ORIGINAL ARTICLE

CONTENTS

The role of maternal ABO blood group and malondialdehyde as diagnostic marker in the development of gestational diabetes mellitus

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ABSTRACT

Aim: The aim of research is to assess whether various blood groups can be associated with the occurrence of gestational diabetes mellitus and if malondialdehyde can be used for the diagnosis of GDM.

Materials and Methods: A case control study started from September 2022 to June 2023 enrolled 200 pregnant women aged between 15-45 years, cases included 100 patients selectively collected with a confirmed diagnosis of gestational diabetes mellitus, and 100 were healthy normal in Kerbala obstetrics and gynecology hospital, both patient and controls group categorized according to blood group type.

Results: Pregnant women with gestational diabetes mellitus are mostly of blood group AB in comparison with pregnant women without Gestational diabetes mellitus, and both A and B were significantly higher among control pregnant women, additionally, two groups' blood group O levels were almost identical. Malondialdehyde mean was significantly higher in pregnant women with Gestational diabetes mellitus with p-values 0.001. Statistically significant differences could not be detected in the mean levels of MDA across the various blood groups (p= 0.505).

Conclusions: This research reveals that mother's ABO blood group has a role in the development of GDM and the blood group AB is more likely to develop GDM, blood group A & B are less likely to develop GDM and may be regarded as a protective factor. MDA can be used for the diagnosis of GDM but further studies are required to support this finding.

KEY WORDS: Gestational diabetes mellitus, pregnancy condition, spontaneous hyperglycemia

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INTRODUCTION

Gestational diabetes mellitus (GDM) is a common pregnancy condition characterized by the occurrence of spontaneous hyperglycemia throughout pregnancy [1]. Throughout a normal pregnancy, the pregnant body experiences a sequence of physiological alterations to meet the needs for the developing child. An essential adapting metabolic pathway entails insulin sensitivity [2]. As pregnancy advances, several substances that stimulate insulin resistance such as estrogen, progesterone, leptin, cortisol, placental lactogen, and placental growth hormone are included [3]. Although it can happen in many settings, β-cell malfunction and persistent insulin resistance describe the vast majority of GDM cases (~80%). Insulin-resistance is already present throughout pregnancy, and it becomes much worse at this time. Consequently, women who are impacted tend to have a higher level of insulin-resistance compared to healthy pregnant women. As a result, they have a bigger decrease in glucose utilization and an increase in glucose synthesis and concentrations of free fatty acids (FFA). β-cells are believed to degrade as a result of more insulin being produced because of higher energy expenditure and insulin resistance, which gradually depletes the cells [4, 5]. The ABO blood type system is considered the most significant in humans. Typically, the blood type of a person does not undergo any changes from the moment of embryo creation. Multiple studies have shown a correlation between the ABO blood type and many health conditions such as infection, cancer, cardiovascular illness, and nervous system disorders [6]. Prior research has also investigated the correlation between ABO blood group and difficulties during gestation [7, 8]. The association between ABO blood type and negative pregnancy outcomes such as preeclampsia, venous thromboembolism, postpartum hemorrhage, and gestational diabetes mellitus is a subject of debate. Insulin-resistance and the onset of type-II

DM are heavily correlated with the ABO antigens, which in turn affect a wide variety of biomarkers. Included in this set of biomarkers are interleukin-6, E-selectin, P-selectin, tumor necrosis factor-α, and soluble intercellular adhesion molecule-1 [9]. The majority of researches examining the correlation between ABO blood type and gestational diabetes mellitus provide inconclusive results, while there have been reports indicating that those with AB blood type may have a lower chance of developing gestational diabetes mellitus [10]. Several researches have shown that individuals with AB blood type have a higher susceptibility to gestational diabetes mellitus [11]. Currently, the available data about the relation between ABO-blood type and GDM is insufficient and contradictory. Oxidative stress refers to the ongoing condition of oxidative damage in a cell, tissue, or organ, resulting from reactive oxygen species (ROS). The majority of reactive oxygen species originates from endogenous sources, namely as by-products of normal and essential activities, such as the production of energy by mitochondria. The level of oxidative stress was shown to be higher in pregnant affected with GDM compared to those with a normal pregnancy [12]. Oxidative stress has been linked to the development of several disorders, including GDM [13]. It delineates a disparity between cellular pro- and anti-oxidants. Damage to cells may occur when oxidative stress upsets the delicate balance of DNA, lipids, and proteins. Both free radical and nonradical forms of oxygen are referred to as reactive oxygen species. These forms include the superoxide anion (O_2–), hydroxyl radical (•OH), and hydrogen peroxide (H_2O_2) [14]. An excessive amount of glucose in the body is linked to oxidative stress. Pregnant with GDM were shown to create an excessive amount of free radicals and have poor systems for removing these harmful molecules [15]. Reactive oxygen species hinder the process of insulin-stimulated glucose absorption by disrupting the functioning of both GLUT4 and IRS-1 also, slows down muscle and liver glycogen production. When reactive oxygen species break down phospholipids in pathological conditions like diabetes mellitus, malondialdehyde (MDA) is the byproduct. MDA is the primary and widely researched molecule resulting from lipid peroxidation, recognized for its mutagenic and hazardous properties, moreover, MDA may be generated enzymatically as an incidental byproduct during the production of thromboxane A2 [16]. Lipid peroxidation produces malondialdehyde (MDA), which has been used as a biomarker to measure oxidative stress in various biological samples (e.g., blood, urine, and exhaled breath condensate, or EBC) in people with various diseases (e.g., cancer, heart disease, lung disease, neurodegenerative disease, etc.) Moreover, the identification of these final substances in inflammatory illnesses indicates that lipid peroxidation has a substantial impact on this particular form of illness [17].

AIM

The aim of our research is to assess whether various blood groups can be associated with the occurrence of gestational diabetes mellitus and if malondialdehyde can be used for the diagnosis of GDM.

MATERIALS AND METHODS

The present study is a case control study started from September 2022 to June 2023 enrolled 200 pregnant women aged between 15-45 years, cases included 100 patients selectively collected with a confirmed diagnosis of gestational diabetes mellitus, and 100 were healthy normal in Kerbala obstetrics and gynecology hospital. The patients were categorized into four categories according to the blood group type, blood group O (51) patient, AB (25) patient, A (13) patient and Blood group B (11) patient. The control group included 100 healthy pregnant women also collected from the obstetrics and gynecology hospital in Kerbala governorate. The control group were divided into four groups depending on the blood group type. Blood group O (41) women, AB (8) women, A (25) women and Blood group B (26) women. The study was conducted under the endorsement of the scientific and ethical committees in the Faculty of Medicine, University of Kufa, Iraq.

INCLUDED PATIENTS

All pregnant women at the second and the third trimester who have a confirmed diagnosis of gestational diabetes mellitus.

EXCLUDED PATIENTS

Pregnant women who have diabetes (both type I and II), pregnant women in the first trimester with diabetes, pregnant women in a baby with congenital anomalies, obese pregnant women, pregnant women who smoke.

COLLECTED SAMPLES

After skin sterilization five milliliters of blood were aspirated from anti- cubital vein split into two halves, the first two milliliters of blood used for the detection of the blood group type, allowing the reminder blood three milliliters to clot for 10-20 min., at room temperature, in gel tubes to obtain serums by centrifuging at (2000-

Table 1. Body mass index and age difference in patients and controls

Variable	Studied group				P -values
	Pregnant with GDM		Control pregnant		
	Mean ±SD	Ranges	Mean ±SD	Ranges	
Age (years)	33 ±7.4	18-45	32.6 ±7.7	18-45	0.655
BMI (kg/m^2)	23.5± 2	19.5-28.2	23.8 ±2.1	19.5-28.2	0.397
Student T test, significant ≤0.05					

Table 2. Blood groups distribution in patients and controls

Variable		Studied group		P value
		Pregnant with GDM	Control pregnant	
Blood group	A	13(33.3%)	26(66.7%)	<0.00*
	AB	25(75.8%)	8(24.2%)	
	B	11(31.4%)	24(68.6%)	
	O	51(54.8%)	42(45.2%)	
Total		100	100	200
*Chi-Square test, significant ≤0.05				

Table 3. Level of malondialdehyde in patients and controls.

Variable	Studied group				P value
	Pregnant with GDM		Control pregnant		
	Mean ± SD	Range	Mean ± SD	Range	
MDA	186.6 ± 103.4	78.3-716.6	73.4 ± 21.7	30.6-112	<0.001*
*Student T test, significant ≤0.05					

3000) r.p.m., for 20 minutes, after that the obtained serum stored at -15 C to perform the biochemical tests for malondialdehyde (MDA).

BODY MASS INDEX CALCULATION

Women's weight before pregnancy and height are used to calculate their BMI. The BMI formula is (body weight divided by the body height in square meters) and expressed in (kg/m^2) [18].

$BMI = weight/ (height)^2$

DETERMINATION OF BLOOD GROUP TYPE BY ANTI A, ANTI B, ANTI AB MONOCLONAL REAGENTS

The hemagglutination principle is utilized by the manual technique, which can be done on a plate or in a tube. When exposed to the antibody-containing reagent, test red blood cells that carry the antigen agglutinate.

MEASUREMENT OF MALONDIALDEHYDE (MDA) BY ENZYMES LINKED IMMUNOSORBENTS ASSAY (ELISA-KITS)

We used the Competitive-ELISA approach with ELISA-kit. These kits come with micro-plates that have already coated with MDA, throughout the procedure, the detection biotinylated Ab, which is specific to MDA. By spectrophotometric measurement of the changes in color is taken at a wave-length of 450±2 nm. After that, we compare the samples' optical densities (ODs) to the standard curve to find the MDA concentrations in the samples.

STATISTICAL ANALYSIS

Data is analyzed using SPSS version 26, which is a statistical tool for the social sciences. Presenting descriptive statistics in the form of frequency tables, the representation of continuous variables includes portraying them as the mean value offset by the standard deviation. As an alternative, categorical variables were described by numbers and percentages. We utilized the chi-square test to identify associations between two categories of data, the Student T used to identify associations between categories of data and continuous variables. Statistical analysis by Receiver operative characteristic curve (ROC) used to evaluate the performance of MDA in detecting GDM. Statistical significance was determined by a P-value of 0.05 or less.

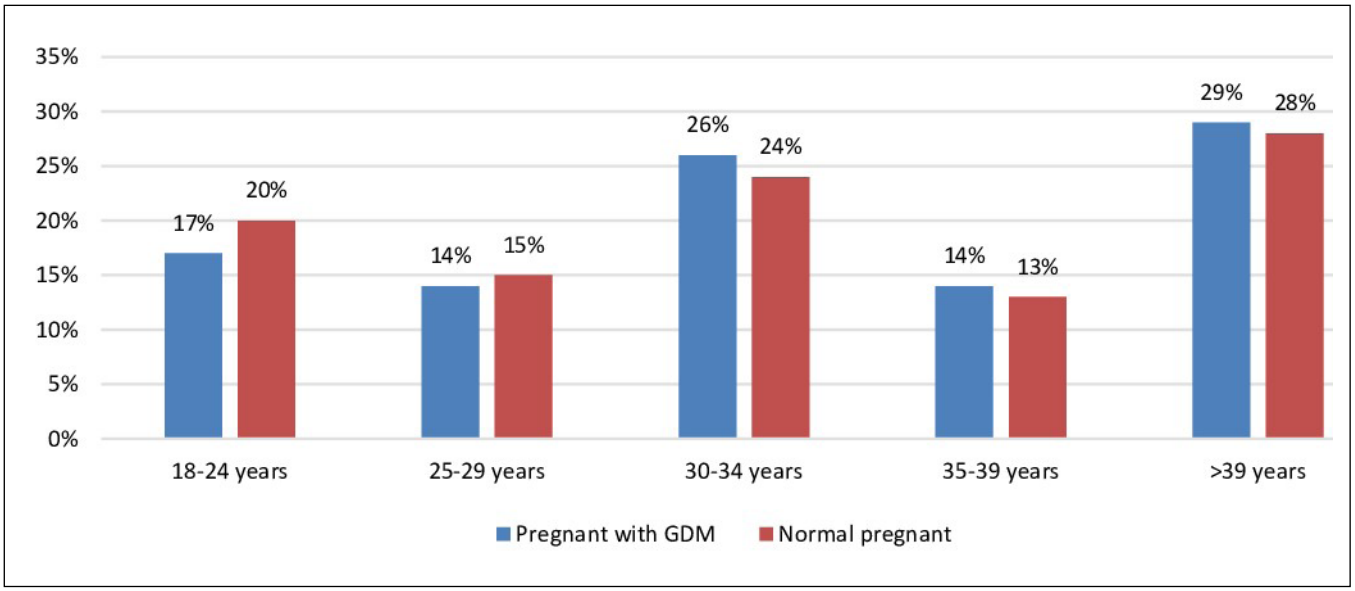

Fig. 1. Age distribution between two studied groups.

RESULTS

THE DIFFERENCE IN AGE AND BODY MASS INDEX IN PATIENTS AND CONTROLS

When comparing the two groups, we found that they were very comparable in age and BMI (p higher than 0.05) (Table 1, Fig.1).

BLOOD GROUPS DISTRIBUTION IN PATIENTS AND CONTROLS

Pregnant women with gestational diabetes mellitus (GDM) are mostly of blood group AB in comparison with pregnant women without GDM, and both A and B were significantly higher among control pregnant women (Table 2). Additionally, the two groups' blood group O levels were almost identical.

LEVEL OF MALONDIALDEHYDE IN THE TWO GROUPS

The studied MDA marker mean was significantly higher in pregnant women with GDM with p-values 0.001 (Table 3, Table 4, Fig.2).

EVALUATION OF MALONDIALDEHYDE'S DIAGNOSTIC UTILITY USING ROC CURVE

The ROC curve for MDA was shown in fig. 3, obtained from the values of OGTT and MDA tests with an area under the curve 0.972 and p value <0.001., with cut of value of 78.7 had sensitivity of 99% and specificity of 41%.

DISCUSSION

During pregnancy if appropriate steps not performed to control the early stage of GDM, the health of mothers and infants would suffer greatly. The most significant human blood grouping system is ABO. Numerous studies have shown that the blood group type is linked to a wide range of illnesses. The relationship between GDM and ABO-blood group is debatable at the moment [19].

BODY MASS INDEX AND AGE DIFFERENCE BETWEEN PATIENTS AND CONTROLS

This study reveals there is no valuable (p>0.05) differences in the mean age and BMI in the two groups. Since obesity is a cause for gestational diabetes mellitus, we intentionally excluded obese pregnant women from the research to focus on the ABO blood type impact, thus this suggests that the two groups were comparable in age and body mass index. Comparable findings were observed in a French cohort, where pregnant women with and without GDM were found to be of similar ages [20]. But according to case-control research carried out in Thailand, age was a crucial factor linked to the development of GDM, with those over 30 having a considerably higher risk of the disease than those under 30 [21]. Likewise, a Turkish cohort study also showed that pregnant women with and without GDM differed significantly in age [22]. Therefore, it is still unclear whether age is a predictor of GDM, and further research that accounts for confounding variables is needed to confirm this link. Known risk factors for gestational diabetes mellitus include advanced maternal age,

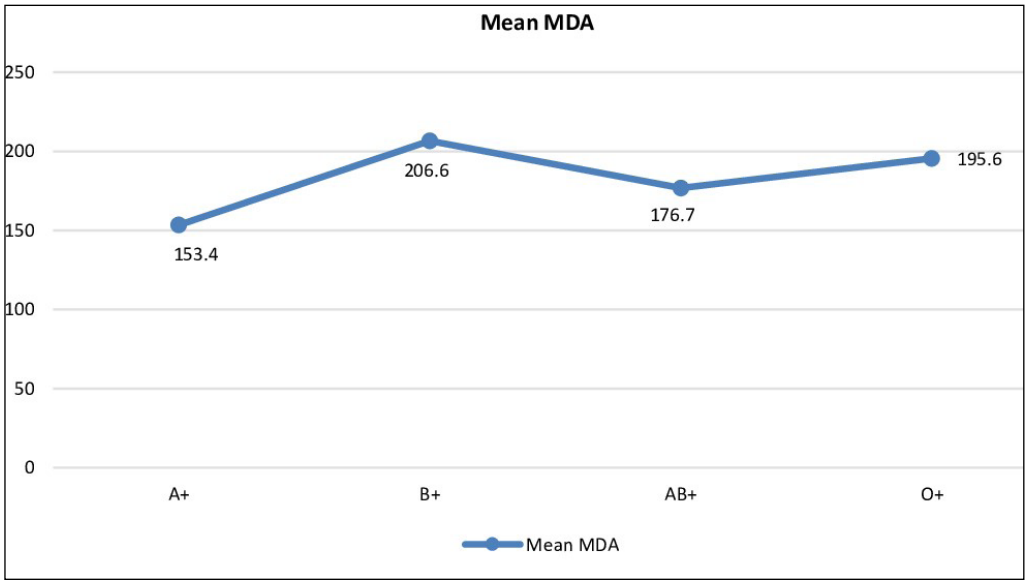

Fig. 2. Mean level of MDA among different blood groups category.

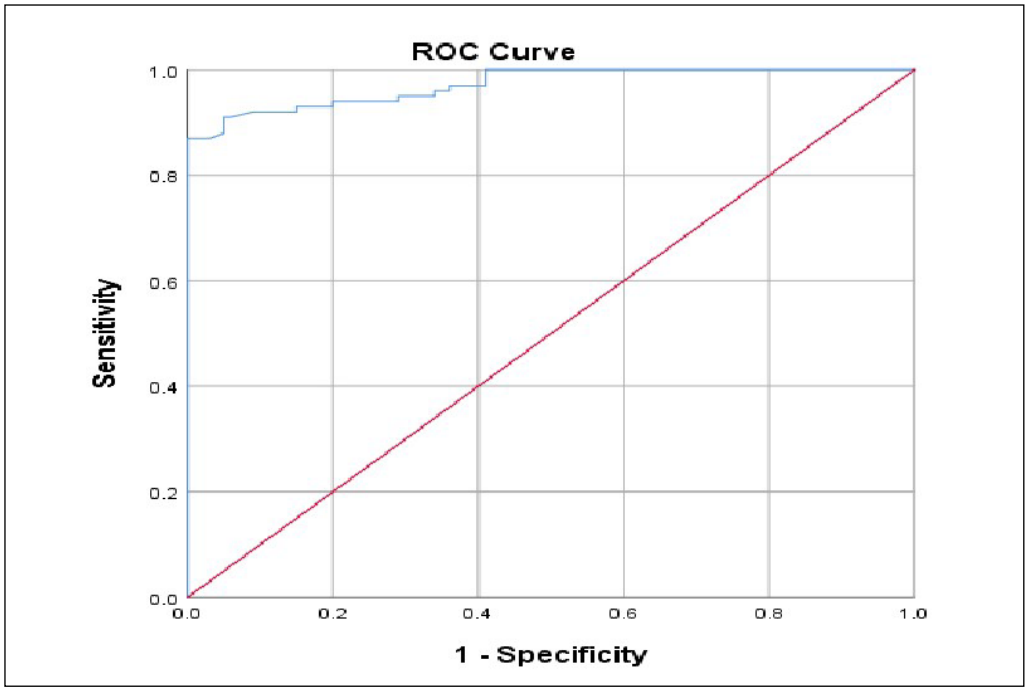

Fig. 3. ROC curve for MDA.

high BMI, excessive (GWG), ethnic race, polycystic ovarian syndrome (PCOS), low stature, high parity, previous large-for-gestational (LGA) deliveries, and a family history of diabetes (DM) [23]. Mother's age is a well-considered risk factor for GDM, since prior research has consistently indicated that older pregnant women have a higher likelihood of developing GDM [24]. However, there is disagreement over what constitutes a reasonable cut-off point. Americans Diabetes Association (ADA) state that age greater than 25 years considered as a risk factor, according to retrospective research of Japanese women (138,530), the incidence of GDM increase by age, in women over forty exhibiting a relative risk of 15.1% [25].

DISTRIBUTION OF BLOOD GROUPS IN PATIENTS AND CONTROLS

The pregnant with gestational diabetes had a significantly greater percentage of blood groups AB (p value ≤ 0.05) than the pregnant women in the healthy control group. These findings suggested that the ABO blood type could be linked to some unfavorable pregnancy outcomes. Patients with AB-blood group were found to have higher risk of gestational diabetes by Karagoz [26]. Another study by Shimodaira suggested that AB-blood group elevate the risk for gestational DM [11]. Additionally, blood group O was found to be roughly the same in both groups, despite the fact that the percentage of

blood types A and B was much greater in the control pregnant women. These findings may indicate that people with blood groups A and B are less likely to acquire GDM. Like our study [26] investigation revealed that participants who have blood group B had a much-decreased chance of acquiring DM; as a result, blood group B may be thought of as a protective factor for the incidence of DM. It should be noted that the blood groups were normally distributed in Karbala Blood Bank for 2023 as follows (O^+ 26.75 percent, O^- 5.11 percent, AB^+ 8.33 percent, AB^- 1.18 percent, A^+ 26.81 percent, A^- 3.62 percent, B^+ 24.83 percent, B^- 3.37 percent).

THE LEVEL OF MALONDIALDEHYDE IN PATIENTS AND CONTROLS

In line with a study by Chen X. and Scholl T. [6], which found that gestational diabetes subjects had significantly higher MDA levels than those of uncomplicated pregnancies. We found in our study that the mean MDA was much more in GDM pregnant women than in healthy pregnant women. Higher levels of malondialdehyde show that GDM is linked to more oxidative stress than healthy pregnancy, in a study [27] the elevated MDA levels in the GDM participants point to the possibility of oxidative stress [27]. MDA levels, a marker of oxidative stress, have increased in the participants' investigations by Pandey M et al. [28]. According to a study of Adeniji A and Oparinde D [29] patients with GDM had significantly higher MDA levels than those with uncomplicated pregnancies. According to the current study, there is more oxidative stress in GDM, which may increase the production of free radicals. Non-enzymatic protein glycation is one of the suggested mechanisms for oxygen free radical creation at higher glucose concentrations during pregnancy, and it may cause the production of oxygen free radicals. Enhanced oxidative stress and mitochondrial electron transport chain flow and oxidative activities of the fetus. A major byproduct of lipid peroxidation is hydroperoxides which have been demonstrated to modify prostaglandin production, which may be the cause of the emergence of embryopathy associated with diabetes [30]. Another study of Grissa O et al. [31] provides more evidence that the reactive oxygen species produced during GDM combine with free lipid to produce lipid peroxidation products, which in turn induce harmful stress to cells by producing MDA. Similarly, lipid peroxidation and oxidative stress have been shown to significantly increase in some GDM studies [32, 33].

DIAGNOSTIC ACCURACY OF MALONDIALDEHYDE BY RECEIVER OPERATING CHARACTERISTIC CURVE

An earlier work of Lappas M et al. [13] emphasized the crucial role oxidative stress plays in the development of GDM. An imbalance between the antioxidant and oxidizing systems is implicated in the development of gestational DM and its accompanying effects this is according to mounting evidence. It has been noted that pregnant GDM patients have decreased antioxidative enzymes and an increase in circulating oxidative stress brought on by hyperglycemia. Both the mother and the fetus may suffer negative consequences from increased oxidative stress [34]. Under pathological circumstances, such as diabetes mellitus, reactive oxygen species induced phospholipid breakdown produces malondialdehyde, the byproduct of lipid peroxidation. It may be readily quantified in plasma and is frequently used to show oxidative stress and lipid peroxidation [35]. The ROC curve is a graph that may be used to analytically evaluate how well a binary diagnostic classification system is doing. One further way to evaluate a test's diagnostic efficacy is to compare its findings to those of other tests using the ROC curve. Understanding the concept of sensitivity and specificity, which are used to assess a diagnostic test's performance, is a prerequisite to comprehend the ROC curve. The percent of people who test +ve for a target disease that genuinely have it is known as sensitivity, while the percent of people who test -ve for the disease is known as specificity [36]. ROC-curve that has the greatest AUC was thought to perform the best diagnostics-performance when comparing the results of two or more diagnostic tests [37]. Figure 3 displays the ROC curve analysis for MDA in our study, with a p value of less than 0.001 and an AUC of 0.972, with threshold level of 78.7, exhibited 99% sensitivity, with 41% specificity. Since there is no information available regarding the MDA ROC curve for GDM diagnosis, our work may be considered one of the first in this area. The big AUC value suggests a high accuracy of the MDA test, meaning it can be used to predict GDM in women in the future.

CONCLUSIONS

This research reveals that mother's ABO blood group have a role in the development of GDM Blood group AB is more likely to develop GDM while blood group A & B are less likely to develop GDM and may be regarded as a protective factor. The other finding is that MDA can be used for the diagnosis of GDM but further studies are required to support this finding.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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