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Original Research Article

An Analytical Assessment of the High Maternal Blood Lipid Levels during Early Pregnancy and its Association with Increased Risk of Congenital Heart Disease in Offspring

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Conflict of interest: Nil

Abstract

Aim: This study aimed to investigate whether maternal blood lipid levels during early pregnancy are associated with the occurrence of congenital heart disease (CHD) in their offspring.

Methods: The present study was conducted in the Department of Obstetrics & Gynecology, Madhubani Medical College & Hospital, Madhubani, Bihar, India and Cases were mothers pregnant with a fetus with CHD were included. Controls were women giving birth to healthy infants during the same period of time, and were matched based on gestational week at the first prenatal examination of the cases. Initially 335 controls were selected, after further careful investigation of medical records, three of the controls had a family history of CHD, 23 had their blood test performed after the 14th week of gestation, and 12 controls used lipid-altering medication, and 3 family history of CHD so were excluded. Finally, a total of 200 cases and 300 controls were included in the study.

Results: In general, maternal age of the CHD mother was slightly older compared with the control group. Most of the women were within the normal BMI range. Gestational weeks and BMI were comparable between cases and controls. More mothers with CHD infants were pregnant through assisted conception compared with controls. There were no differences for the sex of the child and parity. There were 40/200 (20%) infants with severe CHD and 160/200 (80%) infants with mild CHD. All suspected cases during pregnancy had undergone neonatal echocardiography.

Conclusion: Elevated maternal lipid profile was associated with increased risk of CHD in offspring.

Keywords: Congenital Heart Defect, Early Pregnancy, Maternal Lipid Profile, Pregnancy, Risk Factor

Introduction

Congenital anomalies are the leading cause of fetal and childhood mortality. [1,2] In Europe, 2.2% of all infants are affected by major nonsyndromic congenital anomalies

(MNCA). [3] There is evidence that the maternal lipid profile could be of importance in the development of congenital anomalies. [4,5] Pregnancy complications and adverse outcomes in the newborn have been related to elevated maternal triglyceride (TG) levels during pregnancy, as well as to high and low levels of maternal total cholesterol (TC). [5-7] It was also reported that high maternal levels of TC, TG and apolipoprotein B (ApoB) are associated with congenital heart disease and neural tube defects. [8] In addition, extremely low and extremely high TC levels were found in mothers of infants with congenital anomalies. [5]

Massive breakthroughs have been achieved in cardiovascular diagnostics and cardiothoracic surgery over the past century, leading to an increased survival of newborns with CHD. Consequently, more patients with CHD reach adulthood, creating a completely new and steadily growing patient population: patients with grown-up congenital heart disease (GUCH). The prevalence of CHD is estimated to be 4 per 1,000 adults. [9] Patients with GUCH often need long-term expert medical care and healthcare-related costs are high. [10]

With the improvement in living standards and an increasing number of women with advanced maternal age, there is an increase in the prevalence of chronic metabolic diseases, such as obesity, diabetes, hypertension, and hyperlipidemia, among pregnant women. Previous research has suggested that an abnormal maternal lipid profile is associated with elevated risk of CHD in offspring.8,11 However, in one study the maternal lipid levels were determined at around 16 months after the index pregnancy, [8] whereas most of the congenital anomalies develop during organogenesis (first trimester of pregnancy). Another study tested maternal triglyceride (TG) levels during early pregnancy, but only limited cases of CHD were included. [11]

In contrast, a large case–control study based on the multicenter population-based National Birth Defects Prevention Study reported that maternal periconceptional dietary fat intake did not increase the odds of CHD after adjusting for total energy intake. However, the food-frequency questionnaire, which is used to assess nutrient intake during the year before pregnancy, was completed at an average of 11 months after delivery. [12] It remains controversial whether maternal lipid profile is associated with CHD risk in offspring. So far, the majority of the research has used food-frequency questionnaires to assess energy intake. In addition, retrospective studies are hampered by recall biases.

Therefore, we conducted this study to investigate whether an abnormal maternal lipid prolife during the early week of pregnancy was associated with CHD in the offspring, to provide some evidence for developing possible intervention strategies.

Materials and Methods

The present study was conducted in the Department of Obstetrics & Gynecology, Madhubani Medical College & Hospital, Madhubani, Bihar, India

Cases were mothers pregnant with a fetus with CHD were included.

Controls were women giving birth to healthy infants during the same period of time, and were matched based on gestational week at the first prenatal examination of the cases.

Initially 335 controls were selected, after further careful investigation of medical records, three of the controls had a family history of CHD, 23 had their blood test performed after the 14th week of gestation, and 12 controls used lipid-altering medication, and 3 family history of CHD so were excluded. Finally, a total of 200

cases and 300 controls were included in the study.

Exclusion criteria

Known consanguinity, abnormal or unknown genetic testing results, family history of CHD, incomplete information on the mother–child pair, and intake of any lipid-lowering agents before sample collection.

Methodology

Data regarding clinical information, ultrasound examination, genetic testing, and pathological results were extracted from electronic medical records. The following variables were collected: maternal age, gestational week, parity, sex of the child, weight and height before pregnancy, method of conception, folic acid, vitamin B12, thyroid abnormality, homocysteine (Hcy), triglyceride (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), free fatty acid, Apolipoprotein-A1 (Apo-A1), Apolipoprotein-B (Apo-B), fasting blood glucose, and hemoglobin A1c (HbA1c). Body mass index (BMI) was calculated as weight/height2.

Perinatal outcome

Pregnant women who came to the hospital for their first prenatal examination at 8–14 weeks of gestation were asked to complete basic information, and fasting blood was drawn for biochemical examination. At 20–24 weeks of gestation an ultrasound examination was performed to screen for fetal abnormalities. Fetal heart abnormalities were evaluated by cardiac screening examination according to the 2013 International Society of Ultrasound in Obstetrics & Gynecology Practice Guidelines.6 After delivery, all newborns underwent routine clinical examination, and screening for CHD was performed using oxyhemoglobin saturation monitoring. Cardiac auscultation was performed daily by the neonatologist until discharge from the hospital. [13] If neonatal CHD was suspected, neonatal echocardiography was performed. The diagnosis of CHD before and after birth was mainly confirmed by echocardiography and/or autopsy. When fetal CHD was suspected on screening, further detailed ultrasonographic examination was performed by senior sonologists. If the diagnosis was confirmed, amniocentesis was performed for karyotyping and human whole-genome single nucleotide polymorphism genotyping was carried out, after counseling, to exclude chromosomal abnormalities. For those who did not undergo prenatal invasive diagnostic procedures, general condition and clinical signs of the newborns were observed by neonatal pediatricians. At the same time, a family history of neonates was considered. Classification of CHD severity was based on previous definitions.[15] In general, we combined critical cases (defects causing death or needing intervention before age 28 days) and serious cases (defects requiring intervention before age 1 year) of CHD as severe CHD, and significant cases (defects persisting beyond age 6 months, but not classified as critical or serious) and non-significant cases (defects not physically appreciable and not persisting after age 6 months) as mild CHD. For combinations of cardiac defects, the major diagnosis was based on either the most hemodynamically significant structural anomaly or the one requiring the earliest intervention.

Biochemical analyses

Fifteen milliliters of fasting peripheral blood samples were collected between 8 and 14 weeks of gestation. Samples were collected and allowed to clot for 30 minutes before centrifugation at 1000 g for 5 min. All fasting blood samples were processed within 2 hours of collection. TG, TC, HDL-C, LDL-C, free fatty acid, Apo-A1, Apo-B, and fasting blood glucose were analyzed by an automatic

biochemical analyzer (Hitachi 7180, WAKO) using commercially available kits. TG and TC were determined by the cholesterol oxidase method (WAKO), HDL-C and LDL-C were tested by the direct assay method, Apo-A1 and Apo-B were tested using the immune transmission turbidity method (Shanghai Beijia Biochemistry Reagents Co., Ltd), and free fatty acid was tested using the enzyme peroxidase end-point method (DiaSys Diagnostic). Blood glucose reagent (hexokinase method) was provided by the Japan Wako Company. TOSOH HLC-723G8 automatic glycosylated hemoglobin analyzer and original matching reagents (high-performance liquid chromatography, TOSOH Co., Ltd) were used to detect HbA1c. Folic acid and vitamin B12 were measured using the Architect i2000chemiluminescence immunoassay analyzer. Serum Hcy measurements were carried out by Liquid Chromatography Coupled to Tandem Mass Spectrometry (LC/MS/ MS) using an API 3000 LC/MS/MS system (Applied Biosystems). Inter-assay coefficients of variation were <10% for all these assays.

Statistical analyses

All significance tests were two sided; a p value of <0.05 was considered statistically significant. Statistical analyses were conducted using Stata version 16.0 (StataCorp.).

Results

Table 1: Baseline characteristics of mothers pregnant with a fetus with congenital heart disease and control mothers

	CHD N=200	Control n=300	P value
Maternal age (years)a (median, range)	31 (20–42)	30 (21–43)	0.01
Gestational week at blood drawinga (median, range)	11.4 (8.3–13.5)	11.4 (8.0–13.2)	0.90
Parity			
Nullipara	160 (80%)	210 (70%)	0.002
Multipara	40 (20%)	90 (30%)	
Sex of the child			
Males	100 (50%)	156 (52%)	0.50
Females	100 (50%)	144 (48%)	
Multiple birth			
Yes	24 (12%)	3 (1%)	<0.001
No	176 (88%)	297 (99%)	
BMIa , kg/m2 (median, range)	21.03 (14.36–33.25)	20.83 (14.83–38.53)	0.29
BMI < 18.5	24 (12%)	45 (15%)	0.52
18.5 ≤ BMI < 24	140 (70%)	210 (70%)	
24 ≤ BMI < 28	30 (15%)	36 (12%)	
BMI ≥ 28	6 (3%)	9 (3%)	
Gestational diabetes mellitus			
Yes	30 (15%)	15 (5%)	<0.001
No	170 (85%)	285 (95%)	
Thyroid abnormality			
Hypothyroidism	12 (6%)	15 (5%)	0.20
Hyperthyroidism	4 (2%)	3 (1%)	
Thyroid inflammation	8 (4%)	24 (8%)	
Normal	176 (88%)	258 (86%)	

Pregnant through assisted conception			
No	168 (84%)	288 (96%)	<0.001
Yes	32 (16%)	12 (4%)	

Table 1 presents the general characteristics of the study population. In general, maternal age of the CHD mother was slightly older compared with the control group. Most of the women were within the normal BMI range. Gestational weeks and BMI were comparable between cases and controls. More mothers with CHD infants were pregnant through assisted conception compared with controls. There were no differences for the sex of the child and parity.

Table 2: Number and percentage of congenital heart disease subtypes

CGD Subtypes	N%
Ventricular septal defect (VSD)	110 55%
Pulmonary stenosis (PS)	25 12.5%
Tetralogy of Fallot (TOF)	20 10%
Coarctation of the aorta (COA)	4 2%
Coronary arterial fistula (CAF)	4 2%
Transposition of the great arteries (TGA)	2 1%
Hypoplastic right heart (HRH)	2 1%
Total anomalous pulmonary venous drainage (TAPVD)	2 1%
Double aortic arch (DAA)	2 1%
Atrioventricular septal defect (AVSD)	2 1%
Tricuspid regurgitation (TR)	2 1%
Hypoplastic left heart (HLH)	1 0.5%
Double outlet right ventricle (DORV)	1 0.5%
Ebstein's anomaly	1 0.5%
Bicuspid aortic valve (BAV)	1 0.5%
Complex congenital heart disease	21 10.5%
Total	200 (100%)

There were 40/200 (20%) infants with severe CHD and 160/200 (80%) infants with mild CHD. All suspected cases during pregnancy had undergone neonatal echocardiography. During the prenatal screening, we missed six fetuses with major CHD, including two cases of tetralogy of Fallot, two cases of total anomalous pulmonary venous drainage, one case of coarctation of the aorta, and one case of transposition of the great arteries.

Table 3: Biomarkers in blood of mothers pregnant with fetuses with congenital heart disease and control mothers in the first trimester of pregnancy

	CHD N=200	**Control n=300**	**Reference Values**	**P value**
Total cholesterol, median (range), mmol/L	4.41 (2.92–6.28)	4.36 (2.58–8.21)	3.10–5.69 mmol/L	0.70
Triglycerides, median (range), mmol/L	1.35 (0.50–3.81)	1.15 (0.44–2.84)	<1.70 mmol/L	<0.000 1
Free fatty acids, median (range), mmol/L	0.53 (0.12–1.20)	0.50 (0.10–1.24)	0.10–0.45 mmol/L	0.06
HDL-cholesterol, mean ± SD, mmol/L	1.01 ± 0.19	1.01 ± 0.17	0.80–2.35 mmol/L	0.99

LDL-cholesterol, mean ± SD, mmol/L	2.77 ± 0.49	2.79 ± 0.39	<3.12 mmol/L	0.35
Total/HDL-cholesterol, median (range)	4.43 (3.61–6.64)	4.45 (3.75–5.08)		0.46
Apolipoprotein A-1, Median (range), g/L	1.19 (0.54–2.23)	1.13 (0.70–1.78)	1.00–1.60 g/L	0.01
Apolipoprotein B, median (range), g/L	0.78 (0.48–1.29)	0.75 (0.46–2.79)	0.60–1.10 g/L	0.01
HbA1c, median (range), %	5.0 (3.9–5.8)	5.0 (4.0–15.8)	≤6.5%	0.57
Blood glucose,e mean ± SD, mmol/L	4.49 ± 0.38	4.49 ± 0.34	<5.1 mmol/L	0.94
Vitamin B12, median (range), pg/mL	489 (190–1450)	480.50 (13.90–2000.00)	187–883 pg/ml	0.74
Vitamin D, mean ± SD, ng/mL	19.10 (4.50–43.00)	16.90 (5.20–42.60)	9.5–55.5 ng/ml	<0.0001
Folate, median (range), ng/mL	16.10 (3.80–24.36)	16.30 (4.70–20.00)	3.1–20.5 ng/ml	0.67
Total homocysteine,e mean ± SD, µmol/L	7.55 ± 2.29	6.93 ± 1.90	5–15 µmol/L	0.003

Table 3 presents blood biomarkers in the total group. Levels of TG, Apo-A1, and Apo-B were significantly higher in the case group than the control group (all $p < 0.05$). There were no significant differences of HbA1c and fasting blood glucose levels between the two groups. No difference was observed for folate level between the two groups, but the Hcy level was significantly higher in the case group ($p = 0.003$) and the vitamin D level was significantly higher in the CHD group ($p < 0.0001$).

Discussion

Congenital heart defects (CHDs), defined as gross structural abnormalities of the heart or intrathoracic vessels, affect 5 to 15 per 1000 live births. [15,16] CHDs constitute the most common congenital anomaly subgroup among newborns and have emerged as one of the most important causes of infant mortality. [17,18] Furthermore, CHDs have a significant impact on child and adult morbidity and disability. [19] One important area for focus in obstetrics is prenatal diagnosis of severe CHDs. Such early diagnosis permits optimal care during pregnancy, during delivery, and in the newborn period (including surgical correction of the defect) and pregnancy termination for lethal and very severe heart defects. It is therefore important to identify pregnant women at higher risk for CHDs to facilitate targeted screening. It also allows targeted preconception counseling to improve reproductive outcomes and to promote primary prevention. It is important to have reliable information about worldwide CHD birth prevalence because this may lead to better insight into its etiology. In addition, dedicated care could be better planned and provided. Variation in CHD occurrences over time and worldwide has been suggested, but a complete overview is missing.

The main functions of TG are to supply and store energy, and to fix and protect the internal organs. A pilot study from India has reported that a high level of maternal TG was associated with increased risk of neural tube defects. [20] A cohort study from Amsterdam found that both decreased and elevated TG levels in early pregnancy were associated with increased risk of congenital anomalies, especially cardiovascular congenital anomalies, in offspring. [12]

As a major apolipoprotein of HDL-C, Apo-A1 plays a role in regulating reverse cholesterol transport and HDL particle metabolism. Therefore, Apo-A1 may have a significant effect on the maternal lipid profile during pregnancy, and may play a supportive role in regulating maternal and fetal cholesterol homeostasis. [21] Apo-B and Apo-B/Apo-A1 can predict atherosclerotic lipid disorders and cardiovascular risk in adults. A previous study has reported that Apo-B as well as Apo-B/Apo-A1 are associated with an almost twofold increased CHD risk,8 while in another study, the association of Apo-B and Apo-A1 with congenital anomalies in offspring was absent. [12] In our study, we found that the maternal levels of Apo-B and Apo-A1 were significantly higher in the CHD group compared with the control group, and after adjusting for possible confounders, the association between Apo-A1 and CHD risk in offspring remained. During the early development stage, transfer of maternal cholesterol through the placenta is crucial when the fetus cannot synthesize its own cholesterol, and changes in maternal Apo-A1 level during early pregnancy may affect the transport of cholesterol, and so affect fetal development.

Cholesterol is an important nutrient for normal fetal development. In addition, it is an essential component of cell membranes and steroid hormones. Previous research has reported a trend of increased microcephaly risk among neonates of mothers with low serum cholesterol. [22] In contrast, a study from the Netherlands reported that abnormal cholesterol, LDL-C, and TC/HDL-C was associated with increased risk of CHD in offspring. [8] Our study did not observe significant differences of cholesterol level between the cases and controls, but TC/HDL-C was significantly higher in the case group. After adjusting for possible confounders, maternal TC/HDL-C remained a risk factor for CHD in offspring. A meta-analysis including 61 prospective observational studies has suggested that TC/HDL-C is a very strong predictor of ischemic heart disease mortality, which is more informative than TC. [23]

In the current study, maternal levels of folate were relatively high and were comparable between cases and controls. Genetic and biochemical studies have shown that the preventive effect of folate is through lowering the level of hyperhomocysteinemia, which is a biomarker of oxidative stress and can induce the occurrence of CHD. [24,25] We found that the Hcy level was significantly elevated in the case group, indicating that oxidative stress may have increased in mothers of offspring with CHD. [26]

Conclusion

Our study reported that mild dyslipidemia in early pregnancy was closely associated with the occurrence of CHD in offspring. In combination with previous research, it is possible that lipid metabolism in early pregnancy may influence the outcome of pregnancy. However, the association needs to be further investigated by large prospective studies as well as mechanistic studies to establish its causality. However, prevention is the key. Based on current knowledge, it is helpful to encourage lifestyle intervention strategies such as regular exercise, healthy diet, and weight control that may improve the lipid profile in the preconception period and early pregnancy, which may achieve better health in offspring.

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