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Evaluating Markers of Oxidative Stress in Managing Gestational Diabetes Mellitus: A Cross Sectional Study in Iraq

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Authors' contributions

This work was carried out in collaboration between all authors. Author DJ designed the study, performed the statistical analysis and wrote the protocol. Author HA managed the analyses of the study and wrote the first draft of the manuscript. Authors EA and MA managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: The objective of the present study was to evaluate the association of oxidative stress markers and antioxidants in gestational diabetes when compared to non-diabetic pregnant women.
Methodology: This is a cross-sectional study, conducted in Al-Husayniya Medical Centre, Baghdad, Iraq and included 73 participants attending the Maternal and Childhood Unit for the period between January 2008 and May 2010.
Results: Serum 8-Hydroxy-2-Deoxyguanosine was significantly greater in the gestational diabetes mellitus group compared to control group (57.2±17.6ng/dl versus 19.8±7.8ng/dl respectively, *P<.05*). The increase in 8-Hydroxy-2-Deoxyguanosine was associated with a significant elevation in serum total cholesterol and high density lipoprotein cholesterol and a significant reduction in serum superoxide dismutase in the gestational diabetes mellitus group compared to the control group at *P<.05*. A significant

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negative correlation was noted between 8-Hydroxy-2-Deoxyguanosine and superoxide dismutase among all the participants included in this study (r=0.66 at *P<.05*).
Conclusions: The current study proves the importance of measuring markers of oxidative stress (expressed by serum 8-Hydroxy-2-Deoxyguanosine & serum lipids) and antioxidants (expressed by serum superoxide dismutase) in managing cases of gestational diabetes mellitus and provides a useful way of assessing the disease progression and/or remission in response to the treatment.

Keywords: Gestational diabetes; oxidative DNA damage; lipid peroxidation; antioxidants.

1. INTRODUCTION

One of the most important complications of pregnancy is gestational diabetes mellitus (GDM). This clinical disorder is characterised by an impaired glucose level which is first detected during pregnancy [1]. GDM can increase maternal morbidity due to persistent hyperglycaemia [2]. In addition, it increases the risk of foetal malformation like acute respiratory distress, foetal macrosomia, foetal anomalies and platelet hypercoagulability [1].

Previous studies proved that tight glycaemic control can prevent the development of diabetes complications [3]. This is because of the presence of other risk factors which affect the pathogenesis of GDM like the degree of oxidative stress. There are growing evidences showing that oxidative stress can play an important role in the development of maternal and foetal complications of diabetic pregnancies [3].

Oxidative stress occurs when there is an imbalance between generation of free radicals (reactive oxygen and nitrogen species) and the antioxidants scavenging ability [4]. Both of which are disturbed in hyperglycaemia [5]. These free radicals cause lipid peroxidation destructing cellular membrane lipids and oxidative DNA damage, which may lead to the loss of cell survival [6]. Oxidative DNA damage is measured by 8-Hydroxy-2-Deoxyguanosine (8-OHdG), which is produced by oxidation of the nucleoside deoxyguanosine [7]. The accumulation of 8-OHdG in serum provides evidence of increased oxidative DNA damage in patients with diabetes mellitus [8].

Superoxide dismutase (SOD) is one of the main antioxidant enzymes, acts through inhibiting the free radicals reactions (which cause oxidative stress), and maintain iron ions by superoxide [9]. SOD catalyses the dismutation of superoxide anion into hydrogen peroxide and molecular oxygen, thus forming an important antioxidant system [10].

The current study assessed the extent of oxidative DNA damage (as expressed by measuring serum 8-OHdG) and the antioxidant level (by measuring serum SOD) in GDM women compared to the non-GDM pregnant women. Glucose monitoring and serum lipids were also included in the study to complete the picture.

2. METHODOLOGY

2.1 Protocol

The current study was reviewed and approved by the Scientific and Ethical Committee of Al-Nahrain University, College of Medicine. Informed consent was obtained from each subject.

Participants were drawn from the Maternal and Childhood Unit, Al-Husayniya Medical Centre, Baghdad, Iraq during the period of the study between January 2008 and May 2010. Only participants where complete data was available as required for this study were included in the analysis. Participants were divided into two groups: a) Control group (Control group): Including pregnant women with normal blood glucose levels and no family history of diabetes. b) Gestational diabetes group (GDM group): Including pregnant women who were diagnosed with diabetes mellitus for the first time during the current pregnancy according to the recommendations of the World Health Organisation for definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia [11]. Participants in the two groups were comparable for age, smoking habit, diet, and physical activity. Women with gestational diabetes were requested not to take any diabetic medication in the morning of the day of the test before blood sampling. Anticubital maternal venous blood samples were collected prior to 11 am to reduce any possible hyperglycaemic events in those with GDM.

2.2 Measurements of Oxidative Stress

Serum SOD was measured using the SOD assay kit-water soluble tetrazolium salt (Dojindo Molecular Technologies, Rockville, Maryland, USA) [12,13]. Oxidative DNA damage was measured using the serum 8-OHdG ELISA kit (Cayman Chemical, MI, USA) [6]. The test utilizes an anti-mouse immunoglobulin G (IgG)-coated plate and a tracer consisting of an 8-OHdG-enzyme conjugate. This format has the advantage of providing low variability and increased sensitivity compared with assays that utilize an antigen-coated plate.

2.3 Statistical Analysis

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

3. RESULTS

General characteristics of participants included in this study are summarised in Table 1 which show the disease status, age, gestational weeks, body mass index (BMI), fasting blood glucose level (FPG), glycosylated haemoglobin (HbA1c), and blood pressure.

Using an independent samples t-test, FPG and HbA1c levels were statistically significantly elevated in the GDM group compared to the Control group (*P*<.001). BMI also showed a significant increase in the GDM group (*P<.05*), Table 1. This was associated with a significant change in serum lipids. Total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and TC/HDL-C ratio were significantly elevated in the GDM group compared to the Control group (*P<.05*) Table 2.

There was a significant reduction in serum SOD in the GDM group (30.3±3.3U/ml) compared to the Control group (45±6.2U/ml), (*P*<.001) Table 2, Fig. 1. This was accompanied by a significant elevation of serum 8-OHdG in the GDM group (57.2±17.6ng/dl) compared to the Control group (19.8±7.8ng/dl) at (*P*<.001) Table 2, Fig. 2.

The statistical analysis also showed a significant negative correlation between serum 8-OHdG and serum SOD in our participants (r=0.66, *P<.05*).

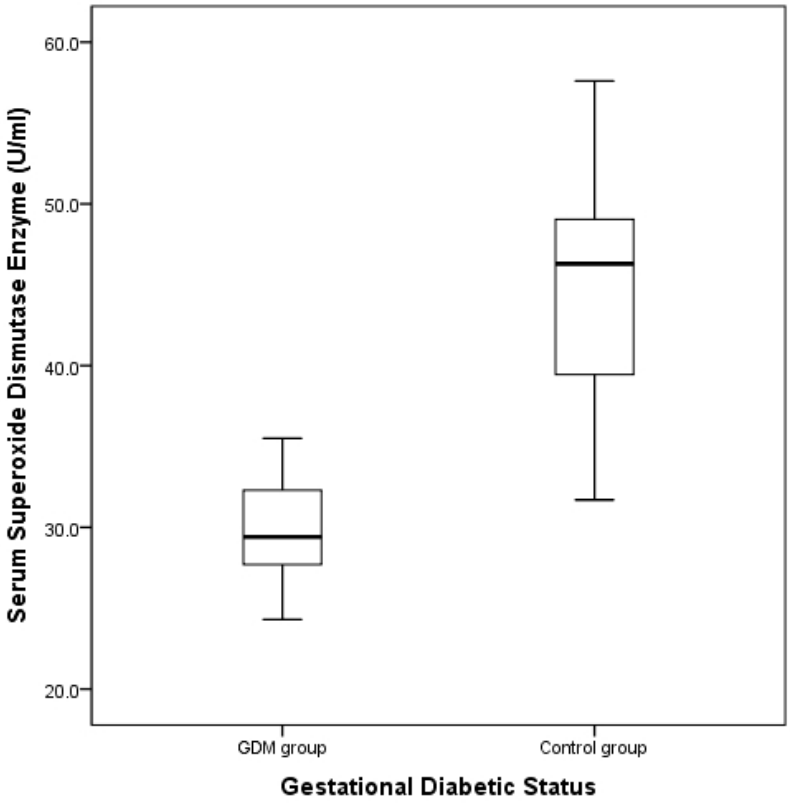

Fig. 1. Significant decrease in serum superoxide dismutase in gestational diabetes mellitus group compared to the control group at *P<.05*

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	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)*

Table 2. Serum lipid profile and oxidative stress markers (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 *
8-OHdG (ng/dl)	19.8±7.8	57.2±17.6 **
SOD (U/ml)	45±6.2	30.3±3.3 **

*HDL-C (high density lipoprotein cholesterol); LDL-C (low density lipoprotein cholesterol); 8-OHdG (8-OH-2-deoxy-Guanosine); SOD (Superoxide Dismutase). * significant difference (P<.05) ** significant difference (P<.001)*

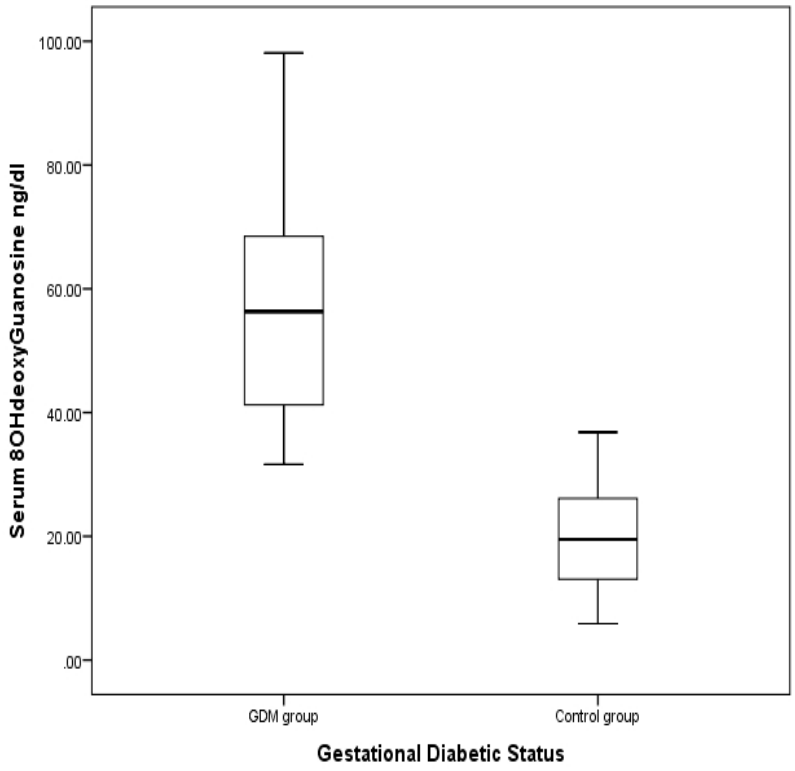

Fig. 2. Significant increase in serum 8-hydroxy-2-deoxyguanosine in gestational diabetes mellitus group compared to the control group at *P<.001*

4. DISCUSSION

Previous studies showed that oral glucose tolerance test is not preferred to be done by the GDM women and also prove to be non-significant in the first trimester before the development of frank gestational diabetes mellitus [14]. This research highlighted the potential of use markers of oxidative stress like 8OHdG and antioxidants like SOD in monitoring the progress of GDM aiming to have a clear understanding of the actual

pathophysiological changes and monitor disease progression. However, more studies need to be done to include more markers of oxidative stress, use of a larger sample size and have a close monitoring of these markers before pregnancy, during pregnancy and after pregnancy.

The increased serum 8-OHdG combined with elevated cholesterol and a decreased serum SOD provides a clear picture of the overall health status of the GDM patient and is a useful indicator to monitor the treatment. The significant negative correlation between serum 8-OHdG and serum SOD indicates the extent of oxidative DNA damage with consumption of the body antioxidant systems.

It has been previously showed that hyperglycaemia observed in type 1 or type 2 diabetes mellitus involves non-enzymatic protein glycosylation and enhanced mitochondrial oxidative events [15]. Previous studies indicated that hyperglycaemia is directly linked to oxidative stress through several pathways including the activation of the sorbitol pathway, the increased formation of advanced glycation end-product formation, activation of protein kinase C isoforms and increased hexosamine pathway flux [16,17]. In our study, women with GDM had higher FPG and HbA1c values, reflecting poor glucose metabolic control. These results may explain a relatively hyperglycaemic status that could enhance free radical production and depress the natural antioxidant defence system. Previously, we showed that pre-diabetic and diabetic patients possess higher oxidative stress as reflected by the increased 8OHdG compared to the normal controls [5,18].

In the current study, serum SOD concentration was significantly decreased in GDM women compared to the non-diabetic normal control women. The possible role of SOD in GDM has not been fully understood. One possible explanation is that SOD scavenges the extracellular superoxide and modulates nitric oxide actions including nitric oxide-dependent uterine relaxation in response to glucose induced oxidative stress [1]. Our results are in accordance with other studies conducted on human beings and experimental animals [19,20]. However, Ruiz et al did not observe any significant change in SOD activity in type 1 diabetes mellitus compared with control group [15]. Other studies have reported that SOD activity was increased in type 2 diabetes mellitus compared with non-diabetic controls [21]. The possible explanation for this variation of SOD activity in diabetes might be related to the incidence and duration of the disease, presence of diabetic complications and the course and type of the treatment [10].

The present study indicated that there is a relative predominance of total cholesterol and LDL-C in GDM, but there was a non-significant change in triglycerides and HDL-C. These findings support the hypothesis that gestational diabetes mellitus affect lipid metabolism and produces more lipid peroxidation [2]. This proves that oxidative DNA damage is already occurring in the early stages of GDM before reaching to the frank hyperglycaemic level compared to the non-gestational diabetes pregnancies. This is in agreement with other studies done previously that measured 8-OHdG in prediabetes and early stages of diabetes mellitus [5,22].

Our study indicated a significant negative correlation between serum SOD and serum 8-OHdG in GDM compared to the Control group. This indicates a close balance between the degree of oxidative stress and the antioxidant reserve systems and it may provide a useful way for monitoring disease progression and the response to the treatment [23].

5. CONCLUSION

The current study proves the importance of measuring markers of oxidative stress (expressed by serum 8-OHdG & serum lipids) and antioxidants (expressed by serum SOD) in managing cases of gestational diabetes mellitus and provides a way of assessing the disease progression and monitoring response to the treatment.

CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this study.

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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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