ORIGINAL ARTICLE

Eastern Journal of Medicine

East J Med 27(4): 507-512, 2022
DOI: 10.5505/ejm.2022.75428

Serum Ischemia Modified Albumin and Oxidative Stress Levels in Patients with Gestational Diabetes Mellitus

Ayşe Arslan

Van Yuzuncu Yil University, Faculty of Health Sciences, Department of Nutrition, Van, Turkey

ABSTRACT

Gestational Diabetes Mellitus (GDM) is a metabolic condition that develops during pregnancy and is associated with complications. This studies aim was to evaluate and measure the oxidative stress markers and antioxidants levels in women with gestational diabetes and compare them with healthy pregnant women.

There were 77 pregnant women who participated in this case-control study. Forty female patients diagnosed with GDM participated in the study as the case group and 37 healthy women as the control group. All biochemical analyzes (IMA, MDA, GSH, CAT) were performed using the spectrophotometric method.

When comparing the two groups, the patients group's serum IMA levels (1.531±0.407 AbsU) were higher than those of the control group's (0.863 ±0.092 AbsU) ($p<0.001$). Similarly, the patient group's MDA levels (1.769 ±0.357 µmol/L) were higher than the control group's (1.025 ±0.018 µmol/L) ($p<0.001$). Conversly, the patient group's GSH levels (0.023 ±0.014 mmol/g) were lower than those of the control group's (0.186 ±0.012 mmol/g) ($p<0.001$). This study also found that both groups' CAT activity levels had no significant statistical difference (GDM-0.072±0.019 and Control-0.075±0.013 U/L) ($p<0.395$).

These results suggest that while the oxidative stress increased, the antioxidant defense levels decreased in women with GDM. It is recommended that patients with GDM are strictly monitored throughout pregnancy, which may help in the management of oxidative stress together with glycemic control.

Keywords: Gestational diabetes, ischemia modified albumin, oxidative stress

Introduction

During pregnancy, one of the most common metabolic disorders that develop is Gestational Diabetes Mellitus (GDM), which, after pregnancy, may be recovered from or may become chronic. GDM is correlated to an amplified risk of maternal and perinatal morbidity (1,2). Although globally the prevalence of GDM varies between 2% and 14%, these rates continue to increase every year (3). Some risk factors for devolping GDM include having a family history of diabetes or stillbirth, an advanced maternity age, being obese, or gaining excessive weight during pregnancy (4).

Oxidative stress is defined as a phenomenon which is characterized by the body's excess production of free radicals as well as the weakening of the body's antioxidant defense system (5), of which the most common free radicals are superoxide anion, hydroxyl and peroxyl radicals. The over production of free radicals in the organism can cause cellular damage and cause depletion of antioxidants (6). Lipid peroxidation's final products can cause DNA and protein damage. The end product of lipid peroxidation is malondialdehyde (MDA), which is an aldehyde that can be measured in plasma and is considered as an indicator of oxidative stress (7). The literature also states that a form of oxidatively modified albumin, ischemia-modified albumin (IMA), increases during normal pregnancy and gestational diabetes and may be an indicator of oxidative stress (8,9). Antioxidants are molecules or enzymes that are found in tissues and blood that have the ability to neutralize or balance free radicals (10). Glutathione (GSH) is the most common cellular antioxidant molecule. It exists in 2 forms, reducible, which is the more dominate form, and oxidized. Previous studies have reported that GSH levels in GDM patients were either lower (11) or higher than a control group (12).

Catalase (CAT; E.C. 1.1.11.16) is a well-known, important antioxidant enzyme which divides the hydrogen peroxide molecule into molecular oxygen

*Corresponding Author: Ayşe Arslan, Ayse Arslan: Assist. Prof. (PhD)- Department of Nutrition, Faculty of Health Sciences, Yuzuncu Yil University, Tusba, Van, Turkey
E-mail: aysearslan@yyu.edu.tr, **Phone:** +90-0533-551-88-38
ORCID ID: Ayşe Arslan: 0000-0003-3876-8376
Received: 15.08.2022, **Accepted:** 28.08.2022

and water (13). In previous studies, it was found that catalase activity decreased (14) or did not change (15) in patients with GDM.

The relationship between gestational diabetes and oxidative stress has not been completely researched and evaluated in the literature, and there is limited data available on IMA levels in GDM patients. This study's goal was to study was to examine oxidative stress markers and IMA levels in pregnant women who had gestational diabetes then compare them with healthy pregnant women without diabetes.

Materials and Methods

In this study, a patient group of 40 pregnant women, whose ages were between 18 and 40, were included who had a diagnosis of GDM from the Endocrinology and Metabolism Diseases Clinic of Yuzuncu Yıl University Dursun Odabaşı Training and Research Hospital. Additionally, the control group of this study had 37 healthy pregnant women. The control group women had no reported complications during pregnancy. The volunteers in the patient group were in their 24-28 weeks of pregnancy, and were diagnosed with GDM in accordance with the American Diabetes Association (ADA) criteria, all of whom underwent a 75 gram, oral glucose tolerance test (OGTT). All patients fasted for a minimum of 8 hours, then blood samples were drawn venously from all patients for biochemical analysis. The collected samples were then centrifuged at 5,000 rpms for 5 minutes so that the serum was separated. The serums were refrigerated at -20 degrees Celcius until the day of processing. The exclusion criteria for this study was GDM patients who had additional conditions, such as hypertension, preeclampsia, placenta previa, hypothyroidism or a history of insulin therapy. The demographic and clinical attributes of both groups (fasting blood glucose, insulin, Hemoglobin A1c (HgA1c), HOMA-IR index were obtained through their hospital records. Patient's ischemia modified albumin, MDA, GSH and CAT analyzes were done using spectrophotometer in the biochemistry laboratory at Yüzüncü Yıl University, Faculty of Science. All patients who participated in the study gave written consent. This study received approval from the ethical committee of Van Yüzüncü Yıl University and followed the guidelines and ethical standards of the Declaration of Helsinki.

Biochemical Analysis

Determination of Catalase Activity

Solutions Used:

1. Preparation of 30 mM H2O2: 34 µl of 30% H2O2 was put into 10 ml of bidistilled water.

2. Preparation of 50 mM Phosphate Buffer: 6.81 g KH2PO4 and 7.1 g Na2HPO4 were dissolved in bidistilled water, the pH of the buffer was adjusted to 7.4 with 1N NaOH and the volume was made up to 1 liter.

Using H2O2 as the substrate, the serum CAT activity was measured. The catalase activity was determined using the Aeibi method (16). First, two tubes were prepared by adding 0.1 ml of enzyme to the first tube and then 0.1 ml of phosphate buffer was added to second tube, after which 1.4 ml 30 mM of H2O2 was added to both tubes, then both tubes were vortexed. Absorbance readings were taken twice at 30 second intervals at 240 nm, and the resulting enzyme activity was reported as units per liter (U/L).

Activity Calculated

$E.U.=(2,3/\Delta X) \times [(\log A_1 / \log A_2)]$

ΔX=30 second

2,3=Optical density of 1 µ mol H2O2 in 1 cm light path

GSH level determination

Solutions used:

1. Phosphate buffer: 0.3 M disodium phosphate was prepared with bidistilled water.

2. Ellman's reagent: 1% sodium citrate was dissolved in 100 ml of bidistilled water. 40 mg of DTNB (5',5'-(2-dithiobis nitrobenzoic acid) was added into it.

In a tube, phosphate buffer (800 µl) was added to the serum (200 µl), after which the absorbance (OD1) was registered for the first time at 412 nm. Then adding Ellman's reagent (100 µl) the absorbance (OD2) was registered for the second time. To calculate the glutathione concentration, mmol/g protein unit was used.

Calculation:

C / 1000 = (OD2-OD1) / 13600 x E1 x 5/2 x ½

13600: Molar extinction coefficient of yellow color formed during the interaction of Glutathione and 5',5'-(2-dithiobis nitrobenzoic acid).

E1: If the bandwidth is greater than 6 nanometers, a derivative extrinsic coefficient is used that corrects for both light path and bandwidth differences. The bandwidth we used was 2 nm.

It will be taken as E1=1 in calculations.

1000: conversion coefficient to mmol.

C: mmol / glutathione (mg/dl)

Determination of MDA level

Solutions used:

1. 0.1 M EDTA solution (Ethylene diamine tetra acetic acid disodium): 37.224 gr EDTA-Na2H2O was dissolved in 1 liter of bidistilled water.

2. 88% BHT solution (Butyl hydroxy toluene): 0.220 gr BHT was dissolved in 25 ml pure alcohol.

3. 0.05 N NaOH solution (Sodium hydroxide): 2 gr NaOH was dissolved in 1 lt bidistilled water.

4. 1% TBA solution (Thiobarbituric acid): 1 g of TBA was added to 100 ml with 0.05 N NaOH.

5. 30% TCA solution (trichloroacetic acid): 30 gr TCA was dissolved in 100 ml distilled water.

6. Phosphate Buffer: 8.1 gr NaCl, 0.194 gr NaH_2PO_4, 2.302 gr Na_2HPO4 are dissolved in bidistilled water and made up to 1 lt. The pH was adjusted to 7.4 with 1N NaOH.

First, 200 µl of serum was drawn into a tube, then phosphate buffer (800 µl), BHT solution (25 µl) and 30% TCA (500 µl) were added to the tube. After mixing the tube in a vortex, it was put in an ice bath for a period of 2 hours, then was brought up to room temperature. It was then centrifuged for a period of 15 minutes at 2,000 rpm, from which 1 ml of supernatant (filtrate) was obtained and transferred to another tube. Then ethylenediaminetetraacetic acid (EDTA) (75 µl) and thiobarbituric acid (TBA) (25 µl) were added to the filtrate. The tube was vortexed and put in a 70 °C hot water bath for a period of 15 minutes, then was cooled down to room temperature. The absorbance rates were read with a UV/Vis spectrophotometer at 532 nm. The MDA level was calculated in µmol/L.

Malondialdehyde level calculation:

C = concentration

F=Dilution factor

A=Absorbance

C = F x 6.41 x A

Determination of ischemia modified albumin level

Solutions used:

1.Cobalt chloride (1gr/L)

2.Dithiothreitol (DTT,1.5 gr/L)

3.%0.9 NaCI

Assessment of IMA levels in serum was determined spectrophotometrically. First, 0.5 ml of cobalt chloride was added to 200 µl of serum, after ten minutes of incubation, 50 µl dithiothreitol was added and mixed. 1 ml of NaCl was added after 2 min. The resulting colored complex was measured by spectrophotometer at a wavelength of 470 nm. (17) The IMA values were measured in absorbance units (AbsU).

Statistical Analysis: The normality of the distribution of the data was tested using the Kolmogorov-Smirnov test. Levene's test was used to test the homogeneity of variances. All quantitative data was stated as the mean ± standard deviation (SD). The T-Test was used in cases where the normal distribution condition was provide in paired group comparisons and the Mann-Whitney U test statistic was used in cases where the normal distribution condition was not provided. The results were considered to be statistically significant when less then 5% ($p<0.05$). All data was analyzed using the IBM® SPSS® software (IBM Corporation, Newyork, NY, USA).

Results

Both groups' demographic and biochemical data are shown on Table 1. There was no significant difference, in terms of maternal and gestational age between the groups. The blood glucose, insulin, HOMA-IR and HbA1c levels were higher in the patient group, but no difference was found in terms of total cholesterol, HDL cholesterol, LDL cholesterol and triglyceride levels between the groups.

The serum CAT, GSH, MDA and IMA values of both groups are shown on Table 2. The serum IMA levels were higher in the patient group (1.531 ±0.407) than control group (0.863 ±0.092) ($p<0.001$). Like wise, the MDA levels were higher in the patient group (1.769 ±0.357), control group (1.025 ±0.018) ($p<0.001$). When the GSH levels between the groups were examined, the GSH levels of the patient group (0.023 ±0.014) were lower than control group (0.186 ±0.012) ($p<0.001$). This study found no significant statistical difference of CAT activity levels between the groups, Patient CAT, 0.072 ±0.019 and Control CAT, 0.075 ±0.013 ($p< 0.395$).

Discussion

The results obtained from our study show that patients with GDM have an increase in oxidative stress markers (IMA, MDA) and decrease in antioxidant defense (GSH). Previous studies have examined the relationship between GDM and oxidative stress markers.

One study found that pregnant women with Type 1 diabetes and GDM had higher plasma and erythrocyte MDA levels. The study also found that the GPX activity was substantially lower in both the Type 1 diabetes and GDM groups than in the healthy control

Table 1. Characteristics and Some Biochemical Data of The Gdm and Control Groups

	GDM (n=40)	Control (n=37)	p
Maternal age (year)	26.68±2.65	26.92± 1.94	0.422
Gestational age	25.55±3.51	26.32±2.60	0.613
Glucose (mg/dl)	88.01±15.46	78.62±8.47	0.001
HgbA1c (%)	5.75±0.72	4.62±0.40	0.001
Insulin (IU/ml).	10.36±6.13	6.70±2.81	0.001
HOMA-IR	2.42±2.31	1.32±0.60	0.001
Total-cholesterol(mg/dl)	225.37±41.14	230.02±58.17	0.258
LDL-cholesterol(mg/dl).	121.35±33.39	129.22±54.43	0.158
HDL-cholesterol (mg/dl)	53.57±12.36	56.76±12.32	0.451
Triglyceride (mg/dl)	178.68±52.23	180.91±64.22	0.805

Values are means ±standard deviation ($p < 0.05$)

Table 2. Serum IMA, MDA, GSH and CAT Values of The Patient and Control Groups

	GDM (n=40)			Control (n=37)			p
	Mean±SD	Min.	Max.	Mean±SD	Min.	Max.	
IMA (AbsU)	1.531±0.407	1.045	2.000	0.863±0.092	0.724	1.016	0.001
MDA (μmol/L)	1.769±0.357	1.025	2.371	1.025±0.018	1.009	1.075	0.001
GSH (mmol/g)	0.023±0.014	0.004	0.061	0.186±0.012	0.147	0.199	0.001
CAT (U/L)	0.072±0.019	0.006	0.092	0.075±0.013	0.039	0.097	0.395

Values are means ±standard deviation ($p < 0.05$)

group (15). Another study compared twenty pregnant women who had gestational diabetes against twenty pregnant women who were healthy found that the serum MDA levels were higher in the patient group, but found no significant difference in CAT activity in the groups (18). In another study on patients with GDM, the study investigated oxidative stress biomarkers and its relationship to pregnancy outcomes, and found that serum MDA levels were higher and that the GSH and SOD levels were lower in patients with GDM when compared to healthy subjects (3). In a study conducted on pregnant women that had Type 1 DM, Type 2 DM and GDM, it was determined that the lipid peroxide levels were higher in each trimester and that the antioxidant capacity was significantly decreased (19).

Various mechanisms have been theorized as the cause of oxidative stress in diabetes patients, but the increase in glucose oxidation due to hyperglycemia is shown as the main source of free radicals (20). Under hyperglycemic conditions, more glucose flows through the glycolytic pathway, which produces more pyruvate and acetyl-CoA, which then leads to more nicotinamide adenine dinucleotide + hydrogen (NADH) production. Since NADH is an electron carrier, an excess of which causes electron pressure in the mitochondrial electron transport chain. Increasing the NADH concentration in mitochondria also increases the NADH oxidation to reduce it. Superoxide radicals are formed as a result of the electron leakage that occurs during the oxidation of NADH to nicotinamide adenine dinucleotide (NAD). Superoxide is the precursor of all other reactive oxygen derivatives, and this increase in the superoxide levels has a role in the formation of oxidative stress. Abnormally high free radical levels and the resulting decrease in antioxidant defense mechanisms may cause damage to cellular organelles and enzymes, an increase in lipid (LDL) peroxidation and the development of insulin resistance. In addition, various factors such as non-enzymatic glycation of proteins and the resulting oxidative degradation of glycated proteins can cause the production of free radicals (21, 20, 22).

The amino-terminal (N-terminal) amino acid sequence of the albumin molecule is the primary binding site of transition metals such as cobalt, nickel, and copper. In conditions such as free radical damage, acidosis, and hypoxia, the N-terminal of albumin is modified, called ischemia modified albumin, which reduces its capacity to bind transition metals (23, 24). A study has found that IMA levels increase due to physiological oxidative stress during pregnancy (8). In a study that investigated the maternal serum IMA levels in normal pregnancies, it was determined that IMA levels were higher in pregnant women than non-pregnant women and correlated with MDA. In the same study, it was suggested that IMA levels may increase markedly in women with various pregnancy-related complications (25). In a study investigating the IMA levels in women with gestational diabetes, IMA levels were found to be higher than in pregnant

women without diabetes, and the study concluded that IMA may be a biomarker of oxidative stress in gestational diabetes (9). This is the only other study in the literature that investigates IMA levels in pregnant women with gestational diabetes and is consistent with our results. It has been suggested in the literature that IMA levels increase due to hypoxia and oxidative stress which is triggered by hyperglycemia in diabetes patients (26).

Our study had some limitations. First is the limited number of cases that constituted the study group. Second, the study was limited to the gestational period and no postpartum follow-up was performed. Third, IMA levels and oxidative stress parameters could not be compared in different trimesters during the pregnancy. Despite these limitations, our study is one of the limited number of studies found in the literature that investigated, especially IMA levels, in gestational diabetes.

In summary, we found that in patients with GDM, the oxidative stress (IMA and MDA) increases and antioxidant defense (GSH) decreases. Oxidative stress may play an important role in the development of pregnancy complications and clinical outcomes of the disease in patients with GDM. The strict monitoring of GDM patients during pregnancy, coupled together with glycemic control, may have a significant positive impact on the management of oxidative stress and the maintenance of mother-infant health.

Acknowledgement: I would like to thanks to Prof. Dr. Halit DEMİR (Yüzüncü Yil University, Faculty of Science, Department of Biochemistry) and Assoc. Prof. Murat Alay (Yuzuncu Yil University, Faculty of Medicine, Department of Endocrinology) for their contributions to our study.

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