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# Evaluation of malondialdehyde, total plasma peroxides, total antioxidant capacity and oxidative stress index in gestational diabetes mellitus

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## Abstract

Oxidative stress has been implicated in the pathogenesis of many pathological cases. it is implicated in both organ and systemic dysfunction. Gestational diabetes mellitus (GDM) is a pathological condition with first onset observed during pregnancy. It causes pregnancy complications and affects 2-7% of pregnant women. For that purpose, this studywas performed with 94 pregnant women with informed consent,51 were diagnosed with GDMand 43 apparently healthy pregnant women. The following parameters were assessed: Malondialdehyde (MDA), Total Antioxidant Capacity (TAC), Total Plasma Peroxide (TPP) and Oxidative Stress Index (OSI). MDA, TPP, and TAC were determined colorimetrically and OSI calculated. Anthropometric indices (weight, height, BMI) and socio-demographic data were determined using standard methods. Data generated were analysed using ANOVA, Student t-test, LSD post hoc and Pearson correlation at $P<0.05$. The TAC level was significantly higher in control groups ($p<0.05$), while TPP and OSI were significantly higher in the GDM group compared to control group ($p<0.05$). A significant negative correlation was observed between TAC and OSI in GDM group ($r= -0.486$, $p=0.005$*), and a positive correlation between MDA and BMI, as well as MDA and Age in GDM group ($r=0.527$, $p=0.002$* and $r=0.375$, $p=0.034$* respectively). GDM patients tend to have a higher TPP level and decreased TAC which may enhance the pathogenesis or pathophysiology of these conditions.

**Keywords:** Oxidative Stress; Diabetes; Pregnancy; Antioxidant; Malondialdehyde

## 1. Introduction

Pregnancy is a state of a woman carrying a fetus or embryo after a successful fertilization of ovum and its successful implantation in the lining of the uterus. It lasts right from the period of conception down to childbirth and this can take about 40 weeks (280 days) for all this processes to be completed. It begins from the first day of the woman's last menstrual period and is divided into three trimesters, each lasting for three months. The nine months of pregnancy can be an exciting part of a mother's life but can become very challenging when faced with complications which might be a threat to the mother's life or that of the baby or both, pregnancy symptoms and complications can range from mild and

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annoying discomforts to severe, sometimes life-threatening illnesses. These complications may arise due to health issues either before or after pregnancy. Complications in pregnancy include but not limited to; Anaemia, Urinary Tract Infection (UTI), Hypertension (High Blood Pressure), Obesity and weight gain, Diabetes and Gestational Diabetes (GDM) etc. [1,2].

Gestational diabetes mellitus (GDM) is defined as glucose intolerance of variable severity with first onset observed during pregnancy. Normally, after delivery the postpartum insulin resistance resolves quickly. Gestational Diabetes Mellitus is the most frequent metabolic disorder of pregnancy occurring in approximately 3 to 5% of pregnancy with an incidence of more than 200,000 cases per year [3].

Prevalence of GDM depends on several factors which include races—as women of African American ethnicity are at 10 times higher risk of suffering from GDM during pregnancy. Other races poised with a high risk of suffering from GDM are Hispanic, Native American and Asian women, family history of DM, etc. There are several criteria for diagnosing gestational diabetes mellitus, for WHO, the cut-off value for GDM (FBS: >7.8 mmol/L, 2h glucose level >11.1mmol/L), for IADPSG cut off (FBS: >5.1mmol/L, 2h glucose level>8.5mmol/L)and ADA (FBS: >7.0mmol/L) [4,5,6]. So it can be seen that the screening cut-off value used also affects with prevalence with IADPSG criteria leading to higher prevalence. Risk factors that can contribute to development of GDM in pregnancy include; advanced maternal age, high parity, polycystic ovarian syndrome (PCOS), multiple pregnancy, family history of diabetes, obesity etc, with research observing that GDM is on the increase with time due to changes in lifestyle associated with urbanization, including dietary changes and sedentary lifestyle, which leads to overweight and obesity. With the increase in the prevalence of obesity and undiagnosed type 2 diabetes mellitus, prevalence of GDM is on the rise [5].

GDM is potentially a serious pregnancy complication which confers some degree of maternal and foetal complication which can be either long term or short term. With the short term effect on mother ranging from hypertension, miscarriages, still birth, premature birth etc and long term effect including development of type 2 diabetes, metabolic syndrome and cardiovascular disease at a later stage in life [7,8].

Oxidative stress can be defined as a state of disrupted balance between reactive oxygen species and the mechanisms of detoxification and repair. Reactive oxygen species (ROS) are formed in every living cell during the physiological process of breathing, and a molecule of ROS contains an atom of oxygen with an unpaired electron. Pregnancy is also a state in which this adaptation and balance may be easily disrupted. There is strong evidence that a chronic inflammatory reaction combined with the presence of a local oxidizing environment may play a vital role in the etiology and development of complications during pregnancy [9,10].

In diabetic condition, several factors such as glucose oxidation, alterations in antioxidant defense system, lipid peroxidation, non-enzymatic glycation of proteins and following oxidative destruction of glycated proteins could result in production of free radicals. Imbalance between the formation and inactivation of oxygen free radicals cause oxidative damage, which is associated with the destruction of membrane lipids and production of lipid peroxides and their products.

Total antioxidant capacity (TAC) demonstrates the balance between antioxidants (neutralizing systems) and the oxidants (oxidative stress). Antioxidants such as catalase, superoxide dismutase, β-carotene, vitamin C, vitamin E, and glutathione peroxidase (GPX) are known to protect against the adverse action of ROS and their derivatives. Some investigators have reported increased lipid peroxidation and significant depletion in antioxidant capacity during the development of Gestational Diabetes [10,11].

Some studies have shown enhanced oxidation products in patients with GDM and reduced anti-oxidant capacity, suggesting that oxidative Stress may contribute to the development and progression of GDM [12]. However, the relation between the different levels of various plasma oxidation markers and the development of GDM during pregnancy has not been systematically characterized [13,14].

## 2. Material and methods

### 2.1. Subjects' selection

In this study, a total of 95 pregnant women were enrolled, aged between 18-45 years. Of the 95 pregnant women, 51 were diagnosed with GDM, while 43 were without diabetes, hypertension, glycosuria and proteinuria (control). Sociodemographic data, family, family history, brief medical history and anthropometric data were obtained from each

subject using a well-structured questionnaire. The research was carried out in accordance with the Ethical Principles for Medical Research involving Human Subjects as outlined in the Helsinki Declaration of 1975 (revised in 2000).

### 2.2. Inclusion and exclusion criteria

The control group included pregnant females without GDM, diabetes, cardiovascular diseases, or hypertension, those not on medication, smokers and without family history of diabetes mellitus.

Those excluded from the study were those whose informed consents were not gotten or those who did not fall within the specified age range (18 – 45 years), non-pregnant females, and pregnant women with other complications other than GDM.

### 2.3. Sample collection

Using aseptical technique, 5 ml of venepuncture blood sample was collected into a clean dry plain sample container and kept away from sunlight. The blood sample was allowed to clot, then dislodged and spurned at 3000 rpm for 10 minutes to obtain serum. The serum was collected and dispensed into a dry, chemically clean serum container after which the samples were stored at -20$^{o}$C until assay.

### 2.4. Determination of analyte: Estimation of Malondialdehyde

Malondialdehyde was assayedusing the method of Burge and Anst, (1978) [15]. Malondialdehyde forms from the breakdown of polyunsaturated fatty acid severs as a convenient index for determining the extent of the peroxidation reaction. Malondialdehyde has been identified as the product of lipid peroxidation that reacts with thiobarbituric acid to give a red species absorbing at 532 nm.

### 2.5. Estimation of total antioxidant capacity

Total antioxidant capacity was determined using the method of Koracevic*et al* (2001) [16]. A standard solution of Fe-EDTA complex reacts with $H_2O_2$ by a Fenton type reaction, leading to the formation of hydroxyl radicals (OH). These reactive oxygen species degrade benzoate resulting in the release of TBARS. Antioxidant from added sample causes suppression of the production of TBARS. The reaction was measured spectrophotometrically at 532 nm using 10S UV-VIS spectrophotometer, (Thermo Scientific, Fischer).

### 2.6. Estimation of total plasma peroxide by fox-2

Total plasma peroxide was determined using the reaction of ferrous-butylatedhydroxyltoulene-xylenol orange complex (FOX-2 reagent) with plasma hydrogen peroxide which yields a colour complex that was measured spectrophotometrically at 560 nm, according to FOX-2 method (Miyazawa, 1989) [17] with minor modifications by Harma*et al*, (2003) [18].

### 2.7. Oxidative stress index (OSI)

The ratio of Total Peroxide (TPP) to Total Antioxidant Capacity (TAC) was the Oxidative Stress Index, an indication of the degree of Oxidative Stress.

$$\text{OSI (\%)} = \frac{\text{TPP (µmol H2O2)}}{\text{TAC (µmol/L)}} \times 100$$

### 2.8. Statistical analysis

Data collected was entered into Microsoft excel spreadsheet and analysed using statistical packages for social science (SPSS) for determination of mean, standard deviation, while comparison of variables was done using analysis of variance (ANOVA) and student t-test, with a probability valueof ($P<0.05$) considered significant.

## 3. Results

Table 1shows the mean Age, BMI, parity, systolic blood pressure (SBP), diastolic blood pressure (DBP), malondialdehyde (MDA), Total plasma peroxide (TPP), Total antioxidant capacity (TAC) and Oxidative stress index (OSI) in control group and gestational diabetes group (GDM). A Significant increase was observed in SBP and TAC in the controls when compared to the GDM groupwhile TPP and OSI were significantly higher in the GDM group when

compared to the control group with P- values (< 0.05), while other parameters showed no significant difference with their P- values (>0.05).

Fig 1shows a correlation plot of oxidative stress (OSI) against Total antioxidant capacity (TAC) in Gestational diabetes group. There was a significant negative correlation (**r = -0.486, P< 0.05**) between OSI and TAC. Fig 2shows a Correlation plot of Malondaldehyde (MDA) against body mass index (BMI) in gestational diabetes group. A significant positive correlation (r = 0.527, p < 0.05) was observed between MDA and BMI. Fig 3shows a correlation plot of malondaldehyde (MDA) against age in gestational diabetes group. A significant positive correlation of (r = 0.375, P < 0.05) was observed between MDA and age.

**Table 1** Mean Age, BMI, Parity, Blood pressure (Diastolic and Systolic), TPP, TAC and OSI in Pregnant women with GDM and Control.

| Parameter | Control N=43 | GDMN=51 | T | P –VALUE |
|---|---|---|---|---|
| AGE (years) | 28.74 ± 4.99 | 28.31 ±4.38 | 0.390 | .698 |
| BMI (Kg/m2) | 27.87 ± 5.24 | 27.92 ± 5.42 | -0.043 | .966 |
| Parity | 2.16 ± 1.13 | 2.03 ± 1.06 | 0.511 | .611 |
| SBP(mmHg) | 110.95 ± 8.92 | 106.03 ± 10.21 | 2.222 | .029* |
| DBP (mmHg) | 69.65 ± 6.62 | 69.41 ± 6.20 | 0.163 | .871 |
| MDA (nmol/L) | 0.71 ± 0.33 | 0.63 ± 0.17 | 1.242 | .218 |
| TPP (µmol/L) | 59.36 ± 15.76 | 89.47 ± 58.22 | -3.827 | .002* |
| TAC (µmol/L) | 745.90 ± 139.88 | 389.49 ± 253.94 | 7.766 | .000* |
| OSI (%) | 8.36 ± 3.07 | 39.26 ± 52.95 | -3.827 | .000* |

*=Significant (P<0.05); Values are given in Mean ± SD, BMI=Body mass index, SBP = Systolic blood pressure; DBP = Diastolic blood pressure; MDA=Malondialdehyde; TPP=Total Plasma Peroxide; OSI=Oxidative stress Index; GDM= Gestational diabetes mellitus.

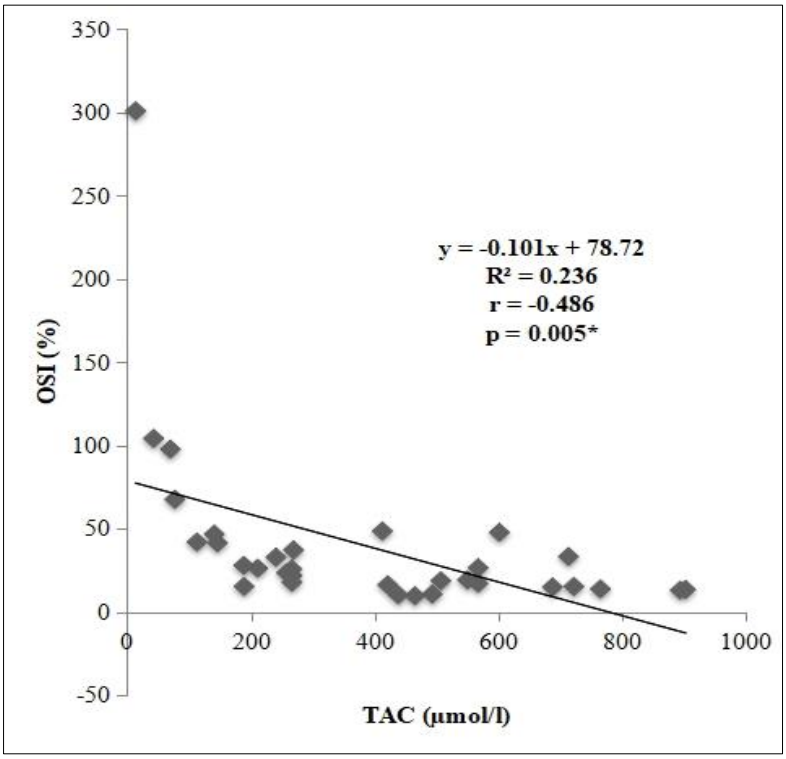


**Figure1** Correlation plot of oxidative stress (OSI) against total antioxidant capacity in Gestational diabetes group

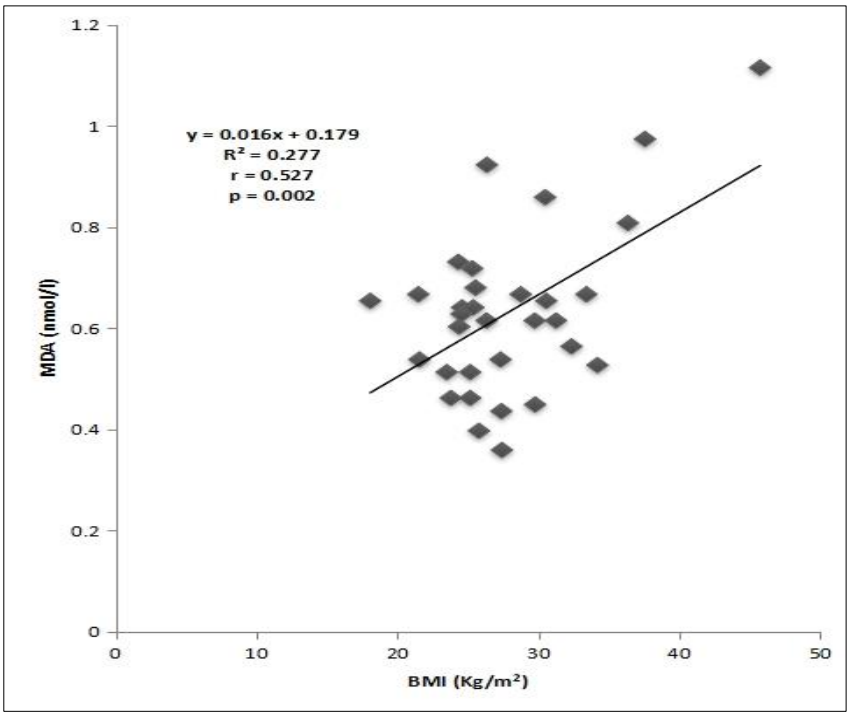


**Figure 2** Correlation plot of Malondaldehyde (MDA) against body mass index (BMI) in gestational diabetes group

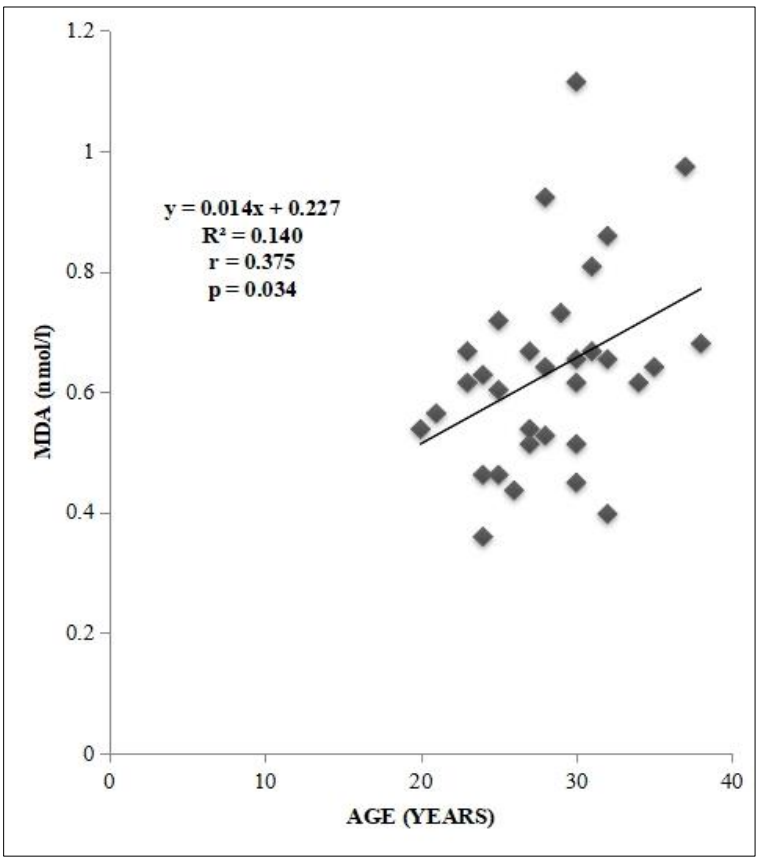


**Figure 3** Correlation plot of malondaldehyde (MDA) against age in gestational diabetes group

## 4. Discussion

Normal human pregnancy is considered as a state of enhanced oxidative stress, while pathological pregnancies conditions including GDM and Pre-eclampsia are associated with a heightened level ofoxidative stress, owing to both overproduction of free radicals and a defect in the antioxidant defenses [11,19]. In the present work, the parameters assessed include: Malondialdehyde (MDA), Total Plasma Peroxide and Total antioxidant Capacity, which serve as oxidative biomarkers.

This study found the levels of Total Plasma Peroxides and Oxidative Stress Index to be significantly higher while Total Antioxidant Capacity was significantly lower in the GDMgroup compared to the control but MDA was not significantly different (p-value>0.05). These findings suggest a great tendency for enhanced and uncompensated free radical generation, which can lead to easier membrane damage and other adverse effect of increased oxidative stress. Increased oxidative stress in pregnant women and possibly their fetuses has been associated with congenital malformations with inadequate antioxidant defense having been postulated as a prime factor in many pathological states [20,21,22].

The significant increase observed in TPP and OSI in this study was also observed inprevious studies [20,11]. Hyperglycemia has been suggested to be the primary causative factor for oxidative stress in GDM [23]. Oxidative stress is generated as a result of free radicals generated during auto-oxidation of glucose generation ROS such as $O^{\cdot-}_2$, $H_2O_2$ and $OH^-$, which further lead to peroxidation of lipids with corresponding accumulation of more free radicals [23,24].

Hyperglycemia favours accelerated glycolysis which in turn uses up NADH converting it to $NAD^+$ , since NADH is associated in the rate limiting reaction in glycolysis involving the enzyme glyceraldehydes-3-phosphate dehydrogenase. Also in the cytosol NADH is oxidized to $NAD^+$ by lactate dehydrogenase (LDH) involved in the reduction of pyruvate to lactate, thus leading to an increased ratio of NADH/$NAD^+$ ratio, since most of the NADH produced has been oxidized to $NAD^+$ which is required for the maintenance of reduced Gluthathione (GSH). Furthermore, competition of Aldose reductase (involved in reduction of glucose to sorbitol) with gluthathionereductase (GSH-R) for NADPH cofactor, further depletes intracellular GSH, thus leading to further elevation of TPP and OSI in study group. This condition can be further amplified when antioxidant defense mechanism is compromised. Hyperglycemia also causes oxidative stress due to production of excessive non-enzymatic formation of glycosylated proteins, associated alterations in the uptake of low density lipoproteins and glucose autooxidation [25,26].

There exists evidence also backing the effect of hyperinsulimenia in the generation of free radicals by an NADPH dependent mechanism involving the activation of phosphatidylinositol 3-kinase and stimulation of proliferative extracellular signal-regulated kinases dependent pathway. Exposure of human adipocytes to insulin has been observed to cause a time and dose dependent accumulation of $H_2O_2$ in-vitro. Insulin resistance which is associated with GDM is also associated with insulin resistance [13,23,27].

Antioxidants are known to play protective roles against oxidative stress. TAC indicates the balance between the neutralizing system and oxidative stress. TAC was also found to be altered in this study with value being lower in GDM group, this observation was in line with previous studies, Although most other studies reported low levels of specific antioxidants in pregnancy. Theoretically, the alteration of antioxidant status are consequences of oxidative stress, glycation of antioxidant enzymes/protein and disturbance of micronutrient status [11, 28,29].

A correlation carried out within the GDM group showed a negative correlation betweenOSIvs TAC, which indicates the defensive role played by TAC in oxidative stress. MDA was also positively correlated with BMIand Age. This can be attributed to the fact that BMI and Age are regarded as risk factors in the progression of GDM.

Damage caused by oxidative stress is an important hallmark of ageing. Oxidative stress increases with increasing BMI and age, as a sequel to an impaired antioxidant status.

Obesity *per se* can induce systemic oxidative stress through various biochemical mechanisms, such as superoxide generation from NADPH oxidases, oxidative phosphorylation, glyceraldehyde auto-oxidation, protein kinase C activation, and polyol and hexosamine pathways. Other factors that also contribute to oxidative stress in obesity include hyperleptinemia, low antioxidant defense, chronic inflammation, and postprandial reactive oxygen species generation [30,31]. The systolic blood pressure of the GDM group showed a significant increase to that of control and since diabetes in general is associated with cardiovascular disorders as well as atherosclerosis, this finding indicated that blood pressure of GDM patient should be paid a close attention.

## 5. Conclusion

From the findings of this study, a significant increase in total plasma peroxide and a failure of compensatory antioxidant functions demonstrated by overall lower antioxidant capacity in GDM patients tend to heighten oxidative stress. Hence oxidative stress may play a significant role in the pathophysiology or pathogenesis of GDM. GDM patients tend to have a higher TPP level and decreased TAC which may enhance the pathogenesis or pathophysiology of these conditions.

## Compliance with ethical standards

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### *Disclosure of conflict of interest*

Authors have declared that no competing interests exist.

### *Statement of informed consent*

Informed consent was obtained from all participants reported in the study.

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