

Serum Homocysteine Level Might Be a Predictor for Persistence of Abnormal Glucose Tolerance in Gestational Diabetes

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Abstract: Background: Increased plasma homocysteine levels are recognized to exert oxidative damage resulting into risk factors for insulin resistance (IR) and vascular disease. Present study was conducted to observe post-partum persistence of abnormal glucose tolerance (AGT) and level of homocysteine after delivery in patient with GDM. Methods: This observational cross-sectional study encompassed 100 subjects (age: 28.58±4.26 years, BMI: 26.7±2.80 Kg/m²; mean±SD) who were diagnosed as GDM during their index pregnancy by any of the internationally acceptable standard criteria. At 6-12 weeks post-partum, each of them underwent anthropometric measurements, 75 gram 2 sample OGTT and measurement of plasma homocysteine level by utilizing the chemiluminescent Immunoassay method. Results: Of the 100 subjects, 32% converted to having AGT during 6 – 12 week of post-partum follow up. Statistically significant difference was found in between the AGT and normal glucose tolerance (NGT) groups in circulating concentration of homocysteine (AGT vs NGT: 9.19±1.15 vs. 6.29±1.31 mmol/L; p=<0.001). Mean age and BMI were significantly higher in the AGT group (p<0.05 for both). Independent association was found between each of the variables age, history of macrosomia and higher homocysteine level with AGT in multiple regression analysis. Conclusion: Our study identified persistence of AGT and elevation of homocysteine levels are more prominent in women with GDM in post-partum period.

Keywords: GDM, Plasma Homocysteine Level, Abnormal Glucose Tolerance

1. Introduction

Gestational diabetes mellitus (GDM) is any degree of glucose intolerance with onset or first recognition during pregnancy, whether or not the condition persisted after pregnancy, and not excluding the possibility that unrecognized glucose intolerance may have antedated or begun concomitantly with pregnancy [1]. There is always a strong possibility of progression to diabetes mellitus sometimes in the postpartum period which will increase the

bulk of diabetes prevalence. So unless proteomic, metabolomics and genetic aspects of GDM are investigated separately as particular entity, the weight and impact of GDM in the abnormal glucose phenomenon cannot be unmasked appropriately.

Homocysteine (Hcy), a sulfhydryl-containing amino acid have long been implicated in the pathogenesis of adverse CVD events through generation of oxidative stress [2]. Early markers of vascular disease such as endothelial dysfunction might be pronounced in GDM which make them susceptible to premature atherosclerosis and coronary heart disease. In

the recent year evidence have accumulated showing a trend of elevated serum homocysteine in GDM mothers during post-partum period specifically in those having persistence of AGT [3]. In patients suffering from diabetes, raised Hcy levels were linked with IR and nephropathy [4]. It could well co-exist as one of the adverse cardio metabolic factors or it could predict development of future DM as well. [2]

Thus current study was anticipated to observe the persistence of abnormal glucose tolerance and level of homocysteine among GDM in post- partum period.

2. Materials and Methods

2.1. Study Subjects

This study encompassed 100 patients diagnosed as GDM on the basis of 75 gm-OGTT during their index pregnancy. Those having history or clinical manifestation of diabetes or use of anti-diabetic drugs before pregnancy, history or clinical manifestation of cardiovascular disease or other co-morbidities (judged on clinical assessment) or having a BMI ≥ 40 kg/m² were excluded from the study. Characters of the study subjects are shown in Table 1.

2.2. Study Design

It was a cross-sectional study carried out at Department of Endocrinology, Bangabandhu Sheikh Mujib Medical University (BSMMU) from May 2017 to December 2018. Subjects were enrolled consecutively on the basis of the OGTT results during index pregnancy. Informed written consent for participation in the study was taken from each of them. All the subjects had been on unrestricted carbohydrate diet for 3 days, and came to BSMMU, endocrine department after an overnight fast (at least 8 hr but not more than 14 hr) to undergo a formal 75-g 2-h OGTT and collection of blood for plasma homocysteine, at 6-12week post-partum. During this period delivery related information were collected. Factors predicting persistence of glucose intolerance like family history of DM, overweight or obesity, high parity, earlier detection of glucose intolerance during pregnancy, high OGTT value at that time, insulin use during pregnancy were ascertained. Clinical evaluation including estimation of height, weight, BMI (kg/m²) and BP (mmHg) was done for each subject by the same investigator. Prior to commencement of this study the research protocol was approved by Institutional Review Board (IRB).

2.3. Analytic Methods

Plasma glucose was assayed immediately by glucose-oxidase method (Dade Behring machine, Germany). The result was deducted from computerized calculation utilizing standard curve derived from known concentrations used by the system. Samples of different subjects were run on different days in different assay runs. Plasma Homocysteine was measured by utilizing the Chemiluminescent Immunoassay method using SIEMENS ADVIA centaur XPon on same day with considering a fixed known

concentration for low level (<6.8 mmol/l) as well as high level (≥ 6.8 mmol/l) was used in every assay run.

2.4. Statistical Methods

All data were processed by SPSS (Windows version 23.0, Intel corporation). Data was expressed as means \pm SD or in frequencies as applicable. Comparison of various quantitative between the subjects with and without AGT in the postpartum was done by unpaired Student's t-test for numerical values as-homocysteine, glucose, age, BMI etc. whereas for qualitative variables Chi Square test was used. Correlation among the variables was done by Pearson's correlation as applicable. Multiple regression analysis was used to find out predictors for the risk of persistence of AGT in postpartum; postpartum AGT as the dependent variable and various factors related with GDM, including homocysteine, as independent variables. P<0.05 was considered statistically significant.

3. Results

3.1. Demographic and Clinical Profile of the Study Subjects

As shown in Table 1, among 100 post- partum subjects mean age was found to be (28.58 \pm 4.26 years; mean \pm SD) and mean BMI was (26.70 \pm 2.80 kg/m²; mean \pm SD). Gestational age of detection was at 23.64 \pm 5.97 weeks (mean \pm SD). 28% of populations were primiparous while 72% were multiparous. Systolic blood pressure on average was (114.30 \pm 9.23 mmHg; mean \pm SD) and diastolic blood pressure on average was (73.20 \pm 8.63 mmHg; mean \pm SD). Macrosomia was found in 27% of population. MR/abortion among 38% of population and IUGR was found in 1% while IUD was found in 7% of total population. Family history of DM was found in 80% and family history of HTN was found in 62% of study subjects. None of them had personal history of DM and 9% of them had personal history of HTN and other diseases were found to be 6% of population.

Table 2 shows the frequency of various categories of glucose intolerance as defined by WHO and ADA criteria (NGT: 68.0% vs. 60.0%; IFG: 4.0% vs. 12.0%; IGT: 22.0% vs. 22.0%; DM: 6.0% vs. 6.0%). Total AGT by WHO criteria was 32 (32.0%) which was 40 by ADA criteria (40.0%).

3.2. Serum Homocysteine in AGT and NGT

Table 3 shows statistically significant difference between the AGT and NGT groups in circulating concentration of homocysteine (AGT vs NGT: 9.19 \pm 1.15 vs 6.29 \pm 1.31 mmol/L; p-value<0.001).

3.3. Frequency of AGT and NGT Under Cutoff 6.38 Mmol/l of Plasma Homocysteine

Table 4 shows out of 32 AGT by WHO criterion, 32 of them had plasma hcy level ≥ 6.38 and out of 68 NGT, 40 of them had plasma hcy less than 6.38 and 28 of them had plasma hcy more than 6.38.

3.4. Age and BMI: AGT vs. NGT

As shown in table 5, women in NGT group were younger as compared to AGT (AGT vs NGT: 29.91±4.92 vs. 27.69±3.79 years, mean±SD; p=0.032), and women with AGT showed higher BMI as compared to NGT (AGT vs. NGT: 27.68±1.32 vs 26.32±3.18 kg/m², mean±SD; p=0.023).

3.5. Multiple Regressions for Persistence of Abnormal Glucose Tolerance in GDM

Table 6 Keeping homocysteine as a dependent variable, except family history of DM (p=0.035) none of the factors, age (p=0.952), BMI (p=0.451), gestational age at detection of GDM (p=0.631), parity (p=0.802), use of insulin (p=0.072), FPG (p=0.714) or 2-hr glucose (p=0.392) during detection of GDM at pregnancy showed any independent predictability over the persistence of AGT at post-partum period of GDM.

Table 1. Baseline characteristics of the study subjects.

Variables	Value (%)
N	100
Age (years, mean±SD)	28.58±4.26
BMI (kg/m ² , mean±SD)	26.70±2.80
Occupation	
Housewife	70 (70.0)
Service/Student	30 (30.0)
Gestational weeks at detection (mean±SD)	23.64±5.97
Parity	
Primiparous	28 (28.0)
Multiparous	72 (72.0)
SBP (mm Hg, mean±SD)	114.30±9.23
DBP (mm Hg, mean±SD)	73.20±8.63
Past Obstetric history	16 (16.0)
MR/Abortion	38 (38.0)
Previous Macrosomia	27 (27.0)
IUGR	1 (1.0)
IUD	7 (7.0)
Family History of DM	80 (80.0)
Family History of HTN	62 (62.0)
History of DM	0 (0.0)
History of HTN	9 (9.0)
†History of other Disease	6 (6.0)

(Within parenthesis are percentages over column total)

BMI: body mass index

SBP: systolic blood pressure

DBP: diastolic blood pressure

Past obstetric history: Previous GDM=5, gestational hypertension=5, hypothyroidism in pregnancy=4, any cardiac problem=2 study subjects

†History of other Disease: Hypothyroidism=3, polycystic ovarian syndrome=2, rheumatologic diseases=1

Table 2. Glycemic status of the studied subjects (N=100).

Glycemic status	Number (%)	
	WHO criteria	ADA criteria
Normal	68 (68.0)	60 (60.0)
IFG	04 (4.0)	12 (12.0)
IGT	22 (22.0)	22 (22.0)
DM	06 (6.0)	06 (6.0)
Total	100	100

(Within parenthesis are percentages over grand total); Groups are mutually exclusive

WHO=World Health Organization ADA=American Diabetes Association

IFG=Impaired fasting glucose IGT=Impaired Glucose Tolerance

DM=Diabetes Mellitus

Table 3. Plasma homocysteine levels in AGT and NGT (mmol/L).

Variable	AGT (n=32)	NGT (n=68)	p-value
Plasma homocysteine (mmol/L)	9.19±1.15	6.29±1.31	<0.001

Data was analyzed using Student's t-test

Table 4. Frequency of AGT and NGT under cutoff ≥ 6.38 mmol/l of plasma hcy.

Homocysteine level (mmol/l)	If ≥ 6.38 (mmol/L) cut-off of homocysteine		Total
	AGT	NGT	
<6.38	0	40	40
≥ 6.38	32	28	60
Total	32	68	100

Table 5. Comparison of risk variables between AGT and NGT.

Variable	AGT (n=32)	NGT (n=68)	p-value
**Age (mean \pm SD, years)	29.91 \pm 4.92	27.69 \pm 3.79	0.032 ^s
**BMI (mean \pm SD, Kg/m ²)	27.68 \pm 1.32	26.32 \pm 3.18	0.023 ^s
*Parity			
Primiparous	7 (21)	21 (30)	0.137 ^{ns}
Multiparous	25 (79)	47 (70)	
*Family H/O DM	28 (87.5)	52 (76.4)	0.198 ^{ns}
*Family H/O HTN	22 (68.7)	40 (58.8)	0.340 ^{ns}
*Personal HTN	5 (15.6)	4 (5.8)	0.112 ^{ns}
*Personal DM	0 (0)	0 (0)	
*Others Disease	1 (3.1)	5 (7.3)	0.406 ^{ns}
*Insulin during pregnancy	11 (34.3)	12 (17.64)	0.064 ^{ns}
**Gestational age at diagnosis of GDM	22.09 \pm 6.70	24.37 \pm 5.49	0.760 ^{ns}

Values within parenthesis are percentage over row total

Data was analyzed using **Student's t-test and was presented as mean \pm SD and *Chi-square test (χ^2) was done to analyze the data

Table 6. Multiple regressions for persistence of abnormal glucose tolerance in patient with post-partum GDM.

Variables	β	SE	P
Constant	-	3.879	0.84
Age	0.017	0.065	0.952
BMI	0.137	0.095	0.451
Gestational age at GDM detection	-0.105	0.037	0.631
Parity	0.069	0.249	0.802
F/H of DM	-0.511	0.783	0.035
Use of insulin	0.367	0.467	0.072
FPG during pregnancy	-0.112	0.314	0.714
Post OGTT 2hr glucose in pregnancy	0.251	0.182	0.392

BMI=Body Mass Index, DM=Diabetes Mellitus

FPG=Fasting Plasma Glucose, OGTT=Oral Glucose Tolerance Test

4. Discussion

Present study suggests that in a GDM mother early post-partum hyperhomocysteinemia might be potential risk factor for the development of diabetes during post-partum period. Homocysteine level was found to be highly significant in the population with AGT compared to NGT population. It is beyond our perimeter to comment on whether high level of homocysteine is caused by previous presence of impaired glucose tolerance in GDM or naïve patient who developed GDM had higher susceptibility to have a higher level of homocysteine. Either way presence of abnormal glucose metabolism is an indicator of several metabolic derangements including IR, hypertension and dyslipidemia. Thus, between 6-12 weeks of postpartum, the measurement of plasma homocysteine would be helpful to identify women with previous history of GDM who at high risk of developing diabetes. Similar findings were found in different studies [5-7].

The pathogenesis of GDM is very similar to that of type 2 diabetes; in which both chronic IR and β -cell dysfunction have roles [8]. GDM has been shown to be associated with

future metabolic dysfunction and diabetes distinct from other various risk factors. GDM mothers may also show early markers of vascular disease such as endothelial dysfunction which make them prone to premature atherosclerosis and coronary artery disease.

Homocysteine is a sulfhydryl-containing amino acid whose detrimental effect on endothelial function is well documented [9]. In addition, high plasma homocysteine levels are known to exert adverse effects through certain mechanisms resulting oxidative damage. Hyperhomocysteinemia [the cutoff point of the homocysteine levels: high (>6.38 mmol/l) or low (<6.38 mmol/l)] is considered in previous studies as an adverse cardiometabolic factor [2, 10]. Hcy decreases insulin sensitivity in adipose tissue by inducing ER stress, promoting pro-inflammatory cytokine production, facilitating macrophage infiltration leading to insulin resistance ultimately causing diabetes mellitus. A trend of raised homocysteine is found in women with GDM during post-partum period, which is more noticeable in women with AGT and shows a noteworthy relationship with history of GDM [11].

Women with GDM are more susceptible to the

development of type 2 diabetes. Previous studies have found 3 to 38% conversion rates in Western populations within the 1st year of postpartum [12]. Limited numbers of similar studies have been done in Asian countries. GDM study Group of Endocrinology Department of BSMMU, Dhaka has observed remarkably higher (50%) rate of persistence of glucose intolerance during 6-12week post-partum, the commonest category of glucose intolerance was IGT followed by DM [10]. However this study encompassed all categories of hyperglycemia in pregnancy including DM in pregnancy (DIP). A recent study in our department found about 20% rate of persistence of abnormal glucose tolerance which included only GDM mothers [11]. In the current study it was about 32% as defined by WHO criteria and it increased to about 40% when extrapolated to ADA criteria. Similar findings are suggested by different researchers [7, 10]. According to Tobias et al. 2011, one third to one half of GDM mothers developed diabetes within 3 to 5 years while 70% developed diabetes over 10 years of follow up. Cumulative incidence rate of diabetes mellitus varied from 2.6% to 70% [13].

Earlier diagnosis of GDM during antenatal period, higher OGTT values at detection, insulin requirement during pregnancy, maternal age, increasing parity, recurrence of GDM, family history of diabetes, pre-pregnancy obesity, weight gain during gestational period and a previous macrosomic infant are reported to be the crucial risk factors for developing diabetes mellitus in the post- partum period [7]. We also analyzed over the impact of such variables related to relevant clinical and biochemical parameters. Of the clinical variables, advancing age, higher BMI, and family history of DM all were statistically significant in the AGT group in comparison to NGT group; on the other hand, insulin used during pregnancy reached near level of significance on persistence of glucose intolerance at 6-12 week post-partum. Similarly studies done in our department found advancing age, higher BMI, earlier gestational age at GDM detection (<20week), multiparity and insulin requirement during antenatal period all were statistically significant in the AGT group [14]. Study done by Nam et al. found older age, high BMI positive family history of DM, high fasting glucose at the time of GDM diagnosis and early gestational age at diagnosis of GDM were found statistically significant in AGT group [15].

However it is not well elucidated whether the increase risk of atherosclerosis and cardiovascular complication in mothers during post-partum period of GDM results from developing or worsening carbohydrate metabolism disorders over time or whether it is linked to other metabolic derangement like hyperhomocysteinemia.

5. Conclusions

Persistence of abnormal glucose tolerance and elevation of homocysteine is more prominent in women in post-partum period of GDM. Hyperhomocysteinemia is significantly more common in AGT than NGT and it could act as a

predictor of future development of DM. Further studies including cohort model with larger sample size are suggested which would be able to detect progression of glucose abnormality over time to confirm our assumption. Also various factors affecting homocysteine levels such as level of vitamin B6, vitamin B12, and folic acid might be included so that we can get an enlighten view regarding the role of homocysteine in the pathogenesis of GDM.

Conflict of Interest

The authors declare that they have no competing interests.

Informed Consent

All patients had informed written consent prior to participation in the study.

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