statistically significant. Spear *et al*. demonstrated that LCAT activity was lower in preterm neonates than the term neonates. This study results were in accordance with those conducted by Parker CR *et al*. (1983) (18), Pardo IMCG *et al*. (2005) [19] and Avinash N. Jadhao, Arun K. Tadas and Swati A. Tadas (2014) (11).

Serum Triglyceride

In this study, triglyceride level was higher in term neonates as compared to preterm neonates. The difference was not statistically significant. Similarly, this study finding is in agreement with those of Donega S *et al.* (15). In their study of new borns of both term and preterm groups. Serum triglyceride values were lower in preterm newborns than in term new borns.

Badiee *et al* [20]. Has reported very high level of cord blood triglyceride in full term Iranian newborn infant compared to

other countries. Vaziri EsfarjaniSh *et al.* [21]. reported that the mean cord blood triglyceride level was meaningfully more than its level in the Nelson textbook of paediatrics. The findings of this study is also similar to the results of Avinash N. Jadhao, Arun K. Tadas and Swati A. Tadas (2014) [11]. The exact reason for this high value of cord blood triglyceride in term neonates compared to preterm neonate has not been elucidated.

Serum VLDL - Cholesterol

It is observed that VLDL – C is higher in term neonates as compared to preterm neonates. The difference was not statistically significant. This finding is in accordance with the study conducted by Yonezawa *et al*. (2009) [22]. They studied that the TG distribution in preterm neonate cord blood and the relationship of VLDL – TG levels with respiratory distress syndrome (RDS). Term neonates had low cord blood TG concentrations distributed equally to the LDL and VLDL fractions. However, preterm neonates had even lower TG concentrations, with VLDL as the dominant carrier. Cord blood VLDL – TG concentrations increased dramatically from 32 to 34 weeks of gestational age. After extensive search the overall pattern of change in TG and VLDL – C is not clearly outlined.

Atherogenic Index of Plasma (AIP)

In this study, the value of Atherogenic Index of Plasma (AIP) in preterm neonates is found to be a little more that in term neonates. But this different is not statistically significant, probably due to the small sample size in the study. This study mimics the study conducted by Pardo *et al.* [19]. Which showed that, the AIP did not differ between genders, but preterm newborns had higher levels than term newborns. In addition, no significant differences were observed in the AIP values between males and females.

The lipid profile in preterm male and female infants was not significantly different from each other, however, the mean levels of all lipid indicators for preterm female neonates were higher than those for male. Among this, the difference in the mean HDL-C values was found to be statistically significant. These findings is in contrast with the study conducted by Kazemi SAN *et al* [23)]. (2010) who found that, all lipid levels were not significantly different between genders.

When breastfeeding starts, a sharp increase in serum levels of lipid profile during the first week of life up to six months of age is reported by various studies. Mean values of a TC rise to 150 mg/dl from 70 mg/dl and those of LDL-C and TG to 100 mg/dl and 58 mg/dl from 30 and 32 mg/dl respectively [24]. After the first year of life, these values rise slowly and around the second year of life, come close to those observed in adolescents and adults. For this reason, newborns have a peculiar lipid profile when compared to neonates, children or adolescents.

7) Conclusion

Altered cord blood lipid profile values were found in preterm neonates in comparison with term neonates. A pro-atherogenic trend to lipid profile in Indian preterm infants is clearly visible. It may be interesting to see whether these susceptible neonates are at increased risk of developing cardiovascular diseases in future. So further prospective and follow up studies involving a larger study group are required. Further studies should also aim at finding a correlation between neonatal birth weight and cord blood lipid profile.

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