Academic Science Journal

Relationship of Serum Iron and Lipemic Levels with Inflammatory Biomarkers and Oxidative Stress in Diabetic Nephropat

Hanan M. Al-Nadawi* and Waseem Yousif M Al-Dulaimy

Department of Chemistry, College of Science, University of Diyala

* hananui56@gmail.com

Received: 6 March 2024 **Accepted: 29 April 2024**

Published: 30 April 2025

DOI: https://dx.doi.org/10.24237/ASJ.03.02.862B

Abstract

Type 2 diabetes is a systemic inflammatory condition with hyperglycemia, insulin resistance, and insulin secretion defects. It increases cardiovascular risk and leads to oxidative stress and inflammation. Diabetic nephropathy, a major microvascular complication, is caused by high iron intake or storage, causing hyperglycemia, insulin resistance, and pancreatic dysfunction. Targeting oxidative stress is crucial for managing DN. The study was conducted at the Ibn Sina Dialysis Center in Baqubah General District, studying iron levels in diabetic nephropathy patients from October 2023 to January 2024. Eighty adults without preconditions. , malnutrition, or inflammatory illnesses were screened. The study included 40 participants as a control group and involved serum lipids, iron, , C-reactive protein, IL-6, glutathione peroxidase, and Dismutase tests. The study also determined BMI, a person's body mass index. Biomarker estimation was performed once in all subjects. Statistical analysis assessed data homogeneity, normal distribution, and normality using an independent samples t-test. The average age of patients was 59.3375±10.0, while the control group had a lower BMI. The study also found significant changes in cholesterol, triglycerides, LDL, and HDL levels between patients and control groups. No significant correlation was found between iron and oxidative stress, lipid profile, or glutathione peroxidase.

Keywords: Diabetes mellitus, hyperglycinemia, Diabetic nephropathy, oxidative stress, lipid profile, Iron.

Introduction

Hyperglycinemia caused by insulin resistance, insulin secretion, or both characterizes type 2 diabetes mellitus (T2D), a low-grade systemic inflammatory disease. Cardiovascular risk in type 2 diabetic patients has been linked to hyperglycemia, inflammation caused by obesity, and dyslipidemia. Curiously, there is mounting evidence that connects these abnormalities to T2D patients' impaired iron metabolism [1–4].

The most common cause of end-stage renal disease (ESRD) globally is diabetic nephropathy (DN), a serious microvascular consequence of diabetes mellitus. The disease progresses to end-stage renal disease (ESRD) due to proteinuria, a decrease in glomerular filtration rate (GFR), and glomerulus structural and functional abnormalities[5]. Although the exact mechanisms by which hyperglycemia causes oxidative stress and inflammation in DN remain unclear, they are known to play a significant impact [6]. Trace amounts of iron are crucial. Important metabolic processes rely on iron, which is a cofactor of multiple enzymes and the main component of oxygen transporters. Many systemic illnesses are associated with iron metabolism [7, 8]. Ingesting or storing a large amount of iron can cause oxidative stress and inflammation by raising reactive oxygen species. This, in turn, can cause hyperglycemia, insulin resistance, and malfunction in the pancreatic β-cells, which can ultimately lead to diabetes mellitus[8]. During the development of diabetic nephropathy, there may be an interaction between diabetes and iron excess. Diabetic people excrete more iron in their urine. An increase in renal iron concentration was documented [9, 10] in diabetic rats that were induced by streptozotocin. A potential mechanism for exacerbating kidney injury.[11]. The combined effect of exogenous iron excess and diabetes on oxidative stress. When it comes to managing and treating diabetic nephropathy, non-invasive assessment of diabetic renal iron overload is crucial. Various inflammatory pathways are activated by persistent hyperglycemia in diabetes, leading to an increase in reactive nitrogen species (RNS) and reactive oxygen species (ROS). This, in turn, causes inflammation and oxidative stress[12]. An rise in oxidative stress biomarkers like malondialdehyde (MDA) and a decrease in antioxidants like glutathione peroxidase (GSH-px) and superoxide dismutase (SOD) are hallmarks of this condition[7].A number of inflammatory cytokines and chemokines, such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6),

interleukin-1β (IL-1β), and monocyte chemoattractant protein-1 (MCP-1), are also increased in DN. These throw off the body's redox equilibrium, which in turn triggers a cascade of events that lead to inflammation, fibrosis, and apoptosis, all of which harm the kidneys. The treatment of DN should thus focus on reducing inflammation and oxidative stress[8].

A higher risk of diabetic complications and increased oxidative stress have been linked to elevated body iron reserves[13]. As a pro-oxidant, iron can use Fenton chemistry to catalyze processes that produce hydroxyl radicals. Dietary iron is more easily absorbed in people with hyperglycemia[14]. In individuals with diabetes, oxidative stress indicators such as 8-hydroxy-2'-deoxyguanosine (8-OHdG) and elevated blood ferritin and transferrin saturation have been associated.

Material and Methods

The iron level in patients with diabetic nephropathy were studied by researchers at the Nephrology and Haemodialysis Unit of Baquba General Hospital's Ibn Sina Dialysis Center from October 20th, 2023, until January 8th, 2024. Eighty adults with no prior hematologic or oncologic conditions, malnutrition, or acute or chronic inflammatory illnesses were found during the initial screening. The hospitals library were categorized according to ages, genders, treatment groups, and long-term illnesses, including cardiovascular disease, hypertension, congestive heart failure, arterial disease, and chronic obstructive pulmonary disease. Forty participants were assigned as the control group in this study. Various blood tests done, which included tracking and documenting levels of serum lipids, iron, ferritin, C-reactive protein, IL-6, glutathione peroxidase, and superoxide dismutase.

- **Blood sampling**

Each patient and healthy control group had their blood drawn using a 5 ml disposable syringe; the blood sample was then divided into two equal portions, each containing 3 ml and 2 ml. The first was taken and placed into a gel tube; after fifteen minutes, it was left to coagulate at room temperature. When not in use, the serum was frozen at -20C after being centrifuged at 3000 rpm for 10 minutes [15].

- **Determine of BMI**

The body mass index (BMI) is a measure of a person's size relative to their weight and height.

Always stated as a number in kilograms per square meter, a person's body mass index (BMI) is the result of dividing their mass (in kilograms) by the square of their height (in meters squared). Mass divided by height is the formula for body mass index (kg/m^2)[16].

- **Biomarker estimation**

All biomarkers in serum samples have been carried out once in all subjects of the two groups (control, and patients).

Statistical analysis: Statistical analysis was conducted to assess data homogeneity, normal distribution, and normality. The data was displayed as the median ± standard deviation, and the probability was evaluated using an independent samples t-test in IBM SPSS version 27.0 A probability < 0.05 was deemed significant.

Results

The results show the clinical demographics, including age and BMI. In our study, the average age of patients was 59.3375±10.0 while for the control group, it was 55.26±4.681. The t-test value was 2.445 with a p-value of 0.0160. The BMI was divided into two subgroups: obese (32.67±2.5) and non-obese (23.16±1.03).

Table 1: Mean± SD of the age and body mass index (BMI) for both the group of patients.

	patient	**Control**	**p-value**
Age Mean±SD	59.3375±10.0	55.26±4.681	p-value= 0.0160 t-test= 2.445
	Obese	**Non- obese**	**p-value**
BMI (kg/m^2) Mean±SD	32.67±2.5	23.16±1.03	p<0.001 t= 7.733

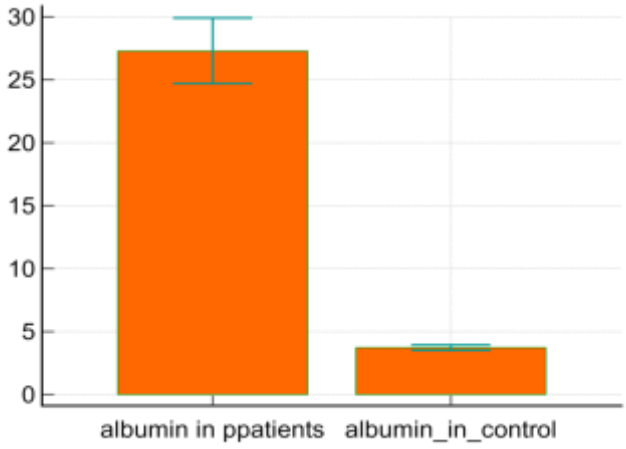
The results show that albumin levels were significantly different in people with diabetes compared to those without the disease. As shown in table 2, the average albumin level in diabetic patients was 27.3067, which is significantly higher than the normal level of 3.7295 ($P < 0.001$), as shown in table 2.

Academic Science Journal

Table 2: Mean ± SD of Albumin levels in studied groups

	Patients	control
Sample	80	38
Arithmetic mean	27.3067	3.7295
95% CI for the mean	24.7097 to 29.9038	3.5003 to 3.9586
Variance	136.1892	0.4860
Standard deviation	11.6700	0.6972
Standard error of the mean	1.3047	0.1131
T-test		
Difference	-23.5773	
Pooled Standard Deviation	9.6387	
Standard Error	1.8990	
95% CI of difference	-27.3385 to -19.8161	
Test statistic t	-12.416	
Degrees of Freedom (DF)	116	
Two-tailed probability	P < 0.0001	

F-test for equal variances, P < 0.001



This study also showed that there are significant changes between lipid variables in patients and healthy people, as shown in table 3. In the table below, the average cholesterol in patients and control groups (204.1125±66.96 and 113.3636±21.2710), respectively, with p-value P = 0.0001. Also a significant difference shown in triglycerid, the results showed a significant between patients and control groups (265.9094 ± 159.0884 and123.0000±20.6446), respectively. LDL and HDL have also a significant study between two groups as shown in Table 3

Academic Science Journal

Table 3: Mean ±SD of lipid levels in studied groups.

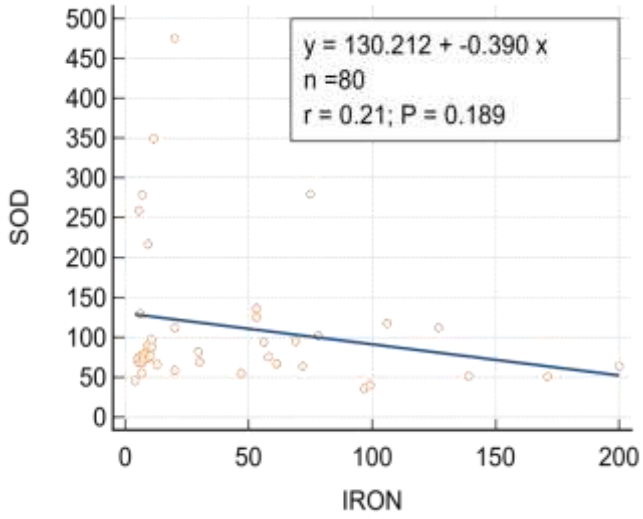
Lipid profile	Patients	Control	p-value	
Cholesterol	204.1125±66.96	113.3636±21.2710	**T-test (assuming equal variances)**	
			Difference	-90.7489
			Pooled Standard Deviation	59.1703
			Standard Error	20.6808
			95% CI of difference	132.5145 to -48.9832
			Test statistic t	-4.388
			Degrees of Freedom (DF)	41
			Two-tailed probability	P = 0.0001
TG	265.9094±159.0884	123.0000±20.6446	**T-test (assuming equal variances)**	
			Difference	-142.9094
			Pooled Standard Deviation	138.7087
			Standard Error	48.4805
			95% CI of difference	-240.8177 to -45.0011
			Test statistic t	-2.948
			Degrees of Freedom (DF)	41
			Two-tailed probability	P = 0.0053
LDL	122.9869± 64.0804	53.6364±22.137	**T-test (assuming equal variances)**	
			Difference	-69.3505
			Pooled Standard Deviation	56.7828
			Standard Error	19.8463
			95% CI of difference	-109.4309 to -29.2701
			Test statistic t	-3.494
			Degrees of Freedom (DF)	41
			Two-tailed probability	P = 0.0012
HDL	27.9437±12.4878	45.4545±6.653	**T-test (assuming equal variances)**	
			Difference	17.5108
			Pooled Standard Deviation	11.3450
			Standard Error	3.9652
			95% CI of difference	9.5029 to 25.5187
			Test statistic t	4.416
			Degrees of Freedom (DF)	41
			Two-tailed probability	P = 0.0001
VLDL	53.1819±31.8177	22.3636±5.4822	**T-test (assuming equal variances)**	
			Difference	-30.8182
			Pooled Standard Deviation	27.7989
			Standard Error	9.7161
			95% CI of difference	-50.4402 to -11.1963
			Test statistic t	-3.172
			Degrees of Freedom (DF)	41
			Two-tailed probability	P = 0.0029

Academic Science Journal

In Table 4 detected a personal correlation between iron and oxidative strees , theses result shown no correlation between irone with SOD and GPx.

Table 4: Personal correlation between iron and SOD,GPx.

	Iron	
	Correlation	Significant value
GPX	0.11	0.502
SOD	0.21	0.189



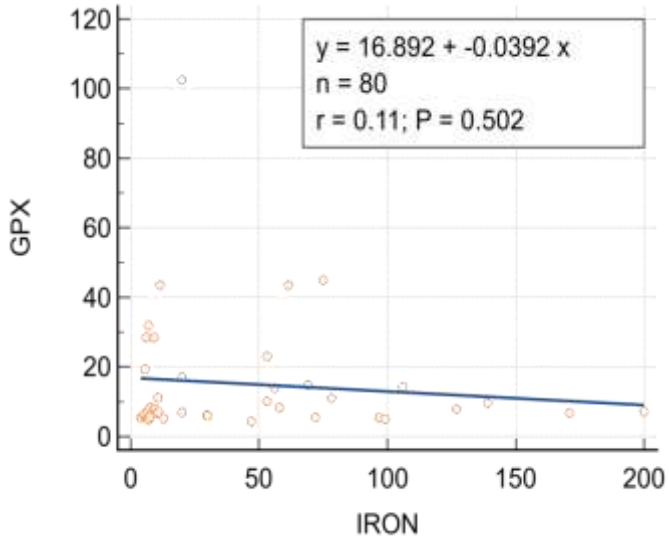
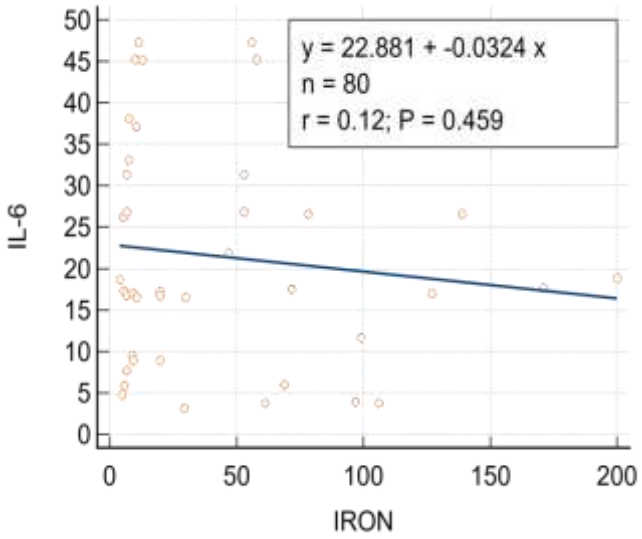
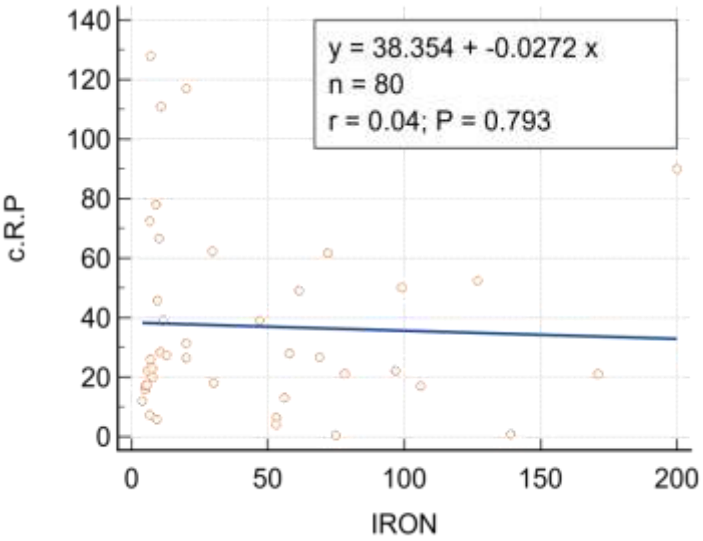


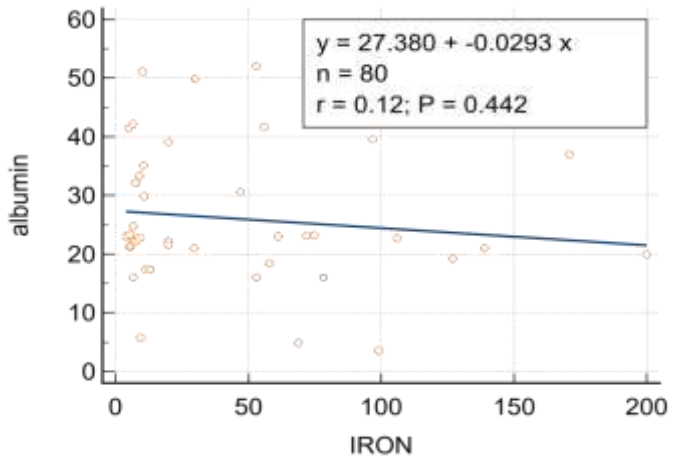
Table 5: No correlation between iron with inflammatory marker and albumin

IRON		
Vairable	Correlation	Significant value
IL-6	0.12	0.459
CRP	0.04	0.793
Albumin	0.12	0.442

Academic Science Journal





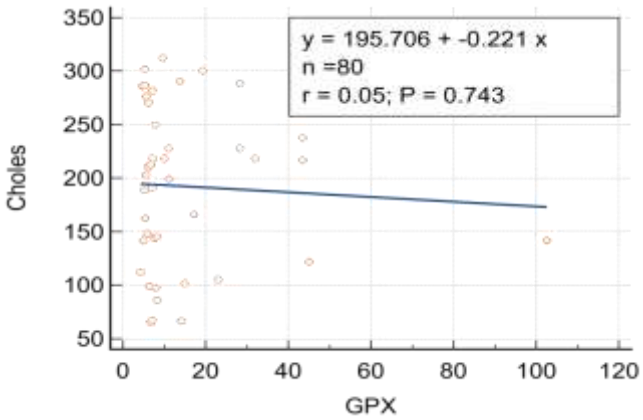


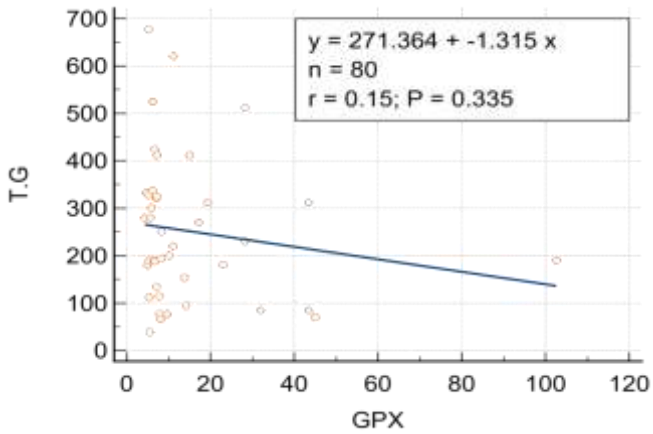
These result shown no significant between lipid profile and glutathione peroxidase as shown in Table 6.

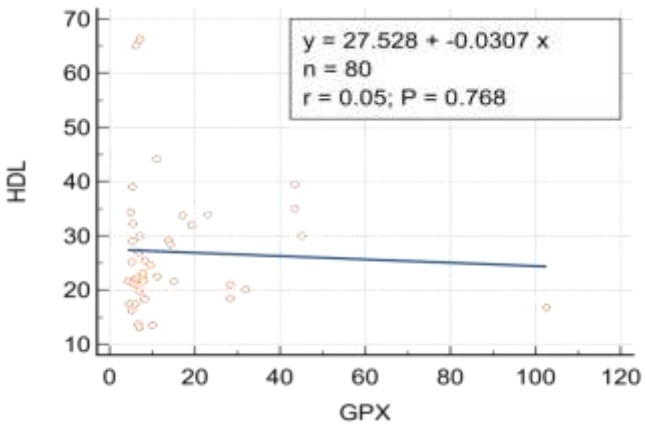
Academic Science Journal

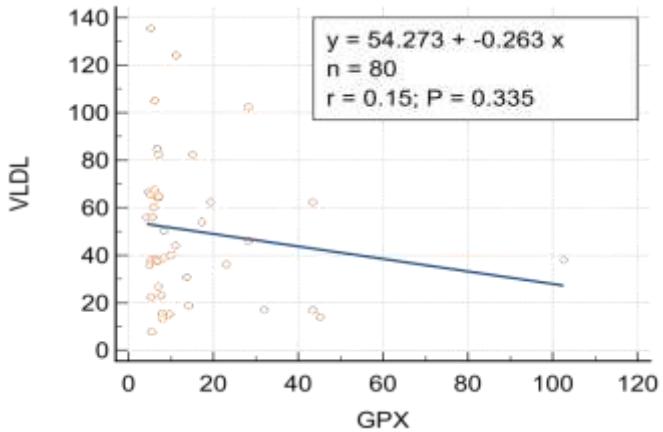
Table 6: personal correlation between lipid profile and GPx

		GPX
GPX	Correlation coefficient Significance Level P n	1
VLDL	Correlation coefficient Significance Level P n	-0.154 0.3351 80
HDL	Correlation coefficient Significance Level P n	-0.047 0.7681 80
Choles	Correlation coefficient Significance Level P n	-0.053 0.7426 80
LDL	Correlation coefficient Significance Level P n	0.010 0.9490 80
T.G	Correlation coefficient Significance Level P n	-0.154 0.3351 80



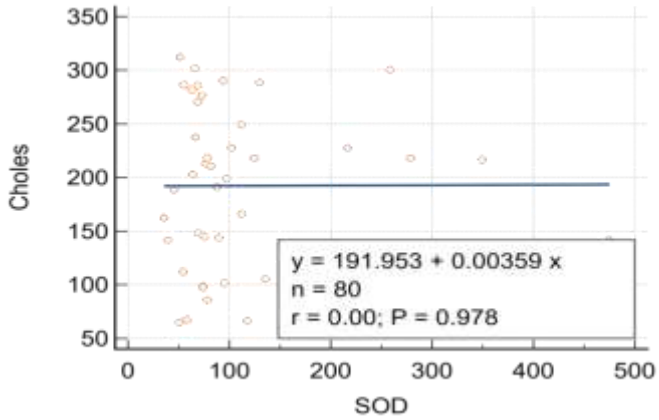


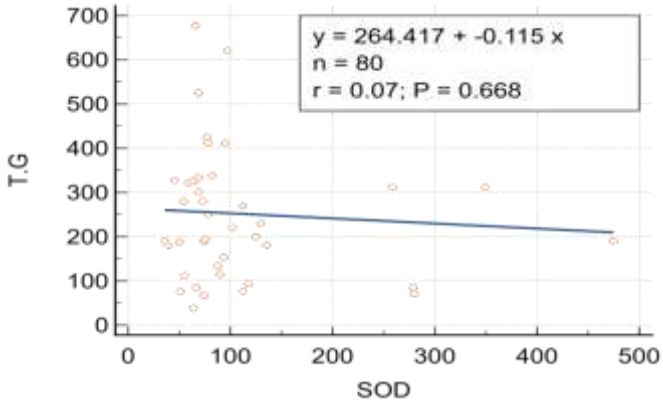


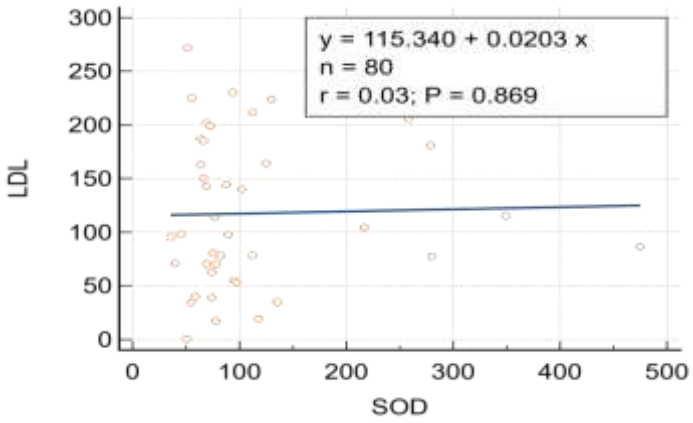


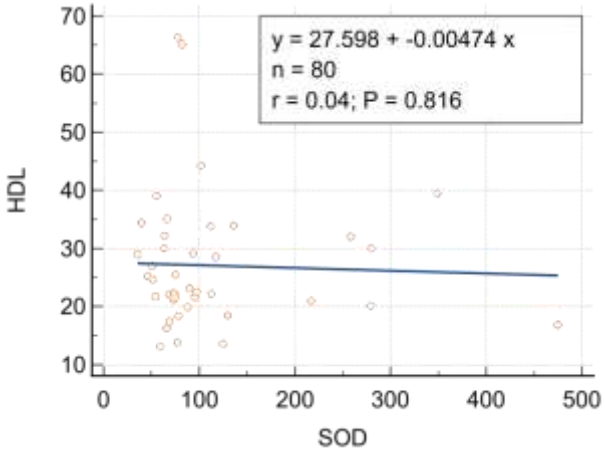
Academic Science Journal

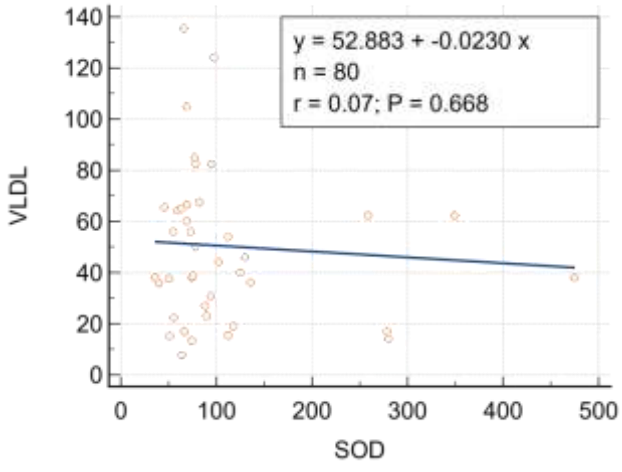
These result shown no significant between lipid profile and superoxide dismutase.











Discussion

Diabetic nephropathy (DN) is one of the most frequent and severe complications of diabetes mellitus (DM) and is associated with increased morbidity and mortality in diabetic patients [17–19]. The authors covered the various morphological and functional abnormalities that can be found in diabetic kidneys, such as the buildup of extracellular matrix, thickening of the glomerular basement membrane, expansion of the mesangial spaces, loss of podocytes, albuminuria, and a decline in the glomerular filtration rate[20]. Renal damage is caused by a complicated interaction between metabolic and hemodynamic variables. The key role of hyperglycemia-induced oxidative stress and inflammation was highlighted in the review. The function of oxidative stress in the onset, maintenance, and progression of micro- and macrovascular problems in type 2 diabetes was the focus of this extensive literature review. It covered possible antioxidant treatment approaches, the pathways that lead to difficulties in diabetes, and the sources of reactive oxygen species that are enhanced in this disease[21, 22].

A study conducted by transient focal cerebral ischemia was induced, this study in a mouse model of ischemic stroke discovered that a lack of monocyte chemoattractant protein-1 (MCP-1) resulted in lower levels of IL-6, IL-1β, and G-CSF in both transcription and plasma.
During the acute phase of ischemic stroke, this showed that MCP-1 unexpectedly regulated the expression of other proinflammatory cytokines [23–25].

Several studies have been their observations that type 2 diabetic individuals had lower levels of the antioxidant enzymes glutathione peroxidase, catalase, and superoxide dismutase, as well as higher levels of malondialdehyde, indicating greater erythrocyte lipid peroxidation. Findings showed that diabetics have compromised antioxidant defenses and increased systemic oxidative[26] stress.

Several studies have been their observations that pregnant women's serum ferritin content and plasma malondialdehyde levels were positively correlated, suggesting a connection between iron status and oxidative stress markers such lipid peroxidation. The study's authors came to the conclusion that iron excess might raise the likelihood of gestational diabetes and oxidative[27] stress.

Increased iron accumulation in parenchyma and the pancreas, loss of acinar cells, inflammation and fibrosis in the pancreas, and raised levels of serum amylase and lipase were all symptoms of iron-induced chronic pancreatitis in this research of mice with iron overload [25]. The reversal of these effects upon iron chelation treatment provides further evidence that iron overload contributes to pancreatic injury through oxidative stress and ferroptosis[28]. This in vitro investigation revealed that hepatic and adipocyte cell lines reacted differently to iron excess. Iron amplified the impact of inflammation and hypoxia on hepatocytes' stress signaling pathways, and it raised oxidative stress markers. Adipocytes, on the other hand, exhibited adaptive responses that limited iron uptake and protected them from iron excess-induced oxidative damage[28, 29].

Conclusion

This study demonstrated that deferoxamine, an iron chelator, reduced inflammation, oxidative stress, and iron accumulation in diabetic nephropathy rat models, thereby alleviating kidney injury and functioning [30]. Deferoxamine showed protective effects against the progression of diabetic nephropathy by reducing renal cortical iron deposits, apoptosis, fibrosis, and pro-inflammatory and pro-fibrotic cytokine production.

Iron, one of the earth's most abundant elements, is crucial for all living things to mature and flourish[30]. Many diseases can result from an imbalance in its amount. It plays an essential role in metabolism, cell growth, proliferation, oxygen binding and distribution, and both mild and strong forms of oxidation. But iron's electron-accepting and -transfer properties make it a potential tissue-damaging oxidative stressor[31]. Systemic and cellular regulators work together to keep the body's iron levels stable. Due to the body's limited iron excretion capacity, iron levels are tightly regulated during gastrointestinal absorption[31]. The iron metabolism systemic regulator is hepcidin. Its production in the liver is influenced by erythropoiesis, inflammation, low oxygen levels, and the body's iron levels[31].

Source of funding: Not available

Conflict of interest: The authors declared no conflicting interests.

References

[1] JM. Fernández-Real, D. McClain, M. Manco, Mechanisms linking glucose homeostasis and iron metabolism toward the onset and progression of type 2 diabetes, Diabetes care,38(11), 2169-76(2015), DOI(https://doi.org/10.2337/dc14-3082)

[2] Y. Feng, Q. Feng, Y. Lv, X. Song, H. Qu, Y. Chen, The relationship between iron metabolism, stress hormones, and insulin resistance in gestational diabetes mellitus, Nutrition & diabetes, 10(1), 17(2020), DOI(https://doi.org/10.1038/s41387-020-0122-9)

[3] P. Krisai, S. Leib, S. Aeschbacher, T. Kofler, M. Assadian, A. Maseli, Relationships of iron metabolism with insulin resistance and glucose levels in young and healthy adults, European journal of internal medicine, 32, 31-37(2016), DOI(https://doi.org/10.1016/j.ejim.2016.03.017)

[4] JS. Choi, I-U. Koh, HJ. Lee, WH. Kim, J. Song, Effects of excess dietary iron and fat on glucose and lipid metabolism, The Journal of nutritional biochemistry, 24(9), 1634-44(2013), DOI(https://doi.org/10.1016/j.jnutbio.2013.02.004)

[5] MK. Sagoo, L. Gnudi, Diabetic nephropathy: an overview, Diabetic Nephropathy: Methods and Protocols, 3-7(2020), DOI(https://doi.org/10.1007/978-1-4939-9841-8_1)

[6] N. Vodošek Hojs, S. Bevc, R. Ekart, R. Hojs, Oxidative stress markers in chronic kidney disease with emphasis on diabetic nephropathy, Antioxidants, 9(10), 925(2020), DOI(https://doi.org/10.3390/antiox9100925)

[7] A. Yiannikourides, GO. Latunde-Dada, A short review of iron metabolism and pathophysiology of iron disorders, Medicines, 6(3), 85(2019), DOI(https://doi.org/10.3390/medicines6030085)

[8] F. Oliveira, S. Rocha, R. Fernandes, Iron metabolism: from health to disease, Journal of clinical laboratory analysis, 28(3), 210-218(2014), DOI(https://doi.org/10.1002/jcla.21668)

[9] JH. Dominguez, Y. Liu, KJ. Kelly, Renal iron overload in rats with diabetic nephropathy, Physiological Reports, 3(12), e12654(2015), DOI(https://doi.org/10.14814/phy2.12654)

[10] K. Chaudhary, A. Chilakala, S. Ananth, A. Mandala, R. Veeranan-Karmegam, FL. Powell, Renal iron accelerates the progression of diabetic nephropathy in the HFE gene knockout mouse model of iron overload, American Journal of Physiology-Renal Physiology, 317(2), F512-F7(2019), DOI(https://doi.org/10.1152/ajprenal.00184.2019)

[11] V. Rao, LV. Rao, SH. Tan, M. Candasamy, SK. Bhattamisra, Diabetic nephropathy: an update on pathogenesis and drug development, Diabetes & Metabolic Syndrome: Clinical Research & Reviews, 13(1), 754-762(2019), DOI(https://doi.org/10.1016/j.dsx.2018.11.054)

[12] A. Singh, R. Kukreti, L. Saso, S. Kukreti, Mechanistic insight into oxidative stress-triggered signaling pathways and type 2 diabetes, Molecules, 27(3), 950(2022), DOI(https://doi.org/10.3390/molecules27030950)

[13] OO. Oguntibeju, Type 2 diabetes mellitus, oxidative stress and inflammation: examining the links, International journal of physiology, pathophysiology and pharmacology, 11(3), 45(2019)

[14] S. Venugopa, Intertwined role of reactive oxygen, nitrogen and carbon in diabetic state, MOJ Cell Sci Rep., 2, 1-5(2015)

[15] H. Cheng, R. Harris, Renal endothelial dysfunction in diabetic nephropathy, Cardiovascular & Haematological Disorders-Drug Targets (Formerly Current Drug Targets-Cardiovascular & Hematological Disorders), 14(1), 22-33(2014)

[16] F. Hu, T. Zhang, Study on risk factors of diabetic nephropathy in obese patients with type 2 diabetes mellitus, International journal of general medicine, 351-360(2020), DOI(https://doi.org/10.2147/IJGM.S255858)

[17] Association AD. 6. Glycemic targets: standards of medical care in diabetes—2021. Diabetes Care, 2021, 44(Supplement_1), S73-S84.

[18] A. Cerise, JM. Chen, JA. Powelson, AJ. Lutz, JA. Fridell, Pancreas transplantation would be easy if the recipients were not diabetic: A practical guide to post-operative management of diabetic complications in pancreas transplant recipients, Clinical Transplantation, 35(5), e14270(2021), DOI(https://doi.org/10.1111/ctr.14270)

[19] D. Care, Care in Diabetesd2018. Diabetes Care, 41(1), S137-S43(2018), DOI(https://doi.org/10.2337/dc18-S002)

[20] M. Sagoo, L. Gnudi, Diabetic nephropathy: an overview, Diabetic Nephropathy, (2020), DOI(https://doi.org/10.1007/978-1-4939-9841-8_1)

[21] JS. Bhatti, A. Sehrawat, J. Mishra, IS. Sidhu, U. Navik, N. Khullar, Oxidative stress in the pathophysiology of type 2 diabetes and related complications: Current therapeutics strategies and future perspectives, Free Radical Biology and Medicine, 184, 114-134(2022), DOI(https://doi.org/10.1016/j.freeradbiomed.2022.03.019)

[22] L. Rochette, M. Zeller, Y. Cottin, C. Vergely, Diabetes, oxidative stress and therapeutic strategies, Biochimica et Biophysica Acta (BBA)-General Subjects, 1840(9), 2709-2729(2014), DOI(https://doi.org/10.1016/j.bbagen.2014.05.017)

[23] NA. Meguid, AA. Dardir, ER. Abdel-Raouf, A. Hashish, Evaluation of oxidative stress in autism: defective antioxidant enzymes and increased lipid peroxidation, Biological trace element research, 143, 58-65(2011), DOI(https://doi.org/10.1007/s12011-010-8840-9)

[24] J. K. Strecker, J. Minnerup, B. Gess, EB. Ringelstein, W. R. Schäbitz, M. Schilling, Monocyte chemoattractant protein-1-deficiency impairs the expression of IL-6, IL-1β and G-CSF after transient focal ischemia in mice, PloS one, 6(10), e25863(2011), DOI(https://doi.org/10.1371/journal.pone.0025863)

[25] N. Qamar, P. John, A. Bhatti, Emerging role of selenium in treatment of rheumatoid arthritis: An insight on its antioxidant properties, Journal of Trace Elements in Medicine and Biology, 66, 126737(2021), DOI(https://doi.org/10.1016/j.jtemb.2021.126737)

[26] H. Steinbrenner, LH. Duntas, MP. Rayman, The role of selenium in type-2 diabetes mellitus and its metabolic comorbidities, Redox Biology, 50, 102236(2022), DOI(https://doi.org/10.1016/j.redox.2022.102236)

[27] M. Pauk, V. Kufner, V. Rumenovic, I. Dumic-Cule, V. Farkas, M. Milosevic, Iron overload in aging Bmp6-/-mice induces exocrine pancreatic injury and fibrosis due to acinar cell loss, International Journal of Molecular Medicine, 47(4), 1-8(2021), DOI(https://doi.org/10.3892/ijmm.2021.4893)

[28] S. Chirumbolo, AP. Rossi, V. Rizzatti, E. Zoico, G. Franceschetti, D. Girelli, Iron primes 3T3-L1 adipocytes to a TLR4-mediated inflammatory response, Nutrition, 31(10), 1266-1274(2015), DOI(https://doi.org/10.1016/j.nut.2015.04.007)

[29] M. Andrews, M. Arredondo, Hepatic and adipocyte cells respond differentially to iron overload, hypoxic and inflammatory challenge, Biometals, 25, 749-759(2012), DOI(https://doi.org/10.1007/s10534-012-9543-9)

[30] D. Banerjee, G. Kaur, B. Chatterjee, H. Joshi, S. Ramniwas, HS.Tuli, Effectiveness of novel iron regulators in the treatment of diabetic nephropathy, (2023), DOI(10.15584/ejcem.2023.3.28)

[31] T. Ganz, Systemic iron homeostasis, Physiological reviews, 93(4), 1721-1741(2013), DOI(https://doi.org/10.1152/physrev.00008.2013)