ZANCO Journal of Pure and Applied Sciences
The official scientific journal of Salahaddin University-Erbil
https://zancojournals.su.edu.krd/index.php/JPAS

ISSN (print):2218-0230**, ISSN (online):** 2412-3986**, DOI: http://dx.doi.org/10.21271/zjpas**

RESEARCH PAPER

Association between some serum oxidative stress biomarkers and lipid profile in type 2 diabetic patients in Erbil City

Hemn Jameel Majeed[1], Gulzar Ismael Ibrahim[2], Pshtiwan Abdullah Yousif[3], Saman Muhsin Abdulkareem[4]
[1,2,3] Department of Chemistry, College of Education, Salahaddin University-Erbil, Kurdistan-Region, Iraq
[4] Department of Biology, College of Education, Salahaddin University-Erbil, Kurdistan-Region, Iraq

A B S T R A C T:

Increased oxidative stress appears to be a negative factor leading to insulin resistance, dyslipidemia, β-cell dysfunction, impaired glucose tolerance, and ultimately leading to type 2 diabetes mellitus. The aim of the study is to investigate some serum oxidative biomarkers, lipids, and their association with hyperglycemia in patients with type 2 diabetes. The 100 participants (50 patients with type 2 diabetes mellitus and 50 healthy individuals) were enrolled in the present study. Anthropometric measures, serum fasting blood sugar, glycated hemoglobin, oxidative biomarkers, and serum lipid profiles were evaluated. The results showed that the level of malondialdehyde, fasting blood sugar, glycated hemoglobin, and lipid profile in patients with type 2 diabetes is higher than those in the control group, while nitric oxide was lower in diabetic than those in the control group. There are also insignificant changes in fasting blood sugar and glycated hemoglobin between baseline and after 3 months follow up. The results showed no linear relationship between oxidative damage and abnormal lipid profiles in patients with type 2 diabetes. It can be concluded that the serum level of malondialdehyde and nitric oxide, along with lipid parameters in patients with type 2 diabetes, can be a useful tool for monitoring of type 2 diabetes.

KEY WORDS: Type 2 Diabetes, Oxidative Stress, Fasting Blood Sugar, Lipid Profile.
DOI: http://dx.doi.org/10.21271/ZJPAS.33.1.11
ZJPAS (2021) , 33(1);107-112 .

INTRODUCTION:

Diabetes mellitus and its complications are among the most important health problems with very high prevalence, morbidity, and mortality (Deshpande et al., 2008). Type 1 and type 2 diabetes are caused by impaired insulin secretion or its function in the target cell, respectively. Insulin allows glucose from the blood to enter the liver, fat, and skeletal muscle cells and regulates carbohydrates, lipids, and protein metabolism. If these actions are not performed well as a result of impaired insulin secretion or function, plasma glucose levels increased (hyperglycemia) and lead to complications of diabetes and threaten the health of a person with diabetes (American Diabetes, 2009, Ibrahim, 2018).

* **Corresponding Author:**
Hemn Jameel Majeed1
E-mail: hemn.majeed@su.edu.krd
Article History:
Received: 16/09/2020
Accepted: 17/10/2020
Published: 20/02 /2021

Type 2 diabetes is linked with some diseases like high blood pressure and dyslipidemia, which are known as risk factors for cardiovascular disease and premature death (Halpern et al., 2010). Microvascular (retinopathy, neuropathy, and nephropathy) and macrovascular disorders, including peripheral vascular disease, ischemic heart disease, and stroke, are the most anatomical derangements and impairments in carbohydrates, fats, and proteins metabolisms and are the main biochemical consequences (Cade, 2008, Abdoulrahman, 2017). Experimental and clinical studies have displayed that oxidative stress and its consequences play a crucial role in the pathogenesis of diabetes and its problems (Oguntibeju, 2019, Matough et al., 2012).

Oxidative stress is defined as an imbalance between the productions of reactive oxygen

species (free radicals) and antioxidant defense systems (Pizzino et al., 2017). Reactive oxygen species (ROS), which includes oxygen radicals and its peroxides, are often generated in low levels by cellular organelles such as mitochondria and normal cell reactions. Under these conditions, they participate in several cellular signaling pathways that mediate cellular proliferation and differentiation; however, overproduction of them induce oxidative damage (Nita and Grzybowski, 2016). Oxygen-free radicals by cellular stress-sensitive pathways has been related to insulin resistance and reduced insulin secretion (Karunakaran and Park, 2013). Free radicals induced damage to macromolecules such as lipids, proteins, and DNA and have been identified as a risk factor in various diseases such as cardiovascular disease, neurodegenerative disorders, and cancer (Nita and Grzybowski, 2016). ROS are also linked to induced the expression of proinflammatory cytokines and decreased nitric oxide release that, in turn, cause endothelial dysfunction (Steven et al., 2019, Marchio et al., 2019). Living organisms, from cell to tissue, have extensive and complex strategies to counter oxidant and free radicals. Enzymatic defenses that include enzymes superoxide dismutases, glutathione peroxidases, catalase and paraoxonase, and non-enzymatic defenses like vitamins C and E, beta carotene, and reduced glutathione (GSH) are the most mechanisms to combat free radicals to reduce their oxidative damage (Jelodar et al., 2018, Ngissah, 2013, Abdulkareem and Nanakali, 2019). ROS overproduction is also linked to the oxidation of low-density lipoprotein cholesterol (LDL-C) and lipid peroxidation, which refers to the oxidation of membrane polyunsaturated fatty acids such as arachidonic acid or linoleic acid by free radical. It can alter the structure and the function of biological membranes. Malondialdehyde (MDA) can be used to monitor the degree of oxidative damage in many diseases like diabetes, atherosclerosis, and chronic inflammation (Bigagli and Lodovici, 2019, Ito et al., 2019). Therefore, this study aimed to assess the serum levels of oxidative stress biomarkers and levels of lipid profile in T2DM patients and healthy controls, and their correlation with HbA1c.

Materials and methods

Subjects

In this study, 50 T2DM patients were compared with 50 healthy adults in terms of serum fasting blood sugar (FBS), HbA1c, lipid profile, and oxidative biomarkers levels. This was conducted in Razgari hospital, Erbil. T2DM patients between the age group of 40-70 years of either sex with 5 years history of diabetes were recruited from the outpatient department of medicine. Healthy individuals between the age group of 40-70 years of either sex were enrolled as a control group. In this study, we excluded patients suffering from inflammation and chronic infection, gestational diabetes, type 1 diabetes, renal disease, liver disease, and thalassemia, as well as those treated with diuretics, antioxidants, and steroids or consumed alcohol or cigarettes.

Estimation of different biochemical parameters

Fasting blood sugar, HbA1c, serum nitric oxide levels, serum malondialdehyde (MDA) level, and lipid profile were measured in all the participants. The glucose oxidase method was used to measure FBS (Saifer and Gerstenfeld, 1958). Serum content of lipid profile includes total cholesterol (TC), total triglyceride (TG), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) were estimated by Roche Cobas 400 integral analyzer. Serum NO was estimated by Cortas and Wakid method (Cortas and Wakid, 1990). The serum MDA level as a marker for lipid peroxidation was measured by Bhutia et al. (2011). The serum glycated hemoglobin (HbA1c) was determined by the resin binding method (Weykamp et al., 2009).

Statistical Analysis

The data were expressed as mean ±SD. An independent *t*-test was used to compare the patient group with the control group. A comparison of baseline and after three months' follow-up was performed by paired *t*-test for the same groups of participants. Significant differences between the patient group (or comparison of mean values) and the control group were denoted by superscript asterisk symbol *, representing significance at $P<0.05$.

Results and Discussion

The results showed that FBS (male: 198±52.1; female: 201±12.3) and HbA1c (male: 8.82±0.91; female: 8.91±0.22) significantly were higher in persons with diabetes than a healthy individual (FBS; male: 100.2±7.4; female: 95±31.2) and (HbA1c; male: 5.31±0.51; female:

5.21±0.51). Also, results showed that the baseline level of these factors and three months of follow up were greater in the patient subjects than in the control subjects (Table 1). There is no significant difference in serum FBS and HbA1c after 3 months of follow-up in diabetic and control groups compared to their baseline levels. There was no significant change in FBS and HbA1c between men and women with diabetes.

Table 1: Age, BMI, FBS, and HbA1c in diabetic and healthy subjects

Subject		Male control	DM (Male)	Female control	DM (Female)
Number of participants		25	25	25	25
Age (year)		52±6.3	54±5.4	53±5.6	54±6.5
Body mass index (Kg/m^2)		23.3±2.3	32.3±3.1***	25.1±2.6	31±4.41**
FBS	**Baseline**	100.2 ± 7.4	198 ± 52.1***	95 ± 31.2	201 ± 12.3***
	3 Months of follow up	98.45 ± 3.5	196 ± 31.3***	96.5 ±6.7	204 ± 43.7***
HbA1c	**Baseline**	5.31 ± 0.51	8.82 ± 0.91*	5.21 ± 0.51	8.91 ± 0.22*
	3 Months of follow up	4.89 ± 0.63	9.14 ± 0.34*	5.04 ± 0.42	9.36 ± 0.75*

*Significant (P<0.05), **highly significant (P<0.01), ***very highly significant (P<0.001).

Well-known complications related to hyperglycemia, such as increased production of the extracellular matrix, cell damage, and impaired vascular function, are involved in the pathogenecity of vascular disease in type 1 and 2 of diabetes (Dalle-Donne et al., 2006). In this study, HbA1c as an index of metabolic control used to evaluate and monitor patients with diabetes. The results showed that HbA1c in the diabetic group is significantly greater than the control group, which is one of the causes of Aamadori reaction (Shinde et al., 2011). The recent increase in the knowledge of free radicals in biology is generating a medicinal revolution that promises a new era of health and disease controlling. Oxygen and nitrogen-free radicals that known as oxidants are highly unstable molecules, which are produced during normal cellular metabolism. Oxygen-free radicals are well known for playing a double role as both detrimental and beneficial species as they can be either dangerous or beneficial to living systems. At physiological level (low or moderate) exerts beneficial effects such as induction of a mitogenic response, defense against infectious agents and the maturation process of cellular structures; at the high level can react with lipids, DNA and proteins and disrupt the normal function of the cells (Akbari et al., 2016, Bigagli and Lodovici, 2019, Matough et al., 2012, Oguntibeju, 2019).

The mean values of serum MDA and NO levels are shown in Table 2. The results stated that the concentration of MDA in patients with diabetes (male: 3.49±0.92; female: 4.11±0.36) was meaningfully higher than the healthy subjects (male: 1.95±0.75; female: 2.65±0.12); the results also showed that the level of MDA did not change considerably after three months of follow-up in the patient group compared to baseline. The level of NO in patients with diabetes (male: 17.41±8.71; female: 19.51±7.30) was significantly less than the control group (male: 39.21±3.17; female: 45.67±3.41); also, after three months of follow-up, this factor did not change significantly in baseline in the patient group and in the healthy subjects. There was an insignificant difference in serum MDA and NO levels between men and women with diabetes.

Table (2): The mean value of serum MDA and NO of control and diabetic groups

Subject		Male control	DM (Male)	Female control	DM (Female)
MDA (nmol /ml)	**Baseline**	1.95 ± 0.75	3.49 ± 0.92*	2.65 ± 0.12	4.11 ± 0.36*
	3 Months of follow up	1.87 ± 0.91	4.36 ± 1.11*	2.43 ± 0.33	4.63 ± 0.41*
NO	**Baseline**	39.21 ± 3.17	17.41 ± 8.71*	45.67 ± 3.41	19.51 ± 7.30*

(µmole /L)	**3 Months of follow up**	38.79 ± 3.65	16.97 ± 7.32*	47.34 ± 3.96	18.97 ± 5.24*

*Significant (P<0.05), **highly significant (P<0.01), ***very highly significant (P<0.001).

In our study, MDA's level in persons with T2DM is markedly higher than that in control subjects. As already noted, MDA is a stable byproduct in lipid peroxidation; on the other hand, evidence suggested that oxidation of lipids in lipoproteins (oxidized LDL) and the plasma cell membrane is linked to the incidence of vascular disease in persons with diabetes (Mahreen et al., 2010, Kaefer et al., 2012). The elevated MDA can lead to adverse physiological consequences such as alteration in the membrane's structure and function, inactivate proteins bound to the membrane such as enzymes and receptors that regulate cellular signaling pathways (Mahreen et al., 2010). The elevated MDA can also be involved in oxidizing LDL, which led to atherosclerosis. Our results also indicated that NO level is significantly less in persons with T2DM than that in control. NO is released by endothelial nitric oxide synthase (eNOS) in endothelial cells of vessels.

It was reported that endothelial dysfunction in diabetes largely decreased NO production and its bioavailability (Takahashi and Harris, 2014). It is clear that a decrease in NO concentration causes vascular stiffness and high blood pressure. In agreement with our results, studies have shown that hyperglycemia induces oxidative damage, and it has a positive correlates with the development of T2DM and its complications (Bigagli and Lodovici, 2019, Matough et al., 2012, Oguntibeju, 2019). In healthy subjects, the levels of oxidative parameters during the three-month follow-up showed no significant change, which could be due to the normal activity of antioxidant systems and normal levels of vitamins such as vitamins C and E.

The mean values (±SD) of the serum lipid profile are presented in Table 3. The outcomes showed that the level of TC, TG, and LDL-C in subjects with diabetes (male: 238.68±41.48, 254.17±38.60, 143.35±32.63, respectively; female: 201.86±30.52, 267.82±0.29, 111.72±12.49, respectively) was significantly higher than the control subjects (male: 174.82±20.24, 156.76±23.19, 88.70±8.88, respectively; female: 161.37±24.27, 127.08±35.24, 86.27±15.16, respectively), while the level of HDL-C in patients with diabetes (male: 23.35±4.30; female: 36.62±4.68) was significantly less than the control group (male: 37.94±4.24; female: 46.62±5.49).

Table 3: The mean values (±SD) of serum lipid profile between control and diabetic group after 3 months of follow up

Groups		**Male control**	**DM (Male)**	**Female control**	**DM (Female)**
TC (mg/dl)	**Baseline**	174.82±20.24	238.68±41.48**	161.37±24.27	201.86±30.52***
	3 Months of follow up	168.34±17.85	241.15±37.64**	158.43±21.32	208.54±29.35***
TG (mg/dl)	**Baseline**	156.76±23.19	254.17±38.60***	127.08±35.24	267.82±0.29***
	3 Months of follow up	159.68±26.76	250.47±37.64***	126.14±41.65	256.36±23.54***
HDL (mg/dl)	**Baseline**	37.94±4.24	23.35±4.30***	46.62±5.49	36.62±4.68**
	3 Months of follow up	34.68±3.68	21.34±3.64***	49.67±4.34	34.65±4.65**
LDL (mg/dl)	**Baseline**	88.70±8.88	143.35±32.63***	86.27±15.16	111.72±12.49***
	3 Months of follow up	92.34±7.68	148.64±32.4***	83.64±12.67	114.34±13.64***

*Significant (P<0.05), **highly significant (P<0.01), ***very highly significant (P<0.001).

Our results showed that high values of TC, TG, and LDL-C, along with a low level of HDL-C observed in a person with T2DM. Like previous studies, our results showed that T2DM is associated with dyslipidemia (Bhowmik et al., 2018). Various mechanisms, including insulin actions on the regulation of lipoprotein lipase and cholesteryl ester transfer protein (CETP), hepatic production of the apoprotein, and peripheral its actions on muscle and adipose cells are involved in diabetic dyslipidemia (Bhowmik et al., 2018).

In this study, elevated lipid profile (TC, TG, and LDL-C) and oxidative stress biomarkers were observed in T2DM cases. As previously reported (Rao and Kiran, 2011), our results showed that there is no linear relationship between abnormal lipid profile and oxidative parameters (data unshow), and these are two independent risk factors in the DM and its complications. One of this study's limitations was not assessed as confounding factors such as dietary behaviors, physical activity level, and medications that affect lipid profile concentrations. Though, this type of study can well identify important relationships that can be explored in future studies. Therefore, the findings of the present study help provide a basis for subsequent studies in our population to investigate the relations between fat disorders, oxidative damage, and the risk of DM.

Conclusion:

The present study results suggest that the serum level of MDA and NO along with lipid biomarkers (especially TC, TG, and HDL-C) in patients with T2DM can be a useful tool for monitoring of T2DM.

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