wjpmr, 2020, 6(12), 343-348

SJIF Impact Factor: 5.922

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Research Article

ISSN 2455-3301

WJPMR

ASSOCIATION BETWEEN OXIDATIVE STATUS AND GESTATIONAL DIABETIC PATIENTS OF IRAQI WOMEN

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Article Received on 03/11/2020 **Article Revised on 24/11/2020** **Article Accepted on 15/12/2020**

ABSTRACT

Background: Risk factors for GDM include obesity, advanced maternal age, type 2 diabetes mellitus (T2DM), family background, prior diagnosis of GDM, recent adverse pregnancy results. At present, the prevalence of GDM is gradually rising, especially in Asia. Highly reactive molecules formed inside the cell are highly reactive oxygen species. **Aim:** The current study aimed to detect the correlation between the oxidants and antioxidants status determined in gestational diabetes mellitus (GDM) patients in holy Kerbala city, Iraq. **Materials and Methods:** This study include 46 gestational outside diabetic patients obtained from gynecological and obstetric teaching hospital, Kerbala health directorate / Kerbala – Iraq in addition to 47 women as apparently control group during Nov., 2019 to Sep., 2020 with matched age ranged between 25 – 45 years. The links between oxidative status and gestational diabetic patients were examined. **Results:** Total reactive oxygen species (TROS) was significantly different between the study groups. It was significantly higher in GDM (70.50 ± 16.01) µmol/L compared to pregnant without GDM (56.05 ± 19.48) µmol/L, with P value ($p< 0.05$) was 0.002. TAO was no significantly different between the study groups. It was no significant different in GDM (1104.50 ± 464.95) µmol/L compared to control (1201.83 ± 422.58) µmol/L with ($P < 0.05$). The GDM patients have higher fasting blood glucose, insulin, HMOA, TROS, HbA1c compared with control. There was a positive significant correlation between HMOA-IR and TROS. There was a significant correlation between HMOA-IR and insulin in GDM patient. Non significant association between the parameters studied in the control group except insulin and HMOA-IR. **Conclusion:** There is a non-significant differences in TAO between control and GDM, TROS was significantly higher in GDM as compared with control. There was strong correlation between HMOA-IR with each of TROS and insulin in GDM patients and control group.

KEYWORDS: GDM, TAO, TROS, FBS, HbA1C, Insulin.

INTRODUCTION

The most prevalent metabolic disease in pregnancy, characterized by elevated blood sugar levels, is gestational diabetes mellitus (GDM), leading to multiple adverse maternal and neonatal outcomes **(Linnenkamp *et al.*, 2014).** Risk factors for GDM include obesity, advanced maternal age, type 2 diabetes mellitus (T2DM), family background, prior diagnosis of GDM, **(Georgiou *et al.*, 2005).** At present, the prevalence of GDM is gradually rising, especially in Asia. Highly reactive molecules formed inside the cell are highly reactive oxygen species (ROS), including either oxygen radicals such as hydroxyl radical (•OH), peroxyl radical (ROO•), superoxide anion radical ($O_2^{\bullet-}$), or reactive non-radicals such highly reactive molecules such as hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$) **(Al-Gubory *et al.*, 2010).** On the other hand, excessive production of ROS can attenuate endogenous antioxidant systems contributing to oxidative stress, which may be associated with oxidative stress in the insulin resistance pathway with detrimental effects on cellular DNA , protein and GDM as reported in T2DM **(Rueangdetnarong *et al.*, 2018)**. We hypothesized that GDM pregnancies have greater levels of markers of oxidative stress. The role of oxidative stress in GDM has attracted researcher's interest. Total antioxidant capacity (TAC) determinations are simple, inexpensive, and capable of evaluating the potential of known and unknown antioxidants and their additives, there are limited studies on total plasma antioxidant capacity (TAC) and their corresponding variables in pregnant women. The creation of oxidative stress produces an imbalance between the synthesis of reactive oxygen species (ROS) and their removal by antioxidant protection mechanisms **(Turek *et al.*, 2015).**

Unhealthy births, including gestational diabetes mellitus (GDM), are associated with increased oxidative stress

due to both the overproduction of free radicals and/or antioxidant defense deficiencies. Regular human pregnancy is considered to be a disorder of elevated oxidative stress. The relative immaturity of the antioxidant mechanism causes embryos and fetuses to be vulnerable to the detrimental effects of oxidative stress. There are only a few scientific trials investigating the possible beneficial effects of antioxidants in GDM **(Lappas et al., 2011)**.

The presented work aimed to detect the correlation between the oxidants and antioxidants status in gestational diabetic women of Kerbala province of Iraq.

MATERIALS AND METHODS

In this current case control study, two groups were enrolled; 46 patients with gestational outside diabetic patients obtained from gynecological and obstetric teaching hospital, Kerbala health directorate / Kerbala – Iraq as a and 47 healthy pregnant women as apparently control group during Nov., 2019 to Sep., 2020 with matched age ranged between (25 – 45) years. All patients were diagnosed with gestational diabetes in the consultant clinic of obstetrics and gynecology hospital, Kerbala, Iraq. The fasting blood glucose, serum insulin level, HbA1c value, oxidative stress markers and body mass index (BMI) of all the study subjects were investigated and measured. Additionally, clinical data including full medical history, and anti-hyperglycemic medicines were recorded for all study samples persons.

Serum total ROS was determined by using a novel method, developed by Erel. Oxidants found in the sample oxidize the ferrous ion–o-dianisidine complex to ferric ion. By glycerol molecules the oxidation reaction is enhanced, which are richly found in the reaction medium. The ferric ion creates a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules found in the sample. The test is calibrated with hydrogen peroxide and the outcome are expressed in terms of micromolar hydrogen peroxide equivalent per liter (µmol H_2O_2 Eq/L).

We quietly mix the content of each tube after addition, allow standing at room temperature for 5 minute, read spectrophotometrically at 560 nm **(Erel, 2005).**

Table 1: The procedure of total ROS determination.

	Blank	Standard	Sample
Distilled water	50 µl	-----	------
Sample	------	-----	50 µl
Hydrogen peroxide	------	50 µl	------
R1	1 ml	1 ml	1 ml
Test tubes were mixed by vortex, and then add:			
R2	250 µl	250 µl	250 µl

The total antioxidant capacity assay of patients and control samples were determined according to reduction of Cu^{++} to Cu^{+}. The reduced form of copper wills selectively appearance a 2:1 complex with the chromogenic reagent. This complex is stable and has an absorption maximum at 450 nm. A known concentration of Trolex is used to generate are ference curve to compare those readings obtained by the sample. Data can be expressed as mM copper reducing equivalents or in Mm Trolox equivalents.

Antioxidant + Cu^{2+} ----------► Cu^{+}

Cu^{+} + 2,9-dimethyl-1,10-phenanthroline ----------► colored complex

The absorbance of the colored complex obtained was measured at 450 nm. Allow dilution buffer, copper solution, and stop solution to equilibrate at room temperature for 30 minutes until the assay is performed. In the dilution buffer given, we dilute both sample and standards 1:40 (e.g. 15 µl serum + 585 µl buffer).

1. In each well placed 200 µl of diluted samples or standards. Reagent blanks should be dilution buffer provided in the absence of standard or sample
2. For a reference calculation read the plate at 450 nm.
3. We add 50 µl of the Cu solution to each well and incubate it at room temperature for 3 minutes.
4. We add 50 µl of stop solution.
5. At 450 nm, the absorbance was measured in the plate for a second time.

The vein puncture was collected from each patient and healthy woman (3 ml) of blood using 5 ml of disposable syringes after 10-12 hours of fasting, (3 ml) of segregated blood was centrifuged, and the separated serum was used for further measurement of fasting blood glucose, TAO, TROS and FBG, insulin and HbA1c%.

RESULTS

The characteristics of population of this study are presented in Table 2. The average age determined was 30.5 ± 6.63 years for the patients and 28.8 ± 6.17 years for the controls. The GDM patients have higher FBG, insulin, HMOA-IR, TROS, HbA1c% as compared with control group.

Table 2: Demographic characteristics of patients and controls.

P value	Control Mean ± SD N = 47	GDM patient Mean ± SD N = 46	Parameter
0.22	28.8 ± 6.17	30.5 ± 6.63	Age (y)
0.09	32.4 ± 5.53	34.4 ± 5.6	BMI (kg/m^2)
0.001	94.56 ± 12.52	164.67 ± 42.80	FBG, mg/dl
0.002	56.05± 19.48	70.50 ± 16.01	TROS, µmol/L
0.34	1201.83 ± 422.58	1104.50 ± 464.95	TAO, µmol/L
0.001	4.07 ± 1.83	14.72 ±12.23	HMOA – IR
0.001	9.35 ± 3.9	37.61± 25.4	Insulin, (µIU/ml)
0.001	5.06 ± 0.53	6.52 ± 1.30	HbA1c (%)

The (mean ± SD) value of TROS was significantly different between the study groups. It was significantly higher in GDM (70.50 ± 16.01) µmol/L as compared with control (56.05 ± 19.48) µmol/L, ($p < 0.05$) as shown in figure 1.

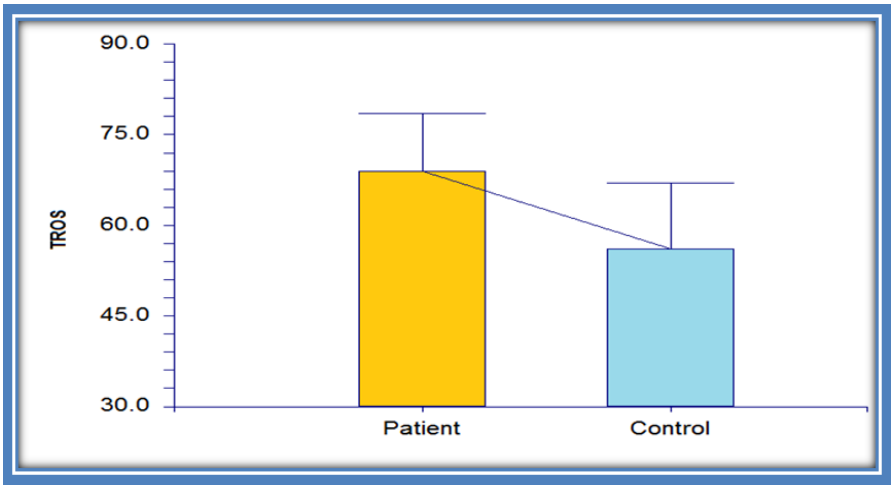

Fig. 1: Mean ± SD of TROS in GDM and control.

The mean ± SD value of TAO was no significantly different between the study groups. It was non significant different in GDM (1104.50 ± 464.95) µmol/L as compared to control (1201.83 ± 422.58) µmol/L and ($p < 0.05$) as shown in figure 2.

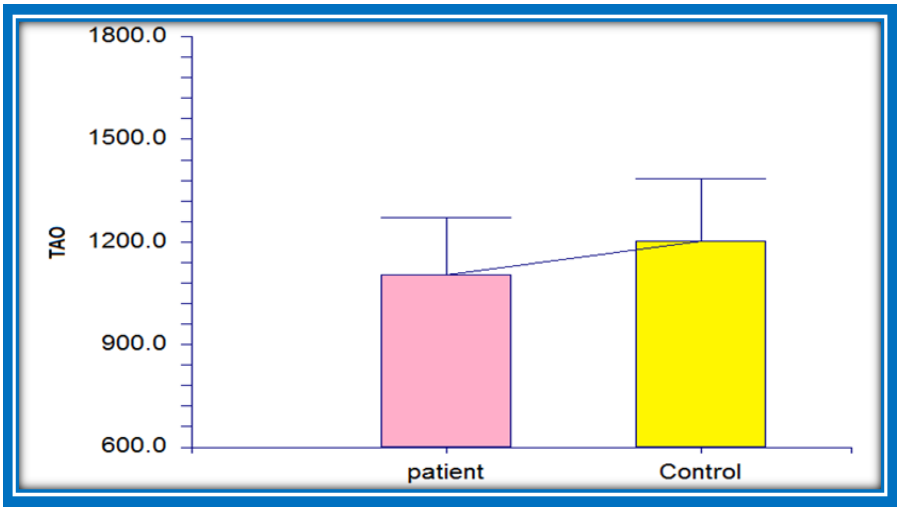

Fig. 2: Mean ± SD of TAO distribution in study Individuals with GDM and control.

There was a positive significant correlation between HMOA-IR and TROS ($r = 0.39$, $P<0.05$) and a positive significant correlation between HMOA-IR and insulin, on the other hand, there were a non-significant association between the others parameters as shown in the table 4

Table 4: Correlation between some parameters in the GDM groups.

	BMI	HbA1c%	Insulin	HMOA-IR	FBG	Total ROS	TAO
BMI	1						
HbA1c%	-0.12821	1					
insulin	0.045901	0.18007	1				
HMOA-IR	0.014615	-0.09708	0.832222	1			
FBG	-0.20609	0.076627	-0.13686	0.104021	1		
TROS	0.037996	0.008171	0.39771	0.394351	-0.17958	1	
TAO	-0.07995	0.097853	0.041914	0.027128	- 0.004688	-0.02481	1

There was a non-significant association between various parameters in the control group except insulin and HMOA-IR group as in the table 5

Table 5: Correlation between some parameters in the control groups.

	BMI	HbA1c	insulin	HMOA-IR	FBG	Total ROS	TAO
BMI	1						
HBA1C	-0.007519	1					
Insulin	-0.278207	0.140289	1				
HMOA	-0.176631	-0.037326	0.334231	1			
FBS	-0.144286	-0.077733	0.010721	-0.171571	1		
Total ROS	-0.05524797	-0.26152843	-0.08042687	-0.032184	-0.25177042	1	
TAO	-0.131376	-0.163963	-0.273015	-0.183871	-0.150982	-0.128911	1

Table 6: Period of infection and level of TAO and TROS.

Parameter	New infected	After period of infection	P value
TAO, µmol/L	1052.57± 93.89	1219.34 ± 55.3	0.034
TROS, µmol/L	69.98 ± 20.22	66.03 ± 11.53	0.872

TROS and period of infection

The mean ± SD value of TROS was no significantly different between the study groups. It was no significantly higher in newly infected with GDM (69.98 ± 20.22) µmol/L compared to patient GDM after period of infection (66.03 ± 11.53) µmol/L, with (P < 0.5), as shown in figure 3.

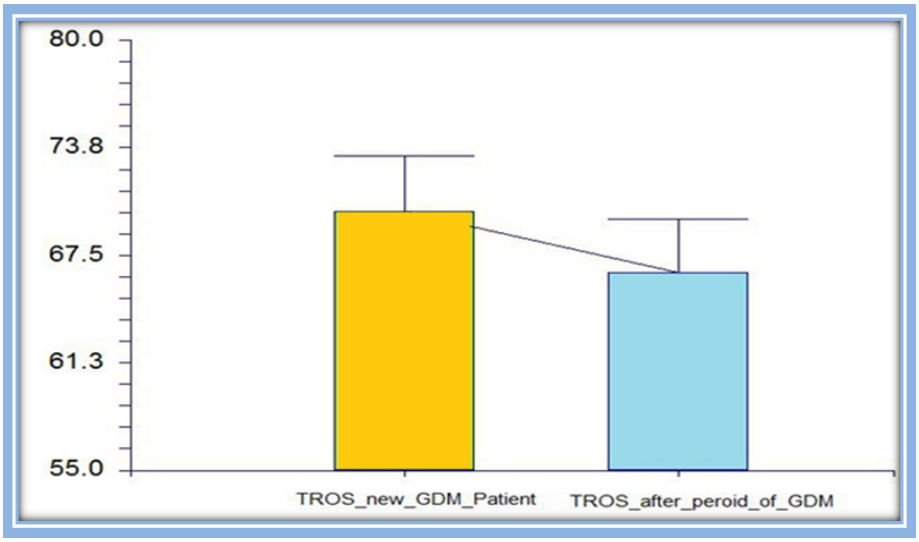

Figure 3: Mean ± SD of TROS distribution in GDM patient according to period of infection.

TAO and period of infection

The mean ± SD value of TAO was no significantly different between the study groups. It was no significantly higher in newly infected with GDM (1052.57 ± 93.89) µmol/L compared to patient GDM after period of infection (1219.34 ± 55.3) µmol/L, (P< 0.005), as shown in figure 4.

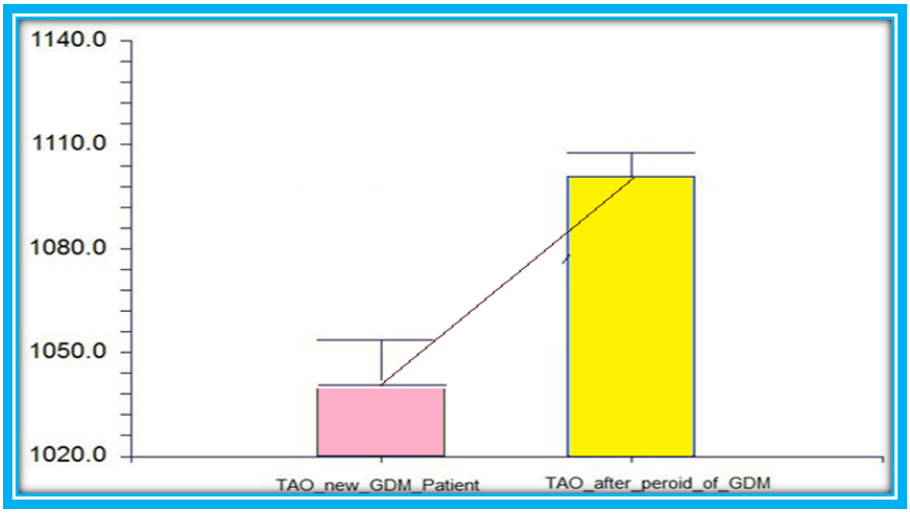

Figure 4: TAO and period of infection.

DISCUSSION

The formation of oxidative stress stems from an imbalance between the syntheses of reactive oxygen species (ROS) and their clearance by antioxidant protection mechanisms. Biological effects of this condition include oxidative disruption to essential cellular components such as nucleic acids, lipids or proteins and, in turn, cell and tissue deficiency. Clinical and laboratory data confirms the notion that oxidative stress is one of the pathological factors associated with gestational diabetes mellitus (GDM), a metabolic condition defined as any degree of onset or first recognition of glucose intolerance during pregnancy. In maternal diabetes, elevated blood glucose concentrations in diabetic pregnancy have been shown to cause oxidative stress by several mechanisms, including increased ROS output in mitochondria and other pathways **(Turek et al., 2015)**.

Total antioxidant potential (TAO) in gestational diabetes had been explored in the data obtained. TAO was conceived as the "cumulative action of all the antioxidants present in plasma and body fluids, thereby establishing a comprehensive function rather than a single sum of observable antioxidants" **(Ghiselli et al., 2000)**. There were no major variations between gestational diabetes and controls in TAO level. The other researchers investigated that diabetes was complicated by increased oxidative stress and reduced detoxification or free radical scavenging capacity complicated by diabetes in pregnancy **(Chaudhary et al., 2003)** as compared to control subjects. The TROS level in our study was elevated in the gestational diabetes groups, compared to the control pregnant. TROS was no significantly different in newly infected with GDM compared to patient GDM after period of infection

Other study indicated that the TAO in GDM patient, overall serum antioxidant protection status in diabetic mothers and their macrosomal babies has been decreased **(Grissa et al., 2007)**, while the presented results found that TAO was no significantly different between the study groups. It was no significant different in GDM patient as compared to control. The observed data of this study found that there was TAO was no significantly higher in newly infected with GDM compared to patient GDM after period of infection.

While the interactions between oxidative stress, antioxidant defense and pathophysiology of diabetes mellitus remain too complicated and not completely resolved. An inverse correlation between TAC and glycated hemoglobin, a relationship was found in a previous report **(Maxwell et al., 1997).** On the other hand, the antioxidant efficiency has been found to be consistent with improved values for the ROS efficiency. These findings represent an optimistic adaptive response capable of ensuring effective defense not only against overproduction of chronic, diabetes-mediated reactive oxygen (ROS) species but also against more oxidative harm **(Mahmoud et al., 2014)**. The presented data agree with other study about significantly association of TROS with GDM, while disagree with previous studies and existing opinions on the role of TAO in gestational diabetes.

CONCLUSION

There is a non-significant differences in TAO between control and GDM, TROS was significantly higher in GDM as compared with control. There was strong correlation between HMOA-IR with TROS and a significant correlation between HMOA-IR and insulin in GDM patients and control group.

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