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Determination of Glucose levels and Total Antioxidant Status of Patients with Gestational Diabetes Mellitus in Kaduna State Nigeria.

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ABSTRACT

Gestational Diabetes Mellitus (GDM) is a form of pregnancy-induced hyperglycemia associated with insulin resistance. It is commonly diagnosed during the second or third trimester and poses significant risks to both the mother and fetus if not adequately managed. This study aimed to evaluate the impact of GDM on total antioxidant status (TAS) among affected individuals.A total of 160 consenting women aged 18–40 years at 24–28 weeks of gestation were recruited. Sixty participants were diagnosed with GDM, while 50 non-diabetic pregnant women served as Control 1. All were attending antenatal clinics at Barau Dikko Teaching Hospital, Yusuf Dantsoho Memorial Hospital, and Gwamna Awan General Hospital. An additional 50 apparently healthy non-diabetic, non-pregnant women (Control 2) were selected from staff of these facilities. Screening was conducted using a 50 g oral glucose challenge test (OGCT), followed by a fasting 75 g oral glucose tolerance test (OGTT). Approximately 5 mL of blood was collected and analyzed using standard laboratory procedures. Data were analyzed using appropriate statistical methods, with significance set at $p < 0.05$. The mean TAS values in OGCT (random blood glucose) and OGTT (fasting blood glucose) samples were significantly lower in GDM and Control 1 groups (5.04 ± 0.19, 5.64 ± 0.14 and 5.62 ± 0.21, 5.39 ± 0.10 µmol/L, respectively) compared to Control 2 (7.14 ± 0.24 µmol/L). Serum glucose levels were significantly elevated in GDM subjects relative to controls. Additionally, significant relationships were observed among adiponectin, TAS, superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase, malondialdehyde (MDA), and vitamin C levels across study groups.These findings highlight the role of oxidative stress in GDM and may provide valuable insights for improving clinical management and therapeutic strategies for affected patients.

Keywords: ***gestational diabetes, antioxidants, pregnancy,vitamin C, glucose***

1. Introduction

Promoting a healthy beginning in life is vital and it could be the greatest impact we can have on the lives of individuals and our society in general (Dennison, Ward, Griffin, & Usher-Smith, 2019). The health of an infant sets a foundation for the rest of his/her life. Every member of the family painfully suffers when a child's

ill-health becomes worsen, with the entire society also bearing the costs of medical expenses, loss of productivity, as well as the potential need for assistance from the public (Paleri, 2022). Therefore, in areas where we know, we can easily intervene to avoid major prenatal challenges that is likely to occur, one of such area lies in the management of Gestational diabetes mellitus. Gestational Diabetes Mellitus (GDM) is the most common metabolic disease in pregnancy and is a condition that displays increased susceptibility to oxidative stress which continuously leads to potential irreversible damage especially during the second half of the pregnancy, which is mostly between twenty four to twenty eight weeks EGA (Rodríguez-Rodríguez et al., 2018). According to a study in Turkey, the prevalence of GDM was found to be 4.8%, 8%, and 13.4% using the National Diabetes Data Group (NDDG), Carpenter-Coustain Criteria (CC&C), and O'Sullivan two-step approach, respectively, while the International Association of the Diabetes and Pregnancy Study Group (IADPSG) single-step approach is 22.3% (Azeez, Abo-Briggs & Adeyanju, (2021). However in Nigeria, a study by (Lindsay, Gibney, McNulty, & McAuliffe, 2012), observed that the overall care and metabolic control of pregnancies of GDM subjects, remains sub-optimal with low as well as late attendance to antenatal clinics as well as poor feto-maternal outcomes.

Pregnant women can be grouped into low, moderate and high risk of developing GDM based on several risk factors which is a very vital tool in the process of screening women for GDM (Nombo, 2018 ; Paleri, 2022). It is interesting to note that, Women younger than 25 years, with normal weight at conception, and no known family history of diabetes are classified as low risk. Women that are obese, greater than 25yrs and whose family has history of glucose intolerance or diabetes are regarded moderate risk subjects. High-risk women include those with glucose in urine and previous GDM cases (Paleri, 2022).

Some of the risk factors implicated in the development of GDM are controllable. They include a negative Body Mass Index (BMI), sedentary lifestyles as well as energy-rich diets. These factors are related to rapid technological developments and urbanization which in turn has drastically resulted to a sharp rise in the number of people coming down with Diabetes Mellitus and which may further increase complications during pregnancy (Gassasse, 2017).

Metabolic process results in the production of free radicals whose accumulation can lead to oxidative stress if there is no antioxidant defense mechanisms to bring about the corresponding reducing response, which is dependent on a number of factors including free radical production, susceptibility of tissue to stress, and strength of the defense and repair system (Gomes, Minasi, da Cruz, & Rodrigues, 2016). Unsaturated fatty acids which are the prime targets of these free radical reactions, plays a vital role in protecting cellular membrane integrity as well as receptor functionality. Thus, cellular lysis is inevitable once these unsaturated fatty acids are compromised by the activities of these free radicals (Livshits et al., 2021). Oxidant/free radicals are species that possess not only very short half-life but with very high reactivity as well as great damaging activities towards macromolecules like proteins, deoxyribonucleic acid (DNA) and lipids (Parast & Paknahad, 2017). Depending on the source from which they are derived (Oxygen or Nitrogen) they are classified as either reactive oxygen or reactive nitrogen species (Banerjee & Vats, 2014; Nasri, Shirzad, Baradaran, & Rafieian-Kopaei, 2015). During a normal metabolism, Reactive Oxygen Species are usually produced as natural by-products and they have vital roles in cell signaling and homeostasis (Jaganjac, Tirosh, Cohen, Sasson, & Zarkovic, 2013). Obesity can crop up during pregnancy and these can give rise to a chronic state of stress in the pregnant subjects (Fisher et al., 2012).

Protective proteins, vitamins, minerals, and enzymes called antioxidants functions to protect cells from the effects of Reactive Oxygen Species (ROS) which are destructive (Parast & Paknahad, 2017). The body cells go through oxidative stress during glucose metabolism, where abnormally high levels of free radicals are usually produced in the system leading to cell damage thus reducing the cell population (Khambule & George, 2019). Suitable balance in the prooxidants / antioxidants exists in a normal cell but the balance can move either towards prooxidants when production of Reactive Oxygen Species (ROS) is increased or it could be towards antioxidants when there is a decrease in the level of antioxidants (Hasain et al., 2020). It has been observed that, the development of vascular complications may be responsible for the hyper production of ROS and Reactive Nitrogen Species (RNS) in DM. However, Antioxidants are well known to adequately protect the body against the negative impact of ROS and its derivatives (Parast & Paknahad, 2017). Research has shown that, during the development of GDM, lipid peroxidation levels are significantly elevated while the reverse is the case for antioxidant status and vitamin E (Sharifipour, Abedi, Ciahkal, Jahanfar, Mohaghegh, & Zahedian, 2020). On the other hand, it was reported that a rich dietary source of antioxidants reduces ROS, modulates oxidative stress, promotes better health, and further improve and build up the total antioxidant status of the body (Joo, Kim, Kim, Jung Han, & Cho, 2021). The collective effects of all reducing agents contained in plasma as well as all body fluids and providing a composed parameter rather than the sum of measurable antioxidants simply termed Total antioxidant status (Pankiewicz, Szczerba, Fijałkowska, Sierdziński, Issat, & Maciejewski, 2022). Basu & Lyons, 2020) reported that the, level of Total Antioxidant Status in Gestational Diabetic Mellitus subjects are still not clearly understood. On the other hand, the

elevated rate of lipid peroxidation and the destruction of the antioxidant defense system may be increased due to the elevated levels of free radicals associated with DM (Yahaya, Salisu, Abdulrahman, & Umar, 2020).
Malondialdehyde (MDA) are widely preferred and is usually used for the identification of free oxygen radicals in various pathological conditions because it is found to be the decomposition product of peroxidized polyunsaturated fatty acids (Ayala, Muñoz, & Argüelles, 2014). However, MDA is often measured as an index of lipid peroxidation and it happens to be one of the several end products of lipid hydroperoxide degradation (Niki, 2014). However, among the several studies which reported a significant variation for MDA levels in women whether pregnant or non-pregnant, a direct relationship was also found between MDA levels and gestational age (Nodine, 2011). Lipid peroxidation has been reported to be potentially harmful, this is because usually it cannot be controlled, it has self-enhancing process that rapidly as well as easily disrupts membrane lipids and other cell components of the system (Ferrante, Carota, Li Volti, & Giuffrè, 2021). Adeniji & Oparinde (2013) reported that, free radicals and reactive oxygen species levels increases during the second trimester of pregnancy for non-GDM patients but the presence of a functional reducing/antioxidant system ensures cellular damage is drastically reduced.
Hyperglycemia induces oxidative stress. Recently, several studies have associated increase in mitochondrial ROS production, elevated glycosylated protein formation from non-enzymatic reactions, and altered low-density lipoproteins uptake and glucose autoxidation with hyperglycemia induced oxidative stress (Ramos-Riera, Pérez-Severiano & López-Meraz, 2023; Nsonwu-Anyanwu, Nsonwu & Usoro, 2020). However, increased free fatty acids levels can also induce oxidative stress via elevated mitochondrial uncoupling and ß-oxidation which can also lead to the increase production of ROS (Jana, Chintamaneni, Krishnamurthy, Wadhwani, & Mohankumar, 2019).Vitamin C which as a hydrophilic molecule has been revealed to possess the capacity to rapidly scavenge for most free radicals with strong affinity for hydroxyl radical (Cederberg & Eriksson, 2005).

Materials and Methods

Study Area

The study area is Kaduna, the capital of Kaduna State. The state is located at the Northern part of Nigeria's High Plains. The vegetation cover is Sudan Savannah type, characterized by scattered short trees, shrubs and grasses. The soil is mostly loamy to sandy type. A substantial amount of clay is found also Kaduna State consists of twenty-three (23) Local Government Areas. It consist of a total of 46,053 km2 (17,781 sq meter) Area rank 4th of 36. Its population (2006 census) recorded a total of 6,113,503, rank 3rd of 36.

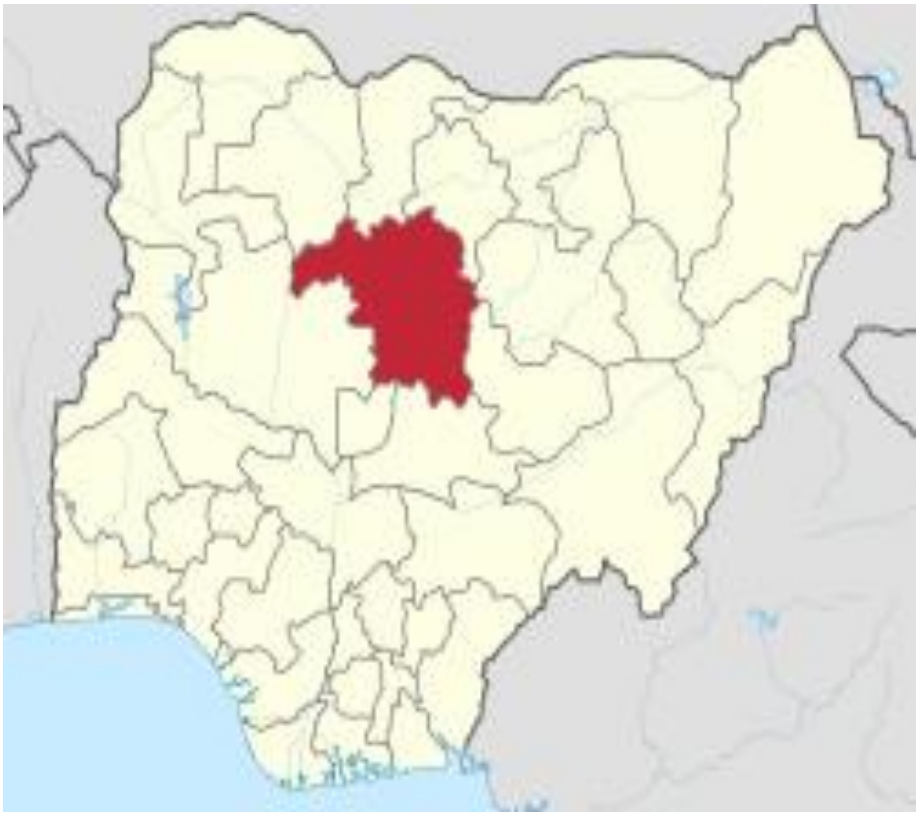

Fig. 1: Location of Kaduna State in Nigeria Coordinates: 10°20′N**7°45′E**

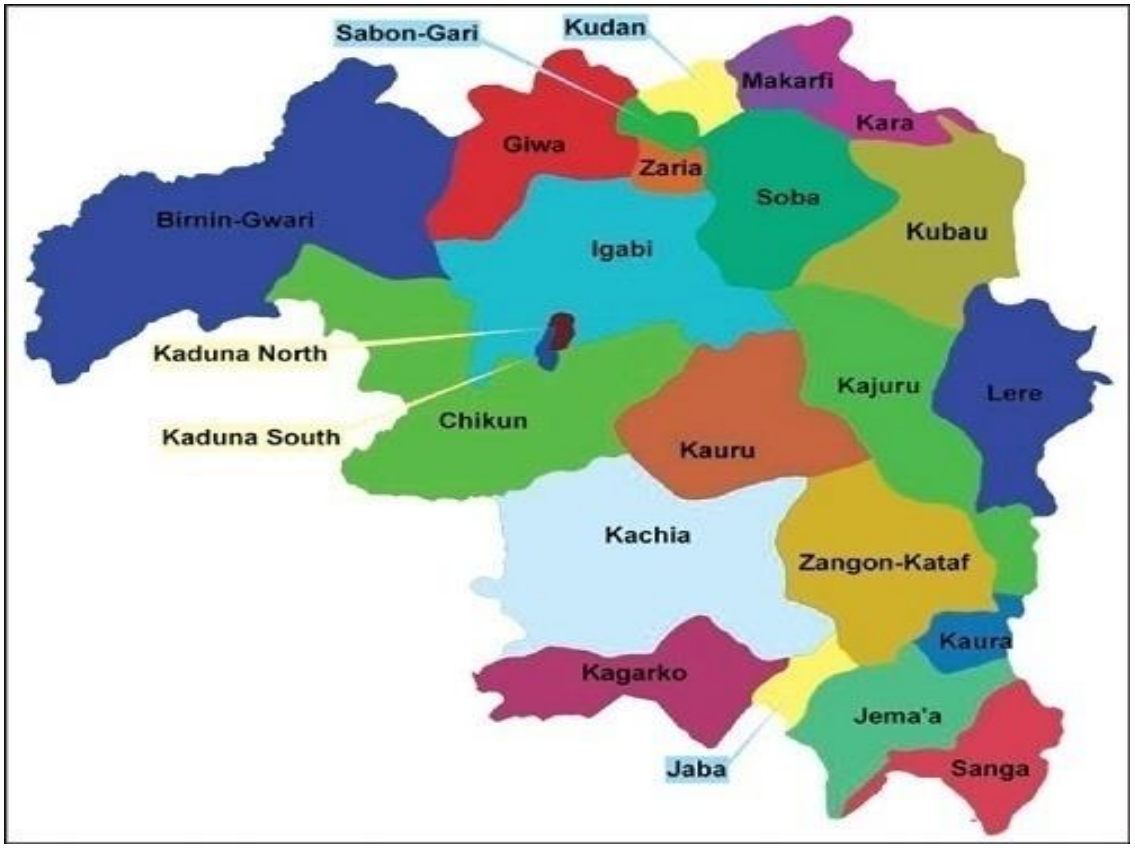

Fig. 2: Map of Kaduna State showing all the Local Government Areas. (Kdsg.gov.ng)

Study Design

This is a case-controlled study, in which a total of 160 subjects between the ages of 18- 40 years were recruited for the study. However, 60 subjects were gestational diabetic women, 50 non-diabetic pregnant women as $Control_1$ all attending Antenatal clinics at Barau Dikko Teaching Hospital, Yusuf Dantsoho Memorial Hospital and Gwamna Awan General Hospital Kaduna State, while 50 apparently healthy non-pregnant non diabetic staff as $Control_2$. Arrangements were made with the clinicians whereby subjects who satisfy the study inclusion criteria were selected. Structured questionnaires (Appendix 1) were administered to the study population. Vital information including the name, age, ethnic group, height, weight, blood pressure and pregnancy induced complications were obtained through personal interview followed by blood sample collection.

Inclusion Criteria

All pregnant women within the age of 18–40 years, permanent residents of Kaduna Metropolis, within 24–28 weeks of gestation according to the WHO diagnostic criteria, who give their consent and underwent GDM universal screening, were included in the study. Women who are non- hypertensive and who agreed to participate were also included in the study, were also included. Apparently healthy aged matched non-GDM pregnant women as well as apparently healthy non-pregnant- non-Diabetic women too were included in the study as $Control_1$ & $Control_2$ respectively.

Exclusion Criteria

Primigravidas, Obesse subjects, women who are ≤18 or ≥45 years, pregnant women with chronic hypertension, multiple gestation, pre-eclamsia and eclamsia were excluded in the study. All those who personally declined to give consent for inclusion were also excluded from the study.

Informed Consent

Informed written consent was obtained from all subjects before inclusion using approved protocol given by the Kaduna State Ministry of Health ethical committee (appendix II).

Ethical Approval

Ethical approval of the study was obtained from the Ethics Committees of the Kaduna State Ministry of Health

in accordance with Helsinki declaration (Appendix III).

Sample Size Determination

The sample size would be determined from a standard formula (Kyriazos, 2018).

$$n = \frac{(Z1 - a)2 \ (P) \ \ (1 - P)}{d2}$$

Where **n**= minimum sample size;
Z_1-a= value of standard normal deviation which at 95% confidence level has been found to be 1.96,
P = the best estimate of the population prevalence obtained from literature review (3.4%)
d = difference between the true population rate and sample that can be tolerated, that is the absolute precision required (in percentage point) on either side of the population i.e. degree of confidence = 0.05

$$\boldsymbol{n} = \frac{(3.8416\ )\ (0.034) \ \ (0.9966)}{0.0025}$$

$\boldsymbol{n}$ = 51
Therefore, a total of 51 with 10% (5) of these subjects will be added to the research for attrition making a total of 56 total subjects but for the purpose of these study 60 samples shall be used.

Sampling Techniques

Sampling

Arrangement was made with the clinicians where those subjects who satisfied the study inclusion criteria were selected. A consecutive sampling method in which subjects meeting the criteria for inclusion, were continuously selected until the desired sample size was obtained. Random 50g oral glucose challenge test was carried out followed by fasting 75g OGTT were performed in pregnant women between 24 and 28 weeks of gestation representing GDM and C_1. Samples were also collected from apparently healthy non-pregnant, non-Diabetic staff and Students that served as control (C_2). Diagnosis of GDM was established according to the diagnostic criteria of the American Diabetes Association 2015. Findings of the blood samples collected from all subjects were fully documented in the proforma (Appendix I). Assessments such as blood pressure (BP), weight (W), height (H) and body mass index (BMI) were also observed

Specimen Collection and Processing

Blood specimen (5ml) for the biochemical measurements, was collected from peripheral vein (antecubital venepuncture). This was done by cleaning the antecubital fossa with methylated spirit and with the application of a tourniquet a few centimeters above the anticubital fossa to distend the veins, an overnight fasting sample of 5ml venous blood was drawn from each subject and dispensed into plane containers. The coagulated whole blood was centrifuged at 1,000 rpm (Revolution Per minute) for 15 minutes, within 30 minutes of collection. The serum was removed, transferred to Bijou bottles. The samples for glucose, antioxidants, Vitamins C was analyzed immediately. Specimens for other parameters that would not be assayed within 24 hours of collection were stored frozen at -80 ^{0}C until the time for analysis.

Equipment

Hettich Universal 32 Centrifuge (Germany) was used to spin blood samples (Appendix IV). Beckman Coulter DU-520, general purpose UV/VIS Spectrophotometer (Germany) was used for the measurements of serum glucose & Bio-rad PR 5100 microplate reader was used for measurements of Adiponectin.

Chemicals/Reagents

The chemicals used in this study were procured from Randox Company Limited. All the chemicals and reagents were of analytical grade or higher.

Analytical Methods

Measurement of Serum Glucose

Serum glucose was measured using enzymatic method .

Principle

Glucose is oxidized to hydrogen peroxide (H_2O_2) and gluconic acid in the presence of glucose oxidase. Hydrogen peroxide in the presence of peroxidase is broken down to water and oxygen. The oxygen released reacts with 4- aminophenazone (4- aminoantipyrine) and phenol to produce a pink colored qsuinoneimine complex that was measured at 505 nm using a spectrophotometer.

$$\beta\text{-D Glucose} + O_2 + H_2O \xrightarrow{\text{Glucose oxidase}} \text{Gluconic acid} + H_2O_2$$
$$H_2O_2 + \text{4-aminophenazone} \xrightarrow{\text{Peroxidase}} \text{Quinone} + 2H_2O$$

Procedure

Into each of clean test tubes labeled test, standard and blank, 0.5 ml glucose solution was placed. Then 0.05 ml of serum sample, standard solution and distilled water was added respectively. These were incubated at 37 ^{0}C for 10 minutes and the absorbances were read at 505 nm.

3.11.3 Measurement of Serum Total Antioxidant Status (TAS)

Serum Total Antioxidant Status (TAS) was measured .

Principle

When MTT dye in phosphate buffered saline (PBS) is incubated with serum for 30 minutes at 37 ^{o}c, the dye is reduced to a blue colored product. The reaction is stopped by the addition of hydrochloric acid in isopropanol and the absorbance read at 580 nm.

Procedure

Briefly, 0.1 mL of sample 2-20 µg/ml) was combined with 1 mL reagent solution containing 0.6 M Tetraoxosulphate (VI) acid, 28mM sodium phosphate and 4mM ammonium molybdate. The mixture was incubated at 95 °C for 90 min. Absorbance was measured at 695 nm after cooling to room temperature. Butylatedhydroxytoluene (BHT) was used as reference compound.

$$Total\ Antioxidant\ capacity\ (\text{umol/ml}) = \frac{(Abs\ Blank - Abs\ Sample)}{Abs\ Blank}$$

Measurement of Serum Catalase (CAT)

Principle

Catalase enzyme activity at pH 7.0 and 25°C can be measured by monitoring the consumption of H_2O_2 substrate at 240 nm.

Procedure

The assay mixture was made up of 1 ml of H_2O_2 solution (800 µmoles) and 1.25 ml of phosphate buffer (0.1M, pH 7.4), to which 1ml of appropriately diluted enzyme preparation (1:50) was quickly added and mixed by gentle swirling. A 1 ml aliquot of the reaction mixture was then withdrawn and added to 2 ml dichromate/acetic acid reagent and the absorbance read at 60 sec interval for 3 minutes at 570 nm.

Catalase activity is expressed in Units/mg protein as calculated from the formula:

$$\text{Catalase activity} = \frac{\Delta\ \text{Abs/min} \times \text{Conc. of standard} \times \text{Volume of assay}}{\text{Abs of standard} \times \text{Volume of enzyme} \times \text{Protein conc.}}$$

Where:

Abs = Absorbance at 570 nm

Δ Abs = Change in Absorbance

Measurement of Serum Malondialdehyde (MDA)

Principle

The assay is based on the reaction of MDA with Thiobarbituric acid (TBA); forming an MDA-TBA adduct, that abso RBG strongly at 532nm

Procedure

A known volume (0.8 ml) of Tris-KCl (0.15M, pH 7.4) was added to 0.2 ml of the sample and then quenched by addition of 0.25 ml of 30% Trichloroacetic Acid (TCA). 0.25 ml of 0.75% Thiobarbituric Acid (TBA) was added and the reaction mixture incubated for 45 min at 80 ^{o}C and then cooled on ice. The resulting pink-coloured reaction mixture was centrifuged at 4000 g for 15 minutes. The absorbance of the clear pink supernatant was then read at 532 nm using distilled water as blank.

Malondialdehyde (MDA) level was calculated using the formula:

$$\text{Malondialdehyde (MDA)} = \frac{\text{Absorbance} \times \text{Vol ume of mixture}}{E_{532} \times \text{Volume of sample} \times \text{mg protein}}$$

Where E_{532} is molar absorptivity at 532 nm = 1.56 x 10^{-5}

Measurement of Serum Super Oxide Di-Mutase Activity (SOD)

Principle

In the presence of SOD enzyme at specific assay pH, the rate of auto-oxidation is inhibited and the percentage of inhibition is linearly proportional to the amount of SOD present within a specific range. Sample SOD activity is determined by measuring ratios of auto-oxidation rates in the presence and absence of the sample and expressed as traditional McCord Fridovich "cytochrome c" units.

Procedure

The diluted sample (100 µL) was added to 1000 µL of 0.05M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer. The reaction was initiated by addition of 150 µL of freshly prepared 0.3mM epinephrine to the mixture. The reference cuvette contained 1000-µL buffer, 150 µL of epinephrine and 100 µL of distilled water. The increase in absorbance at 480 nm was monitored every 0.5 min for 2.5 min.

Superoxide dismutase (SOD) activity was calculated using the formula:

$$\%\ \text{Inhibition} = \frac{\text{Increase in absorbance for substrate}}{\text{Increase in absorbance of blank}} \times 100$$

Where

$$\text{Increase in absorbance/minute} = \frac{A_5 - A_0}{2.5}$$

A_0 = absorbance after 0.5 min

A_5 = absorbance after 2.5 min

1 unit of SOD activity is given as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline to adenochrome during 1 minute.

Measurement of Serum Glutathione Peroxidase (GPX) Activity

Principle

Glutathione Peroxidase catalyzes the reduction of hydrogen peroxide (H_2O_2), oxidizing reduced glutathione (GSH) to form oxidized glutathione (GSSG). GSSG is then reduced by glutathione reductase (GR) and β-nicotinamide adenine dinucleotide phosphate (NADPH) forming NADP+ (resulting in decreased absorbance at 340 nm) and recycling the GSH. Because GPx is limiting, the decrease in absorbance at 340 nm is directly proportional to the GPx concentration.

Procedure

The reaction was initiated by the addition of 200 µL of H_2O_2 (10 mM) to a reaction mixture already containing 800 µL Tris-HCl buffer (0.1 M, pH 7.4), 400 µL GSH (2 mM), 200 µL sodium azide (10 mM) and 50 µL of diluted samples made up to 2 ml with distilled water. The assay mixture was incubated at 37^0C for 5 minutes after which the reaction was terminated by the addition of 500 µL of TCA (10%). After centrifuging the assay mixture at 4000 g for 10 minutes, 250 µL of the supernatant was added to a mixture of 1000 µL of DTNB (0.004%) and 250 µL of disodium hydrogen phosphate (0.3 M) solution. The colour developed was read at 420 nm, a reaction mixture without enzyme was similarly used as the control.

The glutathione peroxidase activity was expressed as Units per milligram protein (Units/mg protein) as calculated from the formula:

$$\text{Glutathione peroxidase activity} = \frac{\Delta\text{Abs/min} \times \text{GSH Standard} \times \text{Total reaction volume}}{\text{Abs of standard} \times 307.32 \times \text{volume of enzyme source} \times \text{protein conc.}}$$

Where:

Molecular weight of GSH = 307.32 g/mole

Abs = Absorbance at 420 nm

Measurement of Serum Vitamin C

Serum vitamin C was determined by spectrophotometric method (Moeslinger, Brunner, Volf, &Spieckermann, 1995; Pathy, 2018)

Principle

Ascorbic acid absorbs RBG maximally at 478.5nm in methanol.

Procedure

To 1ml of serum, 5ml of methanol was added in order to extract vitamin C at 37^oC for 15 minutes using a hot plate. This was allowed to stand and cool for 5 minutes before reading the concentration of the individual

samples at 478.5 nm against a standard curve which was prepared with standard solution of ascorbic acid.

Quality Control (QC)
All analytical tests were done according to the standard operating procedure. It will involve the use of inter and intra-run of control sera along with the samples. Pre-analytical and post analytical precautions would be observed.

Validity of the Instrument
The questionnaire designed by the researcher was shown to the supervisor and face validity and reliability were determined before the questionnaires were administered.

Reliability of the Instrument
The questionnaire was subjected to scrutiny by the researcher`s supervisor. Content validity was established based on assessment of each item of the questionnaire for clarity, consistency and relevance. The result of the pilot study revealed 75% consistency i.e. Cronbach alpha value of 0.75

Statistical Analysis
The data obtained was treated accordingly using Statistical Program for the Social Sciences (SPSS 17.0) for windows (SPSS Inc, Chicago, 1L). Serum antioxidant status, MDA and serum glucose levels of all the subjects were assessed, compared against age and BMI groups of the subjects using the two tail student's t-test. ANOVA was then used to compare between different groups. Other analytes estimated along with the TAS were correlated to find relationship between them and their effect in controlling GDM. Correlation between TAS, SOD, Catalase, GPx and glucose as well as MDA was carried out using Pearson's linear correlation analysis. A p-value of equal to or less than 0.05 ($p \leq 0.05$) was considered significant.

Results

The Mean Blood Glucose Levels of Gdm and Control Subjects.
Table 1 shows mean values of biochemical parameters in GDM and Controls$_1$ & Control$_2$ subjects according to OGCT (RBG) type and OGTT (FBG & 2HBG) types. The Serum glucose concentrations in GDM patients were all significantly increased ($p \leq 0.05$) when compared to Control$_1$ & Control$_2$ subjects. However, inter group comparism of C_1vsC_2 reveals, Serum Glucose (RBG, FBG and 2HBG) levels were all similar ($p \geq 0.05$).

Table 1: The mean blood Glucose Levels of GDM and Control Subjects

Parameter(mmol/L)	**GDM** (n = 60)	**C_1** (n = 50)	**C_2** (n = 50)	**F-value**	**p-value**	**GvC_1**	**GvC_2**	**C_1vC_2**
RBG	8.28±0.11^{a}	5.92±0.16^{b}	5.49±0.15^{b}	126.781	0.000	0.000	0.000	0.036
FBG	6.21±0.14^{a}	4.46±0.10^{b}	4.58±0.10^{c}	70.330	0.000	0.000	0.000	0.492
2HBG	8.70±0.13^{a}	5.82±0.14^{b}	5.62±0.13^{c}	174.401	0.000	0.000	0.000	0.324

n=Number of patients, GDM = gestational diabetes mellitus, Control 1= non GDM pregnant women, Control 2 = non diabetic non pregnant, RBG= Random Blood Glucose, FBG= Fasting Blood Glucose, 2HBG= Two Hours Blood Glucose, SEM=standard error of mean and values with different (^{ab}) superscripts are significantly different ($p \leq 0.05$)

Table 2: TAS and Vitamin C Of Gdm And Control Subjects
The OGCT and OGTT mean values OF TAS and Vitamin C in GDM patients and Controls 1 & 2 are shown in Table 2. The TAS and Vitamin C levels in OGCT and OGTT (FBG) was significantly increased in ($p \leq 0.05$) C_2 than G and C_2 with similar difference existing across all the groups for OGTT (2HBG). Intergroup comparison reveals significant difference between GvC_2 andC_1v$C_2$for both OGCT and OGTT (FBG) with an insignificant difference between GvsC_1, GvC_2 and C_1vC_2.

Table 2: Total Antioxidant Status and Vitamin C levels of GDM patients and controls for OGCT (RBG) and OGTT (FBS & 2HBG) samples

Parameter	**GDM (n = 60)**	**C_1(n = 50)**	**C_2(n = 50)**	**F-value**	**p- value**	**GvC_1**	**GvC_2**	**C_1vC_2**
RBG TAS (µmol/ml)	**5.04±0.19**	**5.62±0.21**	**7.14±0.24**	**26.261**	**0.000***	**0.051***	**0.000***	**0.000***

Vit.C(mg/ml)	**32.53±1.97**	**30.03±2.78**	**73.80±9.31**	**19.854**	**0.000***	**0.742**	**0.000***	**0.000***
FBG TAS(„)	**5.64±0.14**	**5.39±0.17**	**7.14±0.24**	**11.430**	**0.000***	**0.511**	**0.000***	**0.000***
Vit.C(mg/ml)	**34.78±2.29**	**39.79±3.51**	**44.06±1.50**	**3.345**	**0.035***	**0.161**	**0.010***	**0.253**
2BG TAS („)	**5.62±0.21**	**5.78±0.20**	**5.36±0.29**	**0.762**	**0.468**	**0.641**	**0.422**	**0.225**
Vit.C(mg/ml)	**30.70±1.43**	**30.84±3.36**	**25.82±2.43**	**1.338**	**0.265**	**0.967**	**0.152**	**0.159**

n=Number of patients, GDM = gestational diabetes mellitus, Control 1= non GDM pregnant women, Control 2 = non diabetic non pregnant, RBG= Random Blood Glucose, FBG= Fasting Blood Glucose, 2HBG= Two Hours Blood Glucose, SEM=standard error of mean and *= significantly different (p≤ 0.05).

Table 3: Catalase, Mda, Sod And Gpx (Mean±Sem) In Gdm Patients And Control Subjects

For OGCT, CAT was significantly raised in GDM than controls and same trend occurred in OGTT (2HBG) OGTT but in OGTT (FBG), the increase in CAT level was similar (P≥0.05) across the groups. Intergroup relationship reveals significant differences in group GvC_1 and GvC_2. Also for OGCT, MDA was significantly increased (p≤0.05) in GDM patients than C_1 and C_2 but for OGTT,MDA was significantly increased in C_1 than GDM and C_2.Intergroup comparison reveals significant differences in $GvsC_1$ and C_1vsC_2.The level of SOD was statistically increased in C_2 subjects when compared with GDM and C_1 in both OGCT and OGTT samples. However, intergroup ($GvsC_1$, $GvsC_2$ and C_1vsC_2) comparison reveals significant differences in all except that, similarity is seen in $GvsC_2$ for OGCT, Also, GPx was lower in GDM patients. Gluthatione Peroxidase is Significantly increased in GDM and C_1 compared to C_2 in both OGCT and OGTT samples. The intergroup differences for GPx levels exist in $GvsC_2$ and C_1vsC_2 for OGCT and OGTT (2HBG) while for OGTT (FBG), significant differences occur in GvsC1, $GvsC_2$ and C_1vsC_2. All shown in Table 3.

Table 3: Catalase, MDA, SOD and GPx Levels in GDM patients and control subjects80

Parameters	**GDM(n=60)**	**C_1(n = 50)**	**C_2(n = 50)**	**F-value**	**p- value**	**GvC_1**	**GvC_2**	**C_1vC_2**
RBG CAT (IU/mgPr)	4.06±0.40	1.48±0.19	2.10±0.31	17.783	0.000*	0.000*	0.000*	0.199
MDA(µg/mgPr)	362.83±33.03	358.80±34.34	148.31±13.74	16.927	0.000*	0.922	0.000*	0.000*
SOD (µg/mgPr)	1.62±0.18	1.07±0.12	2.16±0.18	7.174	0.001*	0.024*	0.100	0.000*
GPx(µg/mgPr)	222.65±12.72	188.36±10.54	139.08±10.86	13.219	0.000*	0.037*	0.000*	0.004*
FBSG CAT („)	4.61±0.42	3.82±2.12	2.10±0.31	1.353	0.261	0.635	0.107	0.276
MDA („)	233.90±23.80	264.57±34.05	140.0.65±11.87	6.361	0.002*	0.380	0.008*	0.001
SOD („)	1.45±0.14	079±0.11	2.95±0.25	38.316	0.000*	0.008*	0.000*	0.000*
GPx („)	192.41±6.98	138.30±17.93	92.47±3.23	21.896	0.000*	0.000*	0.000*	0.004*
2BG CAT („)	3.72±0.35	1.63±0.22	2.87±0.46	8.887	0.000*	0.000*	0.090	0.018
MDA („)	263.02±26.59	317.23±33.28	162.18±24.60	7.283	0.001*	0.172	0.012*	0.000*
SOD („)	1.41±0.13	1.04±0.12	2.5±0.20	58.330	0.000*	0.086*	0.000*	0.000*
GPx	192.16±7.60	171.77±9.91	86.70±6.42	47.324	0.000*	0.073	0.000*	0.000*

n=Number of patients, GDM = gestational diabetes mellitus, Control 1= non GDM pregnant women, Control 2 = non diabetic non pregnant, RBG= Random Blood Glucose, FBG= Fasting Blood Glucose, 2HBG= Two Hours Blood Glucose, SEM=standard error of mean and values with different (**) superscripts are significantly different (p≤ 0.05).

Discussion

This study was carried out to determine the Antioxidant status, Adiponectin and Lipid Profiles of Gestational Diabetes Mellitus subjects in Kaduna State. A total of 160 subjects consisting of 60 GDM, 50 non-GDM pregnant women and 50 apparently healthy non pregnant women were used for the study. Standard procedures carried out using the 50g OGCT and 75g OGTT according to WHO, 1999 criteria (Rudra, 2019).

The glucose levels for OGCT and OGTT were significantly higher in GDM patients than in non-GDM ($Control_1$) and apparently healthy non-diabetic non-pregnant ($Control_2$) subjects ($p \leq 0.05$). This is similar with other studies by Song, (2022) Parast, (2017) Sun, (2021) Guan, (2021) Agarwal, (2018) Milajerdi, (2019) Abualhamael, (2019) that reported significantly higher concentration of blood glucose in GDM than in non-GDM pregnant control subjects. Furthermore, the statistical difference in the blood glucose levels between intergroup ($Control_1$ vs $Control_2$ as well as GDM vs $Control_1$) for OGCT & OGTT are all similar ($p \geq 0.05$). This finding, even though some other studies had contradictory findings, like studies by Yao, (2019); Si, (2019); Zhang, (2022) have revealed that, blood glucose for OGTT at 1Hr is not significant but at 2HrBG the difference is significant ($p \leq 0.05$). The FBG is significantly higher ($p \leq 0.01$) in GDM than Controls (Zhang, 2017; Marei, 2020; Chen, 2022; Si, 2019). This is expected because of the Gestational Diabetes Mellitus subjects among the study group who are constantly experiencing both metabolic changes as well as hormonal changes going on in their bodies. However, some studies (Bhavadharini, 2016; She, 2022; Rudra, 2019; Eldem, 2021) have revealed, the efficacy of 50g OGCT that is, the 1hour OGCT. Li, (2020) Leblalta, (2022) observed non-significant difference ($p \geq 0.05$) in fasting blood glucose of GDM Patients and non-GDM pregnant Control subjects.

TAS and Vitamin C levels of the OGCT and each OGTT, were significantly lower in GDM than in C_1&$_2$. this is in agreement with the findings of Bogdanet, 2020; Dias, 2021; Vida &Zam-Zam, 2017 Mohammed, 2018

The TAS and Vitamin C levels in OGCT and OGTT (FBG) were significantly increased ($p \leq 0.05$) in $Control_2$ than GDM and non- GDM $Control_1$ with insignificant difference existing across all the groups (GDM vs $Control_1$, GDM vs $Control_2$ and $Control_1$vs$Control_2$) for the OGTT (2HBG) type. Intergroup comparison reveals significant decrease between GvsC$_2$ and C$_1$vsC$_2$ for both OGCT and OGTT (FBG) with non-significant difference between GDMvs$Control_1$, GDMv$Control_2$ and non-GDM pregnant $Control_1$vs non-diabetic, non-pregnant $Control_2$. However, the present study is in harmony with previous studies by Usluoğullari, (2017) Karacay, (2010) Farrar, (2017) Herath, (2021) Pieczyńska, (2019) Mohammed, (2018) and Atiba, (2014) with findings of significant decrease ($p \leq 0.05$) in serum TAS in GDM than in normal pregnant women and non-pregnant group. On the other hand, (Nuzzo, 2021; Sheldon, 2016; Beyazit, 2020) reported significant increase ($p \leq 0.05$) in Plasma levels of TAS in diabetic women when compared with the control groups. This increase could be as a result of the enhanced generation of oxidative stress which causes a decrease in the level antioxidant capacity in diabetic patients. Also, in line with the results of this study, reports showed that higher maternal serum level of TAS could be a significant predictor of developing GDM (Nuzzo, 2021).

However, the women diagnosed with GDM by the 75g OGTT (2HBG), the mean values of TAS were similar ($p \geq 0.05$) in GDM patients, non-GDM pregnant $Control_1$ and non-pregnant non-diabetic $Control_2$ subjects. Further studies by (Ott, 2018; Khan, 2020; Usluoğullari, 2017; Ozler, 2019; Khalafallah, 2016; Uysal, 2015) revealed similar ($p \leq 0.05$) level of TAS in both GDM and Control subjects. In addition, this study is in agreement with the study of Manoharan, (2019) and Rajendran, (2022) that revealed increased serum TAS in GDM subjects with lower TAS activity. This reveals the link between TAS and oxidative stress occurring in GDM Patients as well as a predictor for developing GDM (Ozler, 2019 and Demirci-Çekiç, 2022). Demirci-Çekiç, (2022) also revealed that, the difference in the level of TAS in GDM and Controls is not significant. This could be due to the various degrees of oxidative stress the mother goes through during pregnancy.

Vitamin C level was significantly lower in GDM subjects compared to non-GDM pregnant $Control_1$ and non-pregnant non-diabetic $Control_2$ groups. Other findings revealed that, antioxidant capacity is lower in women with GDM than apparently healthy pregnant controls, possibly related to lower intakes of vitamins (Parast, 2017; Lyu, 2022). This finding is similar with other reports of (Machairiotis, 2021; Parast, 2017), that revealed significantly low level of Vitamin C in GDM patients than apparently healthy pregnant controls. However, there is a number of conflicting reports in which vitamins C was found to be similar ($p \geq 0.05$) in both GDM and non-GDM pregnant $Control_1$ and non-pregnant non-diabetic $Control_2$ subjects (Daneshzad, 2020; Machairiotis, 2021). The level of Vitamin C is highly beneficial to the pregnant subjects since it is likely to reduce the chances of becoming diabetic (Aljanahi, 2020)

The levels of CAT and GPx were significantly higher in GDM subjects than C_1&$_2$, MDA was significantly higher in the OGCT only but SOD was significantly lower in GDM subject than C_1&$_2$ for the OGCT and each of the OGTT type. These is in agreement with the findings Basu, 2021, Zygula, 2019 & Chatzakis, 2022. For the OGCT group, CAT was significantly raised in GDM than in non-GDM $Control_1$ and non-pregnant non-Diabetic $Control_2$ and same trend occurred in OGTT (2HBG) OGTT but in OGTT (FBG) type, there was an increase in CAT level ($p \geq 0.05$) across the groups. The result of CAT, in the present study is in harmony with a

previous study revealed by (Rajatharangani, 2018). A review by (Newsholme, 2019) conversely found that Catalase level significantly decreased in GDM patients than pregnant Control subjects in Hungary. In similar studies, significantly lower blood Catalase level was observed in pregnant women with GDM than apparently healthy non-diabetic pregnant Control subjects (Chatzakis, 2022). Zejnullahu, (2021) also reported significantly low levels of CAT in in healthy pregnant subjects as compared to the non-pregnant control subjects. Usually, the development of GDM is in the second and third trimesters of the pregnancy but reports on the relationship of CAT versus GDM are very conflicting. It has been reported that oxidative stress is high in the second trimester of pregnancy and the Catalase activity was also low during this period due to utilization to mob of free radicals (Du, 2020; Gaurav, 2020). In addition, the blood Catalase activity has been reported to be low in pregnant women with GDM compared to non-pregnant and pregnant non diabetic healthy control women which is contrary to findings in this study (Basu, 2021; Zygula, 2019). Catalase is one of the crucial antioxidant enzymes that mitigates oxidative stress to a considerable extent by destroying cellular hydrogen peroxide to produce water and oxygen (Nandi, 2019). Deficiency or malfunction of catalase is postulated to be related to the pathogenesis of many age-associated degenerative diseases like diabetes mellitus (Sies, 2020). An intergroup relationship reveals significant differences in group GDM vs $Control_1$ and GDM vs $Control_2$.

Also, for OGCT type, MDA was significantly increased ($p \leq 0.05$) in GDM patients compared to $Control_1$ and $Control_2$ subjects. The level of MDA, in the present study is in harmony with earlier studies by (Qin, 2022; Ozler, 2019 & Basu, 2021) with findings of significant increase ($p \leq 0.05$) of MDA, in GDM subjects than normal pregnant women. The same trend was discovered in a cross- sectional study revealed by (Karacay, 2010; Isong, 2022; Javid, 2020; Zheng, 2020, Manoharan, 2019; Bulut, 2021) with significantly higher level of MDA in GDM patients than apparently healthy pregnant control subjects. Similarly, in a study by Zejnullahu, (2021) significantly higher level of MDA was recorded in healthy pregnant subjects as compared to the non-pregnant control subjects. This finding could be due to increase in the oxidative radical in both pregnancy and gestational diabetes disease (Phoswa, 2021), but for OGTT type, MDA was significantly decreased in GDM as compared to the non-diabetic pregnant $Control_1$ and non-diabetic non-pregnant $Control_2$ subjects. This could be due to increased oxidative stress during pregnancy since the increase has also been revealed in non-diabetic pregnant subjects too (Jamilian, 2019). Intergroup comparison reveals significant differences in GDM vs non-GDM pregnant $Control_1$ and $Control_1$ vs non-pregnant non-diabetic $Control_2$.

Likewise, the level of SOD was statistically decreased in GDM subjects when compared with non-GDM $Control_1$ & non-diabetic non- pregnant $Control_2$ in both OGCT and OGTT samples. The same trend was discovered in a cross- sectional study revealed by (Zhang, 2019) with higher level of SOD in pregnant controls than GDM patients. However, intergroup (GDM vs non-GDM pregnant $Control_1$, GDM vs non-pregnant non-diabetic $Control_2$ and non-GDM pregnant $Control_1$vs non-pregnant non-diabetic $Control_2$) comparison reveals significant differences in all but similarity was seen in GDM vs $Control_2$ for OGCT. This finding is consistent with the study by Zejnullahu, (2021) who reported significantly low levels of SOD and GPx in healthy pregnant subjects as compared to the non-pregnant control subjects. More also, Javid (2020) SOD and GPx significant decrease in Diabetic patients more than the apparently healthy control subjects.

As observed in other studies performed on GDM subjects using 75g OGTT, the result of SOD, in this study is in agreement with previous studies by (Özmen *et al*, 2022; Zhang, 2019) with findings of significant decrease ($p \leq 0.05$) in serum SOD level, in women with GDM than the controls. Decreased SOD activity during pregnancy in diabetic groups may be linked to increased hydrogen peroxide since it is well known that ROS, especially superoxide anion and hydrogen peroxide (H_2O_2), inhibit SOD activity (Costa, 2021). Further studies by (Lian, 2021; Feng, 2020) revealed similar ($p \leq 0.05$) level of SOD in both GDM and Control subjects.

The GPx level was significantly increased in GDM and non-GDM pregnant $Control_1$ compared to non-diabetic non-pregnant $Control_2$ in both OGCT and OGTT samples. The intergroup comperism for GPx levels revealed that there exist significant differences in GDM vs non-diabetic non-pregnant $Control_2$ and non-GDM pregnant $Control_1$vs non-diabetic non-pregnant C_2 for OGCT and OGTT (2HBG) types while for OGTT (FBG) type, significant differences occur only in GDM vs non-GDM pregnant $Control_1$, GDM vs non-diabetic non-pregnant $Control_2$ and $Control_1$vs non-diabetic non-pregnant $Control_2$. This study agrees with the report that revealed increased in the level of MDA during progression of normal pregnancy (Li, 2016; Arribas, 2016). This study is consistent also, with the findings of Adeniji, (2013) who revealed increase in serum MDA in GDM subjects with lower TAS activity this reveals the link between TAS/MDA in oxidative stress occurring in GDM Patients. For MDA-OGCT sample is may be recommended for GDM analysis. Similar findings like these, was revealed by Atiba, (2013) Mahmoud, (2014) Arribas, (2016) showing that, serum GPx level in women with GDM increased during the gestational period. However, on the contrary other reports revealed GPx be significantly lower ($p \leq 0.05$) in GDM than non- diabetic pregnant Control subjects (Zhang, 2022; Khambule, 2019; Özmen et al, 2022). Similarly, a study by Zejnullahu, (2021) revealed significantly low levels of GPx in healthy pregnant subjects as compared to the non-pregnant control subjects. More also, Javid (2020) GPx significant decrease in Diabetic patients more than the apparently healthy control subjects. This

could be due to ongoing oxidative stress. Hence the need for continuous monitoring of blood glucose as well as antioxidant supplementation. GPx is significantly raised in GDM and non-GDM pregnant Control$_1$. This increase in GDM patients could be due to ongoing oxidative stress which is likely to be more in the pregnant subjects with GDM than normal non-GDM pregnant subjects.

Conclusion

Fasting (FBG) and postprandial (2HBG) states appear to influence antioxidant levels and malondialdehyde (MDA) in subjects with gestational diabetes mellitus (GDM), suggesting that metabolic activity promotes the generation of oxidants while simultaneously depleting antioxidant defenses. This insight may support healthcare providers in improving the clinical management and therapeutic strategies for patients with GDM.

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