Gene Polymorphism of Superoxide Dismutase in Gestational Diabetes Mellitus in Pregnant Women

Mai M. Madkour[1*], Abd El-Aziz F. Abd El-Aziz[1], Fardous F. El-Senduny[1], Afaf M. Elsaid[2] and Abd El-Aziz A. El-Refaey[3]

[1]Department of Chemistry, Mansoura University, Mansoura, Egypt

[2]Department of Pediatrics, Mansoura University, Mansoura, Egypt

[3]Department of Obstetrics and Gynecology, Mansoura University, Mansoura, Egypt

[*]**Corresponding author:** Mai M. Madkour, Department of Chemistry, Mansoura University, Mansoura, Egypt, Tel: 01066907311; E-mail: maimadkour211@mans.edu.eg

Abstract

Background: Gestational diabetes mellitus is the most common metabolic disorder that develops during pregnancy. GDM can cause major health problems for the mother both during pregnancy and after the baby is born. In addition, the metabolism of the mother's kids is abnormal. The study aimed to investigate superoxide dismutase 1 genetic variants in pregnant women diagnosed with gestational diabetes using amplification refractory mutation system analysis.

Results: Our study demonstrated that the frequencies of genotypes in women with GDM were SOD1-AG (90.0%). The study revealed there is a significant difference in the frequency of the genotype of SOD1 between GDM and healthy pregnant women.

Conclusions: To the best of our knowledge, this is the first study to indicate that the genotype of SOD1 (A/G rs2070424) has a significant impact on the development of gestational diabetes mellitus in Egyptian women.

Keywords: Gestational diabetes; Superoxide dismutase 1 gene; Genetic polymorphism; Oxidative stress

Abbreviations: GDM: Gestational Diabetes; SOD1: Superoxide Dismutase 1; ROS: Reactive Oxygen Species; SNPs: Single-Nucleotide Polymorphisms; T-ARMS-PCR: Tetra-Primer Amplification Refractory Mutation System

Introduction

Gestational Diabetes Mellitus (GDM) is defined as any degree of glucose intolerance that develops or is detected during pregnancy. In the second or third trimester, pregnant women are diagnosed [1]. This condition is associated with adverse pregnancy outcomes, including fetal macrosomia, stillbirth, neonatal metabolic disturbances, and related problems. GDM is not caused by a lack of insulin but by other hormones produced during pregnancy that can make insulin less effective, a condition referred to as insulin resistance [2].

Oxidative stress is a general term used to describe the steady state of oxidative damage in a cell, tissue, or organ caused by Reactive Oxygen Species (ROS). The majority of ROS is produced endogenously as a byproduct of normal and necessary reactions, such as energy generation from mitochondria. This oxidative stress was found to be greater in women with GDM than in normal pregnant women [3]. A complex and integrated antioxidant system plays a crucial role in protecting cells or tissues from damage as a result of ROS. The expression and activity of antioxidants are changed during oxidative stress [4].

Human Cu–Zn Superoxide Dismutase (SOD1) gene is located on chromosome 21 (21q22.1), a homo-dimeric metalloprotein consisting of 153 amino acids with the two subunits linked non-covalently [5]. The SOD1 polymorphism (rs2070424) is distinguished by the substitution of Adenine (A) to Guanine (G) at codon 251 in intron 3 of the gene. This polymorphism has been linked to a reduction in antioxidant capacity [6]. Actually, the SOD1 gene encodes an enzyme that catalyzes the dismutation of superoxide into O_2 and H_2O_2 to eliminate free superoxide radicals produced in the body. The delicate balance between the elimination of ROS and limiting oxidative stress is disrupted by genotype related differences in enzyme activity.

The study aimed to evaluate the Single Nucleotide Polymorphisms (SNPs) in SOD1 (A/G rs2070424) and correlate its genotyping to gestational diabetes mellitus. To the best of our knowledge, this is the irst study to report the association of their genotypes with gestational diabetes in Egyptian women.

Materials and Methods

This case-control study was conducted with 70 women with gestational diabetes and 70 healthy pregnant women. They were recruited from the obstetrics and gynecology department,

faculty of medicine, Mansoura university in the period from July 2017 to March 2019. All of the women in the study were at least 18 years old and had been diagnosed at a gestational age of more than 24 weeks.

Pregnant women with type 1 diabetes, previous macrosomia (a baby weighing more than 4000 g at birth), polycystic ovary syndrome, and other serious medical conditions (hypertension, renal disease, moderate to severe anaemia, thyroid disorder) that interfered with maternal and perinatal outcomes were excluded.

Sample collection and DNA extraction

Each patient's fasting blood was drawn and dispensed into an EDTA containing tube. A blood sample was divided into two portions (3 mL and 2 mL). After centrifugation at >2000 g for 10 minutes, the first portion was used to collect plasma. The plasma was extracted and used for biochemical analysis as well as a native-PAGE investigation of SOD activity. The second portion was used to extract DNA. The DNA quantity and quality were measured by reading the absorbance at λ 230 nm and λ 260 nm.

Amplification refractory mutation system (ARMS)-PCR analysis Tri-ARMS-PCR analysis of superoxide dismutase 1 gene (SOD1)

Primers used in this study (F1, F2 and R) are provided in Table 1. In the SOD1 gene (A/G rs2070424).

200 ng of genomic DNA, 3 μL of an allele primers (F1 and RP) or G allele primers (F2 and RP) (Table 1) and 8 μL 2 × Taq Master Mix (EmeraldAmp GT PCR Master Mix, Cat. No.RR310A) were used in an 18 μL polymerase chain reaction (PCR). The PCR protocol was as follows: 95°C for 3 min, then 35 cycles at 95°C for 30 sec, annealing at 60°C for 30 sec, extension at 72°C for 30 sec, and a final extension at 72°C for 10 min. The agarose gel electrophoresis (2%) was performed at 125 V and a 100 bp MaestroGen DNA ladder (Cat. No. 02001_500) was used to investigate and confirm the quality of the PCR products (Cleaver Scientific Ltd., UK).

Table 1: Primers used in Tri-ARMS-PCR of SOD1 gene (A/G rs2070424).

Primer	Sequence (5′-3′)	Fragment size
F1	TAGCTTTGTTAGCTATGCCA	A allele: 480 (F1+R)
F2	TAGCTTTGT TAGCTATGCCG	G allele: 480 (F2+R)
R	ATCTTTAGAA ACCGCGACTA	
F1=Forward primer 1, F2=Forward primer 2, R=Reverse primer		

Biochemical measurements

Antioxidant levels were estimated by determining catalase, GR activity and plasma levels of MDA using kit supplied by biodiagnostic (Cairo, Egypt). Plasma levels of catalase, superoxide dismutase and glutathione reductase activity can be determined according to the methods of Fossati, and Ponti, et al. and goldberg and spooner respectively [7-10]. Satoh and Tsikas techniques can be used to detect plasma malondialdehyde and nitric oxide levels respectively [11].

SOD activity gel method

The SOD activity gel test was developed by a method that is based on the suppression of NBT reduction by SOD. This test is based on the ability of $O_2\bullet-$to interact with NBT and convert the tetrazolium (yellow colour) in the gel to a blue aggregate. SOD active areas generate a clear region (achromatic bands) that competes with NBT for $O_2\bullet-$ [12-14].

Statistical analysis

Statistical analysis was done using the software package, SPSS version 22 and excel. data were expressed as mean ± SD. The significance of the difference between women with gestational diabetes and healthy pregnant women was determined using ANOVA. Take into account that P–values of less than 0.05 were statistically significant. Frequencies of either genotype or allele of SOD1 polymorphism between two groups were analyzed by the Fisher exact test and hardy Weinberg equilibrium.

Results

Plasma catalase, superoxide dismutase and glutathione reductase levels were significantly decreased in women with gestational diabetes when compared with the control group. On the other hand, the levels of malondialdehyde and nitric oxide were significantly higher than normal pregnant women (Table 2).
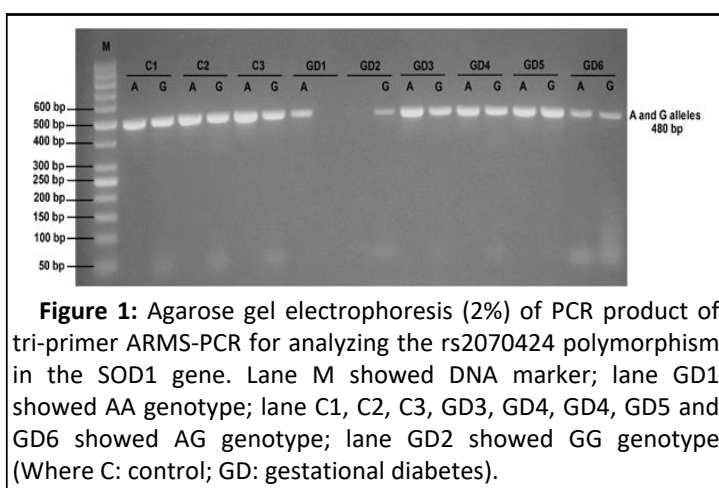
Table 2: Comparison of antioxidant enzymes and oxidative stress markers for gestational diabetes mellitus in pregnant women with healthy pregnant women.

Parameters	GDM group (n =70)	Control group (n =70)	OR (95% CI)	P value
CAT (U/L) mean ± SD	337.39 ± 133.06	875.54 ± 100.6	0.65 (undefined)	p<0.001[*]
SOD (U/mL) mean ± SD	1.43 ± 0.38	2.32 ± 0.14	undefined	p<0.001[*]
GR (U/L) median (IQR)	6.43 (4.02-10.45)	30.54 (22.51-40.21)	0.54 (0.44-0.65)	p<0.001[*]
MDA (nmol/mL) mean ± SD	13.35 ± 4.30	5.36 ± 2.83	2.15(1.77-2.59)	p<0.001[*]
NO (mg/L) median (IQR)	4.81 (3.53-6.79)	2.04 (1.77-2.78)	undefined	P<0.001[*]

Genetic polymorphism and genotype frequencies

Analysis of *SOD1* gene: The genetic polymorphism in the *SOD1* gene (A251G) was investigated, and the genotypes were shown in Figure 1. The frequencies of the allele and genotype of the *SOD1* gene (rs2070424) between women with gestational diabetes and healthy pregnant women were listed in Tables 3 and 4. Genotype frequencies of the rs2070424 A/G polymorphism differed significantly between women with gestational diabetes and control (p=0.025), while allele frequencies did not show any significant difference between women with gestational diabetes and control. Moreover, the distribution of genotypes of *SOD1* for GDM and control groups was in alignment with the Hardy-Weinberg equilibrium (χ^2=44.84, p<0.001; χ^2=70, p<0.001), which was analyzed by Fisher's exact test (Table 5).

Figure 1: Agarose gel electrophoresis (2%) of PCR product of tri-primer ARMS-PCR for analyzing the rs2070424 polymorphism in the SOD1 gene. Lane M showed DNA marker; lane GD1 showed AA genotype; lane C1, C2, C3, GD3, GD4, GD4, GD5 and GD6 showed AG genotype; lane GD2 showed GG genotype (Where C: control; GD: gestational diabetes).

Table 3: Allele frequencies of *SOD1* gene (rs2070424) in the current study.

Allele	GDM group	Control group	P value
	(n=70)	(n=70)	
A	71 (50.7%)	70 (50%)	0.905
G	69 (49.3%)	70 (50%)	

The data was expressed as frequency and percentage. GDM: Gestational Diabetes Mellitus.

Table 4: Genotype frequencies of *SOD1* gene (rs2070424) in the current study.

Genotype	GDM group (n=70)	Control group (n=70)	P value
A/A	4 (5.7%)	0	
A/G	63 (90.0%)	70 (100%)	0.025[*]
G/G	3 (4.3%)	0	

Table 5: Fisher exact test for Hardy-Weinberg equilibrium in the current study.

	GDM group (n=70)	Control group (n=70)
HWE	χ^2=44.84	χ^2= 70
P -Value	p<0.001[*]	p<0.001[*]
GDM: Gestational Diabetes Mellitus; χ^2: *chi-Square* Test; HWE: Hardy-Weignberg Equilibrium; [*]statistically significant if p<0.05.		

The major risk of GDM was evaluated by the co-dominant, dominant, recessive, and over dominant models as shown in Table 6. In the co-dominant model (A/A *vs.* A/G *vs.* G/G), there was a statistically significant difference between GDM and healthy pregnant women (p=0.025). The dominant model (A/A *vs.* A/G+G/G) showed a significant risk of GDM when compared with the A/G+G/G genotype (p=0.04). The recessive model (A/A +A/G *vs.* G/G) did not display any significant risk of GDM compared to the A/A+A/G genotype (p=0.08). Furthermore, the over dominant (A/A+G/G *vs.* A/G) showed a significant risk of GDM compared to the A/G genotype (p=0.006).

Table 6: Association between genotypes of *SOD1* and response status in the current study.

Model	Genotype	GDM group (n=70)	Control group (n=70)	OR (95% CI)	P value
Co-dominant	A/A	0	4 (5.7%)	undefined	0.025*
	A/G	70 (100%)	63 (90.0%)		
	G/G	0	3 (4.3%)		
Dominant	A/A	0	4 (5.7%)	undefined	0.04*
	A/G+G/G	70 (100%)	66 (94.3%)		
Recessive	A/A+A/G	70 (100%)	67 (95.7%)	undefined	0.08
	G/G	0	3 (4.3%)		
Over dominant	A/A+G/G	0	7 (10%)	undefined	0.006*
	A/G	70 (100%)	63 (90%)		
The data was presented as percentage and frequency. GDM: Gestational Diabetes Mellitus; OR: Odds Ratio; 95% CI: 95% Confidence Interval for the difference in means between the two groups; P is significant when <0.005.					

Correlation between antioxidant enzymes, oxidative stress markers and *SOD1* (rs2070424) SNP

The association between antioxidant enzymes, oxidative stress markers and the *SOD1* gene (rs2070424) SNP in the population under study was presented in Table 7. The study population (GDM and control groups) was divided into three groups according to genotypes in AA, AG, and GG. There wasn't any signficant difference in the biochemical parameters between the three groups, except that the level of MDA, and nitric oxide showed significant differences between AG and GG (p=0.039, p=0.014) respectively.

Table 7: Correlation between antioxidant enzymes, oxidative stress markers and *SOD1* (rs2070424) SNP in the current study.

Parameters	AA	AG	GG	Test of significance	Significance within groups
Catalase mean ± SD	409.63 ± 151.27	331.11 ± 134.65	372.91 ± 28.94	F=0.761 P=0.471	P_1=0.598 P_2=0.720 P_3=0.258
Superoxide dismutase mean ± SD	1.41 ± 0.61	1.44 ± 0.38	1.23 ± 0.06	F=0.391 P=0.678	P_1=0.383 P_2=0.567 P_3=0.879
Glutathione reductase Median (IQR)	10.05 (2.61-17.48)	6.43 (4.02-10.45)	4.02 (4.01-13.66)	KW P=0.754	P_1=0.951 P_2=0.721 P_3=0.449
Malondialdehyde Median (IQR)	11.26 (7.96-17.69)	12.08 (10.49-17.14)	9.66 (7.91-9.71)	KW P=0.109	P_1=0.039* P_2=0.289 P_3=0.578
Nitric oxide Median (IQR)	3.79 (3.41-4.40)	5.17 (3.75-7.28)	3.29 (2.82-3.52)	KW P=0.015*	P_1=0.014* P_2=0.108 P_3=0.109

F: one way ANOVA test; KW: Kruskal Wallis test; IQR: Interquartile Range; P_1: Difference between AG and GG; P_2: difference between AA and GG; P_3: difference between AA and AG; *statistically significant if p<0.05.

SOD native gel

The native PAGE gel assay facilitated the identification of SOD isoenzymes in normal pregnant women and women with gestational diabetes. Figure 2 showed superoxide dismutase isoenzymes (Mn-SOD and CuZn-SOD) activities in native gel. The stain intensity of the bands in the control lane was higher, whereas the stain intensity of the bands in the gestational diabetes group lanes was lower. Therefore, the activity of SOD isoenzymes was decreased in women with gestational diabetes compared to normal pregnant women.
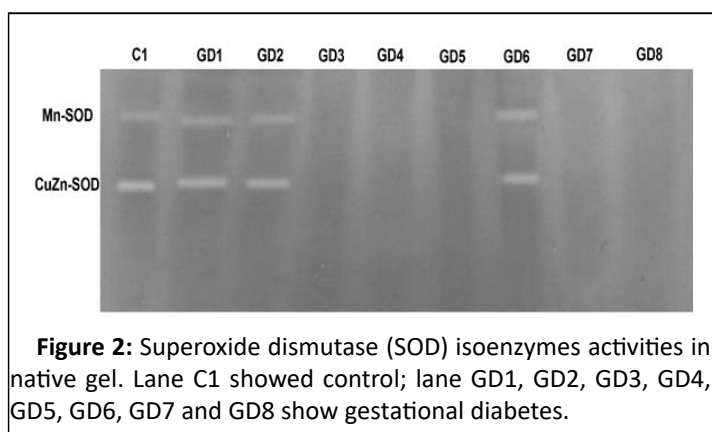

Figure 2: Superoxide dismutase (SOD) isoenzymes activities in native gel. Lane C1 showed control; lane GD1, GD2, GD3, GD4, GD5, GD6, GD7 and GD8 show gestational diabetes.

Discussion

Gestational diabetes mellitus is the most common illness among pregnant women all over the world. Insulin resistance distinguishes it as a complicated metabolic condition [15]. The study aimed to find whether there was a link between superoxide dismutase 1 A251G SNP (rs2070424) with the incidence of gestational diabetes in Egyptian women.

The antioxidant defense system includes the enzymes CAT, SOD, and GR. They are in charge of regulating the level of free radicals in cells [16]. In this study, MDA and nitric oxide levels increased, whereas CAT, SOD, and GR activity decreased in women with GDM compared to normal pregnant women.

It was discovered that there was a substantial rise in MDA levels in other studies [17]. MDA levels rise and anti-oxidant enzyme levels fall as pregnancy progresses [18]. The GDM group had higher MDA concentrations throughout the pregnancy, with significant differences in the first and second trimesters [19]. When comparing women with GDM to controls, a significant increase in lipid peroxidation was found [20].

Previous studies have reported conflicting results related to NO levels in pregnancy. It was found that NO levels dropped during pregnancy, whereas NO levels remained constant throughout the pregnancy in other studies [21-23].

It was discovered that SOD activity was found to be significantly lower in pregnant females with gestational diabetes as compared to normal pregnant females [24]. Furthermore, superoxide dismutase activity is lower in pregnant women than in non-pregnant women in the third trimester of normal pregnancy [25,26]. The reduction in SOD levels was demonstrated by another study [27].

Another research revealed that CAT activity did not alter throughout pregnancy, despite the fact that CAT activity is greater during pregnancy [28,29]. Reduced CAT activity might be attributed to lower lipid peroxidation rates in the third trimester of pregnancy. In agreement with another study, blood catalase activity in gestational diabetes is decreased [30]. The decrease in CAT as an inducible enzyme may be due to the lower level of H_2O_2 generated by SOD.

In addition, it was discovered that the GDM group's plasma GSH concentration was lower than that of healthy pregnant women [31]. This is in line with the results of other research. A gradual decrease in the activity of glutathione reductase

throughout the three trimesters of pregnancy was observed [32].

Superoxide dismutase1 is an intracellular enzyme that primarily protects cells against cytosolic generated superoxide. The expression of cytoplasmic *SOD1* is stable and its activity is often considered as an internal control for SOD gene expression. *SOD1* A251G polymorphisms cause changes in *SOD1* enzyme levels or activities, which can lead to decreased protection against oxidative stress [33]. This study aimed to demonstrate that the genotype frequencies of the *SOD1* gene (A/G rs2070424) differed significantly between women with gestational diabetes and normal pregnant women.

In the Egyptian population, the CC genotype of *SOD1* (rs2234694) SNP was more prone to T2DM [34]. It was revealed that 64.10% of the population has the wild type (AA) *SOD1* gene in the south-south pregnant Nigerian population. 10.26% of the women had the homozygous genotype and 25.64% of the population had the heterozygous genotype. In addition, wild-type *SOD1* genes were most prevalent in the population of women studied [35]. Data regarding the *SOD1* gene polymorphism is available for some populations, including North Indians, Bangladeshis, the Finns, the Romanians, the New Zealanders, and the Czechs, but it is lacking among African populations. There is little information about the frequency or implications of *SOD1* [36].

SOD1 allelic variations (rs17880135) have been linked to the occurrence of diabetic nephropathy [37]. Another study found that significant differences between the allele and genotype frequencies for the *SOD1*+35A/C polymorphism were observed in type 1 diabetes mellitus as compared to controls [38]. In Mexican women, the number of mutant carriers (GA+GG) of SOD (rs2070424) was substantially greater in the obese population than in the normal weight group [39].

However, contradictory results indicated that *SOD1* (rs2234694) SNP was not related to cardiovascular disease in T2DM patients from North Finland. Similarly, it was found that *SOD1*+35A/C gene polymorphism may not be associated with the susceptibility to T2DM among the South Indian population [40]. Another study has also reported that the (CC) genotype and C allele were completely absent among the North Indian population and that there was no association between diabetes *and SOD1*+35A/C gene polymorphism [36].

The current study found the *SOD1* gene was associated with blood glucose, MDA, and nitric oxide. The absence of *SOD1* is associated with the development of impaired glucose tolerance that results from impaired cell dysfunction and reduced cell volume. ROS reacts with polyunsaturated fatty acids to induce the release of toxic and reactive aldehyde metabolites such as MDA, one of the end products of lipid peroxidation. The mutant SOD1 may have the capacity to catalyze the production of ROS such as peroxynitrite and also contribute to increased cellular expression of iNOS synthase.

Conclusion

It was concluded that *SOD1-AG* (90.0%) genotype were shown to be the most common in women with GDM. To the best of our knowledge, this is the first study to show that there is a significant difference in the frequency of the genotype of *SOD1* (A/G rs2070424) in the development of gestational diabetes mellitus in Egyptian women.

Ethics Approval and Consent to Participate

This study was approved by ethical standards of the institutional research board, faculty of medicine and Mansoura university. The patient provided written informed consent.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

MMM contributed to the methodology, analysis, investigation and writing original manuscript. FE contributed to the methodology, acquisition, analysis and investigation. AE contributed to the methodology, analysis and investigation. AAE contributed to interpretation of data, supervision, reviewing and editing of the manuscript. AFA contributed to designing the work, supervision, reviewing and editing of the manuscript. All authors have read and approved the manuscript for publication.

Acknowledgment

References

1. Shen Y, Jia Y, Zhou J, Cheng X-Y, Huang H-Y, et al. (2020) Association of gestational diabetes mellitus with adverse pregnancy outcomes: our experience and meta-analysis. Int J Diabetes Dev Ctries 40:357-370

2. Park S, Kim MY, Baik SH, Woo JT, Kwon YJ, et al. (2013) Gestational diabetes is associated with high energy and saturated fat intakes and with low plasma visfatin and adiponectin levels independent of prepregnancy BMI. Eur J Clin Nutr 67:196-201

3. Murthy KS, Bhandiwada A, Chandan SL, Gowda SL, Sindhusree G (2018) Evaluation of oxidative stress and proinflammatory cytokines in gestational diabetes mellitus and their correlation with pregnancy outcome. Indian J Endocrinol Metab 22:79-84

4. Zhu C, Yang H, Geng Q, Ma Q, Long Y, et al. (2015) Association of oxidative stress biomarkers with gestational diabetes mellitus in pregnant women: a case-control study. PLoS One 10:1-12

5. Doucette P A, Whitson L J, Cao X, Schirf V, Demeler B, et al. (2004) Dissociation of human copper-zinc superoxide dismutase dimers using chaotrope and reductant: insights into the molecular basis for dimer stability. J Biol Chem 279:54558-54566

6. Celojevic D, Nilsson S, Behndig A, Tasa G, Juronen E, et al. (2013) Superoxide dismutase gene polymorphisms in patients with age-related cataract. Ophthalmic Genet 34: 140-145

7. Aebi H (1984) Catalase *in vitro*. Meth Enzymol 105:121-126

8. Fossati P, Prencipe L, Berti G (1980) Use of 3, 5-dichloro-2-hydroxybenzenesulfonic acid/4-aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. Clin Chem 26:227-231

9. Ponti V, Dianzani M, Cheeseman K, Slater T (1978) Studies on the reduction of nitroblue tetrazolium chloride mediated through the action of NADH and phenazine methosulphate. Chem Biol Interact 23:281-291

10. Goldberg D, Spooner R (1983) Assay of Glutathione Reductase. Verlag Chemie 258–265

11. Satoh K (1978) Method of lipid peroxidation determination in serum. Clin Chim Acta 90:37-43

12. Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95:351-358

13. Tsikas D (2005) Review Methods of quantitative analysis of the nitric oxide metabolites nitrite and nitrate in human biological fluids. Free Radic Res 39:797-815

14. Weydert CJ, Cullen JJ (2010) Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissue. Nat Protoc 5:51-66

15. Grupe K, Pfeifer MA, Dannehl F, Liebmann M, Rustenbeck I, et al. (2020) Metabolic changes during pregnancy in glucose-intolerant NZO mice: A polygenic model with prediabetic metabolism. Physiol Rep 8:1-13

16. Yuksel S, Yigit AA (2015) Malondialdehyde and nitric oxide levels and catalase, superoxide dismutase, and glutathione peroxidase levels in maternal blood during different trimesters of pregnancy and in the cord blood of newborns. Turk J Med Sci 45:454-459

17. Adeniji A O, Oparinde D P (2013) The profiles of lipid peroxidation and anti-oxidant activities in gestational diabetes mellitus and normal pregnancies in Nigerian population. Open J Obstet Gynecol 3:472-476

18. Kodliwadmath S, Sadashivadu B, Kodliwadmath M (1989) Serum Malondialdehyde and ceruloplasmin Levels in toxaemia of pregnancy. J Obstet Gynaecol India 5:648-651

19. Arribas L, Almansa I, Miranda M, Muriach M, Romero F J, et al. (2016) Serum malondialdehyde concentration and glutathione peroxidase activity in a longitudinal study of gestational diabetes. PLoS One 11:1-13

20. Rodrigues F, De Lucca L, Neme WS, Goncalves TL (2018) Influence of gestational diabetes on the activity of delta-aminolevulinate dehydratase and oxidative stress biomarkers. Redox Rep 23:63-67

21. Hata T, Hashimoto M, Kanenishi K, Akiyama M, Yanagihara T, et al. (1999) Maternal circulating nitrite levels are decreased in both normal normotensive pregnancies and pregnancies with preeclampsia. Gynecol Obstet Invest 48:93-97

22. Brown M A, Tibben E, Zammit VC, Cario GM, Carlton MA (1995) Nitric oxide excretion in normal and hypertensive pregnancies. Pregnancy Hypertens 14:319-326

23. Jr AS, Allman KG, Young D, Redman CW (1997) Elevated levels of serum nitrate, a stable end product of nitric oxide, in women with pre-eclampsia. Br J Obstet Gynaecol 104: 538-543

24. Chaudhary L, Tandon O, Vaney N, Agarwal N (2003) Lipid peroxidation and antioxidant enzymes in gestational diabetics. Indian J Physiol Pharmacol 47:441-446

25. Wisdom S J, Wilson R, McKillop J H, Walker JJ (1991) Antioxidant systems in normal pregnancy and in pregnancy-induced hypertension. Am J Obstet Gynecol 165:1701-1704

26. Davidge ST, Hubel CA, Brayden RD, Capeless EC, McLaughlin MK (1992) Sera antioxidant activity in uncomplicated and preeclamptic pregnancies. Obstet Gynecol 79: 897-901

27. Shang M, Zhao J, Yang L, Lin L (2015) Oxidative stress and antioxidant status in women with gestational diabetes mellitus diagnosed by IADPSG criteria. Diabetes Res Clin Pract 109:404-410

28. Ademuyiwa O, Odusoga OL, Adebawo OO, Ugbaja R (2007) Endogenous antioxidant defences in plasma and erythrocytes of pregnant women during different trimesters of pregnancy. Acta Obstet Gynecol Scand 86:1175-1182

29. Djordjevic A, Spasic S, Jovanovic-Galovic A, Djordjevic R, Grubor-Lajsic G (2004) Oxidative stress in diabetic pregnancy: SOD, CAT and GSH-Px activity and lipid peroxidation products. J Matern Fetal Neonatal Med 16:367-372

30. Goth L, Toth ZN, Tarnai I, Berces M, Torok P, et al. (2005) Blood catalase activity in gestational diabetes is decreased but not associated with pregnancy complications. Clin Chem 51:2401-2404

31. Jamil D, Al-Aubaidy H, Smith L, Jelinek H (2014) Oxidative DNA damage in gestational diabetes mellitus: correlation with antioxidants in an Iraqi cohort. Int J Biochem Res Rev 4:410-419

32. Patil S B, Kodliwadmath M V, Kodliwadmath SM (2007) Study of oxidative stress and enzymatic antioxidants in normal pregnancy. Indian J Clin Biochem 22:135-137

33. Silig Y, Tas A, Sahin-Bolukbasi S, Caglayan G,Sari I (2017) Superoxide Dismutase 1 (*SOD1*) A251G Polymorphism. Turk J Biochem 42:181-185

34. Ghattas MH, Abo-Elmatty DM (2012) Association of polymorphic markers of the catalase and superoxide dismutase genes with type 2 diabetes mellitus. DNA Cell Biol 31:1598-1603

35. Imaobong A, Ubong A, Glory S, Offiong E, Ndodo N (2017) Genotyping of A251g, copper-zinc superoxide dismutase (Cuznsod) polymorphism, in term pregnant women In Uyo, South-South, Nigeria. Int J biotechnol biochem 3:19-22

36. Vats P, Sagar N, Singh T P, Banerjee M (2015) Association of Superoxide dismutases (*SOD1* and *SOD2*) and Glutathione peroxidase 1 (*GPx1*) gene polymorphisms with type 2 diabetes mellitus. Free Radic Res 49:17-24

37. Mohammedi K, Maimaitiming S, Emery N, Bellili-Munoz N, Roussel R, et al. (2011) Allelic variations in superoxide dismutase-1 (*SOD1*) gene are associated with increased risk of diabetic nephropathy in type 1 diabetic subjects. Mol Genet Metab 104:654-660

38. Flekac M, Skrha J, Hilgertova J, Lacinova Z, Jarolimkova M (2008) Gene polymorphisms of superoxide dismutases and catalase in diabetes mellitus. BMC Med Genet 9:30-39

39. Hernandez-Guerrero C, Hernandez-Chavez P, Romo-Palafox I, Blanco-Melo G, Parra-Carriedo A, et al. (2016) Genetic polymorphisms in SOD (rs2070424, rs7880) and CAT (rs7943316, rs1001179) enzymes are associated with increased body fat percentage and visceral fat in an obese population from Central Mexico. Arch Med Res 47:331-339

40. Nithya K, Angeline T, Isabel W, Asirvatham A (2016) *SOD1* gene +35A/C (exon3/intron3) polymorphism in type 2 diabetes mellitus among south indian population. Genet Res Int 2016:1-5