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ORIGINAL ARTICLE

Asymmetric dimethylarginine level in hyperglycemic gestation

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Abstract We aimed to evaluate plasma asymmetric dimethylarginine (ADMA) concentrations and its relation with insulin sensitivity/resistance indices in pregnant women with different degrees of carbohydrate intolerance. This study included a two step approach; 50 g glucose challenge test (GCT) followed by 100 g oral glucose tolerance test (OGTT) was used for diagnosis of carbohydrate intolerance within 24–28th weeks of gestation. Pregnant women with positive GCT but negative OGTT (AGCT group, $n = 30$) and gestational diabetics (GDM group, $n = 58$) were compared to healthy pregnant controls ($n = 50$). Plasma ADMA concentration and its relationship with glucose and insulin levels and insulin sensitivity/resistance indices (HOMA-IR, QUICKI, ISI_{OGTT}) were evaluated. Both AGCT and GDM groups were found to have similarly higher plasma ADMA levels than control subjects (3.60 ± 1.21; 4.00 ± 1.70; 2.65 ± 0.82 μmol/l, respectively, $P = 0.001$). ADMA was significantly but slightly correlated with insulin sensitivity/resistance indices and moderately correlated with 2-h insulin level. The 2-h insulin value of the OGTT was the independent influencing constant for ADMA ($R = 0.57$, $P = 0.0001$). In conclusion, plasma asymmetric dimethylarginine level was higher in cases with abnormal glucose challenge test but normal OGTT as well as in gestational diabetics, compared to pregnant women with normal glucose tolerance. The elevated ADMA level in pregnant women with carbohydrate intolerance may possibly be due to elevated insulin level.

Keywords Asymmetric dimethylarginine (ADMA) · Gestational diabetes · Abnormal glucose challenge test · Insulin resistance

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Introduction

Gestational diabetes mellitus (GDM) is a state of impairment in carbohydrate metabolism that is first recognized during pregnancy. American Diabetes Association estimated its incidence as 7% of all pregnancies; however, the estimation of true incidence is difficult due to the lack of uniform diagnostic criteria [1]. It is well recognized that GDM is associated with elevated maternal and fetal complications like preeclampsia, fetal macrosomia, shoulder dystocia, and elevated cesarean section rate. Even it has impact on future health of mother with elevated type 2 diabetes mellitus and cardiovascular risk [2, 3].

In clinical practice two step 50 g glucose challenge test (GCT) followed by 100 g oral glucose tolerance test (OGTT) are being used currently for screening and diagnosis of GDM. Widely accepted diagnostic criteria belongs to "National Diabetes Data Group" or "Carpenter and Coustan" [4]. At present, the status of carbohydrate metabolism and risks of pregnant women with high glucose levels exceeding the critical threshold value of GCT, but with a normal 100 g, 3-h OGTT has not been evaluated clearly yet. In few studies pregnant women with abnormal

GCT but normal OGTT has been described as either "borderline glucose intolerance" [5] or "mild gestational hyperglycemia" [6, 7] which is not well defined with either "National Diabetes Data Group" or Carpenter and Coustan's criteria [4].

Studies performed in small cohorts have reported the sign of vascular endothelial dysfunction in vitro and in vivo during pregnancies complicated by GDM. Endothelium derived nitric oxide (NO) is a potent vasodilator and the main regulator of endothelial function. Production of NO by endothelial-derived NO synthase; which uses arginine as a substrate; is important for preserving organ blood flow by regulating vascular tone in pulmonary, cardiovascular, immune, gastrointestinal, and neurologic systems [8]. The synthesis of NO is selectively inhibited by guanidine-substituted analogs of arginine, including monomethylarginine (MMA) and asymmetric dimethylarginine (ADMA) [9]. Since physiological concentrations of ADMA are approximately tenfold higher than that of MMA, ADMA can be regarded as the predominant endogenous inhibitor of NO biosynthesis [10]. Modification of ADMA concentrations has been shown to change vascular NO production and thereby vascular tone and systemic vascular resistance [11, 12].

Plasma ADMA levels have been shown to be elevated in hypertension, hyperlipidemia, diabetes mellitus, hyperhomocystinemia, cardiovascular diseases, and preeclampsia, which were linked to an impairment of the NO-pathway and endothelial dysfunction [11, 13–16]. However, data about the ADMA level in pregnancy and in GDM is scanty and there is no data about ADMA level in pregnant women with abnormal glucose challenge test (AGCT).

In the current study, we aimed to evaluate the plasma ADMA concentrations and its relation with insulin sensitivity/resistance indices in pregnant women who have different degrees of carbohydrate intolerance defined with a two step approach.

Materials and methods

This prospective case control clinical trial was conducted in the Obstetrics and Gynecology and the Endocrinology outpatient clinics of Inonu University, School of Medicine, between January 2008 and January 2009. The study was approved by the institutional ethic committee and a signed written informed consent form was obtained from all participants before enrolment. All of the subjects must meet all of the following inclusion criteria: (1) gestational age of 24–28 weeks, (2) singleton pregnancy, (3) absence of pregnancy complications (i.e. gestational hypertension, preeclampsia/eclampsia, intrauterine growth restriction).

Exclusion criteria from the study were the presence of one of the following conditions: (1) presence of any chronic maternal disease that may influence endothelial function and ADMA levels (i.e. hypertension, heart disease, renal failure, pulmonary disease, and type 2 diabetes mellitus), (2) a prior history of preeclampsia/eclampsia, (3) a prior history of gestational diabetes.

Gestational age was calculated according to the last menstrual period in cases with a reliable and regular menstrual history or early obstetric ultrasound performed before 20 weeks of gestation.

Study design and interventions

Age, weight, and height of each patient were recorded and body mass index (BMI) was calculated as: BMI = weight in kilograms/square of height in meters. All the participants underwent 50 g GCT followed by 100 g 3 h OGTT. Fifty grams of glucose was administered orally regardless of the time of the day or the fasting state. Venous plasma glucose and insulin levels were measured at the first hour of the glucose load. A value of plasma glucose $\geq$140 mg/dl (7.8 mmol/l) was accepted as the threshold value for the positive glucose challenge test [17]. OGTT was performed within 7 days of GCT, after a 12-h fasting. Plasma glucose and insulin levels were analyzed in blood samples collected before and 1, 2, and 3 h after the 100 g oral glucose load, at 8 am in the morning. Before the 100 g glucose load, venous blood was taken and centrifuged and the separated plasma was stored at −80°C for later determination of ADMA level. The cut-off values used for plasma glucose level on fasting, 1, 2, and 3 h were 95, 180, 155, and 140 mg/dl, respectively. GDM diagnosis was established when the two plasma glucose levels exceeding the cut-off values were present in OGTT. Women with one abnormal value were excluded from the study. The subjects were classified into three groups according to the OGTT and GCT results; Group 1: women with normal GCT (served as the control group); Group 2: women with abnormal glucose challenge test but normal OGTT (AGCT group), and Group 3: women with gestational diabetes (GDM group).

Insulin resistance was assessed with HOMA-IR [18] and insulin sensitivity was assessed with QUICKI [19] and ISI_{OGTT} [20] as:

$$\text{HOMA-IR} = \text{Fasting plasma glucose (FPG)(mmol/l)} \times \text{Fasting plasma insulin (FPI)}(\mu\text{U/ml})/22.5.$$

$\text{QUICKI} = 1/[\log(I_0) + \log(G_0)]$ (I_0 represents the inverse log sum of fasting insulin and G_0 represents the inverse log sum of fasting glucose).

$$\mathrm{ISI_{OGTT}} = 10000/\sqrt{(\mathrm{FPG} \times \mathrm{FPI})} \times (\text{Mean glucose level}\,(G) \times \text{mean insulin level}\,(I)).$$

Biochemical analysis

Plasma glucose levels were measured by hexokinase method (Olympus autoanalyser, Olympus Diagnostica, GmbH-Irish Branch-Lismeehan, Ireland Republic) and plasma insulin levels were measured by chemiluminescent enzyme immunoassay method (Immulite 2000 autoanalyser, Diagnostic Products Corporation, Los Angeles, CA, USA).

Asymmetric dimethylarginine (ADMA) assessment was conducted through high (performance) pressure liquid chromatography (HPLC) (SPD-20A, Shimadzu Corporation, Japan) by using EUREKA [Head Quarter: Via E. Fermi 25 60033 Chiaravalle (AN) Italy] kit and the chromatograms were analyzed by a fluorescent detector. Intra-assay and inter-assay coefficient of variations for ADMA were 7% and 6.9%, respectively.

Statistical analysis

Statistical analyses were carried out by employing the Statistical Package for Social Sciences software 13.0 for Windows package software (SPSS, Inc., Chicago, IL, USA). Data was expressed as means ± standard deviation (SD). Normality of distribution for continued variables in groups was determined by the Shapiro–Wilk test. The ANOVA was used to compare parametric data and least significant difference (LSD) test was used for comparison of variables. The correlations of insulin resistance/sensitivity indices and all the glucose and insulin levels on OGTT with the ADMA level and BMI and of ADMA level with BMI were assessed by using Pearson's correlation test. A stepwise multivariate regression analysis was performed to assess the independent variables (glucose and insulin values during the OGTT, insulin resistance/sensitivity indices and pre-gestational BMI and BMI at the time of GCT) influencing the plasma ADMA levels. A P value of less than 0.05 was considered to be statistically significant.

Results

Control, AGCT, and GDM groups consisted of 50, 30, and 58 cases, respectively. Mean age was similar in control, AGCT, and GDM groups (28.6 ± 5, 30.3 ± 4.9, and 30.3 ± 4.9 years, respectively; $P = 0.189$). The mean BMI of AGCT group was significantly higher than the control group's both pre-gestationally and at the time of GCT. The mean pre-gestational BMI of GDM group was significantly higher than control group; however, mean BMI was higher than both control group and AGCT group at the time GCT (Table 1).

Mean glucose level on GCT increased gradually with increasing degree of carbohydrate intolerance in control, AGCT, and GDM groups (107.0 ± 19.1, 157.1 ± 17.3, and 206.2 ± 32.4 mg/dl, respectively; $P = 0.0001$). It was significantly higher in GDM group compared to AGCT ($P = 0.0001$) and control groups ($P = 0.0001$) and in AGCT group compared to control group ($P = 0.0001$). The mean insulin level obtained 1 h after 50 g glucose load also increased gradually in control, AGCT, and GDM groups (47.7 ± 29.3, 54.6 ± 25.0, and 68.4 ± 46.1 μIU/l, respectively; $P = 0.023$), however, the difference was significant for GDM group only compared to control ($P = 0.007$) and AGCT groups ($P = 0.01$) (Table 1).

Oral glucose tolerance test (OGTT) revealed significantly higher mean glucose levels on fasting, 1, 2, 3 h in GDM group compared to AGCT and control groups; however, there was no difference between AGCT and control groups (Table 2). Fasting, 1- and 2-h mean insulin levels were significantly higher in GDM group compared to AGCT and control groups and 3-h value was significantly higher in GDM group compared to control group only. For the AGCT group 1- and 2-h mean insulin levels were significantly higher than the control group and the mean insulin level on fasting and 3 h were similar to control group (Table 2).

All insulin sensitivity/resistance indices; HOMA-IR, QUICKI, $\mathrm{ISI_{OGTT}}$, and fasting insulin concentration were significantly different in gestational diabetic women compared to the other two groups while there was no statistically significant difference between AGCT and control subjects. The mean plasma ADMA levels increased gradually in control, AGCT, and GDM groups (2.65 ± 0.82, 3.60 ± 1.21 and 4.00 ± 1.70 μmol/l, respectively; $P = 0.001$), however, the difference was significant in GDM and AGCT groups compared to control group ($P = 0.0001$ and 0.02, respectively) while its levels were similar in AGCT and GDM groups (Table 1).

Asymmetric dimethylarginine (ADMA) levels showed significant but weak positive correlations with 2-h glucose and 1-h and 3-h insulin levels and HOMA-IR and negative correlations with QUICKI and $\mathrm{ISI_{OGTT}}$. However, the positive correlation with 2-h insulin level was considerable (Table 3).

We did not find a correlation between ADMA level and pre-gestational BMI and BMI at the time of GCT. However, BMI showed significant positive correlations with fasting, 1- and 2-h insulin levels and a significant negative correlation with QUICKI and $\mathrm{ISI_{OGTT}}$ (Table 4). In the stepwise multivariate regression analysis; with the

Table 1 Baseline characteristics of the groups

Group	Control ($n = 50$)	AGCT ($n = 30$)	GDM ($n = 58$)
Age (year)	28.6 ± 5	30.3 ± 4.9	30.3 ± 4.9
Pregestational BMI (kg/m^2)	22.8 ± 2.1[a]	24.8 ± 2.7	25.9 ± 3.1
BMI at GCT (kg/m^2)	24.6 ± 2.4[a]	28.6 ± 3.0	30.6 ± 3.3[b]
Glucose at GCT (mg/dl)	107.0 ± 19.1[a]	157.1 ± 17.3	206.2 ± 32.4[b]
Insulin at GCT (μIU/l)	47.7 ± 29.3	54.6 ± 25.0	68.4 ± 46.1[b,c]
ADMA (μmol/l)	2.65 ± 0.82[a]	3.60 ± 1.21	4.00 ± 1.70
HOMA-IR	1.8 ± 1.1	3.2 ± 4.9	7.8 ± 4.6[b,c]
QUICKI	0.16 ± 0.01	0.15 ± 0.02	0.13 ± 0.01[b,c]
ISI_{OGTT}	7.1 ± 3.2	5.5 ± 2.8	2.02 ± 1.01[b,c]

[a] $P < 0.05$ for both AGCT and GDM groups versus control

[b] $P < 0.05$ for GDM versus AGCT

[c] $P < 0.05$ for GDM versus control

Table 2 Insulin and glucose values during the course of OGTT

Group	Control	AGCT	GDM
Fasting glucose (mg/dl)	87.5 ± 8.4	81.4 ± 5.8	95.6 ± 12.6[b,c]
Glucose 1-h (mg/dl)	149.0 ± 10.5	164.4 ± 23.0	210.1 ± 25.6[b,c]
Glucose 2-h (mg/dl)	133.5 ± 10.9	129.4 ± 21.9	176.1 ± 27.3[b,c]
Glucose 3-h (mg/dl)	108.7 ± 15.7	96.3 ± 23.0	116.1 ± 33.9[b,c]
Fasting insulin (μIU/l)	8.5 ± 4.8	15.3 ± 22.6	34.2 ± 21.42[b,c]
Insulin 1-h (μIU/l)	66.0 ± 43.8[a]	78.9 ± 39.1	121.6 ± 40.5[b]
Insulin 2-h (μIU/l)	41.2 ± 12.6[a]	88.6 ± 52.2	131.8 ± 46.6[b]
Insulin 3-h (μIU/l)	32.4 ± 14.2	41.3 ± 41.1	58.7 ± 18.0[c]

[a] $P < 0.05$ for both AGCT and GDM groups versus control

[b] $P < 0.05$ for GDM versus AGCT

[c] $P < 0.05$ for GDM versus control

Table 3 Parameters correlating with ADMA

Parameter	r value	P value
ISI_{OGTT}	−0.343	0.024
HOMA-IR	0.327	0.032
QUICKI	−0.323	0.034
Glucose 2-h[a]	0.299	0.044
Insulin 1-h[b]	0.366	0.016
Insulin 2-h[b]	0.570	0.0001
Insulin 3-h[b]	0.336	0.028

[a] Glucose level on the 2-h of OGTT

[b] Insulin level on the 1-h, 2-h, 3-h of OGTT

probability of F-to-enter ≤0.05 and F-to-remove ≥0.1; the 2-h insulin level of the OGTT was found to be the independent influencing variable for ADMA ($R = 0.57$, $R^2 = 0.325$, $F = 19.69$ with $P = 0.0001$).

Table 4 Parameters correlating with BMI at time of glucose challenge test

Parameter	r value	P value
HOMA-IR	0.364	0.008
QUICKI	−0.279	0.045
ISI_{OGTT}	−0.356	0.010
Fasting insulin[a]	0.367	0.007
Insulin 1-h[a]	0.409	0.003
Insulin 2-h[a]	0.276	0.048

[a] Insulin levels on fasting, 1-h and 2-h of OGTT

Discussion

This study demonstrated that pregnant women with different levels of carbohydrate intolerance had higher plasma ADMA concentrations than pregnant women with normal glucose tolerance (NGT). There is limited data about the ADMA level in pregnancy and to our knowledge only two very recent studies evaluated the ADMA level in GDM in comparison with pregnant women with NGT [21, 22]. While Akturk et al. [22] showed a significant elevation, Telejko et al. [21] found a non-significant elevation in ADMA level in the GDM group compared to pregnant women with NGT.

In the current study, in addition to gestational diabetes, we evaluated the pregnant women with abnormal GCT but normal OGTT, presuming that this group has insulin resistance similar to gestational diabetics based on our previous clinical experience [23]. In the previous study conducted through our clinic we found comparable impaired insulin indices with gestational diabetics in AGCT group [23]. Similar results were reported by recent studies as well [24, 25]. We for the first time demonstrated in this study that AGCT group had also elevated ADMA levels compared to pregnant women with NGT.

In normal pregnancy systemic resistance falls mainly through NO-mediated endothelium dependent vasodilatation [26]. Further, Telejko et al. [21] showed that ADMA level decreased in healthy pregnant women compared to non-pregnant women, indicating the role of ADMA in the physiological adaptation to pregnancy.

Akturk et al. [22] found the elevated ADMA level in the third trimester related to the glucose levels of the GCT performed at 24–28th weeks of gestation, in GDM. However, the underlying mechanism of elevated ADMA level in GDM is not clear. We demonstrated an intermediate positive correlation between ADMA level and 2-h insulin level of OGTT. It was also the independent predictor of serum ADMA concentration. The other correlations with insulin and glucose levels and the insulin sensitivity/resistance indices were also significant but weak.

It has been proposed that in the early stages of carbohydrate intolerance, serum insulin levels elevate to supraphysiologic levels to maintain glucose homeostasis [27]. Herein the timing of our testing which can be suggested as the early stage of obvious diabetes within the gestational process, with relatively low glycemia but high insulinemia, may be reason of weak correlation of ADMA with sensitivity/resistance indices but moderate correlation with 2-h insulin values. On the other hand small number of cases within the groups may be the other confounding factor.

Nonetheless the pregnant women with different levels of carbohydrate intolerance had higher plasma ADMA concentrations than pregnant women with normal glucose tolerance. But rather than glycemia, serum insulin concentration seems to be more predictive for elevated ADMA concentration in every states of gestational carbohydrate intolerance.

In non-pregnant women elevated ADMA concentrations were found to be related to insulin resistance in obese subjects [28]. Body mass index in synergy with insulin resistance was the strongest responsible factor for the elevated ADMA concentration generally in all the studies conducted through obese subjects [29, 30] and improved with weight loss [29, 31]. Interestingly in polycystic ovarian syndrome, which is typically associated with obesity and insulin resistance, ADMA level was found to be increased independent of obesity [32]. However, we did not find a correlation of plasma ADMA level with either pre-gestational or gestational BMIs. The lack of a correlation could be due to relatively lower BMIs of our patients, or it could be the result of the BMI calculation based on total body weight but not fat weight which has to be lightened with further investigations.

Another limitation of our study can be the grouping bias. On the light of recent data one can assume the threshold values for GCT, high. But this is not under the scope of this study. Additionally the mentioned values used in this study still have a wide acceptance over the world [33].

In conclusion, we found the asymmetric dimethylarginine level higher in gestational diabetes mellitus as well as in cases with abnormal glucose challenge test but normal oral glucose tolerance test, compared to pregnant women with normal glucose tolerance. The elevated ADMA level in pregnant women with carbohydrate intolerance may possibly be due to the elevated insulin level. However, given the sample size of the current study is small; it can be considered a preliminary study. ADMA level and the influencing factors in pregnant women with carbohydrate intolerance should be further evaluated in prospective case control studies with larger sample size which may yield a new era for diagnostic and therapeutic interventions.

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