RELATIONSHIP BETWEEN SERUM MALONDIALDEHYDE (MDA) LEVELS AND CARDIOVASCULAR RISK FACTORS IN DIABETIC PATIENTSIN ZARIA, KADUNA STATE, NIGERIA

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ABSTRACT

Background: Diabetes Mellitus (DM) is associated with hyperglycaemia, dyslipidaemia and oxidative stress. Oxidative damage, indicated by elevated levels of Malondialdehyde (MDA), plays a vital role in development of cardiovascular diseases (CVD) in diabetic patients. Serum Malondialdehyde (MDA) has been used as a biomarker of lipid peroxidation and has served as an indicator of free radical damage.

Aim: The purpose of this study was to evaluate serum Malondialdehydelevels and investigate its relationship to Cardiovascular risk factors in diabetic patients in Zaria, Kaduna State, Nigeria.

Methods: We assessed serum Malondialdehyde levels and lipid profile in 139 men and women with type 2 diabetes in Ahmadu Bello University Teaching Hospital (ABUTH), Zaria, Nigeria, a cross-sectional study. The subjects consisted of 36 males (26 %) of mean aged 53±0.1 SD and 103 females (74 %) of mean aged 52±0,2SEM. Diabetes mellitus status was confirmed biochemically according to World Health Organization diagnostic criteria for classification of diabetes mellitus. Concentrations of serum MDA was measured using the method of Draper and Hardley. Data for selected clinical/demographic variables were obtained from fasting blood samples and an interviewer-assisted questionnaire.

Results: The mean serum MDA and TG concentration were higher in diabetic individuals than in control subjects (2.1±0.1vs 0.7±0.0 (p=0.01)); (1.5±0.1vs1.1±0.0 (p=0.01)) respectively. There is no statically significant difference in the mean values of serum TC, HDL, LDL, and TC:HDL in diabetic patients and controls (p>0.05). There were no correlations between serum MDA and lipids (TC, TG, HDL, LDL,TC:HDL), FBG, HBA1c,BMI, BP and Duration of Diabetes in diabetic patients (p>0.05)

Conclusion: There are increases in free radical activity and lipid peroxidation in individuals with type 2 diabetic mellitus. In addition, MDA associate independently with cardiovascular disease

Key words: Oxidative stress, Malondialdehyde, Diabetes Mellitus, Lipid peroxidation Cardiovascular Disease

INTRODUCTION

Diabetes mellitus (DM) is a chronic, metabolic disease characterized by elevated levels of blood glucose, which leads over time to serious damage to the heart, blood vessels, eyes, kidneys and nerves (WHO, 2020).

About 422 million people worldwide have DM, the majority living in low-and middle-income countries, and 1.6 million deaths are directly attributed to DM each year. Oxidative/free radicals are species with very short half-life, high reactivity and damaging activity towards macromolecules like proteins, carbohydrates, nucleic acid and lipids (Tiganis, 2009; Palanisamy *et al.,* 2009; Xiao-Qiang *et al.,* 2021; Alkadi.,2020). These species may either be oxygen derived reactive oxygen species) or nitrogen derived (RNS — reactive nitrogen species). Under normal physiological conditions, there is critical balance in the generation of oxygen free radicals and antioxidant defense systems used by organisms to deactivate and protect themselves against free radical toxicity. Impairments in the oxidant/antioxidant equilibrium create a condition known as oxidative stress (Medina *et al.,* 2007; Palanisamy *et al.,* 2009; Aikaterini *et al.,* 2022). It was reported that oxidative stress and free radicals are factors that can influence the pathogenesis of diabetes mellitus and it seems that the compounds produced may have a role in the destruction of beta cells (Javad *et al.,* 2014).

DM produces disturbances of lipid metabolism, especially an increased susceptibility to lipid peroxidation. Lipid peroxidation is an autocatalytic free radical-mediated destructive process whereby poly-unsaturated fatty acids in cell membranes undergo degradation to form lipid hydroperoxides (Tangvarasittichai *et al.,* 2009). By-products of lipid peroxidation include conjugated dienes and malondialdehyde (MDA).Lipid peroxidation is responsible for increased incidence of atherosclerosis, a major complication of DM (Moussa, 2008). Studies have shown that Type 2 diabetic individuals are at increased risk of free radical activity which is associated with the increased activity of free radical-induced lipid peroxidation and accumulation of lipid peroxidation products as well as being associated with a substantially higher prevalence of atherosclerositic and cardiovascular mortality (Tangvarasittichai *et al.,* 2009). It was reported that Coronary artery disorder (CAD) has been featured as the leading cause of death in diabetic patients (Rasoul *et al.,* 2009).

Old age, smoking, systemic hypertension, diabetes, dyslipidemia, decreased physical exercise and poor diets are a good example of cardiovascular disease (CVD) risk factors (Tzoulaki *et al.,* 2016). Dyslipidemia manifested as high LDL, high TG and low HDL is an important risk factor for development of CVD. The Current guidelines is focus on lowering LDL cholesterol with statins in both primary and secondary preventive situations **(**NCEPEPo and ToHBCi, 2002; Grundy *et al.,* 2005); Brunzell *et al.,* 2008**).** This approach is supported by evidence from the recent analysis of 14 statin large perspective studies including 90,056 patients. The result of the analysis revealed that, decreasing LDL by 39 mg /dl was associated with about one fifth reductions in the 5-year incidence of major CV events (Unit ES, 2005).

Oxygen free radicals and lipid peroxides have been implicated in the pathogenesis of a large number of diseases such as DM, cancer, rheumatoid arthritis and atherosclerosis. MDA is a three-carbon dialdehyde that can exist in various forms in an aqueous solution (Tangvarasittichai *et al.,* 2009).It is also highly toxic by product formed in part by lipid oxidation from free O_2radicals, and many studies reported its higher levels in DM, reacting both reversibly and irreversibly with proteins and phospholipids with serious consequences (Slatter *et al.,* 2000).CVD remains the main cause of morbidity and mortality among patients with DM. DM contributes to atherosclerosis and heart failure through many mechanisms (Low Wang *et al.,* 2016). Framingham Heart Study (1974) showed a direct association between diabetes and heart failure (Singer *et al.,* 1992).

There is evidence that increased oxidative stress in DM contributes to the development of diabetic complications (Baynes JW and Thorpe SR, 1999). Oxygen derived free radicals interact with the lipid bilayer in cell membranes leading to its peroxidation. A plethora of adverse physiological consequences of elevated MDA levels includes leakiness of cell membranes by altering structural integrity of membrane; inactivation of membrane bound enzymes, inactivation of surface receptor molecules leading to cell-regulating errors, the involvement of oxidized LDL in the foam cell formation leading to atherosclerosis has been documented (Rashida *et al.,* 2010).MDA is a stable end product of lipid peroxidation and its measurement is considered a reliable marker of oxidative damage.

The hypothesis of the present study is that serum MDA levels is increase and its associate with cardiovascular disease risk factors in Type 2 diabetic patients. The aim of this study was to evaluate serum MDA levels as a biomarker of lipid peroxidation and its relationship with cardiovascular disease risk factors in Type 2 diabetic patients in Zaria.

METHODS

The study was conducted in the Departments of Chemical Pathology and Medicine of Ahmadu Bello University Teaching Hospital (ABUTH), Zaria, Nigeria. A total of 253 subjects were recruited for the study. We sourced the subjects (138) with mean age of 52±1.0 years from diabetic patients attending Medical Out-patient Department (MOPD) Clinic and 115 apparently healthy subjects with mean age of 51±0.9 years. The apparently healthy subjects (controls) were recruited from the population of the study area. Diabetes mellitus status and subject selection was confirmed biochemically according to World Health Organization diagnostic criteria for classification of diabetes. The approval of the study was obtained from the Ethical Committee of the faculty of medicine, Ahmadu Bello University, Zaria, in accordance with Helsinki declaration. Written informed consent was obtained from every participant.

Blood Pressure and Anthropometric Measurements

Systolic and diastolic blood pressure was recorded using aspignomamometer and steatoscope. Blood pressure was recorded as obtained from the right arm of seated subjects at 1-min intervals after a 10-min rest period. Weight was measured to within 0.1 kg before breakfast and following a 12-h fast, with subjects wearing light clothing and no shoes, using (HANA) a mechanical bath room scales (Model:BR). Height was measured to within 0.1 cm using a wall-mounted stadiometer. BMI was calculated as weight in kilograms divided by the square of height in meters^2.

Serum /Plasma Biochemistry

Hettich Universal 32 Centrifuge (Germany) was used to spin the blood specimens. Serum MDA concentration was assayed using an enzymatic method of Draper and Hardley (1990). Serum MDA concentrations were read spectrophotometrically at 532 nm on Beckman Coulter DU-520 general purposes UV/VIS Spectrophotometer (Germany). The reagents that were used for the measurements of MDA were procured from BDH Chemicals Limited (Poole Dorset, England). All the chemicals were of analytical grade (99.9 %). Serum glucose concentrations, cholesterol, triacylglycerol and high density lipoproteincholesterol (HDL) were also analyzed with the Selectra XL Series, Vital Scientific, Netherlands; Serial no.6-8096; analyzer using a commercial enzymatic kit (ELiTech Group Empowering IVD, Sees, France). Low density lipoprotein cholesterol (LDL) concentrations were calculated using Friedewald equation (Friedewald *et al.,* 1972). Artherogenic indices (ratio) were calculated as the concentration of total cholesterol divided by the concentration of high density lipoprotein cholesterol.

Glycaed Haemoglobin (HbA1c) in EDTA treated plasma was assayed using a commercially available Kit (Labcare Diagnostic, Gujarat, India) by the Ion Exchange Resin Method. Beckman Coulter DU-520 general purposes UV/VIS Spectrophotometer (Germany) was used to measure the concentrations of HbA1C.

Statistical Analyses

We used SPSS software for Windows (version 16; SPSS, IL) to perform statistical analyses. Serum MDA, lipids, glucose and glycated haemoglobin concentrations obtained from diabetic patients were compared with those of apparently healthy individuals (controls) using two-tailed student's t-test. Correlation analysis was carried out using Pearson's linear correlation analysis. $P< 0.05$ was considered statistically significant.

RESULTS

The mean values of serum MDA, TG, FBG and HbA1cwere higher in diabetic patients than those of controls (p=0.01). Whereas serum TC, HDL, LDL and TC:HDL in diabetic patients were similar to those of controls (p>0.05). The mean values of weight, BMI, systolic and diastolic blood pressure were higher in diabetic patients than those of controls (p<0.05).There were no correlations between serum MDA and lipids (TC, TG, HDL, LDL,TC:HDL), FBG, HBA1c, BMI, BP and Duration of Diabetes in diabetic patients (p>0.05)

Table 1: Clinical parameters (mean ± SEM) in diabetic patient and control

Subject	n	Age	Height	Weight	BMI	Systolic	Diastolic	D(Year)
		(year)	(m)	(kg)	(kg/m^2),	(mmHg)	(mmHg)	
Patient	13852±1.0	1.6±0.01		75±`1.4	29.2±0.5	139±2.1	88.±1.3	6±STD0.5
Controls	115`	51±0.9	1.7±0.01	71±1.2	25.0±0.4	124±1.6	79±1.1	NA
p-value	p=0.87	p=0.95	p=0.02	p=0.01	p=0.02	p=0.03	NA	

n= Number of subjects, BMI = Body Mass index, D= Duration mellitus, SEM = Standard error of mean and NA = Not applicable

Table 2: Serum Malondialdehyde and other biochemical analytes in diabetic patients and control

Subjects	n	TC	TG	HDL	LDL	TC:HDL	MDA	FBG	GHbA1C
		(mmol/L)	(mmol/L)	(mmol/L)	(mmol/L)		(µmol/L)	(mmol/L)	(%)
Patients	138	4.5±0.1	1.5±0.1	1.1±0.0	2.7±0.1	4.0±0.1	2.1±0.1	8.7±0.4	7.7±0.2
Controls	115	4.4±0.1	1.1±0.0	1.3±0.0	2.6±0.1	3.7±0.1	0.7±0.0	4.9±0.1	5.9±0.2
p-value	p=0.94	p=0.01	p=0.73	p=0.81	p=0.18	p=0.01	p=0.01	p=0.01	

n= Number of subjects, TC= total cholesterol, TTG= Triglyceride, HDL = High density lipoprotein, LDL = Low density lipoprotein TC: HDL = Total High Density lipoprotein MDA = Malondialdehyde, FBG= Fast Blood Glucose, GHbA1c = Glycated haemoglobin SEM = Standard error of mean

Table 3: Correlation analysis in diabetic patients (Pearson's linear Correlation)

Independent Variables	Dependent Variable	Patients	
		r	p-value
Biochemical CVD Risk Factors			
MDA (µmol/L)	FBG (mmol/L)	0.013	0.723
TC (mmol/L)		0.214	0.147
TG (mmol/L)		0.098	0.148
HDL (mmol/L)		0.125	0.314
LDL (mmol/L)		0.126	0.281
TC:HDL		0.020	0.814
Clinical CVD Risk Factors			
BMI		0.015	0.910
Systolic Blood Pressure (mmHg)		0.015	0.621
Diastolic Blood Pressure (mmHg)		0.211	0.421

BMI = Body Mass Index, CVD= Cardiovascular disease, r= Coefficient of correlation, p= probability value, MDA = Malondialdehyde , TC= Total cholesterol, TG= Triglyceride, HDL = High Density Lipoprotein, LDL = Low Density Lipoprotein, TC: HDL= Total cholesterol and High Density Lipoprotein ratio, and FBG= Fasting Blood Glucose.

DISCUSSION

Numerous studies have indicated that oxidative stress plays a major role in the pathogenesis of Type 2 diabetes mellitus (T2DM). Free radicals are formed disproportionately in diabetes mellitus (DM) by glucose degradation, non-enzymatic glycation of proteins and subsequent oxidative degradation which may play an important role in the development of complications in Type 2 diabetic patients.

In the present study, the serum MDA levels were significantly elevated in Type 2 diabetic patients. This is in agreement with the reports of other researcher such as (Tangvarasittichai *et al.,* 2009; Nakhjavani *et al.,* 2010; Bhutia *et al.,* 2011; Begum and Dsouza, 2015; Deepak *et al.,* 2019;Kehdr and Badran, 2020). The significant elevation of serum MDA levels in Type 2 diabetic patients demonstrates that there was lipid peroxidation which is an indication of free radical damage in T2DM in the present study. The findings of the present study were also in accordance with Mahreen *et al.* (2010) (a study performed on a sample of Indian subjects). The study Participants were divided into 3 groups, 30 diabetic T2DM without any complications, 30 T2DM patients with myocardial infarction (MI) and 30 healthy subjects as control group. The results demonstrated significantly higher levels of MDA in the diabetic group compared to the control. In addition to that, a significantly higher MDA level was observed in the T2DM group with myocardial infarction compared to the T2DM without complications. They offer two probable explanations for these findings either the longer disease duration or increased glycation of serum proteins which leads to increased activation of receptors for advanced glycation end products which initiates the process of atherosclerosis (Mahreen *et al.,* 2010). Experimental study have also shown that Type 2 diabetic individuals are at increased risk of free radical activity which is associated with the increased activity of free radical-induced lipid peroxidation and accumulation of lipid peroxidation products as well as being associated with a substantially higher prevalence of atherosclerositic and cardiovascular mortality (Tangvarasittichai *et al.,* 2009). According to Javad *et al.*(2014) antibodies to MDA adducts macromolecules have been detected in the serum of patients with Coronary Artery Disease (CAD) and linked with the progression of the disorder.

It is well documented that an increased level of MDA in diabetics suggests that peroxidative injury may be involved in the development of diabetic complications. The increase in lipid peroxidation is also an indication of decline in defense mechanisms of enzymatic and non-enzymatic antioxidants (Saddala *et al.,* 2013). Increased MDA level in plasma, serum, and many other tissues has been observed in diabetic patients (Moussa S., 2008).Baynes and Ramesh *et al.*in 1991 and 2012 respectively reported that lipid peroxidation in diabetes induced many secondary chronic complications including atherosclerosis and neural disorders. Yang *et al.*(2009) reported greater serum lipid peroxidation evaluated in terms of MDA in hyperglycemic mice and proposed that the increase in lipid peroxidation exacerbated the occurrence of myocardial infarction through NADPH oxidase activation.

According to Noberasco *et al.* (1991)the significant increase in MDAdue to increased free radical production and decrease in GSH and GPx levels of patients with type 2 DM suggests permanent structural membrane alterations in diabetic patients. Rani *et al.*(2005) also reported lower total antioxidant status values in type 2 diabetic group compared to the control group and concluded that there is an increased oxidative stress in diabetics compared to their non-diabetic counterparts and emphasized the importance of assessing these markers for early diagnosis and therapeutic interventions. The pro-oxidant-antioxidant imbalance in diabetes maybe due to either acceleration of cellular reactions leading to increased free radical production, such as non-enzymatic protein glycation, glucose oxidation and increased sorbitol pathway, or reduced antioxidant defense potential (Ozdemir *et al.,* 2005). Free oxygen radicals in DM cause peroxidative breakdown of phospholipids that leads to accumulation of MDA.

MDA plays a key role in modifying low density lipoprotein (LDL) which mediates the pathophysiological changes by non-enzymatic and auto oxidative glycosylation (Bhutia *et al.,* 2011). The absolute level of atherogenic lipoproteins in circulation is a major factor in the risk of cardiovascular diseases, and this oxidative modification of blood lipids further increased this risk. Oxidized LDL particles are more readily internalized by macrophages and thereby enhance foam cell formation which, in the vascular wall favours smooth muscle cell proliferation, increases platelet adhesion and the anticoagulant activity of the endothelium. These consequences of oxidative stress can promote the development of complications in diabetic patients including cardiovascular diseases (CVD).

The adverse physiological consequences of elevated MDA levels includes leakiness of cell membranes by altering structural integrity of membrane; inactivation of membrane bound enzymes, inactivation of surface receptor molecules leading to cell-regulating errors. These could also explain the reason of elevation of serum MDA seen in the present study. Overproduction of ROS interferes with the structure of PUFAs and causes loss of fluidity in the biological membranes. MDA may oxidize PUFAs which is responsible for different levels of cell destruction (Mahmut *et al.,* 2007). The present study found a significantly higher TG level in diabetic than the control group which is in-line with Tangvarasittichai *et al.* (2009) who observed significantly higher TG levels in diabetics than the control group. The significant elevation of serum TG levels in Type 2 diabetic patients observed in the present study indicates dyslipidaemia. Significant increases in serum TG may be caused by insulin resistance. This equally demonstrates the presence of insulin resistance in Type 2 diabetic subjects in this study. The precise pathogenesis of diabetic dyslipidaemia is not known. However, a large body of evidence suggests that insulin resistance has a central role in the development of this condition.

The main cause of the three cardinal features of diabetic dyslipidaemia is the increased free fatty-acid release from insulin-resistant fat cells. The increased flux of free fatty acids into the liver in the presence of adequate glycogen stores promotes triglyceride production, which in turn stimulates the secretion of apolipoprotein B (ApoB) and VLDL cholesterol (Arshag, 2009). This may explain the increase levels of TG seen in the present study.

The increase in the levels of TG is pointing-out towards oxidative stress as polyunsaturated free fatty acids (PUFA) undergo degradation to form lipid hydroperoxides (Tangvarasittichai *et al.*, 2009). These latter compounds decompose to form a wide variety of products: low-molecular mass hydrocarbons, hydroxyl aldehydes and fatty acids. Others include ketone, alkenals, alkanals and MDA. The characteristic features of diabetic dyslipidaemia are a high plasma triglyceride concentration, low HDL cholesterol concentration and increased concentration of small dense LDL-cholesterol particles. Atherosclerosis and its complications are a major cause of mortality and morbidity among patients with type 2 diabetes mellitus. This increased risk of cardiovascular complications has many causes including dyslipidaemia, hypertension and cigarate smoking (Seng *et al.*, 1999).

The results of the present study also showed lower levels of HDL in diabetics than control but no significant difference between both groups, which agree with findings by Soliman, (2008) and Tangvarasittichai *et al.*(2009).

With respect to TC when comparing between the two groups, it did not differ significantly. This was contrary to the findings of Tangvarasittichai *et al.* (2009), Soliman, (2008) and Rani *et al.* (2005).

In regards to LDL levels there was no significant difference between the two groups, which contradicts findings by Soliman (2008) and Tangvarasittichai *et al.*(2009).

The present study did not found correlations between MDA and lipid profile parameters (TC,TG, HDL, LDL and TC/HDL ratio). This was similar to the findings of Hemn *et al.* (2021). However, Shalash *et al.*, (2020), Tangvarasittichai *et al.,* (2009) and Hamad *et al.*,(2009) disagree with the results of the present study. With regards to HDL, the report of Hamad *et al.*(2009) is in-line with the findings of the present study as they found no significant relation between HDL level and MDA in type 2 diabetic patients while it contradict the findings of Shalash *et al.* (2020) and Tangvarasittichai *et al.*(2009) as they found significant inverse correlation between serum MDA level and HDL. Many other studies found significant negative correlation between MDA level and HDL in type 2 diabetic patients (Sharma *et al.,* 2017; Aldebasi *et al.*,2013).

The present study found no correlations between MDA and fasting blood glucose or glycated haemogloin. This is in agreement with the findings of Shalash *et al.* (2020),who found no significant correlation between MDA level and fasting blood glucose (FBG) or glycatedhemoglobin in both cases and the control group, but they found significantly higher levels of MDA indiabetics compared to the control group.

The present study found no correlations between MDA and blood pressures. This is in agreement with the findings of Ekeanyanwu *et al.* (2016) who found no significant correlation between MDA levels and blood pressure. In addition, the results of the present study demonstrated significant difference in MDA levels between the two groups. This finding coincides with findings of Dominguez *et al.* (2010) and Shalash *et al.* (2020).

CONCLUSION

It can be concluded from the findings of this study that: there is increase in free radical activity, oxidative stress, lipid peroxidation and Dyslipidaemia among diabetic patients in Zaria.

It was also concluded that there were weak association between MDA and CVD risk factors among diabetic patients in Zaria.

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REFERENCES

Alkadi, Hourieh(2020). A Review on Free Radicals and Antioxidants. *Infectious Disorders - Drug Targets (Formerly Current Drug Targets - Infectious Disorders),*20(1):16-26(11).

Aikaterini Andreadi[1] [2]Alfonso Bellia[1] [2]NicolaDi Daniele[1] [2]Marco Meloni[1] [2]Renato Lauro[1] David Della-Morte[1 2 3] [4]Davide Lauro[1 2](2022).The molecular link between oxidative stress, insulin resistance, and type 2 diabetes: A target for new therapies against cardiovascular diseases. *Current Opinion in Pharmacology* 62:85-96.

Aldebasi YH, Mohieldein AH, Almansour YS, Almutairi BL. Dyslipidemia and lipid peroxidation of Saudi type 2 diabetics with proliferative retinopathy. Saudi Med J.2013;34(6):616-22.

Arshag D Mooradian (2009). Review: Dyslipidaemiain Type 2 diabetemellitus. *Nature Clinical Practice Endocrinology and Metabolism 5150-159.*

Baynes JW (1991). Role of oxidative stress in development of complications in diabetes. Diabetes. 40(4):405-12.

Brunzell JD, Davidson M, Furberg CD, Goldberg RB, Howard BV, Stein JH, *et al*(2008). Lipoprotein management in patients with cardiometabolic risk: consensus statement from the American Diabetes Association and the American College of Cardiology Foundation. Diabetes care. 31(4):811-22.

Begum MJA, Dsouza H (2015). Myeloperoxidase, Malondialdehyde and serum Lipids in Type 2 diabetes mellitus.*Journal of Investigational Biochemistry,* 4:13.

Bhutia Y, Ghosh A, Sherpa ML, Pal R, Mohanta PK (2011). Serum malondialdehyde level: Surrogate stress marker in the Sikkimese diabetics. *Journal Natural Science Biology Medicine* 2: 107-12.

Detection NCEPEPo, Adults ToHBCi (2002). Third report of the National Cholesterol Education Program (NCEP) Expert Panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III): National Cholesterol Education Program, National Heart, Lung, and Blood ….

Dominguez LJ, Galioto A, Pineo A, Ferlisi A, Ciaccio M, Putignano E, *et al*. Age, homocysteine, and oxidative stress: relation to hypertension and type 2 diabetes mellitus. Journal of the American College of Nutrition. 2010;29(1):1-6.

Deepak Basia, Amrita Kulhria and Ravi Tandon. (2019). Correlation of Oxidative Stress Markers, Antioxidants, Vegf and Apoa-I in Patients of Type 2 Diabetes Mellitus without Retinopathy.*International Journal of Advanced Research in Medicine* 1(1): 37-41

Ekeanyanwu RC, Ejiogu RN, Egbogu MC (2016). Lipid peroxidation and non-ezymatic antioxidant status in hypertension in diabetic and non-diabetic patients in Nigeria: a comparative study. Biomedical Research, 27(1): 250-256.

Friedewald WT., Levy RT., Fredricson DS. (1972). Estimation of the concentration of LDL-C in plasma without use of the Preparative Ultracentrifuge. *Clinical Chemistry,* 18:499-502.

Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al (2005). Diagnosis and management of the metabolic syndrome: an American Heart Association/ National Heart, Lung, and Blood Institute scientific statement. Circulation, 112(17): 2735-52.

Hamad MS, Khalaf SJ, Sarhat ER. Relationship between malondialdehyde activity (MDA) & Lipid Profile in Diabetic Patients. Tikret Journal of Pharmaceutical Sciences. 2009; 5(1): 27-32.

Hemn Janeel Majeed, Gulzar Ismael Ibrahim, Pshtiwan Abdullah Yousif, SamanMuhsin Abdulkareem (2021).Association between some serum oxidative stress biomarkers and lipid profile in type 2 diabetic patients in Erbil City. *Biology and Medical Researches,* 33(1).DOI: https://doi.org/10.21271/ZJPAS.33.1.11

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Kehdr Y., Badran M(2020). Assessment of levels of selected trace elements as predictors of oxidative stress in type 2 diabetic patients using Multivariate Statistical Analysis.*J. Elem.*26(1): 47-58. DOI: 10.5601/jelem.2020.25.3.1995.

Low Wang CC, Hess CN, Hiatt WR, Goldfine AB (2016). Clinical update: cardiovascular disease in diabetes mellitus: atherosclerotic cardiovascular disease and heart failure in type 2 diabetes mellitus–mechanisms, management, and clinical considerations. Circulation. 133(24):2459-502.

Mahreen R, Mohsin M, Nasreen Z, Siraj M, Ishaq M (2010). Significantly increased levels of serum malonaldehyde in type 2 diabetics with myocardial infarction. International journal of diabetes in developing countries. 30(1):49.

MahmutBulut, SalihSelek, H. SerdarGergerlioglu, Haluk A. Sayas, H. RamazanYilmaz, Murat Yuce, GiyasettinEkici (2007). Malondialdehyde levels in adult attention-deficit hyperactivity disorder. *Journal of Psychiatry Neuroscience* (32 1(6): 435—438.

Medina LO., Veleso CA., Borges EA., Isoni EA., Calsolari MR., Chaves MM. Nogueira Machado JA(2007). Determination of the Antioxidant Status of Plasma from type II DM. *Diabetes Research Clinical Practice*, 77: 193- 297.

Moghadam RH, Latiffah A (2009). Investigation of lipid profiles and lipid peroxidation in patients with type 2 diabetes. European Journal of Scientific Research. 28(1):6-13.

Moussa SA(2008). Oxidative Stress in Diabetes Mellitus. *Romanian Journal Biophysics*, 18(3):225-236.

Nakhjavani M, Esteghamati A, Nowroozi S, Asgarani F, Rashidi A, Khalilzadeh O (2010);. Type 2 diabetes mellitus duration: an independent predictor of serum malondialdehyde levels. *Singapore Med J.* 51(7): 582. Noberasco G, Odetti P, Boeri D, Maiello M, Adezati L (1991). Malondialdehyde (MDA) level in diabetic subjects. Relationship with blood glucose and glycosylated hemoglobin. *Biomed Pharmacother*45(4-5): 193-6.

Ozdemir G, Ozden M, Maral H, Kuskay S, Cetinaip P, TarkunI (2005). Malondialdehyde, glutathione, glutathione peroxidase and homocysteine levels in type 2 diabetic patients with and without microalbuminuria. *Annals of Clinical Biochemistry* 42(Pt 2):99-104.

Palanisamy P., Govindasamy B., Ganesan S., Raman L(2009). Evaluation of Oxidative Stress and Antioxidant Status in Patients with diabetes Mellitus. *Journal of Applied Science Research,* 5(7): 770-775.

Ramesh B, Karuna R, Sreenivasa RS, Haritha K, Sai MD, Sasis BRB, *et al* (2012). Effect of Commiphoramukul gum resin on hepatic marker enzymes, lipid peroxidation and antioxidants status in pancreas and heart of streptozotocin induced diabetic rats. Asian Pacific journal of tropical biomedicine. 2(11):895-900.

Rani HS, Madhavi G, Rao VR, Sahay B, Jyothy A. Risk factors for coronary heart disease in type II diabetes mellitus. Indian journal of clinical biochemistry. 2005;20(2):75-80.

Rasoul Z., Haleh R., Zohreh M., Mahdi T., Reza R., Mohamad KD (2009). Evaluation of Coronary Artery Disorders in Diabetic Patients with no or Atypical Cardiac Symptoms. *European Society of Endocrinology* 20:345.

Saddala RR, Thopireddy L, Ganapathi N, Kesireddy SR (2013). Regulation of cardiac oxidative stress and lipid peroxidation in streptozotocin-induced diabetic rats treated with aqueous extract of Pimpinellatirupatiensis tuberous root. Experimental and Toxicologic Pathology. 65(1-2):15-9.

Sharma D, Garg S, Mehndiratta M, Madhu S, Puri D. Relationship of serum apolipoprotein AV levels, oxidative stress and inflammatory biomarkers with hypertriglyceridemia in type 2 diabetes mellitus. International journal of endocrinology and metabolism. 2017; 15(2).

SengKheeGan, Yuen M., Welborn M (1999). Hyperlipidaemia in diabetes. *Australian Prescriber* 22(3): 1-4.

Soliman G. Blood lipid peroxidation (superoxide dismutase, malondialdehyde, glutathione) levels in Egyptian type 2 diabetic patients. Singapore medical journal. 2008; 49(2): 129.

Shalash, M.[(1)], Badra, M. [(1)], Imbaby, S. [(2)], ElBanna E. [(3)] (2020). Malondialdehyde In Type 2 Diabetics And Association With Cardiovascular Risk Factors. *Journal of the Medical Research Institute*, 41(2): 21-30.

Tzoulaki I, Elliott P, Kontis V, Ezzati M (2016). Worldwide exposures to cardiovascular risk factors and associated health effects: current knowledge and data gaps. Circulation.133(23):2314-33.

Tiganis T(2009). Antioxidants Could Trigger type II Diabetes, So Use Them in Moderation Say Researchers. *Medical News Today*; 10:260-272.

Tangvarasittichai Surapon, Praparporn Poonsub, Orathai Tangvarasittichai, ViruchSirigulsatien. (2009). Serum levels of malondialdehyde in type 2 diabetes mellitus Thai subjects. *Siriraf Medical Journal* 6 1(1).

Unit ES(2005). Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90 056 participants in 14 randomised trials of statins. Lancet (London, England). 366(9493):1267-78.

World Health Organization (2020). Classification of diabetes mellitus.

Xiao-Qiang Wang[ab1]Wenjing Wang[b1]MengyunPeng[ac]Xian-ZhengZhang[a](2021). Review: Free radicals for cancer theranostics. *Biomaterials*,266:120474.

Yang Z, Laubach VE, French BA, Kron IL (2009). Acute hyperglycemia enhances oxidative Sstress and exacerbates myocardial infarction by activating nicotinamide adenine dinucleotide phosphate oxidase during reperfusion. The Journal of thoracic and cardiovascular surgery. 137(3):723-9.