

Previous History of Gestational Diabetes Mellitus and the Risk of Early Onset Subclinical Atherosclerosis

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Abstract

Background: Women with history of gestational diabetes mellitus (GDM) are at higher risk for developing type 2 diabetes mellitus and metabolic syndrome. The aim of the present study was to investigate whether this population of women also are at increased risk for early subclinical atherosclerosis.

Methods: Twenty women with previous history of GDM and 20 unaffected women were recruited in the study. Two groups were matched based on their age, BMI and parity. The maximum duration from affected pregnancy was set at 5 years. The carotid intimal-medial thickness (CIMT), multiple cardiovascular risk factors along with fasting blood levels of glucose, insulin, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, vitamin B12 and folic acid were measured in two groups.

Results: The mean fasting blood glucose ($p=0.03$) and insulin ($p=0.03$) levels were significantly higher in women with pGDM compared to the control group. Women with pGDM were presented with significantly higher levels of CIMT than control group (0.510 mm vs. 0.478 mm, $p=0.037$). CIMT positively correlated with fasting insulin ($r=0.376$, $p=0.02$).

Conclusion: Women with pGDM are at increased risk for premature atherosclerosis. This might be due to the persistence of disturbance in glucose homeostasis after delivery in this group of women.

Keywords: Carotid intima-media thickness, Gestational diabetes mellitus, Subclinical atherosclerosis, Type 2 diabetes mellitus

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Introduction

Gestational diabetes mellitus (GDM) identified as different degree of the glucose intolerance with onset of first recognition during pregnancy. Approximately 135,000 cases of GDM (3-8% of all pregnancies), are diagnosed annually in the U.S (1). In Iran, the incidence of GDM has been reported to be varied between 4-7% (2-3).

GDM is a heterogeneous disorder which factors such as age, obesity and genetic background could contribute to the severity of the disease. Although the GDM resolves after delivery, many of women with previous GDM (pGDM) have an increased risk of developing type 2 diabetes mellitus (DM) in the years following delivery. The chance of developing type 2 DM increases up to 70% after 28 years of delivery in these women (4), which is almost seven times more than control group. Women with GDM are also at increased risk of developing other cardiovascular risk factors (5-6) such as obesity, hypertension, dyslipidemia, and the metabolic syndrome. Higher lipid and blood pressure levels have been found at 5-6 years follow-up of these women (7). Hypertension, hyperlipidemia and DM are the major risk factors of coronary heart disease. Coronary heart disease (CHD) is a clinical manifestation of atherosclerosis which is responsible for 7.2 million annual deaths worldwide (8). The atherosclerosis process begins early in the life and remains asymptomatic for many years. About 50% of men and 64% of women in the US die suddenly due to CHD without having prior symptoms of CHD (9); however, if this asymptomatic subclinical atherosclerosis left undiagnosed and untreated, it can lead to fatal clinical events such as myocardial infarction and stroke.

One of the non-invasive measures that could be used to detect the subclinical atherosclerosis is Carotid intima-media thickness (CIMT). CIMT as measured by B-mode ultrasound represents the combined thickness of the intimal and medial layers of the carotid artery and has been validated to be a surrogate marker of subclinical atherosclerosis disease (10-11). CIMT is also shown to be related to the vascular disease mortality and morbidity risk (12).

In view of the above considerations, it is apparent that the diabetes mellitus, HTN and

hyperlipidemia are considered as established risk factors for cardiovascular disease (CVD) and subclinical atherosclerosis. However, it is uncertain that women with pGDM who do not subsequently develop the traditional risk factors of CVD are also at increased risk of subclinical atherosclerosis. Assessment of vascular function reflected by measurement of CIMT has been performed in few studies during and after GDM complicated pregnancy. Tarim *et al* (12) studied 30 Turkish women with GDM and 40 normoglycemic women of similar age and BMI in the second trimester of pregnancy. They observed that CIMT in women with GDM was significantly higher compared with non-GDM pregnant women. Another study of CIMT in women with a history of GDM involved 28 women with and 24 women without a history of GDM 2 years after delivery. None of the enrolled women were diabetic. The mean common CIMT was greater among the women with prior GDM (13). However, to our knowledge, no study regarding this issue in national level has been performed to evaluate the risk of premature atherosclerosis development in women with history of GDM.

The aim of the present study was to evaluate if women with pGDM and without clinical manifestations of coronary heart disease are at increased risk of developing subclinical atherosclerosis as measured by CIMT.

Methods

Study population

This historical cohort study was conducted in the endocrine and metabolism research center of Tehran University of Medical Sciences. The participants were selected from outpatient women referred to Dr. Shariati hospital, Teheran, Iran. The investigation was conducted between August 2008 and August 2009 and involved 20 non-pregnant women with the history of GDM and 20 non-pregnant unaffected women as control subjects. Two groups were matched based on the age, BMI, parity. Study protocol was approved by the ethics committee of endocrinology and metabolism research center which was compatible with declaration of Helsinki. An informed written consent was obtained from all the participants. Using instructed

questionnaire, the maternal data including age, parity, habits and pregnancy events were collected.

The inclusion criteria were non-menopause non-pregnant women, aged between 20-44 years old. Patients with pre-existing HTN, HTN, DM, and symptomatic CVD were excluded from the study. Indeed patients were excluded if the duration between indexed pregnancy and enrollment was more than 5 years.

Screening of the patients to not having the diabetes mellitus was performed with measuring the FBS and random blood sugar. Patients were excluded if the fasting blood sugar or blood sugar were higher than 126 and 140 mg/dl, respectively.

Laboratory measurements

Height was measured with a stadiometer. Weight was measured on a calibrated beam balance. Blood pressure was measured using a standard calibrated mercury sphygmomanometer on the right hand after participants had been sitting for at least 5 minutes. Venous blood samples were collected in the morning after 12 h of fasting. The blood samples were centrifuged and then serum was collected for measuring the biomedical indices.

Plasma levels of glucose, triacylglycerol, total cholesterol, high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL), creatinine and BUN were measured by a colorimetric method using pars azmoon kit in an auto-analyzer. The intra and inter-assay coefficients of variation (CVs) for all these measurements were less than 3.5%. Insulin concentration of the serum was assessed by immunoassay (ELISA) using a Bioscience kit (DRG) with intra and inter-assay CVs were: 5.2% and 7.2% respectively. Plasma Folic acid and vit B12 concentrations were measured by immunoassay (ELISA) using a Bioscience kit (DRG). The intra and inter- assay CVs for folic acid were 6.9% and 7.5% and for vitamin B12 were 5.7% and 7.9%.respectively. Plasma total Homocysteine concentration was detected by HPLC (High-performance Liquid Chromatography) (KNAUER, Germany), coupled with fluorescence detector. The method was validated over a linearity range of 1-100 $\mu\text{mol/L}$ of the plasma. The intra-assay and

inter-assay CV were 3.9% and 5.8%, respectively.

Ultrasonographic analysis of the carotid artery was performed with a high-resolution ultrasound scanner, equipped with a linear array 13 MHz transducer (MyLab 70 XVision, biosound esaote USA). The measurements of CIMT were carried out between 8-10 AM, by one physician who was blind to the nature of the group. The investigator performing the ultrasonography assessed the CIMT thickness of maximum 3 volunteers in one day. The subjects first were rested in the supine position in a dark room with stable temperature for 10 minutes. For ultrasound scanning of the carotid, the subject's head was placed in a slightly bended position and transducer was placed in an angle of 90° of the vessel wall. In order to localize the possible plaques, first a fast cross sectional scanning was made starting from the proximal part of common carotid artery (CCA) throughout the bifurcation to the internal and then external carotid artery. This process was followed by a longitudinal scanning of the CCA. In this step, the dynamic sequence images were stored for the following measurement of CIMT. A segment of the artery was magnified to identify a distinct lumen-intima and media-adventitia interface of the artery wall. The carotid intima-media thickness was defined as the distance between the leading edge of the lumen-intima interface and the leading edge of the media-adventitia interface. The scanning procedure was made first on the right CCA and then on the left CCA. For detection of CIMT, a specialized software (vascular tools 5, Medical Imaging Applications LLC, USA) was employed. The regions of interest were defined as 1.0 cm distal to the bifurcation, the bifurcation and 1.0 cm proximal to the internal carotid artery. On each region of interest near and distant walls thickness was measured. The lumen/intima and the media/adventitia borders and the distance in between were identified by the software and reported as CIMT. The CIMT was reported for each subject as the average of 12 measurements (6 measurements from the right and 6 from the left carotid artery).

Statistical analysis

Results are reported as the mean \pm Standard deviation. All of the statistical analyses were

performed using the SPSS version 18 software. Student T-test and analysis of variance (ANOVA) were used to compare the differences between the mean of the parameters in two groups. Differences were considered statistically significant at level of $P \leq 0.05$. The Levene's test was used for assessing the equality of variances. The univariate and multivariate linear regression analysis was used to investigate the correlation between variables and CIMT.

Results

In a historical cohort study, total of 40 women with a mean age of 33.17 ± 6.02 years, including 20 women with pGDM and 20 without pGDM were recruited. The anthropometric characteristics of the study subjects are shown in Table 1.

There was no significant difference between two groups regarding to the age, BMI, parity and education.

Comparison of the plasma levels of total cholesterol, HDL cholesterol, LDL cholesterol, Homocysteine, showed no significant difference between two groups.

Subjects with Previous history of GDM have a significantly higher levels of FBS (93.45 mg/dl vs 87.32 mg/dl , $p=0.03$) and fasting

Insulin ($10.50 \mu\text{IU/mL}$ vs $7.19 \mu\text{IU/mL}$, $p=0.03$) compare to the women without history of GDM (Table 2). Because of the abnormal distribution of folic acid and B12, these two variables were presented as median and interquartile. The median of folic acid in control and GDM groups were 3.20 and 4.25 ng/ml , respectively. The interquartile values of folic acid were 2.32 - 5.95 for control and 2.5 - 7.85 for GDM group. Regarding B12, the median values were 395.5 and 408.5 pg/ml for control and GDM group, respectively. The interquartile values of B12 in control group were 332.2 - 563.25 and in GDM group were 254.25 - 538.75 . No significant difference was observed for two groups in regards to folic acid ($p=0.67$) and B12 ($p=0.46$)

Comparing the mean of the CIMT between two groups, revealed that subjects with pGDM (CIMT= 0.510 mm) had a significantly higher CIMT than control group (CIMT= 0.478 mm . $P=0.037$, Figure 1). After adjustment for age, group, and BMI, in univariate linear regression analysis only fasting insulin levels showed a significant association with CIMT measurement ($p=0.02$). In multivariate analysis insulin ($p=0.013$) and systolic blood pressure ($p=0.02$) had a significant association with CIMT (Table 3).

Table 1. Anthropometric characteristics of the women with and without pGDM

Parameter	Control group (Mean \pm SD)	PGDM group (Mean \pm SD)	P
No of subjects	20	20	
Age (year)	34.27 ± 5.81	31.36 ± 5.61	NS
Height (cm)	159.09 ± 3.70	160.68 ± 6.43	NS
Weight (Kg)	69.82 ± 8.52	70.00 ± 12.14	NS
BMI (kg/m^2)	27.59 ± 3.38	27.29 ± 5.74	NS
Parity	1.95 ± 1.04	1.45 ± 0.73	NS
Waist circumference(cm)	90.59 ± 9.91	87.00 ± 12.14	NS
Systolic blood pressure	72.72 ± 8.22	78.21 ± 7.43	NS
Diastolic blood pressure	115.06 ± 8.01	121.26 ± 9.20	NS

Values are expressed as the mean \pm SD; BMI= body mass index; GDM=gestational diabetes mellitus; NS=not significant

Table 2. Blood biochemistry values (mean \pm SD) of the women with and without pGDM

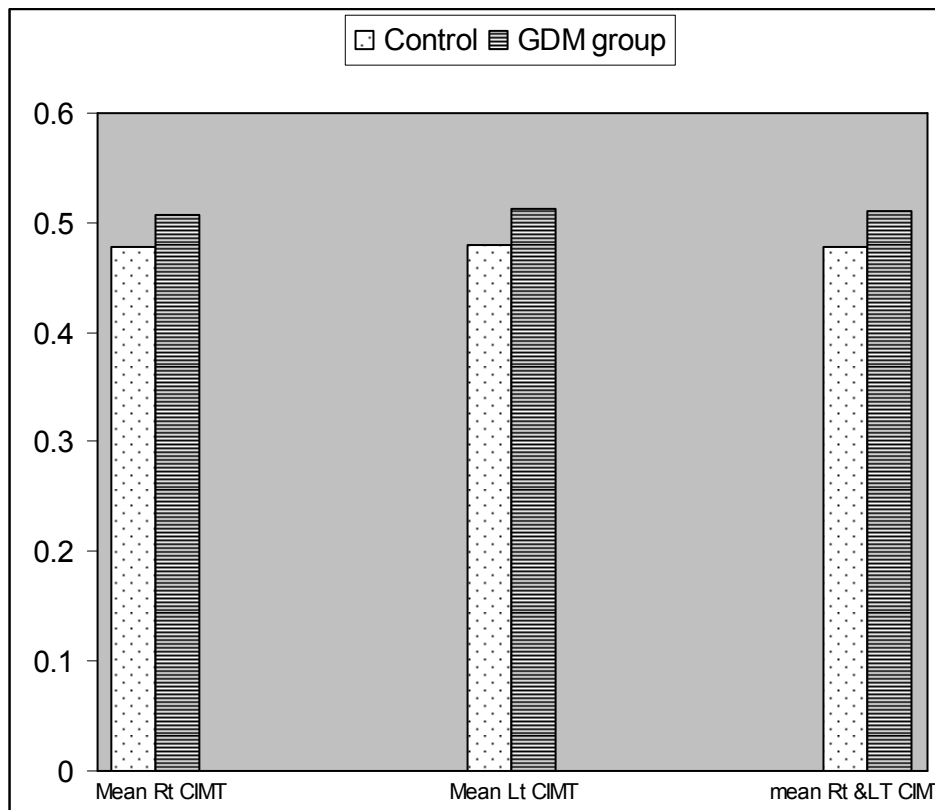
Parameter	Control group (Mean \pm SD)	PGDM group (Mean \pm SD)	P
Triglyceride (mg/dL)	101.41 \pm 42.18	108.86 \pm 57.85	NS
Total cholesterol(mg/dL)	169.36 \pm 28.59	177.23 \pm 31.82	NS
HDL(mg/dL)	50.64 \pm 11.11	51.41 \pm 11.59	NS
LDL(mg/dL)	87.32 \pm 16.81	91.59 \pm 21.10	NS
Fasting blood sugar (mg/dL)	87.32 \pm 7.61	93.45 \pm 10.34	P=0.03
Insulin (μ IU/mL)	7.19 \pm 4.62	5.04 \pm 4.6	P=0.03
Homocysteine	8.92 \pm 2.21	8.51 \pm 2.42	NS

HDL=high density lipoprotein cholesterol; LDL= low density lipoprotein cholesterol;
NS= not significant

Table 3. Univariate and multivariate linear regression analysis for association of mean of common carotid intima-media thickness (CIMT) with different parameters

variable	Univariate analysis		Multivariate analysis	
	β	P value	β	P value
TG	0.06	0.69	0.04	0.8
Cholesterol	0.21	0.18	0.10	0.53
HDL	0.05	0.75	0.004	0.98
LDL	0.18	0.26	0.07	0.65
FBS	0.03	0.83	0.16	0.3
Homocysteine	0.26	0.10	0.19	0.20
Fasting Insulin	-0.37	0.02	-0.37	0.013
Folic acid	0.14	0.93	-0.06	0.66
B12	-0.10	0.95	0.025	0.86
Systolic blood pressure	0.32	0.05	-0.39	0.02
Diastolic blood pressure	-0.04	0.77	-0.18	0.31
Waist circumference	-0.09	0.58	-0.10	0.71

TG= triglyceride, HDL=high density lipoprotein cholesterol; LDL= low density lipoprotein cholesterol; FBS= fasting blood sugar;

**Figure 1. Mean value of common carotid intima-media thickness (CIMT) in women with previous history of GDM and unaffected women**

Discussion

Euglycemic or non-diabetic pregnancy could be described as a metabolic stress which characterized by transient insulin resistance with post-prandial hyperglycemia, elevated serum levels of fasting triglyceride and low density lipoprotein cholesterol (13). GDM which is defined as carbohydrate intolerance with the first onset of diagnosis in the pregnancy imposes the stress on an already compromised metabolic status which makes it reasonable to hypothesize that GDM has the potential to switch a transient metabolic state into a more permanent abnormality in the future. One of the major abnormalities that might happen in patients experiencing the pGDM is the development of early coronary heart disease caused by premature atherosclerosis.

Since the carotid intima-media thickness has been shown to be a surrogate marker of preclinical atherosclerosis (11), in this study, we assessed to see if having a history of pGDM could contribute to development of premature atherosclerosis. We found that despite the patients with pGDM had comparable serum levels of triglyceride, cholesterol, HDL cholesterol and LDL cholesterol to the control group, still CIMT was reported to be significantly higher in this group (0.510 mm vs 0.478mm).

Volpe *et al* revealed in his study including 28 women with and 24 women without pGDM 2 years post partum, the mean of CIMT was greater among the women with prior GDM compared with that of controls (0.57 ± 0.058 mm vs. 0.51 ± 0.051 mm; $p < 0.01$) (14). These findings are in concordance with our finding and point to the idea that patients with pGDM might be more prone to develop the premature atherosclerosis and coronary artery disease. However, it should be mentioned that in their study, the women with pGDM were significantly older and had higher blood pressure (still within normal range) and waist circumference (similar BMIs) compared to the control group while, in our study, the two groups were comparable based on their age, BMI and did not have a significantly different waist circumference.

In previous observational studies, CIMT was shown to be a good indicator of the presence and extent of coronary artery disease (15, 16)

and an association between the increased CIMT measurement and elevated risk of cardiovascular disease and stroke in middle aged and older adults has been reported (17). Hodis and his colleagues (18) reported a relative risk of 2.2 for non-fatal myocardial infarction or coronary death for each 0.03-mm increase /year in CIMT.

It has been shown that CIMT is higher in patients with type 2 diabetes mellitus (12). The mechanism by which the hyperglycemia could contribute to the development of atherosclerosis is not fully understood. It has been shown that acute hyperglycemia even in healthy subjects could disturb the function of endothelium (19). The possible mechanism would be that hyperglycemia could induce the production of oxygen-derived free radicals which result in activation of protein kinase C and formation of glycosylated end product (20, 21). These products decrease production of nitric oxide from endothelial cells which subsequently result in impairment of the vasodilatory and protective capacity of endothelium. As a result, LDL cholesterol infiltrates the subendothelial space. The ongoing inflammatory process after LDL cholesterol uptake leads to leukocytes infiltration of the vascular wall and further growth of the atherosclerotic lesion with a diffuse thickening of the intimal layer (9). The evidence for effect of glucose disturbance on the development of subclinical atherosclerosis also comes from study by Tarim *et al* (12). In their study which was done on 30 women with GDM and 40 euglycemic pregnant women in the second trimester, the CIMT measurement was significantly higher in GDM group (0.582 ± 0.066 mm vs. 0.543 ± 0.049 mm; $p = 0.006$) and CIMT measurement was positively correlated with blood sugar level.

In our study the patients with previous history of GDM had significantly higher level of insulin compared to the control group. Hence in these patients the fasting serum level of glucose was significantly higher than control group. Even though in contrast to study by Tarim *et al*, we could not find a significant correlation between glucose level and CIMT, but we did observe a significant correlation between fasting insulin level and CIMT measurement. Though, in the absence of an OGTT we can not clearly compare the status of glucose homeostasis between two groups of

study, but we may suggest that patients with pGDM suffer at least partially from a disturbance in glucose homeostasis seen several years after delivery compared to the control group. In accordance to this idea, it has been reported by several studies that in contrast to the normal pregnancy a consistently reduced insulin response to nutrients is one of the manifestations of women experiencing GDM (22, 23). A large defect in pancreatic β -cell function was also found in this group of women (24). Moreover, there are many other defects that have been detected in women with pGDM including alterations in the insulin signaling pathway and reduction in insulin-mediated glucose transport (25, 26). However, whether any of these defects are primary or caused by impairment in insulin function and whether these defects are transient or permanent after delivery, need to be elucidated.

The traditional Framingham risk score calculator to assess a person's risk of heart disease does not adequately account for glucose disturbance (it only accounts for established diabetes). Mondy and his colleagues suggested that a simple index of insulin resistance such as HOMA-IR could be a strong predictor of CIMT in HIV patients (27). Our finding of increased CIMT in patients with pGDM may introduce the importance of disturbance in glucose homeostasis, even in the absence of overt hyperglycemia, in determining the cardiovascular disease risk,

It was shown by Sánchez-Vera (28) that GDM increases susceptibility to the LDL cholesterol oxidation. Moreover, Willer and his colleagues (29) demonstrated that women with GDM had

higher levels of serum E-selectin and cVCAM-1, which probably is a manifestation of increased levels of their expressions on the endothelial cells. Based on the fact that the oxidation of LDL cholesterol could initiate the process of atherogenesis, increased levels of E-selectin and cVCAM in women with history of GDM could contribute to the persistence of the atherogenesis process by stimulating the circulating monocytes binding to the endothelial cell surfaces.

In summary we found that young women with previous history of GDM showed an increase in CIMT measurement which could be a sign of preclinical atherosclerosis. This was in spite of the fact that no woman was diabetic or hypertensive and both group were comparable in BMI. Although increased CIMT can be mediated or supported by defects in multiple aspects of metabolic status, we found a positive correlation between insulin level and CIMT. Our limitation in not having the OGTT might be addressed in future studies in order to investigate the relationship between glucose metabolism impairment and preclinical atherosclerosis.

Acknowledgement

This work was supported in terms of design, collection, and management by a research grant from Endocrinology and Metabolism Research Center (EMRC) of the Tehran University of Medical Science. The authors would like to thank Dr. Abbasi and Ms Khooshehchin for their technical assistance. There is no conflict of interest.

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