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Study of maternal and cord serum lipid profile of preterm neonates with respiratory distress syndrome

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Abstract

Introduction: Lipid metabolism has an important role in fetal development during the late stage of gestation. Deficiency or reduced transport of essential and long-chain polyunsaturated fatty acids could inhibit normal fetal growth and maturation and leads to the respiratory distress syndrome. This study was planned to evaluate whether maternal and cord lipid profile have any effect on development of RDS.

Materials and methods: Present study was a hospital based cross sectional study carried out at Neonatal Units of Department of Pediatrics in tertiary care institute of northern part of India from June 2017 to May 2018. Out of 80 preterm neonates, 40 developed RDS and 40 served as controls. Umbilical cord blood and maternal blood was collected just after delivery in both groups to evaluate lipid profile. Chi-Square test and unpaired student t-test were used for statistical analysis. Probability was considered significant if less than 0.05.

Results: Mean birth weight, mean gestational age, parity of mothers, PROM>24 hours, mode of delivery and were statistically insignificant in both the groups. Mean maternal lipid levels of TC, LDL and HDL were significantly higher in normal preterms as compared to RDS group. There was statistically insignificant positive correlation observed between maternal and babies TG, VLDL levels. Serum cord TG, TC, VLDL, LDL and HDL were significantly higher in normal preterms as compared to RDS group.

Conclusion: Levels of all cord serum lipids and maternal lipids were found to be significantly higher in normal preterm babies than preterm with RDS babies.

Keywords: Prematurity, Respiratory distress syndrome, Serum lipids, Umbilical cord.

Introduction

According to WHO preterm birth is defined as birth before 37 completed gestational weeks, and can be further subdivided into extremely preterm (<28 weeks), very preterm (28 – <32 weeks) and moderate or late preterm (32 – <37 weeks).[1] Morbidity rates and fatality risks are very higher when born <28 weeks compared to those born after 32 weeks.[2,3]

Surfactant deficiency resulting in micro atelectasis and low lung volumes is the primary cause of respiratory distress syndrome (RDS).[4] Pulmonary surfactant is a complex mixture of 90 % lipids and 10% proteins. The lipid component includes 80% phospholipids and 10% neutral lipids primarily cholesterol.[5]

Lipid metabolism has an important role in fetal development during the late stage of gestation, including growth and fat accretion in utero, transport of cholesterol to the fetal adrenal for hormone synthesis, increasing amniotic fluid lecithin levels with maturation of pulmonary function and changes in the levels of minor phospholipids in amniotic fluid.[6,7] Deficiency or reduced transport of essential and/or long-chain polyunsaturated fatty acids could inhibit normal fetal growth and maturation and leads to the respiratory distress syndrome postnatally.[8]

Lung cholesterol metabolism is regulated by both low-density lipoprotein (LDL) and high-density lipoprotein (HDL). In addition, several factors significantly affect neonatal serum lipids, especially gestational age and birth weight.[9] Maternal lipoproteins provide the free fatty acid substrate required for fetal surfactant synthesis in vivo.[10]

Scanty data are available in our country regarding cord blood lipid profile. Hence, this study was planned to evaluate whether maternal and cord lipid profile have any effect on development of RDS.

Aims and objectives

1. To compare lipid profile of mothers whose children develops respiratory distress syndrome and who were normal at birth.

2. To correlate maternal lipid profile with new-born's lipid profile and development of respiratory distress syndrome.

Material and methods

Present study was a hospital based cross sectional study carried out at Neonatal Units of Department of Pediatrics, SMS Medical College Jaipur from June 2017 to May 2018. The study was conducted according to the guidelines and approved by the institutional ethics committee (IRB No. 57673/2017). Written informed consent was obtained from all subjects at the time of enrolment.

Sample size calculation was done using the formula: $(n)=(Z1-\alpha/2)^2\ (\sigma)^2\ /(d)^2$, where n=desired no of samples, Z1-α/2= Standardized 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.[11] Sample size was calculated at 95% confidence interval, 80% study power, α error 0.05% with expected S.D. of ±6.94 in Maternal HDL level of preterm neonate in control and study group. Sample size of 40 eligible preterm neonates who develop respiratory distress syndrome as study group and 40 eligible normal preterm as controls group were included. This sample size in each of two groups was also adequate to detect a difference of at least 5 mg /dl in maternal HDL level in two groups. [11]

Inclusion criteria

Neonates with gestational age≥28 weeks to ≤36 weeks and birth weight ranging from 980 grams to 2260 grams.

For Study Group

- Clinical signs suggestive of RDS used in the study were:
- Cyanosis
- Retractions
- Evidence of acidosis or hypoxemia or hypercarbia on blood gas
- Diffuse alveolar atelectasis on X-Ray

For Control Group

- Healthy preterm neonates with the same gestational age were enrolled as controls.

Exclusion criteria

- History of maternal hypertension either before or during pregnancy
- History of 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 ischemic encephalopathy, sepsis, small for gestation age.

Pre design structured proforma was used for history and data collection. It was made by investigators and validated by senior faculty in department who have vast experience in neonatology. After taking written consent from all parents/attendants and applying inclusion criteria and exclusion criteria, detailed antenatal and natal history was taken. Their mothers were analyzed 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 1 and 5 minute and complete clinical examination. Information of each subject was entered separate proforma for each baby.

All new-borns those fulfilled inclusion criteria were divided in two subsets on the basis of development of respiratory distress syndrome. After an infant met admission criteria, the diagnosis of RDS was established. Control infants met all admission requirements but not develop respiratory distress (Group-A). Preterm babies who developed RDS were included as study group (Group-B) Silverman and Andersen scoring was used for assessment of respiratory distress.[13]

Sample collection

2.5 ml of Cord blood sample from each of enrolled newborn was collected from the placental end of the cord and Maternal blood was taken from peripheral veins of the mothers just after the delivery of the baby. 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 Fried Ewald's Formula. [14] Data were collected and subjected for statistical analysis.

Statistical analysis

Statistical analysis was performed with the 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 36 weeks and birth weights from 980 gms to 2260 gms. Of these neonates 40 developed RDS and 40 served as controls.

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. Mean gestational was 31.45±1.36 weeks in normal preterm group and

30.98±1.49 weeks in preterm with RDS group. In both groups most common mode of delivery was vaginal delivery and parity was more in primi gravida mothers [Table-1].

Mean birth weight, mean gestational age, baby gender, parity of mothers, PROM>24 hours, mode of delivery and dexamethasone administration before delivery were statistically insignificant in both the groups. Apgar score at 5 minute was significantly lower in preterm with RDS group as compared to normal preterm group [Table-1].

Mean maternal lipid levels of total cholesterol (TC), low density lipoprotein (LDL) and high-density lipoprotein (HDL) were significantly higher in group A as compared to group B. Triglyceride (TG) and very low-density lipoprotein (VLDL) were also high but difference was not significant [Table -2].

Maternal lipid levels were correlated with gestational age of babies in this study. There was statistically significant positive correlation observed with maternal TG, VLDL and statistically insignificant with maternal TC, HDL levels. There was insignificant negative correlation observed between gestational age and maternal LDL [Table -3].

In present study authors also correlated maternal lipid levels with cord serum lipid levels. There was statistically insignificant positive correlation observed between maternal and babies TG, VLDL levels and statistically insignificant negative correlation observed between maternal and babies TC, LDL and HDL levels [Table-4].

Authors studied serum cord lipid levels in preterm babies with and without respiratory distress. TG, TC, VLDL, LDL and HDL were significantly higher in group A as compared to group B [Table-5].

Discussion

Factors during pregnancy and delivery as well as certain diseases can influence fetal and neonatal lipid metabolism. It has been postulated that low serum lipid levels are evidence of reduced essential fatty acids and long-chain polyunsaturated fatty acid supply, which could inhibit fetal growth in utero, delaying fetal lung maturation and development of RDS.[8]

Mean weight of babies was 1494.75±201.66 grams, mean gestational age was 31.45±1.36 weeks in normal preterm group and 1450.25±233.23 grams, 30.98±1.49 weeks in preterm with RDS group. In study conducted by Mahmoud NS et al [11] mean weight and gestational age of babies were statistically significant (P value=<0.05) in contrast to present study result. This could be due to difference in sample size and gestational age of preterm babies. They observed mean lipid level of all newborns irrespective of birth weight and gestational age while we studied the lipid level separately in normal preterm and preterm with RDS.

In present study out of 80 neonates, the male to female ratio was 1.2:1 and 0.91:1 in control group and preterm with RDS group respectively. In study conducted by Gunes T et al [8] the male to female ratio was similar to present study but another study conducted by Mahmoud NS et al [11] of 50 neonates observed statistically significant higher preponderance in males in RDS group. Another study done by Loughery CM et al [15] found no significant gender difference in incidence of RDS. This discordance could be simply by chance or due to ethnic and cultural difference in study population.

In our study Apgar score at 5 minute was significantly lower in RDS group as compared to normal preterm group (P value <0.01). Similar results were seen in study conducted by Mahmoud NS et al [11](P value <0.05).

In present study, parity status 1 and 2 were maximum followed by parity status ≥ 3 in both the groups proportionally. In an international study conducted Mahmoud NS et al [11] also observed results similar to present study.

In other studies conducted by Mahmoud NS et al [11] and Maksoud HMA et al [16] found that mode of delivery were not statistically significant similar to present study observations.

In present study, statistically insignificant (P value= 1.000) difference was observed in both the groups in terms of history of premature rupture of membranes similar to study done by Mahmoud NS et al.[11] Boskabady H et al [17] observed that ruptured membrane >24 hours was significantly higher in mothers of infants with RDS than control in contrast to present study results.

In study conducted by Mahmoud NS et al [11] observed statistically insignificant difference in both the groups in terms of dexamethasone received by mother which was consistent with present study results.

In present study, mean baby TG (P = <0.001), TC (P = <0.001), VLDL (P = <0.001), LDL (P = <0.002), HDL (P = <0.001) levels were statistically significantly higher in normal preterm babies as compared to preterm with RDS babies which were in agreement with the results of studies done by Duruvasan S et al ,[18] Gunes T et al [8] and Maksoud HMA et al [16]

Mahmoud NS et al.[11]and Gunes T et al.[8]also reported statistically insignificantly higher TG compared to preterm with RDS neonates. This could be due to high variability of sample size i.e., N=50 and 166 respectively in the two studies. In contrast to the present study, Yanagawa R et al [19] 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

In study done by Duruvasan S et al [18] also concluded statistically insignificantly higher (P value=0.10NS) mean serum LDL. In contrast to current study Mahmoud NS et al [11], Gunes T et al [8] and Duruvasan S et al. [18] Maksoud HMA et al [16]also reported statistically insignificantly higher mean serum HDL level. This could be due to different population characteristics & sample size.

Compared with the values of non-RDS infants, lower levels of cholesterol and HDL cholesterol found in infants with RDS indicated a limited ability to metabolize VLDL, probably related to lipoprotein lipase impairment. Voyno Yasenetskaya TA et al [20] demonstrated that both LDL and HDL cholesterol stimulate primary cultures of type II cells to secrete phosphatidylcholine, the major phospholipid component of pulmonary surfactant.

In present study mean maternal TG (P = 0.159), VLDL (P = 0.102) levels were statistically insignificant and TC (P = 0.0005), LDL (P = 0.044), HDL (P = 0.0001) levels were statistically significantly higher in normal preterm babies group as compared to preterm with RDS babies group. A study done by Gunes T et al [8] recorded maternal lipid levels which were in agreement with current study observations. Another study conducted by Mahmoud NS et al [11] concluded statistically significantly higher levels of maternal TG and VLDL levels in contrast to present study observation.

In our study LDL and HDL were non significantly higher in male babies in both the groups. TG, TC and VLDL were non significantly higher in male babies in normal preterm while TG, TC, VDL were non significantly higher in female babies in preterm with RDS) (P =

>0.005). Other studies conducted earlier by different authors were not consistent on gender distribution [21-23]. Umran Tohmaz R et al [24], Kazemi SA et al [25] reported that gender has no effect on serum lipid levels.

In current study mean cord blood TG, TC and VLDL, LDL, HDL levels were statistically insignificantly higher in all gestational age but TG, TC and VLDL significantly higher in gestational age 28-30 weeks, 31-33 weeks and LDL in 28-30 weeks age groups in normal preterm as compare to preterm with RDS group. Gunes T et al [8]observed that TC, LDL and HDL levels were statistically significantly and VLDL, TG insignificantly higher in all gestational age in normal preterm as compare to preterm with RDS group.

In present study, there was a statistically insignificant poor positive correlation between gestational age and maternal TG, TC, VLDL, HDL and poor negative correlation in maternal LDL levels in preterm with RDS.

In current study, there was a statistically insignificant poor positive correlation between gestational age and babies TG, TC, VLDL, HDL and LDL. In study conducted by Mahmoud NS et al [11] observed a statistically insignificant poor positive correlation between gestational age and baby TG, VLDL, HDL levels which were in agreement with current study results but baby TC (r= 0.720, P=<0.001) and LDL (r = 0.409, P <0.05) levels were statistically significant. Kharb, S et al [26] observed a significant negative correlation between gestational age and cord TG.

In Preterm with RDS babies, there was a statistically insignificant poor positive correlation between maternal TG & babies TG levels, maternal VLDL & babies VLDL levels. There was a statistically insignificant poor negative correlation between maternal TC& babies TC, maternal LDL & babies LDL and maternal HDL & babies HDL levels. Mahmoud NS et al [11] observed that there was a significant positive correlation between maternal TG & babies TG levels (r = 0.452, P <0.05), maternal TC & babies TC (r = 0.44, P <0.05) and maternal VLDL & babies VLDL (r= 0.35, P <0.05). There was a statistically insignificant poor positive correlation between maternal LDL & babies LDL.

In a study done by Gora A et al [27] in low birth babies observed a significant poor negative correlation between maternal TG and babies TG levels (r = -0.194, P = 0.04). They reported a statistically insignificant poor positive correlation between maternal TC and babies TC, maternal VLDL and babies VLDL, maternal LDL and babies LDL, maternal HDL and babies HDL in LBW babies.

Ghiasi A et al [28] also found that there was a positive correlation between maternal TC and neonatal serum TC levels (r = 0.23, P = 0.042). Maternal LDL-C level was positively correlated with neonatal HDL-cholesterol (HDL-C) (r=0.24, P=0.035), total cholesterol (TC) (r=0.29, P=0.01)

Limitations of study

- The sample size of our study was small.
- Confirming the diagnosis of RDS in preterm infants is difficult, because the diagnosis is based upon the presence of a combination of clinical findings.
- Gastric shake test could have been done in addition to clinical diagnosis of RDS
- Maternal lipid levels were not compared with babies anthropometric indices

Conclusion

- Levels of all cord serum lipids were found to be significantly higher in normal preterm babies than preterm with RDS babies. Levels of all maternal lipids were also higher in normal preterm babies than preterm

babies with RDS but significant difference was seen in TC, LDL & HDL. Long-term follow-up will be required to show whether the cord serum levels are related to the late consequences of preterm birth. We suggest that cord and maternal serum lipid levels might be with the potential to detect those infants at risk for RDS that develops postnatally. Further studies on large number of cases and follow up is required to verify our results and to evaluate the level of maternal and babies lipid profile in association with development of RDS

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Legend Tables

Table 1: Demographical profile of cases studied

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 (%)	
Baby gender	Male	18		45.0%	21	52.5%	0.655
	Female	22		55.0%	19	47.5%	
Parity	1	25		62.5%	27	67.5%	0.749
	≥2	15		37.5	13	32.5%	
PROM>24 hours	Yes	23		57.5%	24	60%	1.000
	No	17		42.5%	16	40%	
Mode of delivery	LSCS	12		30.0%	14	35.0%	0.811
	NVD	28		70.0%	26	65.0%	
Dexamethasone before delivery	Yes	22		55.0%	15	37.5%	0.128
	No	18		45.0%	25	62.5%	

SD- Standard deviation, PROM- Premature rupture of membrane, LSCS- lower segment caesarean section, NVD- Normal vaginal delivery

Table 2: Mean Maternal blood lipid level of cases studied

	Group A (Normal pre term) [N=40]		Group B (Preterm with RDS) [N=40]		P value
	Mean	SD	Mean	SD	
TG	181.50	52.25	163.08	63.35	0.159 NS
TC	220.46	48.31	187.62	31.43	0.0005
VLDL	36.76	10.41	32.48	12.70	0.102 NS
LDL	136.54	48.99	118.37	27.69	0.044
HDL	51.56	5.67	37.39	3.95	<0.001

TG-Triglyceride, TC- Total cholesterol, VLDL- Very low-density lipoprotein, LDL- Low density lipoprotein, HDL- High density lipoprotein, SD – Standard deviation, NS- Not significant

TC, LDL and HDL were significantly higher in group A as compared to group B. TG and VLDL was also high but difference was not significant.

Table 3: Correlation between gestational age and maternal lipid profile in Preterm with RDS

		M TG	M TC	M VLDL	M LDL	M HDL
Gestational Age	Pearson Correlation	0.306	0.129	0.311	-0.028	0.246
	Sig. (2-tailed)	0.054	0.426	0.0507	0.863	0.124

M= Maternal, TG-Triglyceride, TC- Total cholesterol, VLDL- Very low-density lipoprotein, LDL- Low density lipoprotein, HDL- High density lipoprotein.

There was statistically significant positive correlation observed between gestational age and maternal TG, VLDL and negative correlation with maternal LDL.

Table 4: Correlation between maternal lipid profile and babies lipid profile in Preterm with RDS

		B TG	B TC	B VLDL	B LDL	B HDL
M TG	Pearson Correlation	0.3034				
	Sig. (2-tailed)	0.057				
M TC	Pearson Correlation		-0.0158			
	Sig. (2-tailed)		0.926			
M VLDL	Pearson Correlation			0.2934		
	Sig. (2-tailed)			0.066		
M LDL	Pearson Correlation				-0.0874	
	Sig. (2-tailed)				0.593	
M HDL	Pearson Correlation					-0.1567
	Sig. (2-tailed)		0.990	0.470		0.336

M= Maternal, B= baby, TG-Triglyceride, TC- Total cholesterol, VLDL- Very low-density lipoprotein, LDL- Low density lipoprotein, HDL- High density lipoprotein, SD – Standard deviation.

There was positive correlation observed between maternal and babies TG, VLDL levels and negative correlation observed between maternal and babies TC, LDL and HDL levels.

Table 5: Mean cord blood lipid level of cases studied

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

TG-Triglyceride, TC- Total cholesterol, VLDL- Very low-density lipoprotein, LDL- Low density lipoprotein, HDL- High density lipoprotein, SD – Standard deviation. TG, TC, VLDL, LDL and HDL were significantly higher in group A as compared to group B