



Evaluation of Oxidative Stress level and some Antioxidant enzymes activity parameters in patients with type two diabetes mellitus

DARYA ASSI YOUNUS^{1,9*}, REBAZ M. MUSTAFA^{2,8}, REZHNA ADIL RASHID³,
SAYFADDIN SADRADDIN HAMAD⁴, HEMN RASUL SALIH⁵, DLSHAD
SAADALLA OTHMAN⁶, KAMARAN KAIANI ABDOULRAHMAN⁷

¹Department of Emergency Nursing, Haibat Sultan Technical Institute, Koya, Erbil, Kurdistan Region, 46017, Iraq.

²Department of Chemistry, Faculty of Science and Health, Koya University, Koya KOY45, Kurdistan Region-F.R. Iraq.

³Department of Medical Laboratory Technology, Koya Technical Institute, Erbil Polytechnic University, Koya, Erbil, 46017, Iraq.

⁴Department of Nursing, Koya Technical Institute, Erbil Polytechnic University, Kurdistan Region, 46017, Iraq.

⁵Department of Medicine, Koya University, Koya, Erbil, Kurdistan Region, 46017, Iraq.

⁶Department of Medical Laboratory Technology, Koya Technical Institute, Erbil Polytechnic University, Koya, Erbil, 46017, Iraq.

⁷Chemistry Department, College of Science, Salahaddin University, 46071, Erbil, Iraq.

⁸Volumetric Apparatus and Instrumental Analysis, Modern Surveying Calibration and Testing Labs, Erbil, Kurdistan, Iraq.

⁹Department of Medical Laboratory Technology Taq Taq, Koya Technical Institute, Erbil Polytechnic University, Koya, Erbil, 46017, Iraq.

*Corresponding author E-mail: darya.00598460@gmail.com

<http://dx.doi.org/10.13005/ojc/390621>

(Received : August 09, 2023; Accepted: December 22, 2023)

ABSTRACT

Diabetes is a group of metabolic conditions characterized by abnormalities in the regulation of oxidative stress and elevated blood sugar levels. Focusing on malondialdehyde (MDA) and glutathione (GSH), antioxidant and lipid peroxidation levels in the serum of type II diabetes patients were investigated. The study analyzed one hundred forty serum samples from ninety type II diabetes patients and fifty healthy individuals. MDA and GSH levels represent oxidative status and antioxidant activity, respectively. In addition, lipid profiles, including total cholesterol (CH), triglycerides (TG), low-density lipoprotein cholesterol (LDL), and high-density lipoprotein cholesterol (HDL), were evaluated. Findings revealed significantly higher MDA, total cholesterol, triglycerides, and glucose levels in the diabetic group compared to controls. Conversely, GSH and H2DL levels were notably lower in diabetics. LDL levels did not differ significantly between the two groups. In a group of type 2 diabetes patients, it was found that there is a correlation between glucose levels and MDA concentration. However, no other notable correlations were identified between lipid profile parameters, glucose levels, and MDA or GSH levels. The research highlights the association between diabetes and the elevation of free radicals, as well as a related decrease in antioxidant synthesis, hence contributing to the occurrence of oxidative stress.

Keywords: Type II diabetes mellitus, Oxidative stress, Antioxidants, Lipid profile, Blood sugar.



INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a common metabolic disorder marked by insulin resistance and elevated blood glucose levels^{1,2}. Diabetes is becoming a growing health problem and affects nations regardless of their level of development³. According to the WHO, type II diabetes will be the most common disease in 2030, potentially affecting more than 180 million people worldwide⁴.

Normal metabolism produces reactive oxygen radicals in healthy people. It is believed that an imbalance between the antioxidant system, which acts as a defense mechanism, and the antioxidant system, which favors radicals, causes oxidative stress⁵. In pathophysiological situations such as atherosclerosis, diabetes, cancer, chronic inflammatory diseases, and diseases of the central nervous system. One of the main factors in cellular aging and therefore cell destruction, cell damage and cell death is Oxidative stress^{6,7}. In addition, it can be crucial in the development of micro- and macro-vascular problems⁸. Uncontrolled hyperglycemia results in an elevation of free radical production, specifically reactive oxygen species (ROS) which occurs via processes of protein glycosylation and autooxidation of glucose⁹. The breakdown of unsaturated lipids, along with increased lipid peroxidation, is a well-known mechanism responsible for cell damage resulting from oxidative stress¹⁰. There is strong evidence that diabetes is associated with increased oxidative stress, characterized by increased production of reactive oxygen species (ROS) and impaired antioxidant defense mechanisms¹¹. The toxicity of the fatty acid peroxides produced and the induction of membrane lipid peroxidation by ROS have been proposed to significantly contribute to cellular dysfunction¹². Due to its simplicity, measurement of malondialdehyde (MDA) is the most commonly used test for lipid peroxidation. Therefore, assessment of lipid peroxide levels in the bloodstream provides valuable information about the prognosis of diabetes, complications of which often lead to death¹³. Antioxidants, like glutathione (GSH), act as potent electron donors by providing hydrogen atoms to pair with the unpaired electrons of free radicals¹⁴. This converts reactive free radicals into inactive substances¹⁵. In the present study, the complex balance of oxidative stress and antioxidant markers

with particular emphasis on MDA and GSH levels was investigated through a detailed assessment of the lipid profile in individuals with type 2 diabetes. This research can provide valuable insights into the dynamic interplay of these biomarkers and reveal differences in the biochemical environment compared to the non-diabetic group.

MATERIALS AND METHODS

Patients and setting

In this study, blood samples from 75 individuals with type II diabetes (males and females) and 30 individuals in excellent health were analyzed for the presence of a variety of biomarkers. The investigated components included total serum cholesterol, triglycerides, lipoproteins (specifically HDL and LDL), and decreased GSH and MDA levels. This examination was conducted at the Taq Taq General Hospital in Koya-Erbil, Kurdistan Region, Iraq.

Ethical consideration

This research followed the guidelines of the Declaration of Helsinki. Approval for the study (ID: 3/45/109) was obtained from the Ethics Committee of the Chemistry Department, Faculty of Science and Health, Koya University. Before participating in the study, all subjects provided written informed consent.

Serum sampling

After fasting, five milliliters of venous blood are drawn from both patients and healthy individuals. The collected blood was immediately deposited in yellow tubes and centrifuged at 4000 revolutions per minute (rpm) for 10 min at 4 degrees Celsius. The serum was then refrigerated until it was ready to be analyzed.

Laboratory serum analysis

Following the methodology proposed by^{16,17}, the enzymatic total serum concentration of cholesterol and triglyceride were estimated depending on the enzymatic conversion of cholesterol to quinonimine pigment and the hydrolysis of triglyceride with lipases, then the absorbance spectrophotometrically was measured. The LDL- and HDL-cholesterol levels were also measured according to an outlined method^{18,19}, and serum lipid profiles were measured through the use of standard kits from BIOLAB GOD-PAP (France).

Serum glucose is determined through the use of standard kits from BIOLAB GOD-PAP (France), which depend upon the enzymatic conversion of glucose by the glucose oxidase (GOD) to red-violet quinonimine as an indicator.

The assessment of reduced GSH levels was carried out following the method studied by Buege and Aust²⁰. The concentration of glutathione (GSH) was determined by observing the reaction's final product, which is formed when non-sulfhydrylated (SH) proteins react with the precipitation solution. The addition of sulfhydryl groups to DTNB (5', 5'-) (dithiobis 2-nitrobenzoic acid) produced a solution with a distinct yellow hue. The spectrophotometer measured reduced glutathione in blood samples treated with EDTA anticoagulant at a wavelength of 412 nm after a 24-h period.

The concentration of MDA (malondialdehyde), an indicator of lipid peroxidation, was determined by reacting MDA with thiobarbituric acid (TBA), which produced a mauve substance. Following the procedure outlined in the reference, this product was allowed to come to room temperature before having its absorbance measured at a wavelength of 532 nm using a UV/Vis spectrophotometer²⁰.

Data analysis

The data was analyzed using a T-test on independent samples using the statistical software Social Science Package. The results were presented as the mean plus standard deviation (mean + S.D.). In statistical analysis, findings with a P-value were greater than 0.05 were deemed non-significant (NS), whereas those with a P-value less than 0.05 were deemed significant. In addition, the Person's Correlation coefficient (*r*) was used to evaluate the relationships between the evaluated parameters.

RESULTS

A total of 90 people with type II diabetes took part in our study. The average age was 56 years and ranged from 20 to 90 years. Of the 90 patients, 45 (50%) were men and all were married. 56.6% had a family history of type 2 diabetes. However, 57 (63.3%) patients were hypertensive (Table 1).

Table 1: Sociodemographic of participants

Variable	Frequency (N)	Percentage %
Age		
20–40 years	14	15.5%
40–60 years	40	44.4%
Above 60 years	36	40%
Gender		
Male	45	50%
Female	45	50%
Family history with Types II Dm		
With family history Types II Dm	51	56.6%
Without family history Types II DM	39	43.3%
Comorbidity		
Hypertensive	57	63.3%
Non hypertensive	33	36.6%

The results presented in Table 2 and Fig. 1 show a significant ($P < 0.05$) increase in the levels of cholesterol (284.0 mg/dl) and triglyceride (TG) (390.4 mg/dl) in individuals with diabetes compared to their healthy individuals, which is approximately (163.0 mg/dl) and (110.0 mg/dl) respectively. However, this examined increase in the LDL value from (95.18 to 118.1) mg/dl did not reach statistical significance. Furthermore, HDL levels showed a significant decrease in the sera of diabetic subjects (50.54 mg/dL) compared to the sera of healthy subjects (56.80 mg/dL) serving as controls.

Table 2: Values of lipid profile parameters in individuals diagnosed with type 2 diabetes mellitus

Parameter	Patients (N=90) Mean ± S.D	Control (N=50) Mean ± S.D	P-value
Cholesterol	284.0±38.30*	163.0±5.840	0.002
Triglyceride	390.4±70.60*	110.0±5.850	0.0001
HDL	50.54±1.850*	56.80±2.520	0.049
LDL	118.1±14.90	95.18±5.870	0.141

* $P < 0.05$: significant

Analysis of the data in Table 3 and Fig. 2 showed that serum glucose (257.8 mg/dl) and malondialdehyde (MDA) (0.550 $\mu\text{mol/L}$) levels in diabetic patients are significantly ($P < 0.05$) higher than those observed in healthy controls, while glutathione (GSH) levels (0.004 μM) in the sera of diabetics are significantly ($P < 0.05$) lower than those in healthy individuals (0.030 μM).

Table 3: Serum glucose, malondialdehyde (MDA), and glutathione (GSH) levels in patients with type 2 diabetes mellitus.

Parameter	Patients (N=90) Mean ± S.D	Control (N=50) Mean ± S.D	P-value
Glucose	257.8±9.370*	91.45±1.520	0.0001
MDA	0.550±0.070*	0.210±0.001	0.004
GSH	0.004±0.0007*	0.030±0.001	0.0001

* $P < 0.05$: significant

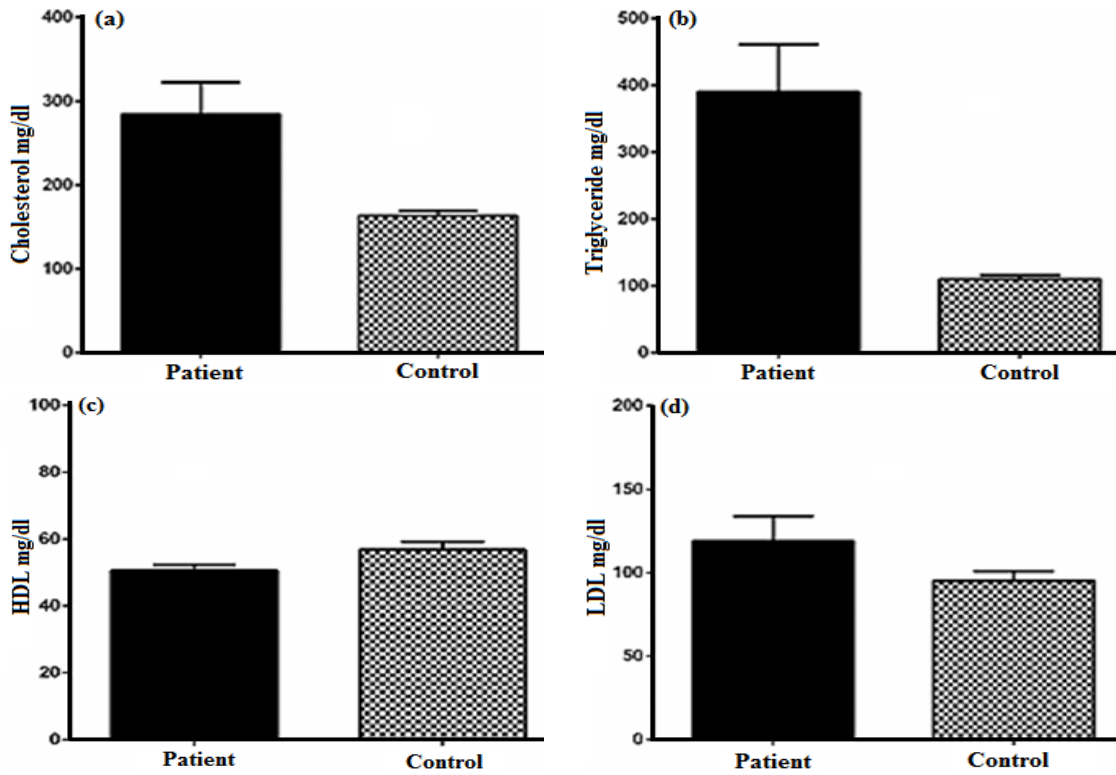


Fig. 1. Comparison of (A) total cholesterol, (B) triglyceride, (C) HDL-C, and (D) LDL-C levels between control and diabetic subjects

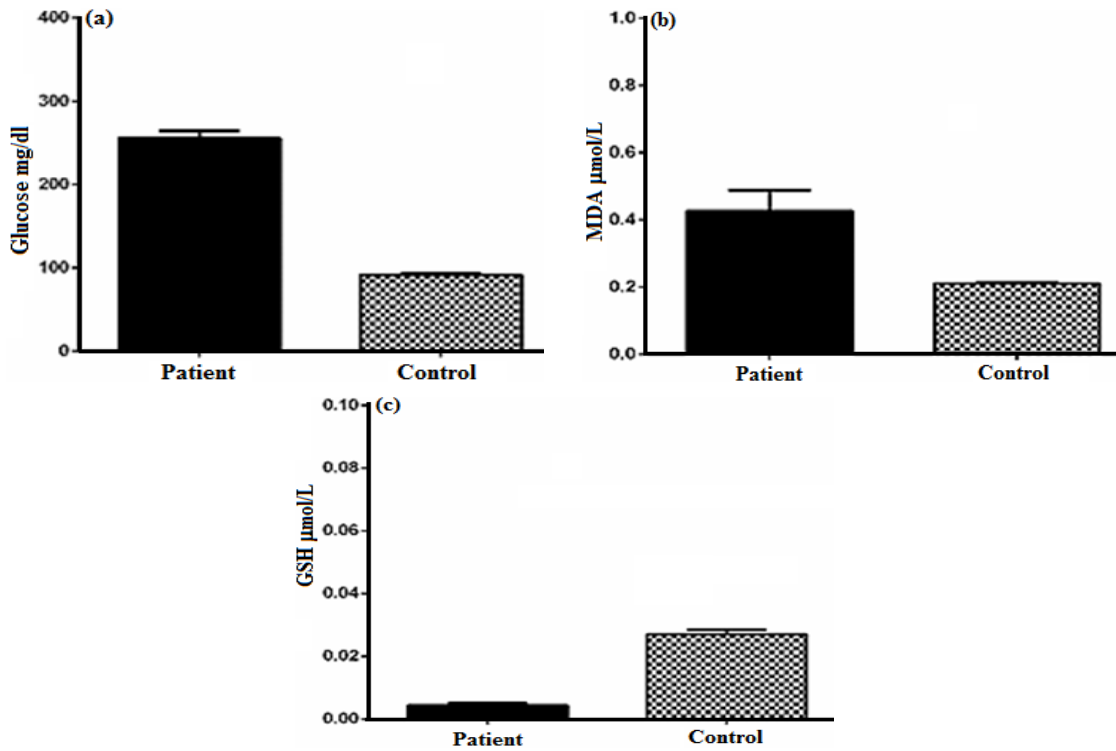


Fig. 2. Comparison of (A) glucose levels, (B) malondialdehyde (MDA) levels, and (C) reduced glutathione peroxidase levels between the control and diabetic groups

The correlation coefficients between oxidative stress (MDA) and antioxidants (GSH) and other evaluated parameters in patients with type 2 diabetes are shown in Table 4. Consequently, there is a non-significant positive correlation between lipid profile parameters and MDA concentration in diabetic patients. Notably, glucose concentration is the only parameter that has a significant positive correlation with MDA. Conversely, GSH levels in diabetics show a negative correlation that, although not statistically significant, is observed in parameters other than LDL such as cholesterol, HDL, triglycerides and glucose levels.

Table 4: Correlations among various biochemical parameters, antioxidants, and oxidative stress indicators in patients with type 2 diabetes.

Diabetic type II	GSH		MDA	
	r	P	R	P
Cholesterol	-0.06205	0.5635	0.2145	0.0745
HDL	-0.04733	0.6596	0.1647	0.1729
LDL	0.02100	0.8452	0.05267	0.6650
Triglyceride	-0.1291	0.2335	0.1480	0.2284
Glucose	-0.06439	0.5488	0.2245	0.0345*

*P<0.05: significant

DISCUSSION

Diabetes mellitus, a common metabolic disease, is closely linked to oxidative stress—a condition that results from an imbalance between the production of reactive oxygen species (ROS) and the body's ability to neutralize their harmful effects.³⁰ The present study aimed to evaluate oxidative stress and related antioxidants (GSH) and some biochemical parameters in a control group and patients with type 2 diabetes. This relationship plays a crucial role in the physiological disorders of diabetes and influences the progression of the disease and the occurrence of its complications. In type 2 diabetes, insulin resistance and a sustained increase in blood glucose levels combine to produce changes in lipid metabolism, resulting in an atypical lipid profile³¹. As characterized by this disorder, this study showed significant differences by increased total cholesterol, triglycerides and low-density lipoprotein cholesterol (LDL-C) in association with decreased high-density lipoprotein cholesterol (HDL-C) in diabetics compared to healthy people. The majority of diabetics suffer from insulin resistance, and several studies have shown that insulin affects the production of apo-lipoproteins in

the liver as well as the regulation of lipoprotein lipase activity and cholesterol transport protein, leading to dyslipidemia in diabetes mellitus²¹. Similar to our results, recently also showed an increase in lipid profile parameters in Iranian patients with diabetes²². Cardiovascular complications of diabetes, as well as diseases such as hypertension, dyslipidemia, and insulin resistance, are thought to result from reactive oxygen species (ROS) production, oxidative stress, and endothelial damage²³. Recently, Hyperglycemia can activate many signaling pathways, making cells sensitive to necrosis, apoptosis, and/or necrosis³¹. In individuals with poorly controlled diabetes, elevated blood sugar levels result in the release of free radicals, most notably reactive oxygen species, produced by the autoxidation of glucose and peroxidation of lipids 5. Studies have shown that oxidative stress not only worsens but is also influenced by dyslipidemia in diabetes³². The process of lipid peroxidation induced by oxidative stress produces reactive byproducts such as malondialdehyde (MDA), which can act as a biomarker indicating oxidative damage to lipids³³. The current study found increased MDA levels in people diagnosed with diabetes compared to non-diabetics who are in optimal health. Mallick *et al.*, discovered a comparable increase in lipid peroxidation of erythrocyte membranes in both male and female diabetics in India²⁴. In the past, it was believed that oxidative stress plays a significant role in the pathogenesis of problems associated with non-insulin dependent diabetes mellitus (NIDDM), especially cardiovascular diseases²⁵. In addition, the highest MDA value was recently measured in patients with diabetic retinopathy in the city of Salah-Addin, Iraq²⁶.

The redox couple that has the greatest importance in antioxidant defense is GSH/GSSG, which also has a central function in metabolic processes and the regulation of the body's homeostasis pathways. Oxidative stress occurs as a result of reduced glutathione (GSH) levels and is associated with aging processes and the development of various ailments and diseases²⁷. When glucose levels are elevated, particularly in hyperglycemia, the use of glucose in the polyol pathway is preferred. This pathway consumes NADPH, a crucial cofactor essential for glutathione (GSH) regeneration facilitated by the enzyme GR³⁴. As a result, hyperglycemia indirectly contributes to the breakdown of GSH. The increased activity in the polyol pathway may deplete NADPH because

the first step involves the reduction of glucose to sorbitol, a process that is dependent on NADPH. This breakdown of NADPH in turn keeps GSH levels in a reduced state³⁵. This study showed a significant decrease in mean GSH levels in diabetic patients compared to a healthy control group, consistent with a previous study that found GSH deficiency in type 2 diabetes patients²⁸. Sekhar, R.V. *et al.*, studied that diabetes patients had GSH deficiency and low GSH levels, which were associated with increased oxidative stress and increased markers of oxidative damage in plasma²⁹.

CONCLUSION

In summary, our study highlights the complicated relationship between diabetes mellitus, oxidative stress and associated complications. Increased oxidative stress in type 2 diabetes is closely associated with altered lipid metabolism, reflected in an abnormal lipid profile with increased total cholesterol, triglycerides and LDL-C, and decreased HDL-C- a pattern consistent with the existing literature and the Prevalence highlights of dyslipidemia in diabetes. Furthermore, our study investigates the complex dynamics of the redox

couple GSH/GSSG and highlights its crucial role in antioxidant defense and metabolic regulation. The study establishes a connection between GSH deficiency, oxidative stress and the development of diabetes-related symptoms and highlights the importance of maintaining redox homeostasis. Regarding lipid peroxidation induced by oxidative stress, malondialdehyde (MDA) emerges as a biomarker indicating lipid damage in diabetes. Elevated MDA levels in diabetics' further support the connection between oxidative stress and lipid metabolism. In conclusion, this study provides crucial insights into the complex relationship between diabetes, oxidative stress and lipid metabolism and lays the foundation for understanding the molecular mechanisms behind diabetes-related complications.

ACKNOWLEDGMENT

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest

The author declare that we have no conflict of interest.

REFERENCES

- Ramakrishna, V.; Jaikhani, R. *Diagnostic pathology.*, **2007**, *2*(1), 1-6. <https://doi.org/10.1186/1746-1596-2-22>.
- Shinde, S. N.; Dhadke, V. N.; Suryakar, A. N. *Indian Journal of Clinical Biochemistry.*, **2011**, *26*, 74-7. <https://doi.org/10.1007/s12291-010-0041-y>.
- Önal, A. E.; Seker, S.; Kaya, I.; Temizkan, N.; Gur, S. O.; Tezoglu, C.; Gungor, G. *International Journal of Gerontology.*, **2012**, *6*(3), 177-81. <http://dx.doi.org/10.1016/j.ijge.2012.01.008>.
- Mohamed, K.; Yates, J.; Roberts, A. *Dental Update.*, **2014**, *41*(2), 144-54. <https://doi.org/10.12968/denu.2014.41.2.144>.
- Ozougwu, J. C., *International Journal of Research.*, **2016**, *1*(8). <https://doi10.36347/SJAMS.2019.v07i10.004>
- Hasanuzzaman, M.; Raihan, M. R.; Masud, A. A.; Rahman, K.; Nowroz, F.; Rahman, M.; Nahar, K.; Fujita, M. *International Journal of Molecular Sciences.*, **2021**, *22*(17), 9326. <https://doi.org/10.3390/ijms22179326>.
- Matschke, V.; Theiss, C.; Matschke, J. *Neural Regeneration Research.* **2019**, *14*(2), 238. <https://doi.org/10.4103%2F1673-5374.244780>.
- Charlton, A.; Garzarella, J.; Jandeleit-Dahm, K. A.; Jha, J. C. *Biology.*, **2020**, *10*(1), 18. <https://doi.org/10.3390/biology10010018>.
- Chandrasegaran, G.; Elanchezhian, C.; Ghosh, K.; Manivel, G.; Hassan, T. *EC Diabetes and Metabolic Research.*, **2022**, *3*(1), 10-7. https://doi:10.25259/IJPP_120_2022.
- Shirzaiy, M.; Dalirsani, Z. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews.*, **2019**, *13*(3), 1991-4. <https://doi.org/10.1016/j.dsx.2019.04.004>.
- Castelli, V.; Paladini, A.; d'Angelo, M.; Allegretti, M.; Mantelli, F.; Brandolini, L.; Cocchiari, P.; Cimini, A.; Varrassi, G. *CNS Neuroscience & Therapeutics.*, **2021**, *27*(4), 403-12. <https://doi.org/10.1111/cns.13610>.
- Su, L. J.; Zhang, J. H.; Gomez, H.; Murugan, R.; Hong, X.; Xu, D.; Jiang, F.; Peng, Z. Y. *Oxidative medicine and cellular longevity.*, **2019**, *13*, 2019. <https://doi.org/10.1155/2019/5080843>.

13. de Dios Alché, *J. Redox Biology.*, **2019**, *23*, 101136. <https://doi.org/10.1016/j.redox.2019.101136>.
14. Parcheta, M.; Wisłocka, R.; Orzechowska, S.; Akimowicz, M.; Chojńska, R.; Lewandowski, W. *Materials.*, **2021**, *14*(8), 1984. <https://doi.org/10.3390/ma14081984>.
15. Wahabi, K.; Perwez, A.; Rizvi, M. A. In *Handbook of Oxidative Stress in Cancer: Therapeutic Aspects.*, **2022**, *11*, 1-16. <https://doi.org/10.1186/2008-2231-20-61>
16. Richmond, W. *Clinical Chemistry.*, **1973**, *19*(12), 1350-6. <https://doi.org/10.1093/clinchem/19.12.1350>.
17. Fossati, P.; Prencipe, L. *Clinical Chemistry.*, **1982**, *28*(10), 2077-80. <https://doi.org/10.1093/clinchem/28.10.2077>.
18. Wilson, P. W.; Abbott, R. D.; Garrison, R. J.; Castelli, W. P. *Clinical Chemistry.*, **1981**, *27*(12), 2008-10. <https://doi.org/10.1093/clinchem/27.12.2008>.
19. Lee, J. S.; Chang, P. Y.; Zhang, Y.; Kizer, J. R.; Best, L. G.; Howard, B. V. *Diabetes Care.*, **2017**, *40*(4), 529-37. <https://doi.org/10.2337/dc16-1958>.
20. Buege, J. A. *Methods in Enzymology.*, **1978**, *52*, 302-10. [https://doi.org/10.1016/s0076-6879\(78\)52032-6](https://doi.org/10.1016/s0076-6879(78)52032-6).
21. Dixit, A. K.; Dey, R.; Suresh, A.; Chaudhuri, S.; Panda, A. K.; Mitra, A.; Hazra, J. *Journal of Diabetes & Metabolic Disorders.*, **2014**, *13*, 1-6. <https://doi.org/10.1186/2251-6581-13-58>.
22. Sadeghi, E.; Hosseini, S. M.; Vossoughi, M.; Aminorroaya, A.; Amini, M. *Diabetes, Metabolic Syndrome and Obesity.*, **2020**, *5*, 2743-50. <https://doi.org/10.2147/DMSO.S259697>.
23. Boydens, C.; Pauwels, B.; Vanden Daele, L.; Van de Voorde, J. *Cardiovascular Diabetology.*, **2016**, *15*, 1-2. <https://doi.org/10.1186/s12933-016-0366-9>.
24. Mallick, A. K.; Maradi, R.; Joshi, V. R.; Shorey, G.; Ahsan, M. *International Journal of Pharmaceutical Sciences Review and Research.*, **2011**, *8*(2), 198-201. <https://doi.org/10.2337/diab.38.12.1539>.
25. de Zwart, L. L.; Meerman, J. H.; Commandeur, J. N.; Vermeulen, N. P. *Free Radical Biology and Medicine.*, **1999**, *26*(1-2), 202-26. [https://doi.org/10.1016/S0891-5849\(98\)00196-8](https://doi.org/10.1016/S0891-5849(98)00196-8).
26. Khalaf, M. R.; Ahmeid, M. S.; Abdulqader, O. J.; Jabar, H. A. *Medico-Legal Update.*, **2021**, *21*(1). <https://doi.org/10.37506/mlu.v21i1.2519>.
27. Al Maskari, M. Y.; Waly, M. I.; Ali, A.; Al Shuaibi, Y. S. *The FASEB Journal [Internet].*, **2011**, *25*(S1). http://dx.doi.org/10.1096/fasebj.25.1_supplement.995.14.
28. Lutchmansingh, F. K.; Hsu, J. W.; Bennett, F. I.; Badaloo, A. V.; McFarlane-Anderson, N.; Gordon-Strachan, G. M.; Wright-Pascoe, R. A.; Jahoor, F.; Boyne, M. S. *PLoS One.*, **2018**, *13*(6), e0198626. <https://doi.org/10.1371/journal.pone.0198626>.
29. Sekhar, R. V.; McKay, S. V.; Patel, S. G.; Guthikonda, A. P.; Reddy, V. T.; Balasubramanyam, A.; Jahoor, F. *Diabetes Care.*, **2011**, *34*(1), 162-7. <https://doi.org/10.2337/dc10-1006>.
30. Bhatti, J. S.; Oxidative stress in the pathophysiology of type 2 diabetes and related complications: Current therapeutic strategies and future perspectives. *Free Radical Biology and Medicine.*, **2022**, *184*, 114-134. DOI: 10.1016/j.freeradbiomed.2022.03.019.
31. Ormazabal, V.; Association between insulin resistance and the development of cardiovascular disease. *Cardiovascular Diabetology.*, **2018**, *17*, 1-14. DOI:10.1186/s12933-018-0762-4.
32. Rochette, L.; Diabetes, oxidative stress and therapeutic strategies. *Biochimica et Biophysica Acta (BBA)-General Subjects.*, **2014**, *1840*(9), 2709-2729. DOI: 10.1016/j.bbagen.2014.05.017.
33. Tsikas, D.; Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges. *Analytical Biochemistry.*, **2017**, *524*, 13-30. DOI: 10.1016/j.ab.2016.10.02.
34. Kumawat, M.; Antioxidant enzymes and lipid peroxidation in type 2 diabetes mellitus patients with and without nephropathy. *North American Journal of Medical Sciences.*, **2013**, *5*(3), 213. doi: 10.4103/1947-2714.109193.
35. Madsen-Bouterse, S. A.; and R. A.; Kowluru, Oxidative stress and diabetic retinopathy: pathophysiological mechanisms and treatment perspectives. *Reviews in Endocrine and Metabolic Disorders.*, **2008**, *9*, 315-327. DOI: 10.1007/s11154-008-9090-4.