Original Article

Correlation of lipid profile and anthropometry with aortic intima-media thickness in newborns of diabetic and non-diabetic mothers

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ABSTRACT

Background: Exposure to diabetes *in utero* has been established as a significant factor for certain component of the clinical syndrome. Although complications of atherosclerosis occur in adult life, the process begins in early childhood. Hence, measuring aortic intima-media thickness (AIMT) in the newborn is a feasible, accurate, and sensitive marker of atherosclerotic risk. **Objective:** The objective of the study was to find an association of AIMT with cord blood lipid levels and anthropometric factors. **Methods:** Babies born to diabetic mothers (gestational diabetes mellitus/type 2 diabetes mellitus (34 weeks–42 weeks) who were taken as one group and babies born to non-diabetic mothers (34 weeks–42 weeks) who were taken as the other group were enrolled in this study. Lipid values were measured of umbilical cord blood, collected immediately after delivery. Atherogenic-indices were calculated; neonatal anthropometric measurements were taken within 24 h after delivery. Abdominal AIMT (aAIMT) was measured within 4 days of delivery using a high-resolution ultrasound B mode by a trained radiologist. Maternal age, parity, height, pre-pregnancy weight, gestational age, and other investigations were taken from maternal records. **Results:** In this study, birth weight (BW) was positively correlated with triglycerides (TG), cholesterol, high-density lipoprotein (HDL), very low-density lipoprotein (VLDL), and atherogenic-indices, whereas, it was negatively correlated with low-density lipoprotein (LDL) ($p<0.01$). A positive correlation was found between aAIMT and BW, abdominal circumference, TG, cholesterol, HDL, LDL, VLDL, and atherogenic-indices ($p<0.001$). **Conclusions:** In this study, infants born to diabetic mothers had higher anthropometry, lipid values, aAIMT compared to babies born to non-diabetic mothers without risk factors.

Key words: *Atherogenic indices, Cord blood lipid profile, Gestational diabetes mellitus*

Atherosclerotic cardiovascular diseases are the major causes of mortality and morbidity both in developing and developed countries. Barker's hypothesis of the fetal origin of cardiovascular diseases suggested that atherosclerotic lesion may have its origin in the neonatal period [1]. Freinkel's theory of fuel-mediated teratogenesis hypothesized that fetal structures may be exquisitely attuned to fine alterations in maternal fuel economy and that these changes could have long-term effects on the metabolic functions of the offspring [2]. Gestational diabetes is defined as carbohydrate intolerance of variable severity with onset or first recognition during pregnancy. Gestational diabetes mellitus (GDM) is known to alter lipoprotein metabolism. Offsprings of diabetic women are at an increased risk of developing cardiovascular risk factors such as hypertension (HTN), impaired glucose tolerance, dyslipidemia, and overweight or obesity [2].

Several other maternal and fetal factors such as obesity, HTN, and high or low birth weight (BW) can also influence fetal plasma lipids which, in turn, may cause long-term metabolic changes [3,4]. Fetal lipid profile will be deranged either due to genetic programming or due to prepartum or intrapartum stress, and this deranged lipid profile can continue into adult life. Abnormalities in lipoprotein composition and concentration are also associated with macrosomia at birth and elevated lipoprotein levels, which may persist after birth and may play a role in the development of atherosclerosis and diabetes in adult life [5].

Hence such children at risk can be identified in the antenatal and postnatal period, and early intervention with lifestyle modifications in the mother and baby can prevent the development of future cardiovascular complications. There is evidence that children with hypercholesterolemia are at increased risk of subsequent atherosclerosis [6]. Therefore, efforts are being made for the screening of hyperlipidemia in the neonatal period as cholesterol levels in the subsequent periods are likely to be influenced by constitutional and environmental factors. A strong independent relationship between childhood and adulthood serum lipids has been reported in earlier studies [7].

Novel imaging techniques applied during the newborn period will allow a more precise assessment of the effect of the intrauterine environment. Aortic intima-media thickness (AIMT) is an early atherosclerotic marker for vascular wall

lesions [8]. This study thus aims to find an association of AIMT with cord blood lipid levels and anthropometric factors which can serve as a starting point for studying atherogenic factors in later life and their implications for adult cardiovascular diseases.

METHODS

This was a comparative corelational study conducted at a medical college hospital in South India. Neonates born between December 2015 and July 2017 to diabetic and non-diabetic mothers were enrolled in the study. Ethics committee consent and approval was taken. Inclusion criteria were the babies born to diabetic mothers (GDM/type 2 diabetes mellitus (DM) (34 weeks–42 weeks) as one group and babies born to non-diabetic mothers (34 weeks–42 weeks) were taken as the other group. Exclusion criteria were multiple births, congenital anomalies, and syndromes, Appearance, Pulse, Grimace, Activity, and Respiration score at 5 min <7, sick neonate, chronic pancreatitis/thyroid disorders/cushings disease/primary hypercholesterolemia in the mother, and maternal drugs which affect lipid levels.

Estimation of sample size was done by assuming the prevalence of gestational diabetes as 2.7% with 5% absolute precision, 80% power and 95% confidence level the required sample size is 42. Using the formula: $n_0 = Z^2pq/e^2$. Where, Z= z statistic at 5% level of significance, e is the desired level of precision (i.e., the margin of error), p is the (estimated) proportion of the population which has the attribute in question, and q is 1-p.

Consent was obtained from the parents of the enrolled newborns. Pregnant women (2 weeks) and those who were having risk factors were screened as early as possible by diabetes in pregnancy study group India criteria using 75 g of oral glucose load. GDM was diagnosed if 2 h postprandial glucose was >140 mg/dl and confirmed by Carpenter and Coustan criteria using 100 g oral glucose and were diagnosed as GDM if fasting glucose was >92 mg/dl and/or a 1 h value 180 mg/dl and 2 h value 155 mg/dl.

Women who had either type 2 diabetes known before pregnancy or a positive glucose tolerance test (GTT) during pregnancy were taken as cases. The first group (cases – 103) included babies born to women having GDM/Type 2 DM with risk factors such as HTN, overweight/obesity, family h/o diabetes, diabetes in a previous pregnancy, previous h/o large babies, cardiovascular disease (CVD), and without risk factors. The second group (controls – 100) included babies born to non-diabetic women with risk factors such as HTN, overweight/obesity, family h/o diabetes, diabetes in a previous pregnancy, previous h/o large babies, CVD, and without risk factors.

Obstetric details were taken from case records including mother's age, weight, height, pre-pregnancy body mass index, weight gain during pregnancy, and maternal investigations such as random blood sugar, GTT, oral glucose challenge test, hemoglobin A1c, GDM/type 2 DM, HTN, pregnancy-induced HTN, family history of diabetes, CVD, diabetes in previous pregnancies, and previous birth of large for date babies. Gestational age at birth was calculated from the last menstrual period supported by ultrasonography (USG) and Ballard scoring.

Neonates' anthropometry and physical examination were done within 24 h of birth. BW was measured using digital electronic weighing scale (accuracy of 0.1 kg) after uncovering, i.e., removing clothes of the baby and before first feeding; length was measured using infantometer.

According to Fenton Centile chart, there are few definitions. Appropriate for gestational age (AGA) is defined as a BW between 10th and 90th percentile for gestational age. Small for gestational age is defined as BW or Crown-heel length <10th percentile or <2 standard deviation (SD) below the mean for gestational age. Large for gestational age (LGA) is defined as BW > 2 SD or above the 90th percentile for gestational age [9,10]. Ponderal index (PI) calculated by = Weight (g) divided by Length (cm^3) × 100. PI > 2.5 is normal, PI < 2.5 suggests intrauterine growth restriction (IUGR); <2 is asymmetrical IUGR and 2–2.5 is symmetrical IUGR [11].

Head circumference (HC) (occipitofrontal) (after 24 h) was measured using a non-stretchable tape passing above the supraorbital ridge and over occipital protuberance. Chest circumference was measured at the level of nipples after removing the clothes of the newborn using a nonstretchable measuring tape. Abdominal circumference (AC) was measured with non-stretchable tape passing through umbilicus soon after birth before feeding. Mid-arm circumference was measured using nonstretchable measuring tape at the point between acromion and olecranon. Cord blood measurements were done as follows: About 5 ml of umbilical venous blood was collected in a clean, dry vial under aseptic precautions immediately after cord clamping from the maternal umbilical end and allowed to clot at room temperature. Serum was separated by centrifugation and analyzed immediately for lipid profile (total cholesterol [TC], triglycerides [TG], high-density lipoprotein [HDL], cholesterol, very low-density lipoprotein [VLDL] cholesterol, and low-density lipoprotein [LDL] cholesterol) by standard enzymatic methods.

Measurement of serum cholesterol was done by CHOD/PAP Trinder's method, HDL cholesterol by phosphotungstate precipitation method and triglyceride was measured by GPO-PAP Trinder's method. LDL cholesterol was calculated by Friedewald formula (LDL = TC–[VLDL + HDL]). The following atherogenic indexes were calculated**:** TC/HDL and LDL/HDL.

Abdominal ultrasound was done within 4 days of birth to record abdominal AMIT (aAIMT) and was correlated with lipid levels. All high-resolution ultrasound B mode measurements were performed on a USG machine using 2–5 MHz Convex and 3–12 MHz linear array transducers. All ultrasound examinations were performed by the same trained radiologist who was working for the past 15 years. Intima-media thickness was defined as the distance from leading edge of the first echogenic line to that of the second line. The first line represents the lumen-intima interference and the second line represents the collagen containing an upper layer of adventitia.

aAIMT was measured in a straight, non-branched 1 cm longitudinal segment of non-branching distal abdominal aorta. The abdominal aorta was first identified in the upper abdomen using 2–5 MHz convex transducer and then followed distally

until aortic bifurcation was reached. The depth (anterior-posterior direction) and location (craniocaudal direction) of the distal 10 mm of the abdominal aorta were measured from these images. This was used to measure aAIMT using 3–12 MHz linear array transducer. Fig. 1 shows the measurement if aAIMT, for assessment of aAIMT, the image was focused on the dorsal wall of most distal 10 mm of the abdominal aorta and gain settings were used to optimize image quality.

Data collected for this study were analyzed statistically for categorical variables; results were presented as a frequency distribution table and graphically wherever necessary. For inferential statistics to compare between cases and controls, to test the difference in means of anthropometric, lipid levels and aAIMT, student's unpaired t-test was applied. To test the equality of mean of the four groups, namely babies born to diabetic mothers with risk factors, babies born to diabetic mothers without risk factors, babies born to non-diabetic mothers with risk factors, and babies born to non-diabetic mothers without risk factors against not equal means was tested using one-way analysis of variance ANOVA.

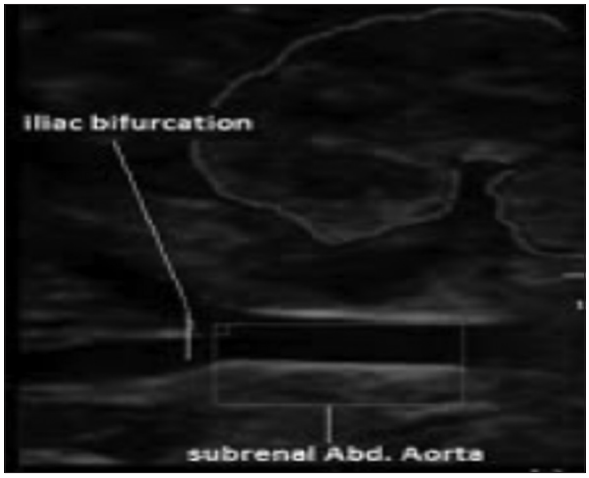

Figure 1: Measurement of abdominal aorta intima-media thickness

Wherever p value has become significant, the *Post hoc* test named Bonferroni's test was applied to observe mean differences.

The Pearson correlation coefficient was computed to find out the relationship between BW, AC, lipid profile, and aAIMT. The results were considered in all the above cases statistically significant when $p<0.005$.

RESULTS

A total of 129 women with diabetes were eligible for the study of which 26 women were excluded due to various reasons such as four intrauterine deaths, four twin pregnancies, eight delivered before 34 weeks of gestation, three congenital anomalies, three birth asphyxia, two delay in sample collection and processing, and two did not give consent. Finally, 103 women were eligible for the study. Out of 103 babies born to GDM/type 2 diabetic mothers, 67 (65%) were term babies and 36 (35%) were late preterm. Out of 100 babies born to non-diabetic mothers, 86 (86%) were term and 14 (14%) were late preterm. Late preterms were more in cases 36 (35%) compared to controls 14 (14%).

In this study, 90 (87.4%) of GDM/type 2 diabetic mothers delivered by cesarean section and 13 (12.6%) by normal vaginal delivery. 36 (36%) of non-diabetic mothers delivered by cesarean section and 64 (64%) by normal vaginal delivery. The rate of cesarean section was higher in diabetic mothers than in non-diabetic mothers. Among GDM/type 2 diabetic mothers, 39 (37.9%) were primigravida and 64 (62.1%) were multiparous, and among non-diabetic mothers, 44 (44%) were primigravida, and 56 (56%) were multiparous. Out of 103 cases, 89 (86.4%) had GDM, and 14 (13.7%) had pre-existing diabetes.

In our study, BW was more among cases compared to controls ($p=0.002$); length was more among cases compared to controls ($p<0.001$). It was observed that the HC was more among cases compared to controls ($p<0.001$) and the same applied to CC also

Table 1: Comparison of neonatal anthropometry, lipid profile, and aAIMT between cases and controls

Parameters	Cases (Mean±SD)	Controls (Mean±SD)	t-value	p-value
BW (kg)	3.18±0.53	2.93±0.62	3.12	0.002
Length (cm)	49.51±2.05	48.29±2.80	3.54	<0.001
HC (cm)	34.36±1.18	33.83±2.41	1.98	0.048
AC (cm)	30.80±1.54	29.89±1.74	3.92	<0.001
CC (cm)	31.51±1.49	30.32±2.10	4.66	<0.001
MAC (cm)	10.38±0.80	9.82±1.01	4.37	<0.001
PI	2.60±0.22	3.01±3.40	-1.24	0.21
Triglyceride (mg/dl)	51.15±13.69	43.20±10.46	4.63	<0.001
Cholesterol (mg/dl)	81.41±17.62	66.21±14.58	6.68	<0.001
HDL (mg/dl)	23.31±4.66	25.64±6.70	-2.87	0.004
LDL (mg/dl)	48.46±14.22	32.67±13.20	8.19	<0.001
VLDL (mg/dl)	10.38±3.82	9.11±2.21	2.90	0.004
LDL/HDL	2.17±0.85	1.34±0.59	8.01	<0.001
TC/HDL	3.61±1.06	2.69±0.72	7.14	<0.001
aAIMT (mm)	0.57±0.08	0.49±0.09	6.48	<0.001

aAIMT: Abdominal aortic intima-media thickness, HDL: High-density lipoprotein, VLDL: Very low-density lipoprotein, LDL: Low-density lipoprotein, PI: Ponderal index, HC: Head circumference

(p<0.001). PI was less among cases compared to controls (p=0.21) while TG was more among cases compared to controls (p<0.001). Cholesterol was more among cases compared to controls (p<0.001). On the contrary, HDL was less among cases compared to controls (p=0.004). LDL and VLDL were more among cases compared to controls (p<0.001) and (p=0.004), respectively. LDL/HDL and TC/HDL ratio was more among cases compared to controls (p<0.001). aAIMT was more among cases compared to controls (p<0.001) and mean BW, length, HC, AC, CC, MAC, TG, TC, HDL, LDL, VLDL, LDL/HDL ratio, TC/HDL ratio, and aAIMT were more in cases compared to controls (p<0.05) (Table 1).

It was observed that the correlation between aAIMT and TG (r=0.5; p<0.001), with cholesterol (r=0.29; p<0.001); LDL (r=0.29; p<0.001); VLDL (r=0.34, p<0.001); LDL/HD (r=0.31; p<0.001); TC/HDL (r=0.34; p<0.001); AC (r=0.43; p<0.001); and BW (r=0.58; p<0.001) was statistically significant. There was statistically significant negative correlation between aAIMT with HDL (r=−0.15; p=0.02).

Among babies born to diabetic mothers with risk factors, correlation between aAIMT with BW (r=0.6; p<0.001); AC (r=0.47; p<0.001); and TG (r=0.22; p=0.04) which was statistically significant. Negative correlation was seen between aAIMT with LDL (r=−0.01; p=0.9) and LDL/HDL(r=−0.01; p=0.9) but was statistically not significant. Among babies born to diabetic mothers without risk factors, positive correlation was found between BW and aAIMT (r=0.48; p=0.04) which was statistically significant. Negative correlation was seen between aAIMT with TC (r=−0.12; p=0.6), with LDL(r=−0.3; p=0.2), with LDL/HDL and TC/HDL (r=−0.3; p=0.1), but was statistically not significant (Table 2).

Among babies born to non−diabetic mothers with risk factors, correlation between aAIMT with TG was r=0.59; (p<0.001), VLDL was r=0.4; (p<0.001), LDL/HDL was r=0.2; p=0.03, TC/HDL was r=0.3; p=0.003, and with BW was r =0.39; p=0.001. Negative correlation was seen between aAIMT with HDL r=−0.15; p=0.1 which was statistically not significant. Among babies born to non-diabetic mothers without risk factors, correlation between aAIMT with TG was r=0.7; (p<0.001), cholesterol was r=0.49; p=0.009, LDL was r=0.59; p<0.001, VLDL was r=0.68; (p<0.001), LDL/HDL was r=0.65; p<0.001, TC/HDL was r=0.7; p<0.001, AC was r=0.6; p=0.001, and BW was r=0.61; p=0.01. Negative correlation was seen between aAIMT with HDL r=−0.31; p=0.1 which was statistically not significant (Table 2).

Table 2 : Correlation between aAIMT with BW, AC, and lipid profile in subgroups of cases and controls

	Diabetic mothers with risk		Diabetic mothers without risk		Total		Non-Diabetic mothers with risk		Non-Diabetic mothers without risk		Total	
	r	p-value	r	p-value	r	p-value	r	p-value	r	p-value	r	p-value
aAIMT and TG	0.22	0.04*	0.45	0.06	0.44	<0.0.01*	0.59	<0.001*	0.7	<0.001*	0.30	0.002**
aAIMT and TC	0.04	0.6	−0.12	0.6	0.40	<0.001*	0.2	0.08	0.49	0.009*	0.30	0.002**
aAIMT and HDL	0.06	0.5	0.45	0.06	0.38	<0.001*	−0.15	0.1	−0.31	0.1	0.29	0.003*
aAIMT and LDL	−0.01	0.9	−0.3	0.2	0.4	<0.001*	0.12	0.2	0.59	0.001*	0.01	0.9
aAIMT and VLDL	0.09	0.3	0.46	0.06	0.26	0.006*	0.4	<0.001 *	0.68	<0.001*	0.65	<0.001*
aAIMT and LDL/HDL	−0.001	0.9	−0.3	0.1	0.03	0.76	0.2	0.03*	0.65	<0.001**	0.27	0.005*
aAIMT and TC/HDL	0.04	0.7	−0.3	0.1	0.15	0.12	0.3	0.003*	0.7	<0.001**	−0.23	0.01*
aAIMT and BW	0.60	<0.001**	0.48	0.04*	0.58	<0.001**	0.39	0.001**	0.61	0.001**	0.45	<0.001**
aAIMT and AC	0.47	<0.001**	0.24	0.3	0.44	<0.001**	0.2	0.08	0.6	0.001**	0.30	0.002**

aAIMT: Abdominal aortic intima-media thickness, TG: Triglycerides, HDL: High-density lipoprotein, VLDL: Very low-density lipoprotein, LDL: Low-density lipoprotein, BW: Birth weight

DISCUSSION

The incidence of diabetes continues to rise, affecting individuals of all ages including children and young adults. This increased prevalence is attributed to lifestyle changes, obesity, and urbanization. In our study, there was a positive correlation between aAIMT and TG with cholesterol, LDL, VLDL, LDL/HDL, TC/HDL, and AC. There was negative correlation between aAIMT with HDL (r= −0.15, p=0.02).

In the study done by Skilton *et al*., infants of diabetic mothers had maximum intima-media thickness (adjusted mean difference between groups 63 μm, 95% CI 4–120 μm, p=0·04) [8], which was statistically

significant. Matteo *et al.* [12] showed that anteroposterior infrarenal abdominal aorta diameter was related to newborn length, HC, and gestational age. Atabek *et al.* [13] showed that intima-media thickness in infants of diabetic mothers (CA-IMT) was not significantly different between the groups and was also not related to atherosclerotic risk factors, but serum lipid and insulin levels were higher in LGA IDM when compared with AGA IDM and controls. They showed that macrosomic IDM were prone to hypertrophic cardiomyopathy but not to atherosclerotic changes in the blood vessels [13].

Mean anthropometric values found were similar to other studies such as Kazemi and Sadeghzadeh [14]. In our study, babies born to GDM/type-2 diabetic mother's had greater weight, length, HC, CC, MAC, and AC, as compared to babies born to non-diabetic mothers [14]. Studies had shown that higher BW may reflect the influence of maternal diabetes in promoting both larger birth size and conferring offspring diabetic risk. Our study showed that mean TG, cholesterol, LDL, VLDL, LDL/HDL ratio, and TC/HDL ratio were more in cases compared to controls. Mean HDL among cases was lower than controls. In the absence of standard cutoff points for lipid levels in the newborns, these values were taken from other studies and they were similar to our study.

Our values were similar to that of study done by Nayak *et al.* [15] (TG, TC, HDL, and LDL were 43.06±15.74 (19–109), 54.21±17.37 (27–150), 22.98±7.86 (8.0–48), 22.65±12.08 (6.4–99.4), respectively), Pratinidhi *et al*., [16] (mean and range of TG, TC, HDL, and LDL were 56.62±31.09 (19–285), 63.03±20.1 (25–125), 24.94±7.23 (14–49), and 97±27.62 (10–97), respectively) lower than Sagar *et al*. and Tailor *et al* [17,18].

Touwslager *et al*. [19] demonstrated that BW, length, and HC of the neonates were associated with impaired endothelial vasodilatation that is an early marker of atherosclerosis. This study also demonstrated that BW, length, and HC were all related to morphological alterations of the newborns' vasculature [19]. Thus, such neonatal parameters when altered could be considered as an expression of morphological and functional early alterations of the cardiovascular system which may increase the cardiovascular risk profile of each individual.

Limitation of this study was the smaller sample size. Further studies with larger sample size and long-term follow-up are required to establish the effect of cord blood lipid levels with aAIMT and anthropometry and metabolic effects occurring in later childhood and adolescents. The degree of maternal glycemic control, diagnostic criteria used to assess GDM and presence of obesity are all confounding factors which play a role while comparing changes in maternal lipids with pregnancy outcomes. Apo-lipoproteins were not measured in this study. High-resolution ultrasound equipment are required for better results.

CONCLUSION

The current study concluded that there is a close relationship between some of the lipid profile parameters and anthropometry at the birth of neonates. Studying for the association of cord blood lipid profile, anthropometry (BW and AC) with aAIMT can serve as a beginning point for studying lipid changes during early life and for correlating them with the cardiovascular diseases in later life by longitudinal studies.

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