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Original Research Article
Oxidative DNA damage in gestational diabetes mellitus: Correlation with antioxidants in an Iraqi cohort

ABSTRACT

Aims: This study aimed to evaluate the degree of oxidative stress in gestational diabetes mellitus when compared to non-diabetic pregnant women.

Methodology: This cross-sectional study included 73 participants (29 gestational diabetic women and 44 control pregnant women) attending the Maternal and Childhood Unit, Al-Husayniya Medical Centre, Baghdad, Iraq. Suitable candidates were invited to participate in the study and were given details about the study plan and aims. Participants were asked to fill and sign the consent form before they can be included in the study. The data was analyzed using SPSS (Version 14) and Microsoft Excel (Office2007, Microsoft). All values were expressed as mean $\pm$ standard deviation (M $\pm$ SD).

Results: Serum 8-Hydroxy-2-Deoxyguanosine was significantly greater in the gestational diabetes mellitus group compared to control group (57.2 $\pm$ 17.6 ng/dl versus 19.8 $\pm$ 7.8ng/dl respectively). The increase in 8-Hydroxy-2-Deoxyguanosine was associated with a significant elevation in serum malondialdehyde level and a significant reduction in plasma reduced glutathione in the gestational diabetes mellitus group compared to the control group at $P=.05$. A significant change in total cholesterol (5.4 $\pm$ 1.1mmol/L) and low density lipoprotein cholesterol (3.3 $\pm$ 0.9mmol/L) were also noted in gestational diabetes mellitus group compared to the control group (4.7 $\pm$ 1.3mmol/L and 2.8 $\pm$ 1mmol/L respectively) at $P=.05$.

Conclusion: An increase in 8-Hydroxy-2-Deoxyguanosine is associated with higher levels of malondialdehyde and a significant reduction in reduced glutathione in gestational diabetes mellitus group, suggesting that significant oxidative stress associated with lipid peroxidation is occurring. Measuring these markers is useful in monitoring gestational diabetes mellitus to prevent the negative outcomes of gestational diabetes mellitus such as increased risk of diabetes and fetal morbidity.

Keywords: Gestational Diabetes, Oxidative DNA damage, Lipid peroxidation, Antioxidants.

1. INTRODUCTION

10 Gestational diabetes mellitus (GDM) is a clinical disorder characterized by an elevated glucose level
11 which is first detected in pregnancy [1] and it accounts for 3-5% of pregnancy complications [1, 2]. It
12 affects pregnant women predominantly at third trimester due to placental hormones action on
13 maternal blood glucose hemostasis [3]. The disorder is associated with high maternal and fetal
14 morbidity [1, 2]. Previous studies have shown that women with a history of GDM are more likely to
15 develop type 2 diabetes (T2DM) mellitus and cardiovascular diseases after pregnancy as compared
16 to women with normal blood glucose level during pregnancy[4]. In addition, type 1 diabetes mellitus
17 (T1DM) is also known to occur following GDM particularly if associated with familial history of T1DM
18 [2].

19 There are several risk factors for development of GDM including (but not limited to) maternal age,
20 previous multiple births, family history of diabetes mellitus and an abnormal high body weight
21 (overweight and obesity) [1]. Therefore, these factors have regularly been used for screening
22 purposes for GDM [1].

23 Johnstone et al. in 1990 showed that controlling plasma glucose level is critical in reducing the
24 incidence of microvascular complications due to GDM [5]. However, reducing plasma glucose level
25 has no effect on reducing the rate of congenital anomalies (like macrosomia) [5, 6]. Fetal
26 malformation may occur not only due to maternal hyperglycemia, but also due to an abnormal level of
27 maternal lipids and amino acids which may accompany a badly controlled diabetes mellitus. Elevated
28 low density lipoprotein-cholesterol (LDL-C) and reduced high density lipoprotein-cholesterol (HDL-C)
29 promote lipid peroxidation through free radical activity, which is increased due to the increased
30 plasma glucose levels.

31 Oxidative stress is common in GDM and occurs due to an imbalance between total free radical
32 production and the body's ability to remove them via its antioxidant systems [7, 8]. Hence previous
33 studies have focused on monitoring a variety of low molecular weight antioxidants and how these are
34 related to GDM [9]. The cumulative action of all antioxidants present in plasma and body fluids is
35 referred to as the total antioxidant status (TAS) which gives an estimation of the sum of measurable
36 antioxidants [3, 10].

37 In normal pregnancy, the degree of oxidative stress and lipid peroxidation products predominates
38 mostly because of the mitochondria- rich placenta and abundant transitional metals such as iron [6,

11, 12]. However, most body cells are capable of counter acting a small increase in oxidative stress, adequately stimulating the activity of antioxidants such as glutathione (GSH). But, when this capacity gets exhausted, the continuing rise of reactive oxygen species (ROS) may cause a severe irreversible damage to cellular components [13]. Oxidative stress is much higher in GDM due to hyperglycemia that favors generation of high amounts of reactive oxygen species and enhances progression of diabetes [6, 14].

Malondialdehyde (MDA) is a biomarker of lipid peroxidation, among other by-products such as pentane, ethane, hexanal and thiobarbituric acid reacting substance (TBARS). 8-Hydroxy-2-Deoxyguanosine (8-OHdG) is a known sensitive marker of oxidative DNA damage and of total systemic oxidative stress [15, 16]. Studies have shown that higher levels of this marker in early pregnancy may be associated with increased GDM risk [17].

Cells possess a wide variety of antioxidants, which under normal conditions are able to minimise the damaging actions of oxygen free radicals. These include enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase (GPX) and small molecules such as vitamin E, vitamin C, carotenoids and glutathione [18]. Antioxidants may be critical to preventing fetal malformations like birth defects in babies of women with diabetes, especially neural tube defects that occur two to five times more often in GDM than in women without diabetes mellitus [6, 19]. Reduced glutathione (GSH) constitutes the principal antioxidant defense system of the body [20] and is a cellular antioxidant found in red blood cells [20]. Patients with diabetes tend to have a smaller amount of GSH and a more oxidized glutathione pool than control subjects of a similar age [21], which weakens the defense against oxidative stress [21].

The current study aims to measure the markers of oxidative stress (as expressed by the level of serum MDA and serum 8-OHdG) and correlate their levels with the antioxidants (as expressed by the level of plasma reduced GSH) in an Iraqi cohort of women whom diagnosed with GDM. This study also aims to compare the extent of oxidative damage present in GDM women compared to non-gestational diabetic women by measuring 8-OHdG levels, lipids, serum MDA and plasma GSH.

2. METHODOLOGY

66 *Protocol:* The study protocol was reviewed and approved by the Scientific and Ethical Committee of
67 the College of Medicine, Al-Nahrain University. Informed consent was obtained from each subject.
68 Participants were drawn from the Maternal and Childhood Unit, Al-Husayniya Medical Centre,
69 Baghdad, Iraq during the period of the study between January 2008 and May 2010. Only participants
70 where complete data was available as required for this study were included in the analysis.
71 Participants were divided into two groups: a) Control group (Control): including pregnant women with
72 normal blood glucose levels and no family history of diabetes. b) Gestational diabetes group (GDM
73 group): including pregnant women who were diagnosed with diabetes mellitus for the first time during
74 the current pregnancy according to the recommendations of the World Health Organization for
75 definition and diagnosis of diabetes mellitus and intermediate hyperglycemia [22]. No exclusions were
76 applied for the GDM group as cardiovascular and/or renal disease occur as a part of disease
77 progression. Participants in the two groups were comparable for age, gender, smoking habit, diet, and
78 physical activity. Women with gestational diabetes were requested not to take any diabetic medication
79 in the morning of the day of the test before blood sampling. All blood samples were collected prior to
80 10 am to reduce any possible hyperglycemic events in those with GDM.

81 *Measurements of oxidative stress:* Serum MDA was measured using the thiobarbituric method of
82 Buege & Aust [23]. Oxidative DNA damage was measured using the serum 8-OHdG ELISA kit
83 (Cayman Chemical, MI, USA) [24]. The test utilizes an anti-mouse IgG-coated plate and a tracer
84 consisting of an 8-OHdG-enzyme conjugate. This format has the advantage of providing low
85 variability and increased sensitivity compared with assays that utilize an antigen-coated plate. The
86 level of plasma GSH was determined using the 5,5-dithiobis-2-nitrobenzoic acid (DTNB) reaction [25,
87 26].

88 *Measurement of serum lipids:* Serum lipids were measured using ELISA Kits from BioMerieux,
89 France.

90 *Statistical analysis:* The data was analyzed using SPSS (Version 14) and Microsoft Excel (Office2007,
91 Microsoft). All values were expressed as mean $\pm$ standard deviation ($M \pm SD$). Statistical analysis was
92 performed using an independent sample t-test, if data was normally distributed; otherwise a Mann
93 Whitney test was applied. In all tests, $p < 0.05$ was considered to be statistically significant.

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3. RESULTS

Table 1 illustrates the baseline values of the 73 participants. Disease status, fasting blood glucose level (FPG), age, gestational weeks, glycosylated hemoglobin (HbA1c), blood pressure and Body mass index (BMI) are shown.

TABLE 1. Characteristics of the subjects included in this study

	Control Group	Gestational Diabetes Group
Number	44	29
Age (years)	24.4 ± 4.7	24.9 ± 5.2
Gestational Weeks at time of blood collection	24.5 ± 3.8	23.3 ± 2.4
Body Mass Index (kg/m²)	25.8 ± 4.3	27.5 ± 2 [*]
Fasting Plasma Glucose (mmol/L)	4.5 ± 0.3	6.1 ± 0.7 ^{**}
HbA1c %	5.7 ± 0.5	7.6 ± 1 ^{**}
Systolic Blood Pressure (mmHg)	116.8 ± 6.3	118.8 ± 6.1
Diastolic Blood Pressure (mmHg)	73.1 ± 7.4	73.2 ± 7.7

^{*}significant difference ($P = .05$)

^{**}significant difference ($P < .001$)

Using an independent samples t-test, FPG and HbA1c were significantly different between the Control group and the GDM group ($p < 0.001$). BMI also showed a significant increase in the GDM group ($P = .05$), (Table 1).

For serum lipids, there was a statistically significant elevation of total cholesterol (TC), (LDL-C) as well as TC/HDL-C ratio in the GDM group compared to the Control group ($P = .05$) (Table 2).

TABLE 2. Serum lipid profile (mean ± SD) in control and gestational diabetes groups (mmol/L)

Serum Lipid Profile	Control Group	Gestational Diabetes Group
Total Cholesterol	4.7 ± 1.3	5.4 ± 1.1 [*]
Triglycerides	1.7 ± 1	1.7 ± 0.8
HDL-C	1.3 ± 0.3 [*]	1.2 ± 0.4
LDL-C	2.8 ± 1	3.3 ± 0.9 [*]
TC/HDL Ratio	3.8 ± 1.1	5.3 ± 3.3 [*]

HDL-C high density lipoprotein cholesterol; LDL-C low density lipoprotein cholesterol.

^{*}significant difference ($P = .05$)

There was also a significant elevation of serum 8-OHdG in the GDM group compared to the Control group ($P < .001$) (Table 3). This was accompanied by a significant rise in serum MDA in the GDM group (2.1 ± 0.8) compared to the Control group ($P < .001$).

TABLE 3 TABLE 3 Biomarkers of oxidative stress (mean ± SD) in control and gestational diabetes groups

Oxidative Stress Biomarkers	Control Group	Gestational Diabetes Group
MDA (nmol/ml)	1 ± 0.4	2.1 ± 0.8 ^{**}
GSH (mg/dl)	24.1 ± 4.4	20.6 ± 5 [*]
8-OHdG (ng/dl)	19.8 ± 7.8	57.2 ± 17.6 ^{**}

^{*}significant difference ($P = .05$)

^{**}significant difference ($P < .001$)

MDA (serum malondialdehyde); GSH (plasma reduced glutathione); 8-OHdG (8-OH-2-deoxy-Guanosine).

The statistical analysis also showed a significant positive correlation between serum 8-OHdG and serum MDA in our participants ($r=0.67$, $P = .05$), (Figure 1).

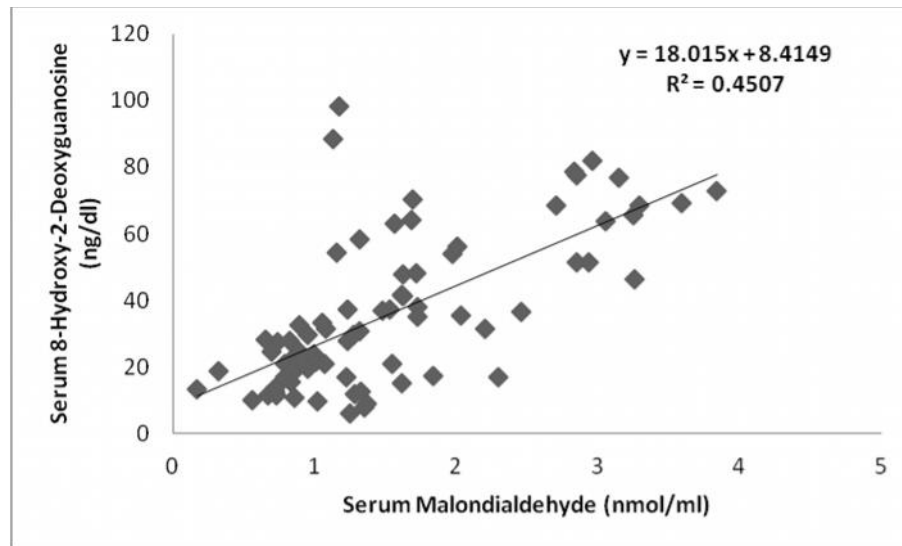


FIGURE 1 Significant positive correlation between serum 8-hydroxy-2-deoxyguanosine and serum malondialdehyde among all participants of the study ($r=0.67$, $P = .05$)

A reduction in the plasma GSH levels was observed in the GDM group compared to the control group ($P = .05$) (Table 3) and is associated with a significant negative correlation to serum 8-OHdG ($r=0.36$, $P = .05$), (Figure 2).

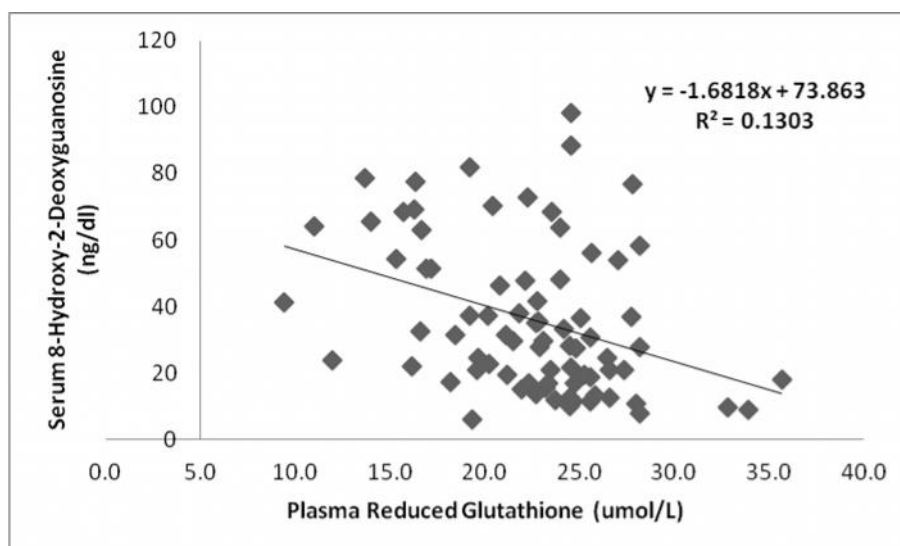


FIGURE 2 Significant positive correlation between serum 8-hydroxy-2-deoxyguanosine and plasma reduced glutathione among all participants of the study ($r=0.36$, $P = .05$)

4. DISCUSSION

This research has demonstrated the efficacy of using emerging biomarkers as a multi-marker approach to characterize **GDM during pregnancy**. Increased 8-OHdG combined with increased MDA and a decrease in GSH provides a unique picture of the health state of the patient and can be used as an indicator for individualized treatment. The significant correlation between 8-OHdG with MDA and GSH indicates the extent of lipid peroxidation and oxidative DNA damage.

Gestational diabetes mellitus need to be diagnosed early during pregnancy for optimum control of maternal blood glucose level which is a critical step to minimize the risk on the developing fetus [1, 2]. Although the oral glucose tolerance test can confirm gestational diabetes mellitus, this test is not appropriate during the first trimester of pregnancy, as gestational diabetes is not quite developed at this stage [27]. However early treatment is necessary to prevent fetal pathology. Previous studies agreed that GDM is characterized by a disturbance in the redox balance [28]. This redox imbalance can be measured by the extent of impaired enzymatic antioxidant activities [29], reduced circulatory antioxidants [30], and increased levels of lipid and protein oxidation [28]. But there is still controversy about the most reliable oxidative stress markers for clinical use in GDM.

The increased serum MDA observed in our study in the GDM group compared to the Control group is in accordance with a study reported by Grissa et al [29] and confirms that the reactive oxygen species generated during GDM react with the free lipid and lead to lipid peroxidation products that cause toxic stress to cells such as MDA. Similarly Suhail et al. (2010) and others have reported a significant increase in lipid peroxidation and oxidative stress in GDM [31, 32]. However Karb (2000) reported a statistically non-significant rise of this biomarker in GDM with a much smaller cohort (8 GDM participants) [33]. An association between MDA and 8-OHdG has previously been demonstrated in various pathologies suggesting the involvement of oxidative stress as a major component of disease progression [34].

Serum 8-OHdG was significantly higher in GDM group compared to the Control group. This indicates that oxidative DNA damage is already occurring in GDM where hyperglycemia is prominent compared to the non-gestational diabetes pregnancies. These finding are generally consistent with a relatively large body of literature documenting positive associations of 8-OHdG with hyperglycemia, impaired glucose intolerance, and type 2 diabetes in men and non-pregnant women [35-37]. In addition, the rise in serum 8-OHdG appeared to be consistent with the increase in serum MDA among all our participants (GDM group and control group) indicating an overall disturbance in oxidative stress as illustrated by an increase in lipid peroxidation and protein damage (nucleic acid damage), (Figure 1). Increased 8-OHdG may occur due to cellular processes associated with ROS and lipid peroxidation [38].

In addition, the GDM group showed a reduction in the plasma concentration of reduced GSH in comparison with the healthy pregnant mothers (Table 3). This is in accordance with the findings from other studies [39, 40]. The low level of plasma GSH in GDM can be explained by the higher activities of glutathione peroxidase enzyme to counteract the high level of lipid hydro-peroxidation and protein damage produced as a result of hyperglycemia compared to the non-gestational diabetes mothers[40]. This is evident by the significant negative correlation between serum 8-OHdG and the plasma GSH among all participants of this study (Figure 2).

5. CONCLUSION

Our study showed that measuring the biomarkers of oxidative stress (as expressed by serum MDA and serum 8-OHdG) and antioxidants (as expressed by plasma GSH) is an important step in monitoring the disease progression at second trimester. Further studies are required to monitor the behavior of these markers during the whole pregnancy aiming to help monitor disease progression and reduce the risk of hyperglycemia-associated pathology on pregnant women and their fetuses.

COMPETING INTERESTS

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this study. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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