Original Article

DOI: 10.7860/IJNMR/2020/45965.2282

Paediatrics Section

Umbilical Cord Serum Lipid Profile of Normal Preterm Neonates and Preterm Neonates with Respiratory Distress Syndrome: A Hospital-based Cross-sectional Study

RAM NARAIN SEHRA[1], ANKUSH GARG[2], SUNITI VERMA[3]

ABSTRACT

Introduction: Respiratory Distress Syndrome (RDS) is a common cause of morbidity and mortality in preterm neonates. Levels of minor phospholipids and lecithin in amniotic fluid are significantly influenced by lipid metabolism.

Aim: To evaluate umbilical cord lipid profile of preterm infants with RDS and normal preterm group without RDS.

Materials and Methods: This was a hospital-based cross-sectional study carried out in neonatal unit of at a Tertiary Care Centre at SMS Medical College, Jaipur, from June 2017 to May 2018. Out of total 80 preterm infants, 40 developed RDS and 40 infants served as controls. Umbilical cord blood lipid profile of neonates were done in both the groups and compared. Chi-Square test and unpaired Student's t-test were used for statistical analysis. Probability was considered significant if less than 0.05

Results: Mean weight of babies was 1494.75±201.66 grams in normal preterm group and 1450.25±233.23 grams in preterm with RDS group (p=0.364). Mean gestational age was 31.45±1.36 weeks in normal preterm group and 30.98±1.49 weeks in preterm with RDS group p=0.140). Low Density Lipoprotein (LDL) and High Density Lipoprotein (HDL) were higher in male babies in both the groups as compared to female babies (p>0.05). Mean cord blood Triglyceride (TG), Total Cholesterol (TC), Very Low Density Lipoprotein (VLDL), LDL and HDL levels were significantly lower in preterms with RDS as compared to normal preterms without RDS which was statistically significant (p<0.05). Mean cord blood TG, TC, VLDL and HDL levels in all gestational age were higher in normal preterm as compared to preterm with RDS (p<0.05), except for LDL on 34-36 weeks age.

Conclusion: Preterm newborns with lower cord serum lipids may develop RDS. The cord blood, which is easily available, can be used for lipid levels at birth to predict RDS.

Keywords: Prematurity, Respiration, Umbilical cord

INTRODUCTION

Preterm birth is one of the leading causes of infant morbidity and mortality worldwide [1]. The rate of preterm births has been increasing over the last several decades [2]. In extremely preterm newborns with respiratory distress, RDS is by far the most common diagnosis. RDS is one of the most common causes of morbidity and mortality associated with premature delivery [3]. RDS is a developmental disorder rather than a disease process per se. Surfactant deficiency which increases surface tension in alveoli, resulting in microatelectasis and low lung volumes is the primary cause of RDS [4].

Pulmonary surfactant is a complex mixture of 90% lipids and 10% proteins [5]. Levels of lecithin and phospholipids in amniotic fluid, use of cholesterol for hormone synthesis, foetal growth and development during late gestation are significantly affected by lipid metabolism [6,7].

Lung cholesterol metabolism is regulated by both LDL and HDL. In addition, several factors significantly affect neonatal serum lipids, especially gestational age and birth weight. Maternal lipoproteins provide the free fatty acid substrate required for foetal surfactant synthesis in vivo [8]. Reduced transport of essential and long chain polyunsaturated fatty acids could inhibit normal foetal growth and maturation including delayed development of foetal lungs, which could lead to the RDS [9].

The purpose of the study was to compare umbilical cord lipid profile of preterm infants with RDS and normal preterm infants without RDS.

MATERIALS AND METHODS

This hospital-based cross-sectional study was carried out at Neonatal Units of Department of Paediatrics, SMS Medical

College Jaipur from June 2017 to May 2018, after getting ethical clearance from research review board of the institute (IRB No. 57673/2017)

Sample size calculation was done using the formula:

$(n)=(Z_{1-\alpha/2})^2\ (\sigma)^2/(d)^2$, where n=desired no of samples, $Z_{1-\alpha/2}$= Standardised value for corresponding level of confidence (At 95% CI, it is 1.96 in two tailed and 1.64 in one tailed test), d=Margin of error or rate of precision, σ=SD which is based on previous study, $n=(1.96)^2\ (6.6)^2/(2)^2=42$, two tailed=$(1.64)^2\ (6.6)^2/(2)^2=29$, one tailed, alpha error 0.05% . Sample size was rounded off to 40 cases and 40 controls with expected HDL values 34.2 mg/dL with SD±6.60 and weight [10]. Total 80 newborns were included in study, 40 newborns were normal at birth and 40 newborns developed RDS.

All newborn that fulfilled inclusion criteria were divided in two subsets on the basis of development of RDS. Preterm babies who developed RDS were included as preterm with RDS study group B and who did not develop RDS as healthy preterm control group A. Silverman and Andersen scoring was used for assessment of respiratory distress [11].

Inclusion criteria: Inclusion criteria at admission was neonates with gestational age ≥ 28 weeks to ≤ 36 weeks and birth weight ranging from 980 grams to 2260 grams. For preterm with RDS (group B) clinical signs suggestive of RDS used in the study were cyanosis, retractions, evidence of acidosis or hypoxaemia or hypercarbia on blood gas, diffuse alveolar atelectasis on x-ray. Healthy preterm neonates (group A) with the same gestational age were enrolled as controls.

Exclusion criteria: Exclusion criteria was history of maternal hypertension either before or during pregnancy, paternal or maternal hyperlipidemia, maternal cardiovascular disease and diabetes mellitus or gestational diabetes, any history of maternal drug use during or before pregnancy (except for vitamins, folic acid, and iron), maternal history of smoking, neonates with congenital malformations, hypoxic ischaemic encephalopathy, sepsis, small for gestation age.

Predesign structured proforma, devised by investigators, was used for history and data collection. After taking written consent from all parents/attendants detailed antenatal and natal history was taken. The mothers were analysed for maternal age, maternal membrane ruptured >24 hours, antenatal steroid administration, Pregnancy Induced Hypertension (PIH), and parity. Newborn examination included birth weight, assessment of gestational age by Modified Ballard score [12], Apgar score at one and five minutes, and complete clinical examination. Information of subjects was entered in separate proforma for each baby.

Sample collection: Primary investigator collected the cord blood sample. Cord blood samples of 3 mL were obtained from umbilical vein immediately after delivery. Samples were taken with all aseptic precautions in plain dry test tube and allowed to clot at room temperature for 20 minutes. Serum was separated by centrifugation (20 min, 2500 rpm) and kept at -20°C in hospital blood bank until the analysis. Serum was used for estimation of lipid profile using enzymatic colorimetric method. Serum LDL was estimated using Friedewald's Formula [13].

STATISTICAL ANALYSIS

Statistical analysis was performed with Statistical Package for the Social Sciences (SPSS), version 21 for windows statistical software package (SPSS inc, Chicago, IL, USA). The categorical data was presented as numbers (percentage) and were compared among groups using chi-square test. The quantitative data was presented as mean and standard deviation and were compared by student's t-test and continuous non-parametric data were compared by Pearson correlation coefficient test. Probability was considered to be significant if less than 0.05.

RESULTS

The study groups consisted of 80 preterm infants with gestational ages ranging from 28 weeks to less than 36 weeks and birth weights from 980 g to 2260 g. Of these neonates, 40 developed RDS and 40 served as controls.

As regard the clinical characteristics of the studied infants in the present study, Apgar scores at five minutes were significantly higher in preterm with RDS as compared to the normal preterm group ($p<0.001$) and no significant differences of mean birth weight ($p=0.364$), mean gestational age ($p=0.140$), mean Apgar score at one minute ($p=0.280$), mode of delivery ($p>0.05$), parity ($p=0.749$) and premature rupture of membranes ≥ 24 hours ($p=1.00$) were observed between the groups [Table/Fig-1].

In the present study, mean cord lipid levels in relation to gender were observed statistically non-significant in both the groups ($p>0.05$), except for TC, which was significantly lesser ($p<0.05$) in females than males in group A. The male to female ratio was 1.2:1 and 0.91:1 in normal preterm group and preterm with RDS group, respectively [Table/Fig-2].

Mean cord blood TG, TC, VLDL, LDL and HDL levels were significantly lower in preterms with RDS as compared to normal preterms without RDS which was statistically significant ($p<0.05$) [Table/Fig-3].

Mean cord blood levels of TG, TC, HDL and VLDL were found to have significant differences in all gestational age subgroups in both RDS and non-RDS group ($p< 0.05$) except LDL which was nonsignificant in 34-36 weeks ($p>0.05$) [Table/Fig-4].

In preterm with RDS babies, there was a poor positive correlation between gestational age and cord blood TG ($r=0.077$, $p=0.635$), TC ($r=0.145$, $p=0.369$), VLDL ($r=0.0792$, $p=0.627$), LDL ($r=0.1702$, $p=0.293$) and HDL ($r=0.0546$, $p=0.737$) levels.

Parameter		Group A (Normal Preterm) N=40		Group B (Preterm with RDS) N=40		p-value
		Mean (mg/dL)	SD	Mean (mg/dL)	SD	
Birth weight (Grams)		1494.75	201.66	1450.25	233.23	0.364
Gestational age (Weeks)		31.45	1.36	30.98	1.49	0.140
Apgar score						
1 Min		6.56	0.59	6.70	0.56	0.28
5 Min		7.70	0.72	8.32	0.57	<0.001
		Number (n)	Percentage (%)	Number (n)	Percentage (%)	
Parity	1	25	62.5%	27	67.5%	0.749
	≥2	15	37.5%	13	32.5%	
PROM > 24 hour	Yes	23	57.5%	24	60%	1.000
	No	17	42.5%	16	40%	

[Table/Fig-1]: Characteristic of study groups.
SD: Standard deviation; PROM: Premature rupture of membranes
Unpaired student t-test was used. Chi-square test used in parity and PROM. Level of significance value was 0.05

Cord blood lipids (mg/dL)	Group A (Normal Preterm) N=40			Group B (Preterm with RDS) N=40		
	Male	Female		Male	Female	
	Mean (mg/dL) ±SD	Mean (mg/dL) ±SD	p-value	Mean (mg/dL) ±SD	Mean (mg/dL) ±SD	p-value
TG	85.46± 53.65	74.52± 41.26	0.482	30.47± 8.38	39.78± 27.62	0.099
TC	148.23± 5.64	125.17± 37.71	0.0192	95.16± 15.66	96.55± 17.73	0.793
VLDL	17.07± 10.75	14.92± 8.29	0.319	6.09± 1.76	8.00± 4.46	0.088
LDL	94.19± 40.85	74.78± 36.61	0.125	65.72± 13.67	64.19± 15.36	0.743
HDL	38.45± 11.34	35.47± 3.56	0.290	25.06± 5.18	24.36± 5.04	0.667

[Table/Fig-2]: Mean cord blood lipid level in relation to gender of normal preterm (Group A) and preterm with RDS (Group B). Unpaired student t-test was used. Level of significance value was 0.05.
TG: Triglyceride; TC: Total cholesterol; VLDL: Very low density lipoprotein; LDL: Low density lipoprotein; HDL: High density lipoprotein; SD: Standard deviation

Although, there was a weak positive correlation between gestational age and cord lipid levels, yet statistically it was not significant [Table/Fig-5].

DISCUSSION

Lipid metabolism changes phospholipids in amniotic fluid and helps in maturation of pulmonary function. It has an important role in foetal lung development by increasing amniotic fluid lecithin levels [7].

The present study resulted that mean cord lipid levels of TG, TC, VLDL, LDL and HDL were lower (statistically significant)

Cord blood lipid (mg/dL)	Group A (Normal Preterm) N=40		Group B (Preterm with RDS) N=40		p-value
	Mean (mg/dL)	SD	Mean (mg/dL)	SD	
TG	80.54	48.19	35.36	17.80	<0.001
TC	137.85	46.21	95.89	16.58	<0.001
VLDL	16.10	9.66	7.10	3.54	<0.001
LDL	85.45	39.73	64.92	14.42	0.002
HDL	37.11	8.78	24.70	5.05	<0.001

[Table/Fig-3]: Mean cord blood lipid level of cases studied.
Unpaired student t-test was used. Level of significance value was 0.05
TG: Triglyceride; TC: Total cholesterol; VLDL: Very low density lipoprotein, LDL: Low density lipoprotein; HDL: High density lipoprotein; SD: Standard deviation

Cord blood lipid (mg/dL)	Gestational age (Weeks)	Group A (Normal Preterm) N=40		Group B (Preterm with RDS) N=40		p-value
		Mean (mg/dL)	SD	Mean (mg/dL)	SD	
TG	28-30	79.98	21.58	31.87	11.54	<0.001
	31-33	85.84	61.21	40.00	23.09	0.005
	34-36	53.32	27.45	32.33	18.15	0.0001
TC	28-30	150.13	48.27	94.05	13.08	<0.001
	31-33	134.31	47.05	96.93	20.13	0.004
	34-36	114.34	25.25	102.33	19.66	0.02
VLDL	28-30	16.01	4.33	6.39	2.28	<0.001
	31-33	17.19	12.26	8.02	4.59	0.005
	34-36	10.45	5.31	6.53	3.72	0.0003
LDL	28-30	99.28	46.30	62.91	10.91	0.001
	31-33	79.65	36.66	66.30	18.10	0.0.042
	34-36	69.00	17.94	70.47	14.14	0.912 NS
HDL	28-30	36.63	3.74	24.71	4.73	<0.001
	31-33	37.91	11.47	24.56	5.93	<0.001
	34-36	34.40	2.24	25.33	2.08	0.002

[Table/Fig-4]: Mean cord blood lipid level according to gestational age of cases studied.
Unpaired student t-test was used. Level of significance value was 0.05
TG: Triglyceride; TC: Total cholesterol; VLDL: Very low density lipoprotein; LDL: Low density lipoprotein; HDL: High density lipoprotein, SD: Standard deviation; NS: Not significant

		TG	TC	VLDL	LDL	HDL
Gestational Age (weeks)	Pearson Correlation (r)	0.77	0.145	0.0792	0.1702	0.0546
	p-value	0.635	0.369	0.627	0.293	0.737

[Table/Fig-5]: Correlation between gestational age and baby lipid profile in Preterm with RDS. Pearson Correlation coefficient test was used. Level of significance value was 0.05
TG: Triglyceride; TC: Total cholesterol; VLDL: Very low density lipoprotein; LDL: Low density lipoprotein; HDL: High density lipoprotein
There was poor positive correlation observed between gestational age and serum cord lipids. which was not significant.

in RDS group as compared to non-RDS group. The results of the present study were similar to previous studies [Table/Fig-6]

Various studies	RDS/ Non-RDS/p-value	TG Mean± SD	TC Mean± SD	VLDL Mean± SD	LDL Mean± SD	HDL Mean±SD
Gunes T et al., (2007) n=166 [9]	RDS	59.9± 12.9	60.5± 9.7	11.9± 3.25	27.0± 5.9	21.3±5.1
	Non-RDS	66.4± 25.8	84.2± 9.7	13.6± 3.4	36.3± 6.26	34.3±8.1
	p-value	>0.05	<0.05	>0.05	<0.05	<0.05
Yonezawa R et al., (2009) n=104 [16]	RDS	15.5± 3.5	77.0± 5.3	8.8± 1.6	29.0± 2.1	39.2± 4.0
	Non-RDS	25.8± 3.8	70.6± 4.3	10.0± 0.9	24.3± 2.1	36.3±2.2
	p-value	0.065	0.304	0.517	0.137	0.648
Mahmoud NS et al., (2012) n=50 [14]	RDS	70.05± 22.0	61.97± 10.2	14.0± 4.4	25.1± 5.6	22.24± 6.8
	Non-RDS	81.7± 17.4	89.7± 10.5	16.2± 3.4	36.5± 6.0	37.1±7.5
	p-value	>0.05	<0.001	>0.05	<0.001	<0.001
Maksoud HMA et al., (2015) n=160 [15]	RDS	43.51± 11.5	63.84± 10.7	-	20.9± 1.97	28.79± 5.5
	Non-RDS	47.33± 11.0	77.25± 11.2	-	26.06± 2.09	29.51± 5.9
	p-value	>0.05	<0.05	-	<0.05	>0.05
Duruvasan S et al., (2015) n=56 [10]	RDS	42.32± 21.42	114.01± 31.43	21.09± 12.80	44.74± 17.88	30.92± 6.48
	Non-RDS	72.00± 38.73	130.46± 24.19	29.97± 13.01	52.99± 19.4	39.14± 11.71
	p-value	0.001	0.033	0.013	0.100	0.002
Present study (2020) n=80	RDS	35.36± 17.80	95.89± 16.88	7.10± 3.54	64.92± 14.42	24.70± 5.05
	Non-RDS	80.54± 48.19	137.85± 46.22	16.10± 9.61	85.45± 39.73	37.11± 8.78
	p-value	<0.001	<0.001	<0.001	0.002	<0.002

[Table/Fig-6]: Comparison of mean cord serum lipid levels with different studies in RDS and non-RDS group.Level of significance value was 0.05 [9,10,14-16]. TG: Triglyceride; TC: Total cholesterol; VLDL: Very low density lipoprotein; LDL: Low density lipoprotein; HDL: High density lipoprotein; SD: Standard deviation; RDS: Respiratory distress syndrome

[9,10,14-16]. In contrast to the present study, Yonezawa R et al., observed similar levels of lipid profile in RDS and non-RDS group. They studied newborns with relatively more advanced gestational maturity as compared to present study and none of the neonates born after 34 weeks of gestation in their study developed RDS [16]. Katragadda T et al., studied cord blood lipid profile of preterm Appropriate for Gestational Age (AGA) and preterm Small for Gestational Age Neonates (SGA) and compared atherogenic index of both groups and concluded that prematurity is a factor associated with a more atherogenic lipid profile. In contrast to the present study, they did not compare RDS and non-RDS babies [17].

Foetal growth directly depends on the nutrients crossing the placenta to baby. Mother adapts her metabolism according to the continuous supply and body need of nutrients. Bansal N et al., reported that maternal cholesterol can cross the placenta and its concentration in mother affects concentration in neonates [18]. Factors during pregnancy and delivery as well as certain diseases can influence foetal and neonatal lipid metabolism. Inhibition of cholesterol synthesis leads to impaired surfactant synthesis [7]. Similar to the present study findings Lane DM et al., found that preterm infants developing RDS had significantly less cord serum lipid levels than the normal term infants. Although in present study, only preterm newborns were included and it did not document difference in cord serum lipid levels [7].

Lower cord serum lipids further inhibits foetal growth in utero and may delay maturation of foetal lungs. Yonezawa R et al., stated that 34 weeks of gestation is a critical period of TG metabolism in neonates because cord blood VLDL-TG increases dramatically from 32 to 34 weeks and development of RDS seemed to be inhibited after this period [16]. Lame DM et al., also suggested that placental transport of lipid components might be abnormal in preterm infants who developed RDS [7].

Similar to the present study, Gunes T et al., concluded that lower lipid parameters in RDS infants are evidences of reduced maternal supply, which could delay lung maturation. Additionally, their study stated that lipoprotein lipase impairment was also responsible for the lower cord lipid levels [9]. Maternal VLDL loading regulates surfactant synthesis in foetal lungs. LDL and HDL cholesterols stimulate primary cultures of type II cells to secrete phosphatidylcholine, the major phospholipids component of pulmonary surfactant [19].

Present study looked for lipid levels in relation to gestational age. Values like TG, TC, HDL, LDL and VLDL were significantly lower in RDS group than Non-RDS group. LDL was significantly lower in RDS group in 28-33 weeks gestation. Present study observations are similar to other studies done by Gunes T et al., and Maksoud HMA et al., [7,15].

In the present study, there was a positive correlation between gestational age and cord lipid levels in preterm with RDS group, which was statistically not significant ($p>0.05$). Mahmoud NS et al., observed statistically nonsignificant results of correlation between gestational age and TG, VLDL, HDL levels except LDL ($p<0.05$) [14].

In the present study, the baseline characteristics including birth weight, gestational age, mode of delivery, parity and Apgar score at one minute were similar in both the groups. These findings were in line with those presented by Maksoud HMA et al., [15]. Mahmoud NS et al., reported significantly lower gestational age and birth weight in RDS group [14]. This difference could be due to different ethnicity, inclusion of wider gestational age (27-36) weeks and wider birth weight range (750-2600) g in RDS group.

In the present study, common mode of delivery was vaginal delivery as compared to cesarean delivery and the difference

was statistically not significant. While Mahmoud NS et al., found equal numbers of vaginal and cesarean deliveries in both the groups [14]. In contrast to the present study, Maksoud HMA et al., found higher rates of cesarean section but statistically not significant [15]. Similar to others studies, the index study did not find statistically significant difference when the gender of study subjects were compared [20-23].

Limitation(s)

Present study did not compare maternal lipid profile with serum cord lipid profile. Gastric aspirate shake test could have been used to supplement the diagnosis of RDS.

CONCLUSION(S)

Lower serum cord lipids of newborns have trends to develop RDS. Mean cord lipid levels are significantly associated with gestational age but not with gender in RDS and non-RDS group. Mean cord blood TG, TC, VLDL, LDL and HDL levels were significantly lower in preterms with RDS as compared to normal preterms without RDS. The cord blood, which is easily available, can be used for lipid levels at birth to predict RDS. Study suggests more studies for further investigations of maternal lipid profile and preterm neonates having low cord lipid levels with RDS would be helpful.

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PARTICULARS OF CONTRIBUTORS:
1. Senior Professor, Department of Paediatrics, SMS Medical College, Jaipur, Rajasthan, India.
2. Junior Resident, Department of Paediatrics, SMS Medical College, Jaipur, Rajasthan, India.
3. Senior Professor, Department of Obstetrics and Gynaecology, SMS Medical College, Jaipur, Rajasthan, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Ram Narain Sehra,
G-63, Tirupati Nagar, Jagatpura, Jaipur-302017, Rajasthan, India.
E-mail: rnsehra@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Jul 18, 2020
- Manual Googling: Dec 01, 2020
- iThenticate Software: Dec 30, 2020 (21%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Jul 18, 2020**
Date of Peer Review: **Sep 26, 2020**
Date of Acceptance: **Dec 14, 2020**
Date of Publishing: **Dec 31, 2020**