

Original Research Article

## Product of Free Radical Injury and Antioxidant Status in Patients with Gestational Diabetes Mellitus (GDM)

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### ABSTRACT

**Objective:** This was to determine oxidant and antioxidant status of patients with GDM

**Methods:** Total of 31 pregnant women with GDM and 51 patients with physiologic pregnancy were successfully recruited into the study. Gestational diabetes mellitus was diagnosed taking into consideration the guidelines of IADPSG and WHO in the course of oral glucose tolerance test (OGTT). Blood sample was also taken for the laboratory analysis of malondialdehyde (MDA), glutathione peroxidase (GPx) and superoxide dismutase (SOD).

**Results:** Higher plasma concentration of MDA though not statistically significant ( $p=0.305$ ) was observed in patients with GDM ( $5.75\pm 0.60$   $\mu\text{mol/l}$ ) than patients with Non GDM ( $4.55\pm 0.55$   $\mu\text{mol/l}$ ). Lower plasma concentrations of GPx ( $2.98\pm 1.10$  Vs  $3.12\pm 0.78$  U/ml,  $p=0.017$ ) and SOD ( $69.33\pm 21.94$  vs  $86.47\pm 24.26$  U/ml,  $p=0.62$ ) were observed in patients with GDM than patients without GDM

**Conclusion:** The study shows release of product of free radical injury (MDA) in excess of antioxidants (GPx and SOD) in patients with gestational diabetes mellitus.

**Keywords:** Gestational Diabetes, free radical, antioxidants

### INTRODUCTION

Gestational Diabetes Mellitus (GDM) is being diagnosed using different guidelines. [1,2] The criteria by International Association of Diabetes and Pregnancy Study Group (IADPSG) diagnose GDM at a fasting plasma glucose greater than 5.1mmol/l while criteria from World Health Organisation diagnose GDM at greater than 6 mmol/l. [1,2] Gestational Diabetes Mellitus is also diagnosed using 2- hour post 75g glucose load (2-HPG) of greater than 8.5 mmol/l (IADSPSG) [1] and greater than 7.8 mmol/l (WHO). [2] International Association of Diabetes and Pregnancy Study Group further recognises 1-hour post glucose load

concentration of greater than 10 mmol/l to diagnose GDM. Researchers have found significant changes in some biochemical parameters like oxidant-antioxidant status using any of the set criteria. [3,4]

Gestational diabetes mellitus is an abnormal glucose metabolism occurring for the first time in pregnancy. [5] This has predicted future development of diabetes mellitus in later life in some patients. [6] Despite all the efforts in place to diagnose, understand the pathogenesis as well as treating this gestational disease, pregnant women are still at the risk of developing GDM. The prevalence of 3.8 – 8.6 % has been observed using various guidelines in

Ekiti State, Nigeria. [7] This is not appreciably different from the prevalence of 3-5% observed in some other parts of the world. [8,9] Recently, there are reports from different parts of the world that antioxidants depletion do play a vital role in the pathogenesis of some clinical conditions like systemic hypertension, [10] diabetes mellitus [11] as well as GDM. [3]

Majority of these clinical conditions from the available reports are not only linked with antioxidant depletion but also free radical (oxidant) injury. [12,13] This is called oxidative stress, that is, imbalance in oxidant - antioxidant in the excess of oxidant. [13] The pathogenesis of GDM like any other types of diabetes mellitus has been linked with free radical generation as well as insufficient antioxidants. [13] The deficiency in antioxidants may not only be as a result of their consumption to ameliorate free radical injury but may also be due to insufficient dietary intake of both enzymatic antioxidants and antioxidant vitamins. Dietary intake of antioxidants is bound to vary from community to community and this may influence their body contents. [14]

In view of the above, the oxidant-antioxidant status in patients with GDM may not also go the same way as presented in some previous reports, hence this study. The study looked into the determination of evidence of free radical attack (malondialdehyde) on cellular membrane lipid and antioxidant status (superoxide dismutase (SOD), glutathione peroxidase (GPx)) of patients with GDM.

## METHODOLOGY

Thirty one (31) newly diagnosed patients with gestational diabetes mellitus using the guidelines of IADPSG (FPG =5.1mmol/l, 1hr=10mmol/l, 2hr=8.5mmol/l) [1] and WHO(FPG =6mmol/l) 2hr=7.8mmol/l) [2] were recruited from the Special Investigation Clinic of Chemical Pathology Department of the Ekiti State University Teaching Hospital, Ado Ekiti. Fifty (50) apparently healthy pregnant

women were recruited from the same clinic as control during routine screening for GDM. None of the recruited subjects had previous history of hypertension, cancer coexisting with pregnancy, glucose intolerance and were all free of symptoms and signs of diabetes mellitus. Both subjects and controls were subjected to the same conditions. Patients were fasted over night for a minimum of 10 hours. Patients after a rest of about 15 minutes about 7.5 mls of venous blood was drawn from antecubital fossa using aseptic procedure of phlebotomy. The blood was divided into 2.5 mls and 5mls and dispensed respectively into fluoride oxalate and lithium heparin specimen bottles. Subsequently, 2.5 mls was collected after an hour and two hours post 75g glucose load into fluoride oxalate bottles. Plasma was extracted into plain specimen bottle from each of the samples above after centrifugation at 3000Xg for 5 minutes. The plasma extracted from three samples collected into fluoride oxalate bottles were used for the laboratory analysis of three-point glucose checks. The plasma extracted from lithium heparin specimen bottle was used for the laboratory analysis of malondialdehyde, glutathione peroxidase and superoxide dismutase. Plasma glucose, glutathione peroxidase and superoxide dismutase analysis were determined using commercially manufactured kit by Randox laboratory, Aldren, USA. Plasma malondialdehyde was determined using method of Satoh et al. [15]

## RESULT

As shown in table 1, total number of eleven patients were diagnosed based on fasting plasma glucose concentration  $\geq 6.0$ mmol/l. Total number of twenty patients were diagnosed based on plasma glucose concentration value of  $\geq 7.8$  mmol/l gotten from sample of 2-hour post 75g glucose load. Furthermore, total numbers of 5 patients were diagnosed using value of glucose concentration of  $\geq 8.5$  mmo/l gotten from sample of 2-hour post 75g glucose load. About 64.5% of the subjects were

diagnosed with 2-hour post 75g glucose load concentration of  $\geq 7.8$  mmol/l

TABLE 1: CRETARIA FOR DIAGNOSIS

Plasma Glucose Concentration	GDM (n= 31)	Non GDM (n=53)
FPG $\geq 6$ mmol/l	11.0	0.0
1-HPG $\geq 10$ mmol/l	0.0	0.0
2-HPG $\geq 7.8$ mmol/l	20.0	0
2-HPG $\geq 8.5$ mmol/l	5.0	0

The comparison of biophysical profile as shown in table 2 above indicates statistical significant values in FPG, 1-HPG, 2-HPG and DBP. Significant higher values were observed in FPG ( $p=0.035$ ) in patients with GDM ( $5.77 \pm 0.99$  mmol/l) than patients with Non GDM ( $3.57 \pm 0.73$  mmol/l), 1-HPG ( $p=0.001$ ) in patients with GDM ( $7.01 \pm 1.35$ ) than patients with non GDM ( $5.36 \pm 0.8$ ) and 2-HPG ( $p=0.000$ ) in patients with GDM ( $6.71 \pm 1.30$  mmol/l) than patients without GDM ( $5.34 \pm 0.80$ )

TABLE 2: COMPARISON OF BIOPHYSICAL PROFILE AND PLASMA GLUCOSE CONCENTRATION

	GDM (n= 31)	Non GDM (n=53)	p-value
Age (yr)	29.68 $\pm$ 4.01	30.94 $\pm$ 3.03	0.273
GA (wk)	27.06 $\pm$ 2.29	29.58 $\pm$ 3.26	0.013
FPG (mmol/l)	5.77 $\pm$ 0.99	3.57 $\pm$ 0.73	0.035
1-HPG (mmol/l)	7.01 $\pm$ 1.35	5.36 $\pm$ 0.8	0.001
2-HPG (mmol/l)	6.71 $\pm$ 1.30	5.34 $\pm$ 0.8	0.000
SBP (mmHg)	113.23 $\pm$ 12.75	121.13 $\pm$ 10.13	0.09
DBP (mmHg)	70.97 $\pm$ 9.78	65.66 $\pm$ 6.36	0.032
BMI (kg/m <sup>2</sup> )	28.46 $\pm$ 3.58	30.32 $\pm$ 2.89	0.091

Table 3 shows higher plasma concentration though not statically significant ( $p=0.305$ ) of MDA in patients with GDM ( $5.75 \pm 0.60$   $\mu$ mol/l) than patients with Non GDM ( $4.55 \pm 0.55$   $\mu$ mol/l). The lower plasma concentrations of GPx ( $2.98 \pm 1.10$  Vs  $3.12 \pm 0.78$  U/ml,  $p=0.017$ ) and SOD ( $69.33 \pm 21.94$  vs  $86.47 \pm 24.26$  U/ml,  $p=0.62$ ) were observed in patients with GDM than patients without GDM

TABLE 3: COMPARISON OF BIOCHEMICAL VARIABLES

	GDM (n= 31)	Non GDM (n=53)	p-value
MDA ( $\mu$ mol/l)	5.75 $\pm$ 0.60	4.55 $\pm$ 0.55	0.305
GPx ( U/ml)	2.98 $\pm$ 1.10	3.12 $\pm$ 0.78	0.017
SOD ( U/ml)	69.33 $\pm$ 21.94	86.47 $\pm$ 24.26	0.62

## DISCUSSION

The diagnosis of GDM in our centre follows the guidelines of WHO. [2]

However, for the purpose of this study in subject selection the criteria of IADPSG [1] were combined. World Health Organisation according to its guidelines diagnoses GDM with fasting plasma glucose of 6mmol/l and above and about 35.5 % of the recruited subjects were diagnosed based on this. The IADPSG diagnoses GDM at a value of 8.5mmol/l and above 2 hours after 75g glucose load and about 16.1 % of subjects were recruited based on this. World Health Organisation diagnoses GDM at a lower value of 7.8 mmol/l and above 2 hours post 75g glucose load and majority of the recruited subjects fell on this guideline. This in a way may justify the use of WHO and to affirm its continuous usage in diagnosing patients with GDM. However, this has not ruled out the need to conduct a research to set specific reference ranges for our centre based on peculiarities of socio cultural as well as environmental factors that could influence different biochemical parameters in our environment. Gestational Diabetes Mellitus has been a disorder of glucose metabolism for ages without appreciable ways of preventing it. The stress of taking regular insulin injection during pregnancy has called for researches. Oxidative stress injury has been implicated in appreciable number of clinical conditions [10,11] of which also there are reports on its roles in the development of GDM. [3,16]

Oxidative stress simply means imbalance in free radicals (oxidants) and antioxidants in excess of oxidants. [10,11] This study did not measure any free radical as they are quite unstable in the system. This study measured plasma malondialdehyde (MDA) which is one of the intermediate products of free radical injury. This comes as a result of free radical attack on membrane lipid in the process of lipid peroxidation. Though, not statistically significant, the increased plasma value of MDA observed in patient with GDM may signify increase free radical production and subsequently injury to the membrane lipid. This finding has been observed in some other studies previously. [3,16] Some in their

findings reported the oxidative stress to be due to reduced antioxidant contents in the system. [3,4] In the present study, despite that our patients were already on 'routine drugs' of which ascorbic acid was part, the plasma MDA was still elevated in the subjects than the controls. This may be due to the production of free radicals overwhelming the available antioxidants or the specific antioxidants to counteract the free radical injury were not adequate in the system. Also our subjects might have needed more than ascorbic acid supplement being given exogenously. Among various antioxidants that could be considered but not given routinely are catalase, glutathione peroxidase (GPx), superoxide dismutase and so on

The significant decrease in plasma value of GPx observed in our study may be a reflection of its inability to adequately compensate for free radical injury. One expects a compensatory increase in antioxidant to counteract the increasing free radical formation and injury. Glutathione peroxidase is an antioxidant enzyme that plays a role in inactivation of free radicals like hydrogen peroxide and intermediate product of peroxidation like lipid peroxides, thereby protecting the body against oxidative stress. It is believed to be one of the most important antioxidant enzymes in humans. This finding is in agreement with some previous studies. [4,17,18] However, the study of Arribas et al in his report gave a contrary finding in which the serum GPx was higher in patients with GDM; [19] the sample size of the study was not different from the sample size of this present study but the diagnosis of GDM in their study was based on plasma glucose values gotten after 100g glucose load. The contrary finding in their study was believed to be because of adequate compensation to free radical injury as well as increased antioxidant intake in the diet of their recruited subjects. Furthermore, study of Mahmoud et al also reported a higher serum value of GPx in patients with GDM [3] and the diagnosis criteria as well as subject selection was the same as this

present study. This unexpected result in their study also was believed to be due to adequate compensation to free radical production and injury. Antioxidants mobilisation and usage from the diet might be adequate.

The reduced plasma SOD observed in subjects with GDM in our study may be as a result of overwhelming use of this antioxidant in counteracting free radical formation and its injury. Superoxide dismutase is an important antioxidant defense in nearly all living cells exposed to oxygen. It helps in degrading hydrogen peroxide which is one of the oxygen related free radicals. The finding of this present study concerning the SOD is in total agreement with the work of Simmi Kharb. [20] This may be due to consumption of this antioxidant in clearing the free radicals from the system.

The present study as described above observed lower antioxidant in the phase of increasing product of free radical injury. This defines oxidative stress in the GDM patients recruited in the study. The available antioxidants (GPx and SOD) were not sufficient to degrade the free radical production. The antioxidant component from the diet of the majority of the subjects may not also be adequate in counteracting the effect of free radical injury.

## CONCLUSION

The study shows production of free radical injury in excess of antioxidants (GPx and SOD) in patients with gestational diabetes mellitus. It is advice that management of pregnancy and GDM should not only focus on antioxidant vitamin supplement but also antioxidant enzymes.

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