

The Profiles of Lipid Peroxidation and Anti-Oxidant Activities in Gestational Diabetes Mellitus and Normal Pregnancies in Nigerian Population

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Abstract: To compare the lipid peroxidation and total antioxidant status in women with gestational diabetes mellitus and normal pregnancy in the environment. This was a 2 years, cross sectional case control study of 25 Gestational Diabetes Mellitus (GDM) and 75 matched normal pregnant women at Ladoke Akintola University of Technology (LAUTECH) Teaching Hospital, Osogbo, Nigeria. The study protocol was reviewed and approved by the Institutional Ethical Review Committee and all participants gave their consent. The fasting serum of recruited patients were analysed for lipid peroxidation product Malondialdehyde (MDA) based on MDA reaction with Thiobarbituric Acid (TBA) with rapid, sensitive and specific Thiobarbituric Acid (TBA) assay while the Total Anti-oxidant Status (TAS) was determined using the capacity of the biological fluids to inhibit the production of Thiobarbituric Acid Reactive Substances (TBARS) from sodium benzoate under the influence of the free oxygen radicals derived from Fenton's reaction. The mean serum MDA was significantly higher in the GDM group ($3.64 \text{ nmol mL}^{-1}$) than the value recorded ($1.33 \text{ nmol mL}^{-1}$) in the control group ($p < 0.0001$). Conversely, significantly lower mean TAS (0.34 vs. $0.66 \text{ nmol mL}^{-1}$) was noticed in the GDM group ($p < 0.0001$). The study showed significant increase in lipid peroxidation and failure of compensatory antioxidant functions in GDM Nigerian women.

Key words: Lipid peroxidation, anti-oxidant status, gestational diabetes mellitus, malondialdehyde, TBA

INTRODUCTION

The World Health Organisation defined Gestational Diabetes Mellitus (GDM) as carbohydrate intolerance resulting in hyperglycaemia of variable severity with onset or first recognition usually during the second half of pregnancy. It affects 3-10% of pregnancies depending on the population studied (WHO, 1999). This definition applies irrespective of whether or not insulin is used for treatment or the condition persists after pregnancy. In Nigerian population, a prevalence of 1.7% had been reported for diabetes in pregnancy with GDM accounting for 61% of them (Ozumba *et al.*, 2004). Pregnancy is a physiological condition characterised by various potentially diabetogenic hormonal changes due to increase in glucocorticoids, human placental lactogen and oestrogens. This can result in insulin resistance and it may be the first life challenge of a woman's ability to respond to a physiological stress and to detect those at greater risk of developing diabetes in the future. Women with GDM are at 3 fold increased risk of developing type 2 diabetes mellitus later in life (American Diabetes Association, 2004) and thus much attention has been

focussed on the interlink between these two metabolic conditions. Whether type 2 diabetes mellitus is a continuum of GDM is speculative. However, both diseases share similar pathophysiology and it is generally known that the higher the prevalence of type 2 diabetes in the population, the higher that of GDM. Oxidative stress which is a general term used to describe the steady state level of oxidative damage to cell, tissue or organ, caused by the elevated Reactive Oxygen Species (ROS) (4) has been linked to type 2 diabetes mellitus however, limited and inconsistent data are available on the involvement of oxidative stress in GDM.

Lipid peroxidation, formation of free radicals and reactive oxygen species are normal occurrences in pregnancy but these happen at low level in normal pregnancy with its peak in the second trimester of pregnancy. In normal pregnancy, the balance between lipid peroxidations, free radicals (prooxidants) and the antioxidants formations are well maintained in order to minimize cellular damage (Wickens *et al.*, 1981; Poranen *et al.*, 1996). When the balance is disrupted towards an overabundance of ROS, oxidative stress occurs.

Hyperglycaemia causes oxidative stress due to increased production of mitochondrial ROS, excessive non-enzymatic formation of glycosylated proteins, associated alterations in the uptake of low-density lipoproteins and glucose autooxidation (Brownlee, 2001; Suhail *et al.*, 2010). Elevated FFA can also cause oxidative stress due to increased mitochondrial uncoupling and β -oxidation, leading to the increased production of ROS (King and Loeken, 2004; Wender-Ozegowska *et al.*, 2004).

Reports in respect of pro-oxidant and anti-oxidant status in GDM have been inconsistent and controversial. While some studies had reported significant increase in lipid peroxidation activities in this condition when compared with normal pregnancy (Carone *et al.*, 1993; Chaudhari *et al.*, 2003; Surapaneni, 2007; Karacay *et al.*, 2010) others found no evidence of greater lipid peroxidation and in fact concluded that total antioxidant capacity was similar in GDM women when compared with normal pregnancy (Bates *et al.*, 1997; Sobki *et al.*, 2004).

In Nigeria, published information about the serum lipid peroxides and antioxidants status in gestational diabetes mellitus women in the environment is scarce. For this reason, this study has been undertaken to establish the serum Malondialdehyde (MDA) and Total antioxidant status in women with GDM and compare with women of normal pregnancy in the environment.

MATERIALS AND METHODS

This was a cross sectional case control study of pregnant patients at Ladoke Akintola University of Technology (LAUTECH) Teaching Hospital, Osogbo, Nigeria. The study was over a 2 years period (2008 to 2010) and the study protocol was reviewed and approved by the Institutional Ethical Review Committee. All participants gave informed consent to participate in the study.

The diagnosis of gestational diabetes mellitus and inclusion in the study were in accordance with the guidelines of American Diabetes Association criteria (American Diabetes Association, 2004) which stipulated two or more results of the 100 g Oral Glucose Tolerance Test (OGTT) glucose levels to exceed the following cut-off values: fasting: $\geq 95 \text{ mg dL}^{-1}$ (5.3 mmol L^{-1}); 1 h: $\geq 180 \text{ mg dL}^{-1}$ (10.0 mmol L^{-1}); 2 h: $\geq 155 \text{ mg dL}^{-1}$ (8.6 mmol L^{-1}); 3 h: $\geq 140 \text{ mg dL}^{-1}$ (7.8 mmol L^{-1}). Patients' recruitment and samples collection for assessment of lipid peroxidation and total antioxidant status were collected same time as for fasting plasma glucose and only processed if diagnosis of GDM was confirmed. Other samples in which GDM was not established were matched along with other normal pregnant patients

recruited and processed as controls. The exclusion criteria were women with pre-pregnancy diabetes mellitus, obesity, chronic hypertension, haemoglobinopathy, dyslipidaemia, multiple pregnancy, liver diseases, history of use of antioxidant medications and patient's refusal of consent to participate in the study. All GDM patients were managed by their primary obstetricians, initially by diet control and when necessary with insulin.

A total of 100 pregnant women 25 gestational diabetes mellitus and 75 normal pregnant patients (controls) were recruited for the study. Each recruited GDM patients patient was matched with 3 normal pregnant patients as controls for age (± 2 years), estimated gestational age (± 1 week) and parity.

Assay for lipid peroxidation and antioxidant status were done with ten millilitre of fasting peripheral venous blood collected into sterile plain bottle and centrifuged at 4,000 rpm for 10 min. The resultant serum was collected into fresh sterile, acid-washed, plain capped bottle and stored at -20°C until analysis. Lipid peroxidation product, serum Malondialdehyde (MDA) level was assayed for using a rapid, sensitive and specific Thiobarbituric acid assay (Botsoglou *et al.*, 1994) based on MDA reaction with Thiobarbituric Acid (TBA) and the Total Anti-oxidant Status (TAS) was determined as by Koracevic *et al.* (2001). In Koracevic Method, a standardised solution of Fe-EDTA complex reacts with hydrogen peroxide by a Fenton-type reaction leading to the formation of hydroxyl radicals ($\bullet\text{OH}$). These reactive oxygen species degrade benzoate, resulting in the release of TBARS. Antioxidants from the added sample of human fluid cause suppression of the production of TBARS. This reaction can be measured spectrophotometrically and the inhibition of colour development defined as the anti-oxidative activity.

Statistics: Data obtained was analysed with Statistical Package for Social Sciences Version 16 (SPSS Inc., Chicago, IL, USA) for continuous variables as mean \pm standard deviation, Student t-test and confidence interval. Level of significance was set at $p \leq 0.05$.

RESULTS

A total of 100 patients were recruited for study, of which 25 were women diagnosed with gestational diabetes mellitus and 75 were normal pregnant women (Controls). The women recruited as controls were matched at ratio of 3 for each woman with gestational diabetes mellitus for age (± 2 years), estimated gestational age (± 1 week) and parity. There was no significant difference

Table 1: Socio-demographic factors of gestational diabetes mellitus women and the normal pregnant controls

Parameters	Gestational diabetes mellitus patients (n = 25) Mean (SD)	Control n = 75 Mean (SD)	t-value	p-value	95% CI
Age (years)	35.65 (2.29)	33.26 (2.32)	4.4749	<0.0001	1.3301, 3.4499
EGA (weeks)	28.64 (6.45)	28.58 (6.39)	0.0406	0.9677	-2.8753, 2.9953
Weight (kg)	74.40 (8.16)	73.35 (5.42)	0.7329	0.4654	-1.7932, 3.8932
Height (m)	1.59 (0.02)	1.62 (0.04)	3.5944	0.0005	-0.0466, -0.0134
BMI (kg/m ²)	29.43 (2.34)	27.94 (2.26)	2.8300	0.0056	0.4452, 2.5348
Fasting plasma glucose (mmol L ⁻¹)	105.21 (10.18)	72.23 (15.21)	10.0963	<0.0001	26.4977, 39.4623
Plasma glucose-1 h (mmol L ⁻¹)	158.43 (21.23)	126.31 (17.32)	7.5775	<0.0001	23.7082, 40.5318
Plasma glucose-2 h (mmol L ⁻¹)	185.36 (24.52)	129.22 (16.74)	12.8328	<0.0001	47.4585, 64.8215
Plasma glucose-3 h (mmol L ⁻¹)	126.65 (14.64)	85.32 (15.23)	11.8616	<0.0001	34.4154, 48.2446

BMI = Body Mass Index; EGA = Estimated Gestational Age

Table 2: Comparison of serum malondialdehyde and total antioxidant status in gestational diabetes mellitus women and normal pregnant controls

Factors	Gestational diabetes mellitus patients (n = 25) Mean (SD)	Control (n = 75) Mean (SD)	t-value	p-value	95% CI
MDA (nmol mL ⁻¹)	3.64 (0.64)	1.33 (0.15)	29.2054	<0.0001	2.1530, 2.4670
TAS (nmol mL ⁻¹)	0.34 (0.10)	0.66 (0.13)	11.2353	<0.0001	-0.3765, -0.2635

MDA = Malondialdehyde; TAS = Total Antioxidant Status

in the mean weight, estimated gestational age and parity distribution at recruitment in both groups. The mean age of patients recruited in the GDM group was significantly higher than in the control (35.65 vs. 33.26 years; $p < 0.0001$) so also was the body mass index which was extremely significantly different in the GDM group than control (Table 1). The mean height of the participants was significantly lower ($p = 0.0005$) in the GDM group than the control group (1.59 vs. 1.62 m).

In Table 2, the mean serum MDA was significantly higher in the GDM group (3.64 ± 0.64 nmol mL⁻¹) than the value recorded (1.33 ± 0.15 nmol mL⁻¹) in the control group ($p < 0.0001$). Conversely, significantly lower mean TAS of 0.34 (0.10) nmol mL⁻¹ was recorded in the GDM group in contrast to mean value of 0.66 (0.13) nmol mL⁻¹ in the control group ($p < 0.0001$).

DISCUSSION

The findings from this study showed increased lipid peroxidation and reduced levels of total antioxidant status in GDM women above those in the normal pregnant women. These are similar to the conclusions from other studies (Suhail *et al.*, 2010; Carone *et al.*, 1993; Chaudhari *et al.*, 2003; Surapaneni, 2007; Karacay *et al.*, 2010). However, some other studies had also reported that no significant difference existed in the lipid peroxidation and the antioxidant status between GDM patients and controls (Bates *et al.*, 1997; Sobki *et al.*, 2004; Dey *et al.*, 2008). The findings from this study showed about 3 fold increase in the lipid peroxidation activities and concurrently, 2 fold decrease in total anti-oxidant status in women with GDM when compared with the normal pregnant women. These findings suggest a great tendency for enhanced and uncompensated free radical generation which can lead to easier membrane damage in GDM patients and other adverse effects of oxidative

stress. Primary effect of increased oxygen free radical in gestational diabetes is believed to alter prostaglandin biosynthesis which may be responsible for the development of diabetes related embryopathy (Sivan *et al.*, 1997). Increased oxidative stress in pregnant women and possibly their fetuses has been associated with congenital malformations (Bis-Gluchowska *et al.*, 2001). Inadequate antioxidant defense has been postulated as a prime factor in pathologic states of many premature infants (Saugstand, 1990). However, this study was not designed to assess the fetal parameters.

If lipid peroxidation status in GDM has been controversial, the antioxidants parameters have not been less, with many conflicting reports in the literatures. While some researchers had reported decline of various antioxidant agents (Suhail *et al.*, 2010; Surapaneni, 2007; Kharb, 2007) when compared with normal pregnant women others reports had claimed no change in antioxidants status of GDM patients (Bates *et al.*, 1997; Sivan *et al.*, 1997). However, the finding indicated a 2 fold decrease in total antioxidants status of GDM patients in the population. It may be reasoned therefore from the finding that addition of supplemental antioxidant nutrients such as vitamins C, E, carotenoids, selenium and others may be beneficial in improving the TAS status in GDM patients. This however, may require further investigations for possible justification in clinical practice.

Patients recruited for this study were matched for age, estimated gestational age and parity. These strict enrolment criteria were to eliminate the confounding effects of these socio-demographic factors. Despite these efforts, patients with GDM were significantly older and were heavier in weight, though of shorter heights. The impact of higher weight and shorter height reflected on the BMI of the GDM group with the mean value of 29.43 kg/m² which is pre-obese by the World Health Organisation classification (WHO Expert Consultation,

2004). Reports in literature had associated maternal pre-pregnancy weight as well as weight gain in pregnancy, especially in the first trimester as independent risk factors for GDM (Hedderson *et al.*, 2010; Horosz *et al.*, 2013). However, in this study, it was not possible to explore these factors because most patients were seen in pregnancy and mostly in advanced gestations. A structured pre-conception care for all mothers to be would definitely be of benefit in properly assessing the influences of these socio-demographic factors.

CONCLUSION

This study demonstrated significant increase in lipid membrane damage activities (lipid peroxidation) as evidenced by rise of serum MDA in GDM women and failure of compensatory antioxidant functions demonstrated by overall lower antioxidant capacity in this group of pregnant women. These further buttress a possible link between oxidative stress and GDM. Also, significant factors noted in these GDM patients were increased maternal age, body mass index and short stature. Further researches on possible effects of antioxidant supplements may be justified.

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