

Comparison of Lipid Profiles from Cord Blood Of Appropriate and Small for Gestational Age Babies in a Tertiary Care Hospital: A Case-control Study

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ABSTRACT

Introduction: Hyperlipidemia and its complications are common health issues in the current era with multifactorial in origin. Foetal malnutrition results in neuroendocrine, pancreatic, and adipose tissue dysfunction, ultimately increasing food intake and decreasing energy utilisation. It leads to an increase in adiposity and insulin resistance and ultimately increase adult diseases in later life.

Aim: To compare the lipid profiles of Small for Gestational Age (SGA) and Appropriate for Gestational Age (AGA) babies.

Materials and Methods: This was a case-control study was conducted in the Department of Pediatrics, Obstetrics and Gynecology of Karwar Institute of Medical Sciences, Karwar, Karnataka, India over a period from (December 2020 to March 2021). A total of 133 deliveries were recruited randomly and babies were divided into cases including those small for gestational age & controls including those appropriate for gestational age. Data was analysed and described using

descriptive (mean, standard deviation, and range) and inferential statistics (students' t-test).

Results: There were 99 appropriate for gestational age (AGA) and 34 small for gestational age (SGA) babies studied. This study found that SGA-babies had statistically significantly lower gestational age (Mean 37.50 ± 2.77 weeks) at birth compared to AGA-babies (Mean 38.55 ± 1.11 weeks) $t = -2.584$, $p = 0.012$. The mean Total Cholesterol (TC) level (63.62 ± 40.48 mg/dL) was higher in SGA-babies compared to AGA-babies (48.69 ± 2.29 mg/dL) and this difference was statistically significant (p -value = 0.007). The mean high density lipoprotein (HDL) levels of SGA and AGA babies were comparable with no statistical significance (21.82 ± 13.26 mg/dL of SGA; 21.49 ± 14.64 mg/dL of AGA; p -value 0.907). The mean very low density lipoprotein (VLDL) level (17.11 ± 5.35 mg/dL) was higher in SGA-babies compared to AGA-babies (9.47 ± 9.35 mg/dL) and this difference was statistically significant (p -value 0.05).

Conclusion: Levels of all lipids were found to be higher in SGA-babies than in AGA-babies.

Keywords: Foetal malnutrition, Newborn, Umbilical cord blood, Hyperlipidemia

INTRODUCTION

It is a well known fact that hyperlipidemia can lead to complications like stroke, coronary artery disease/ cardiovascular disease (CVD), renal failure, atherosclerosis [1-3] Also, it is found that foetal malnutrition/ intrauterine growth retardation and prematurity influence the subtypes of lipoproteins in baby's cord-blood [2-5].

High levels of triglycerides (TG) and apolipoprotein-B lower maturity, as well as enhance apolipoprotein-C1 rich in high density lipoprotein (HDL) in low birth weight newborns and are thought to raise the risk of heart disease later in life [6-8]. It is known that foetal malnutrition results in neuroendocrine, pancreatic and adipose tissue dysfunction; ultimately increased food intake and decreased energy utilisation. Adiposity and insulin resistance increase and ultimately adult diseases in later life. It is also observed that increased rate of hyperlipidemia and coronary heart diseases are found in people having low birth weights or low-normal birth weight, thin or short at birth or with too small placental size [2,5,9,10].

Many studies showed that lipid values are higher in low birth weight and preterm babies as compared to the normal and high birth weight babies and in term babies [11,12]. Very few studies describe the lipids comparison between small for gestational age and appropriate for gestational age babies [13,14]. This study makes an effort to find the relation between lipid profiles of small and appropriate for gestational age babies, which is one of the

steps in correlating the hyperlipidemia of foetal malnutrition and future life metabolic diseases as proposed in famous Fetal Origins of Adult Disease (FOAD) hypothesis [2]. Hence the present study was planned and conducted to compare and contrast umbilical cord-blood lipid profile between small (SGA) and appropriate (AGA) for gestational age neonates.

MATERIALS AND METHODS

This case-control study was conducted in the Department of Paediatrics and Department of Obstetrics and Gynaecology (OBG) of Karwar Institute of Medical sciences, Karwar, Karnataka, India over a period of 4 months from December 2020 to March 2021 after Institutional Ethical Committee approval (IEC NO. IEC/KRIMS/O/06/2020-2; 9-2-2020.). The study was explained to mothers in their vernacular language, an informed written consent was obtained.

Inclusion criteria: For cases, the newborns with antenatally predicted intrauterine growth restriction (IUGR) born (<24 hours post-delivery) to the mothers admitted in Department of OBG and gave informed written consent to participate in the study were included. For controls, the neonates with normal intrauterine growth, born (<24 hours post-delivery) to the mothers admitted were included.

Exclusion criteria: Neonates with perinatal asphyxia or congenital malformations or congenital heart diseases or respiratory distress syndrome, Infants of mother diagnosed with diabetes mellitus

or hypertension or cardiac diseases or thyroid diseases were excluded.

Sample size: Considering mean-VLDL of 12.72 ± 8.958 mg/dl among low-birth-weight babies and 8.27 ± 3.593 mg/dl, with $\alpha=0.05$ and 80% power, a minimum sample size of 78 was calculated and 39 newborns were planned to be included in each group [11]. The newborns were selected using consecutive sampling method based on their birth and admission to hospital.

Procedure

After IEC approval, Study was explained to mothers in their vernacular language, an informed written consent was obtained hence recruitments were done using Consecutive sampling method. After delivery, baby's cord blood (4 mL) from placental end was collected for lipid estimation after cord clamping and before placental delivery. Samples were analysed for Total Cholesterol (TC), Triglycerides (TG), high density lipoproteins (HDL) and low density lipoproteins(LDL) using standard methods: TC by CHOD-PAP method(Cholesterol oxidase-peroxidase 4-aminoantipyrine phenol), TG by GPO method (Glycerol phosphate oxidase), HDL by precipitation method and estimated by CHOD-PAP method, LDL cholesterol by Friedwald formula as follows:

$[LDL=TC-(VLDL+HDL)]$, whereas $VLDL= TG/5$

Normal ranges of lipids were considered as follows: TC ranges from 114 to 203 mg/dL, TG ranges from 29 to 99 mg/dL, HDL ranges from 38 to 75 mg/dL, and LDL ranges from 63 to 129 mg/dL [15].

Anthropometric measurements were taken from all enrolled babies. Birth weight was measured using a calibrated digital weighing scale; birth length was measured using a standard infantometer. Head Circumference (HC) and abdominal circumference (AC) were measured using a nonstretchable measurement tape. Finally ponderal index (PI) was calculated using the standard formula: [16]. PI is low(<2.0) in malnourished infants [17].

$PI=[\text{weight (g)/length (cm}^3)] \times 100$.

The anthropometry was documented for each newborn in order to know the nutritional status like AGA or SGA using Fenton charts. Also Ponderal Index (weight and length) was calculated so that each baby can be defined as AGA (PI >2.0) or SGA/IUGR (PI <2.0) [16,17].

Each newborn babies exact gestational age is assessed using this clinical tool- modified New Ballard scoring system [18]. Following that, the Fenton charts- birth weight against Gestational age was plotted. If plot value falls below 10th Percentile, it defines as baby is SGA (small for gestational age) or IUGR. If falls above 90th percentile, it defines baby as LGA (large for gestational age). In between 10th and 90th percentile, the babies are AGA (appropriate for gestational age) [19]. Hence, a baby can be SGA even if preterm, term or post term. Babies were classified as low birth weight (LBW) (weight <2.5 kg including the weight of 2.49 kg) and normal birth weight (weight >2.5 kg but less than 4 kg) [20].

STATISTICAL ANALYSIS

The data was coded and entered into Microsoft (MS) excel 2019. The data was analysed using statistical software Statistical Package for Social Sciences (SPSS) version 16.0. The results were described using descriptive and inferential statistics. The descriptive statistics included mean, standard deviation and range. The association between two continuous variables were done by using chi-square test and students' t-test. The p-value <0.05 was interpreted as statistically-significant.

RESULTS

Out of 133 babies studied, 99 were AGA and 34 SGA babies. [Table/Fig-1] lists the baseline characteristics of the neonates. Both research groups had similar gender distributions and there was no significant difference (p value- 0.5611), with males accounting for 60(60.6%) of neonates in the AGA group and 18 (52.9%) of neonates in the SGA group. The AGA group had a mean gestational age of 39.22 ± 1.05 weeks, while the SGA group had a mean gestational age of 37.40 ± 1.01 weeks and this difference was statistically significant (p-value- 0.0001). In terms of the mean birth weight of babies, there was a significant difference between the AGA and the SGA groups (2.71 ± 0.31 kg vs. 1.98 ± 0.22 kg, p-value 0.0001); the majority of these neonates were delivered vaginally, with caesarean sections in 15.1% and 20.5% of instances in the AGA and SGA groups, respectively.

Demographic characteristics of newborns	AGA (n=99)	SGA (n=34)	p-value
Male*	60 (60.6%)	18 (52.9%)	0.5611
Female*	39 (39.4%)	16 (47.1%)	
Gestational age (week; mean \pm SD)	39.22 ± 1.05	37.40 ± 1.01	0.0001
Birth weight (kg; mean \pm SD)	2.71 ± 0.31	1.98 ± 0.22	0.0001

[Table/Fig-1]: Comparison of demographic details of AGA and SGA.

This study revealed that low birth weight babies had lesser gestational age with statistical significance (Mean 37.69 ± 2.45 weeks), at the time of birth compared to that of normal birth weight babies (Mean 38.55 ± 1.11 weeks) $t=-2.351$, $p=0.022$ [Table/Fig-2].

Gestational age	Low birth weight (n=51) Mean \pm SD	Normal birth weight (n=82) Mean \pm SD	t-value*	p-value**
GA (in weeks)	37.69 ± 2.45	38.55 ± 1.11	-2.351	0.022

[Table/Fig-2]: Comparison of Gestational Age(GA) among low birth weight and normal birth weight babies.

*Independent t-test; **p <0.05 -statistically significant

The mean-TC level (63.62 ± 40.48 mg/dL) was higher in SGA compared to AGA-babies (48.69 ± 21.29 mg/dL) and this finding was statistically (p-value=0.007) significant [Table/Fig-3].

Biochemical parameters analysis (mg/dL)	AGA (n=99) Mean \pm SD	SGA (n=34) Mean \pm SD	t-value	p-value*
TC*	48.69 ± 21.29	63.62 ± 40.48	-2.055	0.007
TG	38.34 ± 32.21	49.83 ± 36.23	-1.763	0.805
HDL	21.49 ± 14.64	21.82 ± 13.26	-0.117	0.907
LDL	22.45 ± 14.99	26.77 ± 21.99	-1.064	0.293
VLDL*	9.47 ± 9.35	17.11 ± 25.35	-2.540	0.012

[Table/Fig-3]: Comparison of biochemical parameters of AGA and SGA. Independent t-test; *p <0.05 -statistically significant; TC: Total cholesterol; TG: Triglycerides; HDL: High density lipoprotein; LDL: Low density lipoprotein; VLDL: Very low density lipoprotein

The mean Triglycerides (TG) and Low density lipoproteins(LDL) levels (49.83 ± 36.23 mg/dL and 26.77 ± 21.99 mg/dL) were high in SGA-babies compared to AGA-babies (38.34 ± 32.21 mg/dL and 22.45 ± 14.99 mg/dL) even though these differences were statistically not significant (p-value 0.805 and 0.293) [Table/Fig-3].

The mean HDL- levels of AGA and SGA babies were comparable and had no statistical-significance (21.82 ± 13.26 mg/dL of SGA; 21.49 ± 14.64 mg/dL of AGA; p-value=0.907). The mean VLDL-level (17.11 ± 25.35 mg/dL) was higher in SGA-babies than AGA-babies (9.47 ± 9.35 mg/dL) and this result was statistically significant (p-value <0.05).

Serial no.	Authors name Year of the study	Place of the study	Sample size	Values for the parameters assessed			Association of the parameters in SGA/AGA babies (p-values)
					AGA (mg/dL)	SGA (mg/dL)	
1	Limmy Loret Lobo et al., August, 2016 [13]	Secunderabad, India	103 (AGA=52; SGA=51)	TC	54.08±13.77	60.21±15.42	<0.05
				TG	32.67±17.74	42.89±24.90	<0.05
				HDL	19.92±7.46	20±8.91	<0.05
				LDL	27.56±8.94	33.71±14.71	<0.05
				VLDL	6.60±3.60	9.10±6.64	<0.05
2	Puspendra Magon et al., July, 2013 [14]	Pondicherry, India	504 (AGA=420; SGA=84)	TC	83.4±27.4	68.6±20.2	<0.05
				TG	64.8±43.4	61.2±43.4	<0.05
				HDL	32.6±15.7	35.8±18.7	<0.05
				LDL	149.3±65.5	135±56.5	<0.05
				VLDL	183.1±163.8	143±146.2	<0.05
3	Present study	Karwar, India	133 (AGA=34; SGA=99)	TC	48.69±21.29	63.62±40.48	<0.05
				TG	38.34±32.21	49.83±36.23	<0.05
				HDL	21.49±14.64	21.82±13.26	<0.05
				LDL	22.45±14.99	26.77±21.99	<0.05
				VLDL	9.47±9.35	17.11±25.35	<0.05

[Table/Fig-4]: Comparison of biochemical parameters of present study with similar studies.
AGA: Appropriate for gestational age; SGA: Small for gestational age

DISCUSSION

Low birth weight (LBW) and SGA/intrauterine growth retardation (IUGR) have been identified as major Cardiovascular Disease (CVD) risk factors, particularly in developing countries [1,4]. In low-income countries, estimation of serum lipids in neonates could be helpful in predicting chances of lipoprotein disorders and CVD in adulthood [21].

In this study, the authors found that low birth weight babies had lesser gestational age (Mean 37.69±2.45 weeks) at birth than normal birth weight babies gestational age (Mean 38.55±1.11 weeks), which was statistically significant $t=-2.351$, $p=0.022$. The mean TC-level (63.62±40.48 mg/dL) was more in SGA compared to AGA babies (48.69±21.29 mg/dL) and this difference was statistically very significant (p -value=0.007).

Aletayeb SMH et al., [22], Donegá S et al., [23], Sreekarthik KP et al., [24], showed in their study that the mean serum TC-lipid levels were significantly higher in SGA-babies than in AGA babies which were statistically significant. But, studies by Gora A et al., [11] and Magon P et al., [14] highlighted that the mean serum-TC level was more in LBW and SGA babies than in normal birth weight babies; even though this difference was not significant statistically.

The mean-TG and LDL-levels (49.83±36.23 mg/dL and 26.77±21.99 mg/dL) were higher in SGA compared to AGA-babies (38.34±32.21 mg/dL and 22.45±14.99 mg/dL) even though these differences were statistically not significant (p -value 0.805 & 0.293). Studies by Katragadda T et al., [1] Gora A et al., [11], Basha Kalluri M [21], Aletayeb, S. M. H et al., [22], Duggal M et al., [25], showed that the mean TG levels were more in SGA as compared to AGA-babies with statistical significance. On the contrary, a study by Kelishadi R et al concluded that term-neonates had statistically significant higher TG-levels than preterm neonates. This observation could be due to the reason that their study has included neonates of varied GA i.e. near term to late preterm [26].

In a study by Gora A et al., [11], the mean LDL-level was higher in SGA-babies compared to AGA babies and this was statistically not very significant. Similar results are found in studies done by Magon P et al., [14], Bashakalluri M [21], Sreekarthik KP et al., [24], Dr. Duggal M et al., [25], Jain R et al., [27], Yonezawal R et al., [28], conferred that preterm SGA-neonates had higher LDL levels than term babies and it was statistically very significant. The socio-demographic profile difference in the study population could explain this.

The mean HDL-levels of AGA& SGA babies were comparable with no significance statistically (21.82±13.26mg/dL of SGA; 21.49±14.64mg/dL of AGA; p -value=0.907). Similar results were found in studies by Gora A et al., [11], Magon P et al., [14], Aletayeb SMH et al., [22], Sreekarthik KP et al., [24], Yonezawal R et al., [28] concluding that serum concentrations of HDL-cholesterol were much higher in preterm and SGA compared to term and AGA-babies; with no statistical significance.

In the present study, the mean VLDL-level (17.11±25.35mg/dL) was more in SGA compared to AGA-babies (9.47±9.35 mg/dL) and this difference was statistically very significant (p -value<0.05). Similar findings were reported by many studies like Gora A et al., [11], Bashakalluri M et al., [21], Jain R et al., [27], Yonezawa R et al., [28], Diaz M et al., [29] whose findings were statistically significant indicating the higher values of VLDL was a constant association of SGA compared to AGA-babies. Similarly Magon P et al., [14], Aletayeb SMH et al., [22], Duggal M et al., [25], have pointed out that preterm babies had higher VLDL than term babies, and the values were statistically significant.

Earlier researchers did hypothesize that there may be a possible link between lipid profiles of foetus and adults. A study by Ijzerman RG et al., [8] suggested that genetic factors account for the association of LBW with high levels of TC, LDL and apolipoprotein-B. Recent study by Nayak C et al., [30] reported that abnormal intrauterine milieu created by maternal changes during each gestation may bear a significant impact on lipid metabolism in foetuses, which may account for their differences in lipid profile and anthropometry at birth.

Limitation(s)

Number of SGA babies studied was less than sample size calculated. Long term follow up was not done in LBW-babies to evaluate the risk of CVD in later part of life.

CONCLUSION(S)

All lipids were found to be higher in SGA than AGA-babies and this difference was statistically significant for TC and VLDL. It may be interesting to see whether these susceptible neonates do remain at an increased risk for developing cardiovascular diseases in future.

Larger studies with large sample size is required including comparison of mothers' lipid parameters with the baby's lipids and long term follow up of these babies are required.

Authorship contributions: The concept of the study was helped out by V.M, design by V.M and M.U, data collection or processing of this study by V.M., study analysis or interpretation by V.M.,M.U. and R.A., literature search was helped out by V.M. and M.U. while writing part was done by V.M.

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