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Research Article

Assay of neonatal cord blood lipid levels and its correlation with neonatal gestational age, gender and birth weight: a single center experience

Yashaswini Kenchappa[1]*, Narendra Behera[2]

[1]Department of Pediatrics, Sri Balaji Medical College and Hospital, Chrompet, Chennai, tamil nadu, India
[2]Department of Pediatrics, MKCG Medical College, Berhampur, Odisha, India

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***Correspondence:**
Dr. Yashaswini K,
E-mail: yashbmc@gmail.com

ABSTRACT

Background: It is a well-documented fact that atherosclerosis can originate in the fetal period itself. There is evidence that children with cholesterol levels in upper quartiles are at increased risk of subsequent atherosclerosis and numerous antenatal and perinatal factors may influence this atherogenesis. The current study tried to determine the levels of total cholesterol, triglycerides, LDL cholesterol, VLDL and HDL cholesterol in cord blood and correlate levels of these lipoproteins with various factors including fetal gender, gestational age, birth weight and gestational maturity and see whether these factors influence lipid profile in neonates.
Methods: Cord blood lipid profile including assessment of total cholesterol, triglycerides, LDL cholesterol, VLDL and HDL cholesterol was done involving 90 neonates.
Results: The low birth weight group and preterm neonates had higher and statistically significant levels of total cholesterol, triglycerides and LDL. They also had higher levels of HDL and VLDL, but the levels of these lipids were not statistically significant. No significant difference in lipid profile was noted based on gender and gestational maturity.
Conclusions: Our study results reaffirm the relation between cord blood lipid profile and perinatal factors. With the significantly unhealthy lipid profile among the preterm and low birth weight babies we might safely say that the preterm and low birth weight babies are exposed to a more hypercholesterolemic and potentially more atherogenic environment than their term counterparts and early lifestyle modifications may be required to prevent their progression to coronary artery disease.

Keywords: Cord blood, Lipid profile, Hypercholesterolemia, Atherosclerosis, Barker's hypothesis

INTRODUCTION

Atherosclerosis is a major risk factor for coronary artery disease and consequent morbidity and mortality in adult life. The process of atherosclerosis begins early in life and progresses silently for decades.[1] It is a well-documented fact that atherosclerosis can originate in the fetal period itself.[1] The association of low birth weight and adult cardiovascular disease led to the 'fetal origins hypothesis' formulated by Barker, which asserts that the fetus adapts to any in utero stress by cell programming at a critical period in development, eventually manifesting as a systemic organ dysfunction.[2] The fetal programming and the 'fetal origins hypothesis' emphasize the profound and sustained impact of factors related to the fetal health including atherosclerosis on the process of chronic diseases in adulthood.[2]

There is evidence that children with cholesterol levels in upper quartiles are at increased risk of subsequent atherosclerosis.[3-6] Therefore, efforts are being made for screening of hyperlipidemia in the neonatal period as cholesterol levels in the subsequent periods are likely to be influenced by other factors: constitutional and environmental. Factors which influence in-utero atherogenesis include fetal gender, gestational age, birth weight, perinatal stress, and maternal hypertension and diabetes.[3-6] Cord sera have been demonstrated to contain all well characterized adult lipoproteins and apolipoproteins. Hence determination of cord blood lipids becomes a useful investigation in detection of high risk babies who may have altered levels of lipids and lipoproteins compared to that found in healthy neonates.[3,5-7]

Given the understanding that fetal lipid profile will show deranged results either due to genetic programming or due to prepartum and/or intrapartum stress, and that this deranged lipid profile can continue into adult life, it is wise to recognize such children at risk in the antenatal and postnatal period itself and give special attention to them in terms of life style modification to prevent development of future complications; particularly cardiovascular complications. Such screening can be done through determination of cord blood lipid profile. The current study tried to determine the levels of total cholesterol, triglycerides, LDL cholesterol, VLDL and HDL cholesterol in cord blood and correlate levels of these lipoproteins with various factors including gestational age, birth weight and fetal gender and see whether these factors influence lipid profile in neonates.

METHODS

This was a prospective study performed between October 2011 and September 2013 at a secondary care center affiliated to a medical college and hospital at Berhampur, Odisha, India involving the department of pediatrics (special newborn care unit) in collaboration with department of obstetrics and gynecology and department of biochemistry. 90 pregnant women in total: booked antenatal cases expecting vaginal delivery were enrolled randomly in the study after obtaining informed and written consent. Maternal and newborns' details were noted in a predesigned proforma. After obtaining informed and written consent 10 ml of umbilical cord blood was collected into plain vials from the placental end within five minutes of delivery and assessed within four hours of collection. If the collection time to processing time exceeded four hours, samples were refrigerated and assessed within 12 hours of collection.

Exclusion criteria

Women with pre-existent medical illness/complications including diabetes mellitus, eclampsia, preeclampsia, thyroid disorders, cardiovascular diseases and HIV, women receiving glucocorticoid therapy for fetal lung maturation, women with family history of cardiovascular diseases; particularly coronary artery disease, women with instrumental delivery with forceps or vacuum and neonates with one minute APGAR score of less than 7 were excluded from the study as these factors can induce intrapartum or prepartum fetal stress and may impact the cord blood lipid levels. Neonatal groups were divided as in Table 1.[8]

Table 1: Neonatal groups based on various factors.

Based on gestational age	Based on birth weight	Based on gestational maturity
Term neonates: neonates born between 37 and 42 completed weeks of gestation	**Low birth weight (LBW) neonates**: neonates with birth weight less than 2.5 kgs	**Small for gestational age (SGA) neonates**: neonates with birth weight less than tenth percentile of growth for gestational age as defined in the WHO growth chart
Preterm neonates: Those neonates born before 37 completed weeks of gestation	**Adequate for gestational age neonates**: neonates with birth weight between 2.5 kg and 4 kg	**Appropriate for gestational age (AGA) neonates**: neonates with birth weight between tenth percentile and ninetieth percentile of growth for gestational age as defined in the WHO growth chart

Gestational age was confirmed by new ballard scoring system done within 24 hours of birth and was correlated with the age determined as per first trimester antenatal scan.[9]

Statistical analysis

Continuous data was computed as mean±standard deviation (SD). The Student's t-test was applied for comparison of mean values. The relationship of cord blood lipid profile with birth weight and gestational age was determined by regression analysis. Data was analyzed by ANOVA followed by Tukey's test and Bonferroni's multiple comparison test. The significance level was set at $p < 0.05$. Graphpad prism 5.0 software was used for analysis. Microsoft excel sheet was used for data storage and for tables and graphs.

RESULTS

Of the total 90 neonates in the study 46 (51.11 %) were females and 44 (48.88 %) were males. Table 2 depicts mean lipid values based on gender. As seen in Table 2, the total cholesterol, triglyceride, HDL and LDL levels were higher in male neonates, but were not statistically significant.

Table 2: Lipid parameters in cord blood for male and female neonates.

Lipids (mg/dl)	Males (n=44)	Females (n=46)	P-value
	Mean±SD		
TC	113.4±42.03	99.87±30.53	> 0.05
HDL	30.48±9.63	27.24±7.89	> 0.05
TG	115.4±52.36	111.8±41.46	> 0.05
VLDL	22.70±10.35	22.07±8.31	> 0.05
LDL	58.91±27.85	49.13±19.72	> 0.05

Of the total 90 neonates in the study 46 (51.11 %) were of birth weight more than 2.5 kg and 44 (48.88 %) were low birth weight neonates. Figure 1 depicts the significant difference in values of total cholesterol, LDL and triglycerides. HDL and VLDL levels were also higher in low birth weight neonates, but were not statistically significant. The significance value determined shows that the levels of total cholesterol, triglycerides and LDL were higher and statistically significant in the low birth weight group as shown in Table 3.

Of the total 90 neonates in the study 61 (67.8 %) were of term gestation and 29 (32.2 %) were preterm. The preterm group had 13 (14.44%) males and 16 (17.8%) females, whereas the term group had 30 (33.33%) females and 31 (34.44%) male neonates respectively as shown in Figure 2.

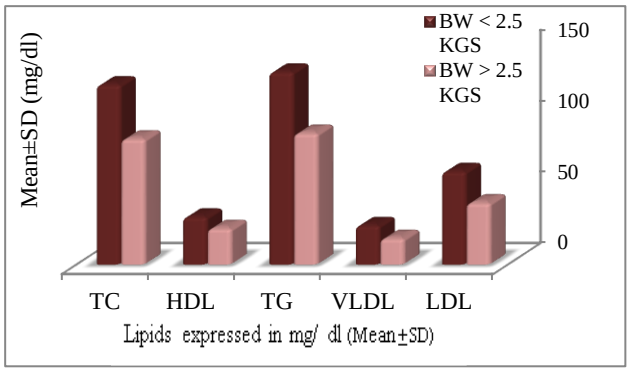

Figure 1: Lipid parameters in cord blood based on birth weight.

As shown in Table 4 statistically significant difference and higher values were noted in the preterm group for total cholesterol, triglycerides and LDL. Higher values of HDL and VLDL were noted in the preterm group in comparison to the term group, but were not statistically insignificant.

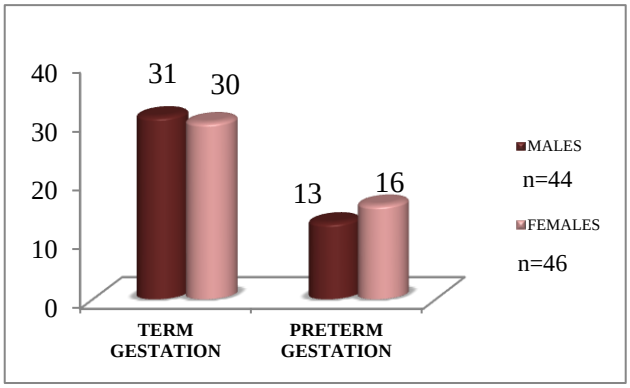

Figure 2: Gender wise breakup of neonates based on gestational age.

Table 3: Lipid parameters in cord blood based on birth weight.

Lipids (mg/dl)	BW ≤2.5 kg (n=44)	BW >2.5 kg (n=46)	P-value
	Mean±SD		
TC	126.0±38.72	87.74±23.34	<0.05 (significant)
HDL	32.89±10.08	24.93±5.23	>0.05
TG	135.5±53.47	92.54±26.24	<0.05 (significant)
VLDL	26.73±10.65	18.22±5.21	>0.05
LDL	65.05±25.09	43.26±18.42	<0.05 (significant)

Table 4: Lipid parameters in cord blood based on gestational age.

Lipids (mg/dl)	Term (n=61)	Preterm (n=29)	P-value
	Mean±SD		
TC	94.28±3.598	132.1±7.565	<0.05 (significant)
HDL	26.26±0.8525	34.21±1.961	>0.05
TG	101.3±4.488	139.4±10.72	<0.05 (significant)
VLDL	20.00±0.9011	27.38±2.129	>0.05
LDL	46.97±2.459	68.52±5.176	<0.05 (significant)

Of the total 90 neonates in the study 49 (54.5%) were small for gestational age (SGA) neonates and the rest 41 (45.5%) were adequate for gestational age (AGA) neonates. 18 (20%) of the total 90 neonates were preterm AGA, 23 (25.5%) were term AGA, 11 (12.25%) were preterm SGA and the rest 38 (42.25%) were term SGA. In the SGA group there were 7 (7.77%) females in the preterm group, 14 (15.44%) females in the term group,

4 (4.44%) males in the preterm group and 24 (26.66%) males in the term group. In the AGA group there were 9 (10%) females in the preterm group, 16 (17.77%) females in the term group, 9 males (10%) in the preterm group and 7 (7.77%) males in the term group as shown in Figure 3. The average birth weight in the AGA group was 2.99 kg in the term group and 2.22 kg in the preterm group, whereas the average birth weight in the SGA group was 2.47 kg in the term group and 1.69 kg in the preterm group. As seen in Table 5 comparison based on gestational maturity showed comparable and near equal lipid values in both the AGA and the SGA group suggesting no statistical difference of lipid values in the two groups.

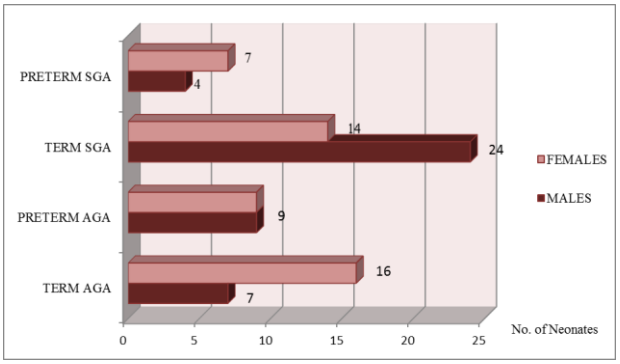

Figure 3: Gender based break up with reference to gestational maturity.

Table 5: Lipid parameters based on gestational maturity at birth.

Lipids (mg/dl)	AGA (n=41)	SGA (n=49)	P-value
	Mean±SD		
TC	106.9±32.19	106.1±40.96	>0.05
HDL	28.63±8.18	28.98±9.51	>0.05
TG	114.6±43.50	112.7±49.95	>0.05
VLDL	22.56±8.69	22.22 ±9.89	>0.05
LDL	54.32±23.62	53.57±25.28	>0.05

Subgroup analysis was done in the AGA and SGA groups based on gestational age. AGA neonates showed higher values of all lipid parameters in the preterm group, of which difference in total cholesterol, triglyceride and LDL values were statistically significant as shown in Table 6.

Similar to the AGA group SGA neonates when compared based on gestational age showed higher values of all lipid parameters in the preterm group, of which difference in total cholesterol, triglyceride and LDL values were statistically significant as seen in Table 7.

Further analysis was done comparing term SGA with term AGA neonates and preterm SGA with preterm AGA neonates to look for comparison based on gestational maturity. As seen in Table 8 similar and comparable lipid values were seen in both the SGA and AGA group with reference to term gestation. As shown in Table 9 no significant differences were noted even in the preterm group suggesting cord blood lipid profile in our study was not influenced by gestational maturity. Though all the lipid parameters showed higher values in the preterm group in both AGA and SGA neonates, statistically significant difference were not noted for any of these values.

Table 6: Lipid parameters in cord blood at birth among AGA neonates.

Lipids (mg/dl)	Term AGA (n=23)	Preterm AGA (n=18)	P-value
	Mean±SD		
TC	92.13±23.43	125.7±32.52	<0.05 (significant)
HDL	25.09±4.91	33.17±4.33	>0.05
TG	100.7±28.9	132.4±52.68	<0.05 (significant)
VLDL	19.78±5.92	25.11±10.43	>0.05
LDL	44.43±18.51	67.94±23.83	<0.05 (significant)

Table 7: Lipid parameters in cord blood at birth among SGA neonates.

Lipids (mg/dl)	Term SGA (n=38)	Preterm SGA (n=11)	P-value
	Mean±SD		
TC	95.58±30.82	142.5±51.54	<0.05 (significant)
HDL	26.97±7.492	35.91±12.61	> 0.05
TG	101.6±38.67	150.8±66.18	<0.05 (significant)
VLDL	20.13±7.712	29.45±13.25	> 0.05
LDL	48.50±19.69	71.09±34.62	<0.05 (significant)

Further analysis was done comparing term SGA with term AGA neonates and preterm SGA with preterm AGA neonates to look for comparison based on gestational maturity. As seen in Table 8 similar and comparable lipid values were seen in both the SGA and AGA group with reference to term gestation.

As shown in Table 9 no significant differences were noted even in the preterm group suggesting cord blood lipid profile in our study was not influenced by gestational maturity. Though all the lipid parameters showed higher values in the preterm group in both AGA and SGA neonates, statistically significant difference were not noted for any of these values.

Of the total 90 women involved in the study 22 (24.44%) were in the 20 to 23 year age group, 48 (53.33%) in the 24 to 27 year age group and the rest 20 (22.22%) in the

28 to 30 year age group. Break-up of individual age group is shown in Figure 4.

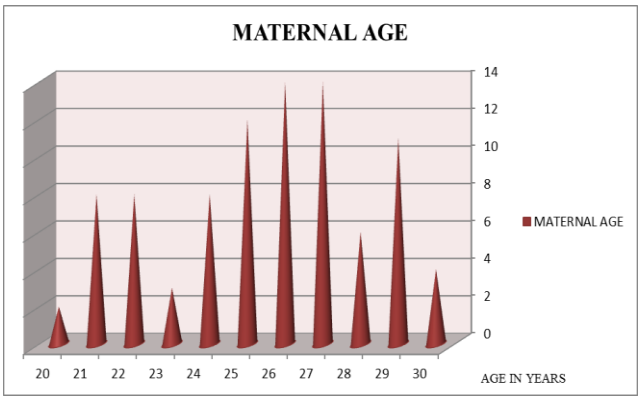

Figure 4: Number of women in each age group.

Table 8: Comparison of lipid fractions in term AGA and SGA neonates.

Lipids (mg/dl)	Term AGA (n=23)	Term SGA (n=38)	P-value
	Mean +/ - SD		
TC	92.13±23.43	95.58±30.82	>0.05
HDL	25.09±4.91	26.97±7.492	>0.05
TG	100.7±28.90	101.6±38.67	>0.05
VLDL	19.78±5.92	20.13±7.712	>0.05
LDL	44.43±18.51	48.50±19.69	>0.05

Table 9: Comparison of lipid fractions in preterm AGA and SGA neonates.

Lipids (mg/dl)	Preterm SGA (n=11)	Preterm AGA (n=18)	P-value
	Mean±SD		
TC	142.5±51.54	125.7±32.52	> 0.05
HDL	35.91±12.61	33.17±9.338	> 0.05
TG	150.8±66.18	132.4±52.68	> 0.05
VLDL	29.45±13.25	26.11±10.43	> 0.05
LDL	71.09±34.62	67.94±23.83	> 0.05

As shown in Table 10, no significant correlation was observed between maternal age and cord blood lipid profile, but inverse relationship was observed between advancing age and the levels of total cholesterol, triglycerides, HDL, VLDL and LDL.

Table 10: Correlation coefficients between lipid profile and maternal age.

Lipids (mg/dl)	r-value	p-value
TC	-0.072	0.531
HDL	0.012	0.497
TG	-0.076	0.841
VLDL	-0.668	0.912
LDL	-0.021	0.471

DISCUSSION

Atherogenesis as the leading cause of coronary artery disease and dyslipidemia as the foremost cause of atherosclerosis is an established fact.[1,10] Intrapartum changes and their bearing on the cord blood lipid profile and the persistence and progress of dyslipidemic changes of neonates into adult life leading to cardiovascular diseases has been a prominent topic of interest since the acceptance of Barker's fetal origins hypothesis.[2,11] Since significant number of studies have contrastingly elucidated the role of gestational age, gender and birth weight in altering the cord blood lipid profile, we set out to determine as to whether gestational age, gender and birth weight significantly impact the cord blood lipid profile.

Our study showed that there was no statistically significant difference in the levels of cord blood lipids in terms of gender, but higher values of total cholesterol, HDL, LDL and Triglycerides were noted in the male neonate. This was in unison with few other studies including Anderson et al and Tsang et al which showed no significant changes in the cord blood lipid profile in terms of gender.[12,13] But contrasting results were noted in studies by Hardell et al, Zhao et al, Simmi Kharb et al and Badiee Z et al who noted significantly higher total cholesterol and HDL levels in their study group.[14-17]

Higher and statistically significant values of total cholesterol, LDL and triglyceride levels were noted in our low birth weight (LBW) study group. Higher triglyceride values were found in the LBW group in the study by Kumar et al and Desai et al.[18,19] Our study also showed higher levels of HDL and VLDL, but their levels were not statistically significant. No effect of birth weight on cord blood lipid profile were noticed in the studies by Mathur et al and Pushpendra et al.[20,21]

Higher and statistically significant values of total cholesterol, LDL and triglyceride levels were noted in our preterm study group. Higher values of HDL and VLDL were seen in the preterm group, but were not statistically significant. Mishra et al observed that the mean triglyceride levels in cord blood of preterm neonates were higher in comparison to term neonates.[22] Similar findings were noted by Haridas et al who showed higher triglyceride levels in cord blood of preterm neonates.[23] But this group had similar total cholesterol levels in the term and preterm group, unlike our study where the total cholesterol levels were higher in the preterm group. Kalra et al showed that total cholesterol, HDL and LDL cholesterol levels were higher in preterm babies than term ones. But the difference was statistically significant only for total cholesterol.[24] Jain et al showed significant increase in cholesterol and triglyceride levels in preterm neonates' cord blood.[25] Pushpendra et al showed statistically significant differences in the levels of total cholesterol, VLDL and LDL with higher levels in favor of the preterm group.[21]

Comparison based on gestational maturity with respect to gestational age showed higher values of all the lipid components in the term SGA group in comparison to term AGA group, but none were statistically significant. Contrasting results were obtained in the study by Oba et al which showed higher and statistically significant values of total cholesterol and HDL with statistically insignificant higher values of LDL and triglycerides in the term SGA group.[26] Findings of significantly higher lipid values in this study may be attributable to a lower significance value of <0.01 set and ours having a higher significance value of p <0.05. Findings of high total and HDL cholesterol were found in the term SGA group in studies by Christensen et al and Diaz et al.[27,28] Similarly Kumar et al showed significantly higher triglyceride levels in the term SGA group.[18] Such significant finding in these studies may be attributable to its larger study sample. Comparison with these studies in the preterm group showed that Oba et al group had significantly higher total cholesterol and HDL in the preterm SGA group; whereas the preterm AGA group had higher triglyceride values.[26] Higher triglyceride value in the preterm AGA group was also noted by Ajay Kumar et al.18 Our study showed no significant difference in the preterm SGA and AGA group. This probably is attributable to higher significance value set i.e. <0.05 compared to other groups with p value of <0.01. In summary 90 neonates were enrolled in the present study. 51.11% of the 90 neonates were females and the rest 48.88% were males. Statistically significant difference in the cord blood lipid profile was not seen based on gender in our study, though the levels of total cholesterol, triglycerides, HDL and LDL were higher in male neonates than in females. 51.11 % of the total neonates were low birth weight and the rest 48.88% had birth weight of more than 2.5 kg. The low birth weight group had higher and statistically significant levels of total cholesterol, triglycerides and LDL. They also had higher levels of HDL and VLDL, but the levels of these lipids were not statistically significant. 67.8% of the total neonates were of term gestation and the rest 32.2 % were preterm. There were 16 females and 13 males in the preterm group, whereas 30 females and 31 males in the term group. The preterm group had higher and statistically significant levels of total cholesterol, triglycerides and LDL. They also had higher levels of HDL and VLDL, but the levels of these lipids were not statistically significant. Assessment based on gestational maturity did not show significant differences in the lipid values. But when these SGA and AGA groups were compared in terms of gestational age i.e. as term and preterm SGA and AGA, higher and statistically significant levels of total cholesterol, triglycerides and LDL were found in the preterm SGA and the preterm AGA groups. There was an inverse relation between advancing maternal age and the levels of total cholesterol, VLDL, LDL and triglycerides in the cord blood.

CONCLUSION

With coronary heart disease as the leading cause of crippling morbidity in the developing world, screening of the at risk babies i.e. preterm and low birth weight babies using cord blood lipid profile helps in primordial and primary prevention of diseases and decreases the disease burden. Cord blood lipid profile definitely has a vital role in giving a better quality of life to these at risk babies. This is in conjunction with Barker's fetal origins hypothesis which states that the adult cardiovascular diseases have their origin in fetal life itself and their progression is modified by environmental factors in later life. Our study results reaffirm the relation between cord blood lipid profile and perinatal factors. The point of interest in the present study is that there is a significantly unhealthy lipid profile among the preterm and low birth weight babies where in, with the values noted at birth, we might safely say that the preterm and low birth weight babies are exposed to a more hypercholesterolemic and potentially more atherogenic environment than their term counterparts. Since this process of vessel damage is not a onetime event and needs the blood environment for a long term to be promotive to the process, it opens up new avenues to venture for further research to track the lipid values as the neonate grows and also to note the natural as well as the dyslipidemic trend.

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