Original Article
Dyslipidemia in pregnancy may contribute to increased risk of congenital heart defects

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Abstract: Objective: To investigate the relationship between the presence of dyslipidemia in pregnant females in China and the risk of congenital heart defects (CHDs). Methods: A total of 54 pregnant females at 24 to 28 weeks (w) of gestation were enrolled in this study between March 2013 and June 2014. The case group included 18 females who had a fetus with cardiac defects, and each case was matched with 2 controls with no pregnancy complications. The mean ages were 29.06 (SD=3.11) and 29.03 (SD=3.9) years in the case and control groups, respectively. The main outcome measures were the levels of total cholesterol (TC), triglycerides (TGs), low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c) and apolipoprotein. Results: The subjects in the case group had significantly higher mean LDL-c (4.15 vs. 3.45) and apolipoprotein B levels (2.23 vs. 1.78) (all $P<0.05$) than those in the control group. After adjusting for potential confounders, a lipid profile with apolipoprotein B>1.81 mg/dl was found to be associated with a higher incidence rate of CHDs (61% vs. 22%). Conditional logistic regression analysis revealed that the CHD risk increased with each increase in the standard deviations of LDL-c and apolipoprotein B. Conclusions: The serum LDL-c and apolipoprotein B levels were significantly higher in the case group than in the control group. An increased apolipoprotein level was associated with a higher incidence rate of CHD. Thus, increases in the LDL-c and apolipoprotein B levels may be involved in the pathogenesis of CHD.

Keywords: Congenital heart defects, pregnancy, lipoproteins, cholesterol, risk factors

Introduction

Congenital heart defects (CHDs) affect approximately six to eight infants per 1000 live births annually and are a leading cause of infant death due to cardiovascular malformations [1]. Despite considerable advances in the understanding of CHDs, the etiology remains unknown in many cases. It is widely accepted that complex interactions between maternal environmental and genetic factors may result in CHDs [2, 3]. Maternal metabolic disorders such as diabetes, obesity and hyperhomocysteinemia are associated with an increased risk of adverse pregnancy outcomes, including CHDs in offspring [4-6]. These conditions have been implicated in the development of cardiovascular disease in adults [7, 8]. In particular, high concentrations of total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-c) increase the risk of cardiovascular disease.

However, little is known regarding how dyslipidemia in pregnancy affects fetal development. Recently, some evidence has been presented suggesting that maternal fat intake and lipid metabolic abnormalities affect fetal heart development.

The maternal intake of fat, riboflavin and nicotinamide increases the risk of having offspring with CHDs [9]. An increase in the maternal lipid level at approximately 16 months after index pregnancy has been reported to be associated with an increased risk of CHDs in offspring [10]. However, the lipid levels after pregnancy differ from those during pregnancy [11].

Thus far, both animal and human studies focusing on the association between dyslipidemia in pregnancy and CHDs in offspring are lacking. Thus, we aimed to investigate the association between the lipid levels in pregnancy and the risk of CHDs in offspring.

Table 1. General characteristics and lifestyle of two groups

Demographics	Case group (n=18)	Control group (n=36)	P-value
Age, y	29.06±3.11	29.03±3.9	0.93
Gestational weeks	26±1.57	26±1.49	0.93
Body Mass Index (BMI), kg/m²	23.38±2.58	23.14±3.11	0.83
Family history of CHD	2 (11)	3 (8)	0.74
Lifestyle			
Periconception use of Cigarettes (any)	3 (17)	5 (14)	0.78
Folic acid and/or (multi) vitamins	9 (50)	19 (53)	0.11
Medication	2 (11)	4 (11)	0.99
Current use of			
Cigarettes (any)	2 (11)	4 (11)	0.99
Folic acid and/or (multi) vitamins	8 (44)	15 (41)	0.38
Medication	3 (17)	5 (14)	0.78

Abbreviations: CHD, congenital heart disease. Values are Mean ± SD or numbers (percentage).

Table 2. Biomarkers in blood from case group and control group

Biomarkers	Case group (n=18)	Control group (n=36)	Reference values[a]
Total cholesterol, mmol/l	6.48±0.97	6.38±0.99	<5.7
Triglycerides, mmol/l	2.49±0.64	2.51±0.74	<1.7
HDL-cholesterol, mmol/l	1.9±o0.43	1.95±0.7	>1.09
LDL-cholesterol, mmol/l	4.15±0.88*	3.45±0.76	<3.6
Total cholesterol/HDL-cholesterol	3.47±0.45	3.46±0.78	
Apolipoprotein A-1, g/l	2.35±0.48	2.32±0.47	<2
Apolipoprotein B, g/l	2.23±0.2*	1.78±0.19	<1.75
Apolipoprotein B/A-1	0.54±0.13	0.46±0.09	
Homocysteine nmol/ml	0.44±0.38*	0.17±0.1	
FPG mmol/L	4.35±0.29	4.35±0.34	<5.6
FINS µU/mL	11.8±8.72	7.9±4.25	<10
HOMA-IR	2.23±1.47	1.56±0.93	

*P<0.05. [a]Reference values according to the Clinical Chemistry laboratory of the the First Affiliated Hospital of Sun Yat-sen University in Guangzhou, China.

Material and methods

Subjects

A total of 54 pregnant females at 24 to 28 weeks (w) of gestation were enrolled in this study; 18 of them had a fetus with cardiac defects, and each case was matched with 2 controls with no pregnancy complications. These controls were scanned on the same day, and their fetuses resulted in the live births of phenotypically normal neonates. All participants were from Guangzhou, China, and they were recruited from among patients who had been referred for antenatal examination and regular antenatal check-ups between 2013 and 2014.

The crown rump length (CRL) was used to estimate gestational age. Maternal demographic and clinical data and serum samples were obtained for determination of the pregnancy-associated levels of lipids (TC, triglycerides (TGs), LDL-c, high-density lipoprotein cholesterol (HDL-c) and apolipoprotein), fasting insulin (FINS) and fasting glucose (FPG) at 24 w and 28 w of gestation. Nuchal translucency (NT) thickness was measured to estimate the aneuploidy risk at 11 to 14 w of gestation. Karyotype and/or newborn examinations were performed to assess the chromosomal status. The CHD status was determined by prenatal imaging and/or postnatal imaging and by physical examination for the controls. The CHD phenotypes consisted of Tetralogy of Fallot (n=3), atrioventricular septal defect (n=1), perimembranous ventricular septal defect (n=5), double outlet right ventricle (n=1), single ventricle defect (n=1), right-sided aortic arch (n=4) and miscellaneous (n=3).

All cases with aneuploidy or no cardiac defects that were diagnosed prenatally or during the neonatal period were excluded. All CHD diagnoses were made by a specialist in fetal echocardiography. For the pregnancies that resulted in live births, the CHDs were confirmed in the newborns. For cases that underwent termination of pregnancy for which an autopsy was not performed, the diagnosis was made based on prenatal examination performed by expert fetal echo cardiologists.
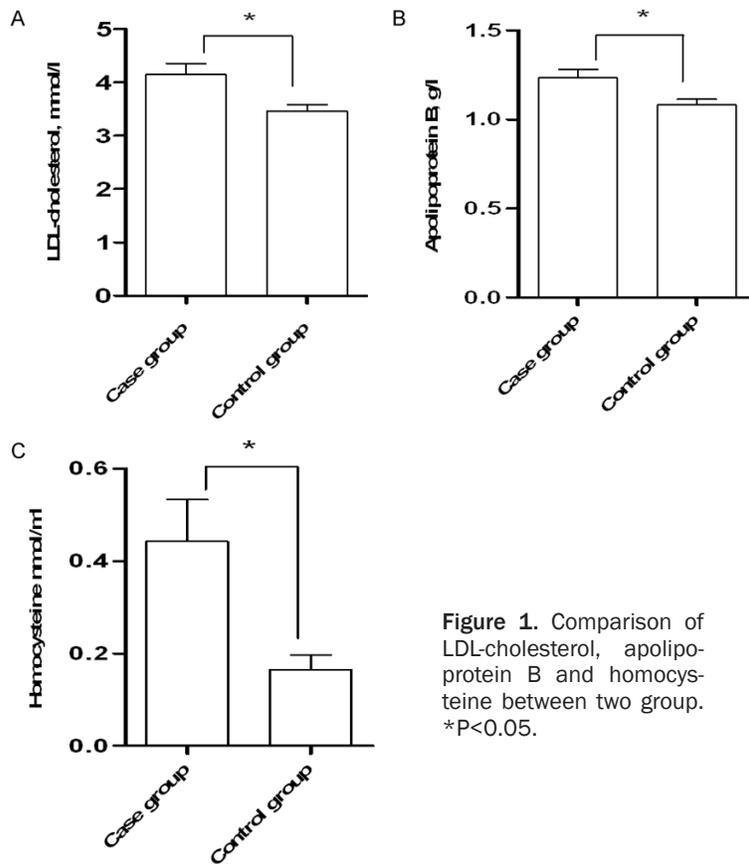
A

B

C

Figure 1. Comparison of LDL-cholesterol, apolipo-protein B and homocys-teine between two group. *P<0.05.

-20°C freezer, and the samples were transferred to racks and stored at -80°C within 24 hours.

The nature and purpose of the study were carefully explained to the subjects and their families before obtaining written informed consent. This study was approved by the Institutional Ethical Board of the First Affiliated Hospital of Sun Yat-sen University.

Biochemical tests

The FPG, FINS, TC, TGs, LDL-c and HDL-c levels were determined using fasting blood samples. All assays were performed at the biochemical laboratory of the First Affiliated Hospital of Sun Yat-sen University. The homeostasis model assessment-insulin resistance (HOMA-IR) index, an index of insulin resistance, was calculated according to the following equation as previously described [12]: HOMA-IR=FINS×FPG/22.5, with FINS expressed in µU/mL and FPG expressed in mmol/l.

Statistical analysis

All participants completed a questionnaire concerning lifestyle behaviors and demographic data. The extracted data included the maternal age, number of gestational weeks, presence of pre-existing diabetes, educational level, ethnicity and the use of alcohol, cigarettes and vitamin supplements. Periconception vitamin supplement use was defined as daily intake during the entire periconception period. The data obtained on vitamin supplements included information on the content (for folic acid only or multivitamin supplement) and frequency of intake. Subjects were considered to use alcohol or cigarettes if they reported any consumption during the questioned periods.

The samples were immediately transferred to the laboratory within 5 minutes of collection. They were left standing at room temperature for 10-15 minutes to allow for clotting before being processed. Then, they were centrifuged at 3000 rpm for 10 minutes to separate the serum. The samples were subsequently stored in a blue box that had previously been numbered. The blue box was temporarily stored in a

The subjects' demographic and clinical characteristics were summarized as the mean and standard deviation (mean ± SD) for continuous data and as n (%) for categorical data by group; differences between groups were compared using the two-sample t-test for continuous data, and dichotomous variables were assessed using the chi-square test. Upper quartiles of the lipid levels were determined based on the control data, and the CHD risk was calculated using the multivariable logistic model by comparing the upper tertile to the lower quartiles as references. Conditional logistic regression analyses with the lipids as continuous variables were performed to estimate the relative risk of CHDs by calculating odds ratios (ORs) and 95% confidence intervals (CIs). All statistical assessments were two-tailed, and a

Table 3. The distribution of lipid level in case group and control group

Biomarkers	Cases/controls	Cut-off value	Case group	Control group	P-values
Total cholesterol, mmol/l	18/36	>7.175	6 (33)	9 (25)	0.519
Triglycerides, mmol/l	18/36	>2.955	5 (27)	8 (22)	0.653
HDL-cholesterol, mmol/l	18/36	<1.61	5 (27)	9 (25)	0.826
LDL-cholesterol, mmol/l	18/36	>4.212	7 (38)	9 (25)	0.292
TC/HDL-cholesterol	18/36	>3.953	2 (11)	9 (25)	0.232
Apolipoprotein A-1, mg/dl	18/36	>2.517	9 (50)	9 (25)	0.066
Apolipoprotein B, mg/dl	18/36	>1.81	11 (61)	8 (22)	0.005
Apolipoprotein B/A-1	18/36	>0.533	7 (38)	8 (22)	0.197

Cut-off values are upper or lower quartile based on control group. Values are numbers (percentage).

Table 4. Associations between lipids as continuous variables and CHD risk

Biomarkers	B	SE	OR (95% CI)	P
Total cholesterol	0.128	0.271	1.137 (0.668-1.934)	0.636
Triglycerides	-0.049	0.43	0.952 (0.4082-2.22)	0.909
HDL-cholesterol	0.014	0.454	1.014 (0.417-2.467)	0.976
LDL-cholesterol	4.64	1.15	1.59 (1.01-2.94)	0.02
Apolipoprotein A-1	-0.072	0.877	0.931 (0.167-5.193)	0.935
Apolipoprotein B	3.696	1.75	40.27 (1.31-1242.46)	0.035

OR, odds ratio; CI, confidence interval. Odds ratios are adjusted for maternal age, BMI, family history of CHD, periconception folic acid and/or (multi) vitamin use, homocysteine.

P<0.05 was considered statistically significant. All statistical analyses were performed using SPSS 17.0 statistical software (SPSS Inc., Chicago, IL, USA).

Results

General characteristics and lifestyle

A total of 54 subjects were enrolled in this study, including 18 subjects in the case group and 36 in the control group. The average ages of the subjects in the case and control groups were 29.06 (SD=3.11) and 29.03 (SD=3.9) years, respectively. Summaries of the subjects' demographic data and clinical characteristics for the two groups are summarized in **Table 1**. The age, number of gestational weeks, body mass index (BMI), CHD family history, smoking status and folic acid use of the pregnant females were comparable between the groups.

Biomarkers

The subjects in the case group had significantly higher mean LDL-c (4.15 vs. 3.45), apolipoprotein B levels (2.23 vs. 1.78) and homocysteine (0.44 vs. 0.17) (all P<0.05) than those in the control group (**Table 2** and **Figure 1**). The TC, TGs, HDL-c, apolipoprotein A-I, FPG, FINS and HOMA-IR levels were comparable between the groups.

Associations between lipids and CHD risk

After adjusting for potential confounders, a lipid profile with apolipoprotein B>1.81 mg /dl was determined to be associated with a higher incidence rate of CHDs (61% vs. 22%) (**Table 3**). Conditional logistic regression analysis revealed that the CHD risk increased with increasing standard deviations of the LDL-c and apolipoprotein B levels (**Table 4**). However, none of the covariates confounded the association between the lipid level and CHD risk in the pregnant females. In addition, the TC, TGs, HDL-c and apolipoprotein A-1 levels in the pregnant females were not associated with the CHD risk.

Discussion

The results of this study demonstrated that increased lipid levels in pregnancy, including LDL-c and apolipoprotein B, were associated with an increased risk of having a child with CHD. The apolipoprotein B level is the strongest predictor of the CHD risk. These findings are very interesting because apolipoprotein B is the main structural protein of atherogenic lipoproteins [13]. Further, this protein reflects the entire spectrum of proatherogenic particles and thus serves as a valid biomarker for the atherogenicity of LDL-c [14, 15]. A growing number of studies have shown that maternal diet

and the presence of a metabolic disorder during gestation are closely related to CHDs in offspring. Abnormal folate metabolism has been linked to CHDs in both human and animal studies [5]. There is extensive evidence of associations between alterations in the homocysteine and methionine levels and the development of CHDs [16]. Recently, Ray et al have shown that there are significant alterations in the levels of acylcarnitine, sphingomyelin, and other metabolites in CHD pregnancies during the first trimester [17].

To the best of our knowledge, an association between an altered gestational lipid profiles and the CHD risk has not yet been reported. Our findings are consistent with a previous study reporting the higher dietary intake of saturated fats by mothers of children with CHDs compared with control mothers [9], as well as with a study demonstrating alterations in the lipid profiles of mothers of children with CHDs at 16 months after the index pregnancy [10]. Taken together, these findings suggest that alterations in the maternal lipid levels may be associated with an elevated risk of congenital heart disease in offspring. However, the specific causality of this association needs to be further studied.

Direct adverse effects of elevated maternal serum lipid levels on embryonic heart development are biologically plausible. Depending on the genetic background of the embryo, exposure to a slight alteration in the maternal lipid profile may have variable effects on genes and tissues, specifically the neural crest, which is involved in cardiogenesis [18]. Normal fetal development requires the availability of both essential fatty acids and long-chain polyunsaturated fatty acids, and the nutritional status of the mother during gestation has been correlated with fetal growth and the metabolic profiles [19, 20]. However, the excessive intake of specific long-chain fatty acids may cause both a decrease in the arachidonic acid level and enhanced lipid peroxidation, thereby reducing the antioxidant capacity [21, 22]. In our study, the level of homocysteine, a biomarker of oxidation, was increased in the mothers of children with CHDs. This finding is in agreement with the results of a previous study showing that the levels of biomarkers of oxidative stress related to the transsulfuration pathway are higher in mothers of children with CHDs than in

controls [23]. In a mouse model, maternal high-fat feeding primes steatohepatitis in adult mouse offspring, which involves mitochondrial dysfunction and altered lipogenesis gene expression [24, 25]. Moreover, in mice, a maternal diet high in saturated fats during gestation and lactation results in dyslipidemia and aortic vascular dysfunction in female offspring by altering hepatic LDL receptor mRNA expression programming [26]. Thus, abnormal maternal lipid metabolism may alter gene expression in offspring. Furthermore, these changes may also exist during heart development. Thus, there may be a vulnerable time window during which even slightly increased maternal lipid levels in combination with excessive oxidative stress in the mother and embryo may cause altered cardiogenesis.

This study has some limitations, including the use of cross-sectional data from a relatively small cohort that were analyzed retrospectively. In addition, we did not investigate the mechanisms underlying dyslipidemia in pregnancy that may contribute to an increased risk of CHDs. Further prospective studies with larger samples are needed to clarify the role and mechanisms of dyslipidemia in pregnancy in association with the CHD risk in the sub-population of pregnant females and in association with specific CHD phenotypes.

In conclusion, the results of this study showed that the LDL-c and apolipoprotein B levels were significantly higher in the case group than in the control group. After adjusting for potential confounders, a lipid profile with apolipoprotein B>1.81 mg /dl was associated with a higher incidence rate of CHD. Taken together, our results suggest that dyslipidemia in pregnancy may contribute to an increased risk of CHDs.

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Disclosure of conflict of interest

None.

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