Impairment of Glucose Tolerance Test During Pregnancy and Serum Assymmetric Dimethylarginine Levels

Gebelikte Bozulmuş Glukoz Tolerans Testi ve Serum Asimetrik Dimetil Arjinin Düzeyleri

Gestational Diabetes and Adma

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Özet

Amaç: Asimetrik dimetilarjinin (ADMA), nitrik oksit sentaz (NOS) inhibitörü olup endotel disfonksiyonunu göstermektedir. ADMA diabetli hastalarda yüksek tespit edilmiştir. Bu çalışmada amacımız, normal glukoz toleranslı, bozulmuş glukoz toleranslı ve gestasyonel diabetli gebe hastalardaki serum ADMA ve Arjinin düzeyleri arasındaki farkları tespit etmek ve serum ADMA ve Arjininin bebek kiloları ile ilişkisini araştırmaktır. Gereç ve Yöntem: Çalışmamıza 50 gr glukoz tolerans testi normal olan 64 gebe (grup 1 ,NGT); 50 gr testi yüksek ve 100 gr oral glukoz testi (OGTT) normal olan 33 gebe (bu grup bozulmuş glukoz toleransı olan grup olarak da adlandırılmaktadır) (grup 2, IGT) ; ve gestasyonel diabetli 8 gebe (GDM, grup 3) dahil edildi. Gebelerin serum ADMA ve Arjinin düzeyleri araştırıldı. Bu üç grup arasında istatiksel analiz yapıldı. Bulgular: Serum Arjinin düzeyleri bozulmuş glukoz toleransı olan grupta (IGT), NGT grubundan anlamlı derecede yüksekti. ADMA düzeyleri GDM grubunda yüksekti ancak istatistiksel olarak anlamlı kabul edilmedi. Arginin ve ADMA düzeyleri arasında korelasyon tespit edildi ($r = 0.219$; $p<0.05$). Ayrıca serum ADMA düzeyleri ile ailesel diabet öyküsü arasında istatistiksel olarak anlamlı ilişki tespit edildi ($r = 0.217$; $p<0.05$). ADMA, Arjinin düzeyleri ve bebek kiloları arasında ilişki bulunamadı. Tartışma: Bozulmuş glukoz toleranslı (IGT) ve gestasyonel diabeti (GDM) olan grupta ADMA yükselme eğilimindedir. Bu yükseliş klinik olarak önemli olmasa da, nitrik oksit eksikliğini gösteren bir durum olup, özellikle ailesel diabet öyküsü olan, beden kitle indeksi artmış ve ileri yaş gebeliklerde daha belirgindir. Buna bağlı olarak da bu gebelerde daha dikkatli monitorizasyon ve takip gereklidir. Artan ADMA ve Arjinin düzeyleri bebek kilosunu etkilemiyor görünmektedir..

Anahtar Kelimeler

Asimetric Dimetilarjinin; Gestasyonel Diabet; Bozulmuş Glukoz Toleransı; Endotel Disfonksiyon; Arjinin

Abstract

Aim: Assymetric dimethylarginin (ADMA), an inhibitor of nitric oxide synthase (NOS), has been linked to endothelial dysfunction. Our aim in this study was to compare ADMA and arginine levels in normal glucose tolerance, impaired glucose tolerance and gestational diabetes groups and investigate the effect on baby birth weight. Material and Method: Serum ADMA and arginine levels were investigated in 64 patients whose 50-g glucose loading test was normal (group 1, NGT); 33 patients whose 50-g test result was high and those whose 100-g oral glucose tolerance test (OGTT) was normal, namely, those with impaired glucose tolerance (IGT, group 2) ; and in 8 patients diagnosed with gestational diabetes mellitus (GDM, group 3). Results: Arginine levels were significantly higher in the IGT group than in the NGT group. ADMA levels were high in the GDM group, but the difference was not statistically significant. There were a statistically significant correlation between arginine and ADMA levels and the ADMA level of those with a diabetes history. No significant relationship was found between ADMA level, arginine and the weight of the infant. Discussions: Although there has not been a clinical status related with nitric oxide deficiency caused by increasing ADMA concentrations, pregnancies with increased body mass indeks (BMI), family history for diabetes and older ages should be carefully monitorized. ADMA tends to increase in patients with IGT and GDM. In addition, blood ADMA and arginine levels do not seem to influence the weight of the infant.

Keywords

Asymmetric Dimethylarginine; Gestational Diabetes; Impaired Glucose Tolerance; Endothelial Dysfunction; Arginine

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Introduction

Gestational diabetes mellitus (GDM) is a metabolic disease characterised by the impairment of glucose tolerance during pregnancy. Although the American Diabetic Association reports its incidence as 7% in all pregnancies, it is difficult to estimate the actual incidence, since the diagnostic criteria for gestational diabetes are not very clear [1, 2]. GDM is marked by macrosomia, preeclampsia, fetal mortality, placental changes, and increased diabetes risk for the mother in the post-pregnancy period [3, 4].

Nitric oxide synthase (NOS) is located in the brain and cerebral arteries and catalyzes the synthesis of nitric oxide (NO) from L-arginine. NO plays an important role in the maintenance of vascular homeostasis by controlling the vascular tonus. It also prevents platelet aggregation, leukocyte–endothelium interaction, and vascular smooth muscle cell proliferation, which are important steps in the development of atherosclerosis [5]. assymetric dimethylarginine (ADMA) is a competitive endogenous inhibitor of endothelial NOS and decreases endothelial NO synthesis and bioavailability [6]. High ADMA values are associated with endothelium dysfunction.

Age, diabetes mellitus (DM), hypertension, carotid arterial intima-media thickness, hyperlipidemia, hyperhomocysteinemia, obesity, inflammation, and sickle cell disease were found to be associated with increased blood ADMA levels [7, 8]. ADMA levels were also reported to be high in metabolic syndrome and preeclampsia [9, 10].

Type 2 diabetes and insulin resistance were found to be associated with endothelium dysfunction [11–13]. This relationship is characterised by a decrease in NO. In recent studies, a relationship between ADMA and insulin resistance has also been found [14, 15]. ADMA levels were also found to be high in patients with a previous history of gestational diabetes and those with gestational diabetes [16–19]. In one recent study, ADMA levels were found to be high in patients with impaired glucose tolerance (IGT), which was related to high insulin levels [2]. In gestational diabetes, micro and macrovascular endothelial dysfunction have been found but clear information could not be obtained [20, 21].

In the present study, which was planned based upon the aforementioned findings, serum ADMA levels of GDM patients, those with IGT, and normal pregnant women were examined, and their serum arginine levels were compared. Our aim was to compare ADMA and arginine levels in pregnant women with IGT who are accepted normal in routine clinical practice and investigate the effect of these parameters on baby birth weights. Drawing upon the fact that GDM leads to placental endothelial dysfunction, infants were investigated. In addition, the relationship between body mass index (BMI), a family history of diabetes, weight gain during pregnancy, and age factors, and serum ADMA and arginine levels were determined.

Material and Method

Pregnant women referred to the pregnancy outpatient clinic of Kecioren Education and Research Hospital between May 2012 and Septembre 2012 were included in the present study and were allocated to one of three groups. Group 1 included 64 patients with normal 50-g glucose loading test results; group 2 (the IGT group) included 33 patients with high 50-g test results and normal 100-g test (OGTT) results; and group 3 (GDM) included 8 patients with the diagnosis of GDM. The diagnosis of GDM was made with at least two high values after a 100-g 3-hour OGTT according to the Carpenter/Cousten criteria [22] (the references, fasting blood sugar: 95; 1-hour blood sugar: 180 mg/dl; 2-hour blood sugar: 155 mg/dl; 3-hour blood sugar: 140 mg/dl). For the 50-g glucose screening test, the cut-off value was considered as ≥ 140 mg/dl blood sugar.

Ethical approval was in accordance with the Helsinki Declaration and the informed consent of the patients was obtained. Patients with a history of diabetes, chronic renal failure, hypertension, and other chronic inflammatory diseases were excluded from the study. Names and family names, ages, gestational weeks, the number of pregnancies, BMI, weight gained until the present weeek of pregnancy, smoking status, family history of diabetes, and the weight of infants after term births were recorded for all patients.

Measurement of ADMA and arginine. Blood samples were obtained from patients and controls, and were transferred to plain tubes. Sera from plain tubes were separated after centrifugation at 3000 g for 10 minutes and used for ADMA and arginine analyses. Samples were stored at –20 °C, and serum ADMA and arginine levels were analyzed on the same day on an Applied Biosystems MDS SCIEX(USA) API 3200 LC-MS/MS system in electrospray ionization (ESI) positive mode with an Agilent Eclipse XDB-C18 analytical colon [23]. In brief, a 100-µL serum sample was deproteinized with acetonitrile and centrifuged. The supernatant was derivatizated with hydrochloric acid (HCl)/n-butanol at 65 °C, dried under nitrogen (N2), dissolved in the mobile-phase solution, and analyzed by chromatographic separation in the LC-MS/MS system. According to this method, the intra-day coefficient of variation (CV) and inter-day CV were 3.9% and 6.2%, respectively.

Statistics: A Kolmogorov–Smirnov test was performed before the analysis and the differences between the groups were compared using the Kruskal–Wallis and Mann Whitney U tests for non-parametric variables, and analysis of variance (ANOVA) for the parametric variables. The SPSS 16.0 program was used for all of the statistical analyses.

Results

According to the statistical analysis, the mean age and BMI in both group 2 and group 3 were significantly higher than in group 1 (control group). Arginine levels were significantly higher in group 2 (IGT) than in group 1. Group 3 (GDM) also yielded higher results. However, the difference was not statistically significant. ADMA levels were also higher in group 3 but they were not statistically significant. The reason for this could be due to the small number of patients in group 3 (Table 1). Arginine levels correlated significantly with ADMA levels ($r = 0.219$; $p<0.05$) and ADMA levels correlated with DM history ($r = 0.217$; $p<0.05$). BMI correlated with age ($r = 0.415$; $p<0.001$) and DM history ($r = 0.191$; $p<0.05$). No significant relationship was found between fetal weight and ADMA and arginine levels.

Discussion

Normal pregnancy is characterised by low systemic vascular

Table 1. Comparison of demographics and the determined parameters between the groups.

	Grup 1 (n=64)	Grup 2 (n=33)	Grup 3 (n=8)	p
Age (years)	24.6 (18-38)	28.2 (19-38)	30.8 (20-38)	<0.001[a,b]
BMI (kg/m2)	25.2(25.9-41.1)	27.3 (17.0-34.9)	30.9(21.9-43.8)	0.002[a,b]
Fetal Weight (gram)	3337 (2310-4290)	3445 (2680-4600)	3652 (3270-3960)	0.083
Smoking	5 (%7.8)	2 (% 6.1)	0 (%0)	1
Weight gain (kg)	6.64 (1-13)	6.59 (2-12)	6.38 (4-10)	0.97
ADMA(micromol/lt)	0.34 (0.01-0.9)	0.34 (0.01-0.83)	0.39 (0.14-0.61)	0.66
Arginine(micromol/lt)	5.1 (0.08-29.8)	9.7 (1.33-60.1)	8.35 (1.24-19.8)	0.03[a]

a. Significant between group 1 and group 2, b. Significant between group 1 and group 3.
Values are represented as median (Min-Max)

resistance and a decrease in blood pressure. NO plays a part in the adaptation of the vessels during pregnancy. NO synthesis is inhibited by ADMA and ADMA significantly increases with endothelial dysfunction [24–26].

Based upon the studies reporting that ADMA is high and NO decreases in patients with diabetes [7, 8], ADMA levels were compared between normal pregnant women and those with IGT and GDM. ADMA levels were found to be higher in the GDM group compared to those in normal pregnant women. This result may be attributed to the low number of cases in the GDM group. Although in previous studies, in pregnant women with IGT, ADMA levels were found to be high [2], in the present study which may be related to the significantly high arginine levels in this group (p = 0.003). According to the results of the correlation analysis (Table 2), arginine levels seem to be related to

Table 2. Correlation analysis results

	Age	Smoking	DM history	Weight gain	fetal weight	ADMA	Arginine	BMI
Age	1.000							
Smoking	-0.082	1.000						
DM history	0.025	-0.056	1.000					
Weight gain	0.038	0.079	-0.102	1.000				
Fetal weight	-0.075	0.079	0.004	0.138	1.000			
ADMA	-0.020	0.054	0.217*	0.001	0.081	1.000		
Arginine	-0.019	0.165	0.101	0.010	-0.047	0.219*	1.000	
BMI	0.415**	-0.003	0.191*	-0.106	0.183	0.029	-0.012	1.000

**Correlation is significant at the 0.01 level *Correlation is significant at the 0.05 level (2-tailed).

ADMA levels (arginine increases as ADMA increases). Arginine is a semi-essential amino acid that should be taken in the diet by infants, pre-term infants, and at certain stages of growth. Arginine suplementation can be recommended in hypoxic conditions (e.g. in fetuses with meconium) [27]. NOS catalyzes the conversion of arginine to NO, and NOS is inhibited by ADMA(5) An increase in arginine levels in the IGT group is thought to be a consequence of the inhibition of NOS. An increase in arginine levels in our study is thought to reflect the tendency of ADMA levels to increase.

ADMA values were found to be related to the BMI of the pregnant women and the DM history in the family. The fact that a past GDM history was found to be associated with high ADMA levels is consistent with these results [16]. In gestational diabetes, the risk continues after birth, and those women are considered to have a high risk of cardiovascular disease [28]. There is a previous study on the importance of ADMA in fetoplacental and neonatal circulation [29]. In this study, placental macro and microvascular endothelial dysfunction was established in diabetic pregnant women, but no clear information could be obtained on this subject [20, 21]. In addition, high serum levels of ADMA detected in the umbilical vein of infants complicated with meconium may indicate that ADMA influences fetoplacental circulation.

In this study, the two-step approach by Carpenter and Coustan was used in the diagnosis of GDM [22]. In the two-step test used for the diagnosis of GDM, in those patients with high values from the 50-g glucose loading test, diagnosis was made with at least two high values in the 100-g 3-hour OGTT. Pregnant women in whom the 50-g test results were found to be high between weeks 24–28 were candidates for the 100-g OGTT. The GDM diagnosis was made when two or more values were above the reference levels (fasting blood sugar 95mg/dl; 1-hour blood sugar 180 mg/dl; 2-hour blood sugar 155 mg/dl; and 3-hour blood sugar 140 mg/dl). Recently, the International Association of Diabetes in Pregnancy Study Group (IADPSG) published a consensus derived from the Hyperglycemia Adverse Pregnancy Outcome (HAPO) Study and suggested that all pregnant women without known diabetes should be given a 75-g OGTT rather than the two-step approach (30). However, the Turkish Endocrinology Organization recommended continuing to use the Carpenter–Coustan criteria until the results of using the 75-g OGTT were evaluated in Turkey. A limitation of this study is the lack of a GDM group diagnosed with 75-g OGTT criteria. Our results in this study might suggest that group 2 patients(IGT group)who are still being followed up as having normal pregnancies, have also been affected by endothelial dysfunction. This finding should perhaps be taken into consideration while the use of the 75-g–50/100-g approaches are being evaluated in Turkey.

Conclusions

In conclusion, a potential rise in ADMA levels is thought to be expressed as an increase in arginine levels in the IGT group. However, when the relationship between ADMA and a family history of DM is taken into consideration, it can be stated that in pregnant women with IGT and GDM, if there is a history of diabetes and their BMI is high, then their ADMA level is liable to increase. Even though ADMA does not appear to influence the weight of infants in the IGT and GDM group, it is not clear whether this is due to high arginine levels or high insulin levels. When fetoplacental macro and microvascular dysfunction is considered in GDM, more detailed prospective studies investigating the relationship between ADMA, arginine, fetal weight and the fetoplacental unit are required. In addition, studies on the compensatory effect of the intake of food that is rich in arginine content on increased ADMA levels in GDM cases will further elucidate the issue.

List of abbreviations:

ADMA: Assymetric dimethylarginin

NOS: Nitric oxide synthase

GDM: Gestational diabetes mellitus

NGT: 50-g glucose loading test was normal

OGTT:100-g oral glucose tolerance test

IGT: impaired glucose tolerance

NO:Nitrite oxide

Competing interests

The authors declare that they have no competing interests.

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