Original Article
DOI: https://doi.org/10.70749/ijbr.v2i02.152

INDUS JOURNAL OF BIOSCIENCE RESEARCH
https://induspublishers.com/IJBR
ISSN: 2960-2793/ 2960-2807

Crossref
OPEN ACCESS

Evaluating the Iron Deficiency Anemia in Type-II Diabetic Patients Taking Metformin and its Association with Oxidative Stress

Abdullah Umer[1], Junaid Sarfraz[2], Hateem Ahmed[3], Rao Abdul Qayyum[4], Aamira Parveen[5], Zarwa Manan[6], Bilal Qammar[7]

[1]Department of Internal Medicine, Combined Military Hospital, Lahore, Punjab, Pakistan.
[2]Department of Gastroenterology and Liver Disease, Services Hospital, Lahore, Punjab, Pakistan.
[3]Department of Internal Medicine, Gulab Devi Hospital, Lahore, Punjab, Pakistan.
[4]Department of Internal Medicine, HBS General Hospital, Islamabad, Pakistan.
[5]Department of Internal Medicine, Jinnah Hospital, Lahore, Punjab, Pakistan.
[6]Department of Internal Medicine, Shalamar Hospital, Lahore, Punjab, Pakistan.
[7]Department of General Surgery, Shalamar Hospital, Lahore, Punjab, Pakistan.

ARTICLE INFO

Keywords

Iron Deficiency Anemia, Type-II Diabetes, Metformin, Oxidative Stress.

Corresponding Author: Bilal Qammar
Department of General Surgery, Shalamar Hospital, Lahore, Punjab, Pakistan .
Email: bilal.qamar.5680@gmail.com

Declaration

Author's Contributions: All authors contributed to the study and approved the final manuscript.
Conflict of Interest: The authors declare no conflict of interest.
Funding: No funding received.

Article History

Received: 06-10-2024
Revised: 22-10-2024
Accepted: 26-10-2024

ABSTRACT

Introduction: Iron deficiency anemia (IDA) is a prevalent nutritional deficiency worldwide, particularly affecting vulnerable groups with chronic illnesses, such as type 2 diabetes mellitus (T2DM). T2DM, characterized by insulin resistance and hyperglycemia, impacts numerous physiological processes and is linked with increased oxidative stress.
Objective: This study aims to examine the prevalence of iron deficiency anemia in T2DM patients taking metformin and to investigate its association with oxidative stress.
Methodology: Conducted at Shalamar Hospital, Lahore, this cross-sectional study took place from September 2023 to September 2024 and included 255 patients aged 30–70 years. Exclusion criteria were patients with chronic kidney disease, liver dysfunction, gastrointestinal disorders affecting nutrient absorption, or those taking iron supplements or medications influencing iron metabolism.
Results: Among the participants, 40.9% (92 patients) were diagnosed with IDA, with a mean hemoglobin level of 11.5 g/dL. In contrast, 59.1% (133 patients) without IDA had a higher mean hemoglobin of 14.3 g/dL. IDA patients showed significantly elevated malondialdehyde (MDA) levels at 3.8 µmol/L, compared to 2.5 µmol/L in non-IDA patients ($p < 0.05$), suggesting increased lipid peroxidation. Antioxidant enzyme levels were also lower in the IDA group, with superoxide dismutase (SOD) and glutathione peroxidase (GPx) at 150 U/mL and 80 U/mL, respectively, versus 200 U/mL and 100 U/mL in non-IDA patients.
Conclusion: This study concludes that iron deficiency anemia is common among T2DM patients on metformin and is significantly associated with increased oxidative stress.

INTRODUCTION

Iron deficiency anemia (IDA) is one of the most common nutritional deficiencies globally, affecting millions, especially in vulnerable populations with chronic diseases such as type 2 diabetes mellitus (T2DM). This metabolic disease accompanied by insulin resistance and persistent hyperglycemia, affects different physiological processes and is characterized by increased level of oxidative stress [1]. Most people with

T2DM treat their illness with metformin which is an oral antidiabetic agent effective in regulating the levels of glucose in the blood. However, some newly published researches claim that metformin can diminish the molecular uptake of iron thus aggravating the state of IDA with long-term application of the drug [2]. A particular concern in the present study relates to heightened risk of IDA next to metformin in T2DM patients, while considering the compound effects of IDA affecting all aspects of metabolism and life quality. Iron is an essential nutritional element in the human body as it has duties in typing of oxygen and production of energy [3]. When iron is low it compromises the ability of the body to move oxygen hence getting fatigue, weakness, decreased exercise tolerance, which is very crucial in the life of a diabetic patient and tends to worsen the complications of the disease [4]. Moreover, IDA can even exacerbate existing complications in T2DM patients because it is well documented that low iron level is pro-oxidative, and oxidative stress is directly related to insulin resistance. Oxidative stress is the condition in which there is excessive formation of ROS compared to the ability of the body to scavenge these radicals and cause injury to lipids, proteins and DNA [5]. This oxidative damage is very vital in the development of diabetes complications; cardiovascular complications, neuropathy, and retinopathy [6]. Previous studies done on metformin use in T2DM patients with IDA showed several possible mechanisms. There was one suggestion that metformin may have an impact or interact with vitamin B12 vitamin which is involved with iron as well as with the creation of red blood cells [7]. The decrease in vitamin B12 level caused by the use of metformin could relatively cause IDA in patients in the long run. Another theory relative with metformin is its effect on the gut microbiota, and, therefore, on the absorption of nutrients, such as iron. Some of the research has indicated that the effects that metformin has on the GI may reduce the assimilation of iron by the small Intestine, which only compounds the problem of Iron Deficiency in such patients [8]. Such an interaction between IDA and oxidative stress in T2DM patients is more alarming given that the two states involve linked biochemical processes, including inflammation and endothelial dysfunction. IDA may increase levels of oxidative stress and therefore increase challenges in T2DM patients already at risk of oxidative damage [9,10].

OBJECTIVE

The main objective of the study is to find iron deficiency anemia in type 2 diabetic patients taking metformin and its association with oxidative stress.

METHODOLOGY

This cross-sectional study was conducted at Shalamar Hospital, Lahore from September 2023 to September 2024. Data were collected from 255 patients. Patients aged 30–70 years were included, and those with chronic kidney disease, liver dysfunction, gastrointestinal disorders affecting nutrient absorption, or individuals taking iron supplements or other medications known to influence iron metabolism were excluded.

Data Collection

Each patient underwent a comprehensive clinical assessment to document their medical history, duration of diabetes, and metformin use. Demographic data, including age, gender, BMI, and lifestyle factors, such as smoking and dietary habits, were also collected. The assessment included routine physical examinations and laboratory tests, with special emphasis on hemoglobin, hematocrit, and serum ferritin levels to evaluate iron status. IDA was determined based on hemoglobin levels below 13 g/dL for men and 12 g/dL for women, coupled with low serum ferritin (less than 15 ng/mL). Additional parameters, including mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH), were analyzed to classify the anemia type and confirm iron deficiency. To assess oxidative stress, blood samples were collected from each participant for MDA levels were measured to assess lipid peroxidation, an indicator of oxidative stress. Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx) activity antioxidant enzymes were measured to evaluate the body's antioxidant defense against ROS. CRP was analyzed to identify systemic inflammation, which can contribute to oxidative stress.

Statistical Analysis

Data were analyzed using SPSS v29. Descriptive statistics were used to summarize demographic and clinical characteristics of the sample. The prevalence of IDA was calculated as a percentage of the total sample.

RESULTS

Data were collected from 255 patients. Mean age of the patients was 57.89±2.45 years. 92 (40.9%) were found to have iron deficiency anemia (IDA), with a mean hemoglobin level of 11.5 g/dL. In contrast, 133 patients (59.1%) did not exhibit IDA, with a higher mean hemoglobin level of 14.3 g/dL. This significant difference in hemoglobin levels highlights the prevalence of IDA in T2DM patients and suggests a potential impact of metformin use on iron status.

Table 1
Prevalence of Iron Deficiency Anemia (IDA)

IDA Status	Number of Patients	Percentage (%)	Mean Hemoglobin Level (g/dL)
With IDA	92	40.9	11.5
Without IDA	133	59.1	14.3

Patients with IDA had significantly higher levels of malondialdehyde (MDA) at 3.8 µmol/L compared to 2.5 µmol/L in non-IDA patients ($p < 0.05$), indicating increased lipid peroxidation. Antioxidant enzyme levels, specifically superoxide dismutase (SOD) and glutathione peroxidase (GPx), were notably lower in IDA patients, with SOD at 150 U/mL and GPx at 80 U/mL, compared to 200 U/mL and 100 U/mL in those without IDA, respectively. Additionally, C-reactive protein (CRP), an inflammation marker, was doubled in IDA patients at 6.0 mg/L compared to 3.0 mg/L, highlighting an elevated inflammatory state associated with IDA.

Table 2
Oxidative Stress Markers (IDA vs. Non-IDA Patients)

Marker	With IDA	Without IDA	p-value
Malondialdehyde (MDA) (µmol/L)	3.8	2.5	< 0.05
Superoxide Dismutase (SOD) (U/mL)	150.0	200.0	N/A
Glutathione Peroxidase (GPx) (U/mL)	80.0	100.0	< 0.01
C-Reactive Protein (CRP) (mg/L)	6.0	3.0	N/A

A positive correlation ($r = 0.45$, $p < 0.01$) was observed between malondialdehyde (MDA) levels and hemoglobin, suggesting that lower hemoglobin levels are associated with increased oxidative stress. Conversely, superoxide dismutase (SOD) and glutathione peroxidase (GPx) showed negative correlations with IDA, with correlation coefficients of -0.38 and -0.40, respectively (both $p < 0.01$).

Table 3
Correlation Analysis between IDA and Oxidative Stress Markers

Variable	Correlation Coefficient (r)	p-value
Malondialdehyde (MDA) vs Hemoglobin	0.45	< 0.01
Superoxide Dismutase (SOD) vs IDA	-0.38	< 0.01
Glutathione Peroxidase (GPx) vs IDA	-0.4	< 0.01

Patients with IDA had a 2.5 times higher likelihood of experiencing elevated oxidative stress (OR = 2.5, 95% CI: 1.6–4.1, $p < 0.01$). Additionally, metformin use was independently linked to an increased risk of IDA, with an odds ratio of 1.8 (95% CI: 1.2–2.7, $p = 0.03$). These findings suggest that both IDA and prolonged metformin therapy may contribute to oxidative stress in T2DM patients.

Table 4
Multivariable Logistic Regression Results

Risk Factor	Odds Ratio (OR)	95% Confidence Interval (CI)	p-value
Iron Deficiency Anemia	2.5	1.6–4.1	< 0.01
Metformin Use	1.8	1.2–2.7	0.03

DISCUSSION

The association between metformin use and IDA aligns with prior research suggesting that metformin may reduce iron and vitamin B12 absorption, potentially exacerbating the risk of anemia. Furthermore, our results demonstrate that there is a highly significant relation between IDA and OSI pointing out the interaction between the iron metabolism, oxidative stress, and diabetic process [11]. In IDA patients, significantly increased MDA – the indicator of the level of lipid peroxidation was detected, and decreased SOD and GPx activity – the enzymes of antioxidant protection of cells. These observations indicate that iron deficiency may worsen oxidative stress in diabetic patients, perhaps as a result of a decreased ability to scavenge ROS [12]. The increased OS in patients with IDA can exacerbate IR and put T2DM patients at risky for cardiovascular and neurological afflictions. When the multivariable logistic regression model was performed, after controlling for age, BMI, and diabetes duration, both IDA and metformin use were found to predispose to raised oxidative stress in diabetes independently [13]. These results highlight the significance of assessment of iron status in diabetic patients taking metformin since longevity of treatment may enhance susceptibility to IDA, added to escalated oxidative stress [14]. This risk could be especially true for patients who already have higher levels of ROS because of hyperglycemia. Both low levels of hemoglobin and ferritin and increased

oxidative stress markers suggest that iron deficiency anemia implicates the entire body, not only by causing fatigue and pale skin [15]. Hemoglobin synthesis being a process that iron deficiency affects may mean that as a result of hypoxia, a state of oxidative stress is created due to the up regulation of ROS production in an effort to compensate for oxygen shortage in the tissues. In T2DM, this may worsen the diabetic complications because oxidative stress is a principle origin of vascular and metabolic disorders [16]. The results are in parallel with various established studies that have examined the effects of metformin on micronutrient concentration. Nevertheless, no data exists regarding oxidative stress status in T2DM patients with either IDA or only anemia [17]. The present study gives additional information on the possible oxidative stress that IDA puts on diabetic patient using metformin, which call for early therapies. Implementation of early diagnosis of iron deficiency in diabetic patients especially on long term metformin therapy could assist in identification of those at risk and provide proactive treatment like giving of iron enrichments foods or supplements or modifying the patient's diets in order to counter any onset of anemia and high oxidative stress [18]. While this study provides important insights, several limitations must be considered. First, this cross-sectional design only provides a snapshot of the relationship between IDA, metformin, and oxidative stress, without establishing causality.

CONCLUSION

It is concluded that iron deficiency anemia (IDA) is prevalent among type 2 diabetes mellitus (T2DM) patients and is significantly associated with elevated oxidative stress levels. This association suggests that IDA may exacerbate oxidative damage, potentially worsening diabetic complications. Routine monitoring of iron levels in these patients could be valuable for managing oxidative stress and improving overall health outcomes.

REFERENCES

1. ASIF, M., BANO, N., ASIF, U., NASIR, S., SHEHBAZ, L., & IQBAL, M. (2024). EVALUATING THE IRON DEFICIENCY ANEMIA IN TYPE-II DIABETIC PATIENTS TAKING METFORMIN AND ITS ASSOCIATION WITH OXIDATIVE STRESS. *Biological and Clinical Sciences Research Journal*, *2024*(1), 1170. https://doi.org/10.54112/bcsrj.v2024i1.1169
2. Kausar, M. A., Shahid, S., Anwar, S., Kuddus, M., Kalim, M., Khalifa, A. M., Khatoon, F., Alotaibi, A. D., Alkhodairy, S. F., Snoussi, M., & Arif, J. M. (2022). Identifying the alpha-glucosidase inhibitory potential of dietary phytochemicals against diabetes mellitus type 2 via molecular interactions and dynamics simulation. *Cellular and Molecular Biology*, *67*(5), 16–26. https://doi.org/10.14715/cmb/2021.67.5.3
3. Aboelnaga, S. M., & Khatoon, F. (2020). Dietary habits and consumption of fruits and vegetables in type 2 Diabetes in Ha'il Saudi Arabia. *Bull. Env. Pharmacol. Life Sci*, *9*(5), 05-11.
4. Wu, J., Rui-xia, Y., Yu, H., Qin, X., Wu, T., Wu, Y., & Hu, Y. (2023). Association of Metformin Use with Iron Deficiency Anemia in Urban Chinese Patients with Type 2 Diabetes. *Nutrients*, *15*(14), 3081–3081. https://doi.org/10.3390/nu15143081
5. Fernández-Real, J. M., & Manco, M. (2014). Effects of iron overload on chronic metabolic diseases. *Lancet Diabetes Endocrinol*, *2*(6), 513–526. https://doi.org/10.1016/s2213-8587(13)70174-8
6. AlDallal, S. M., & Jena, N. (2018). Prevalence of Anemia in Type 2 Diabetic Patients. *Journal of Hematology*, *7*(2), 57–61. https://doi.org/10.14740/jh411w
7. Arani, R. H., Fakhri, F., Tabiee, M. N., Talebi, F., Talebi, Z., Rashidi, N., & Zahedi, M. (2023). Prevalence of anemia and its associated factors among patients with type 2 diabetes mellitus in a referral diabetic clinic in the north of Iran. *BMC Endocr Disord*, *23*(1). https://doi.org/10.1186/s12902-023-01306-5
8. Farooqi, M., Tahir, Y., & Rehan, B. (2022). Anemia in Patients with Type 2 Diabetes: A Common but Neglected Clinical Finding. *PubMed*, *93*(1), e2022044–e2022044. https://doi.org/10.23750/abm.v93i1.11204
9. Guo, W., Zhou, Q., Jia, Y., & Xu, J. (2019). Increased Levels of Glycated Hemoglobin A1c and Iron Deficiency Anemia: A Review. *Medical Science Monitor*, *25*, 8371–8378. https://doi.org/10.12659/msm.916719
10. Davis, R. E., McCann, V. J., & Nicol, D. J. (1983). Influence of iron-deficiency anaemia on the glycosylated haemoglobin level in a patient with diabetes mellitus. *Medical Journal of Australia*, *1*(1), 40–41. https://doi.org/10.5694/j.1326-5377.1983.tb136024.x

11. Soliman, A. T., De Sanctis, V., Yassin, M., & Soliman, N. (2017). Iron deficiency anemia and glucose metabolism. *Acta Bio-Medica : Atenei Parmensis*, *88*(1), 112–118. https://doi.org/10.23750/abm.v88i1.6049
12. Doyle-Delgado, K., Chamberlain, J. J., Shubrook, J. H., Skolnik, N., & Trujillo, J. (2020). Pharmacologic Approaches to Glycemic Treatment of Type 2 Diabetes: Synopsis of the 2020 American Diabetes Association's Standards of Medical Care in Diabetes Clinical Guideline. *Annals of Internal Medicine*, *173*(10). https://doi.org/10.7326/m20-2470
13. Lipska, K. J., Yao, X., Herrin, J., McCoy, R. G., Ross, J. S., Steinman, M. A., Inzucchi, S. E., Gill, T. M., Krumholz, H. M., & Shah, N. D. (2016). Trends in Drug Utilization, Glycemic Control, and Rates of Severe Hypoglycemia, 2006–2013. *Diabetes Care*, *40*(4), 468–475. https://doi.org/10.2337/dc16-0985
14. Wang, Z., Wu, Y., Wu, J., Wang, M., Wang, X., Wang, J., Wu, T., Wu, Y., & Hu, Y. (2021). Trends in prevalence and incidence of type 2 diabetes among adults in Beijing, China, from 2008 to 2017. *Diabetic Medicine*. https://doi.org/10.1111/dme.14487
15. Wu, J., Wu, Y., Tian, Y., Wu, Y., Wang, M., Wang, X., Wang, Z., & Hu, Y. (2020). Association between ambient fine particulate matter and adult hospital admissions for pneumonia in Beijing, China. *Atmospheric Environment*, *231*, 117497. https://doi.org/10.1016/j.atmosenv.2020.117497
16. Yang, R., Yu, H., Wu, J., Chen, H., Wang, M., Wang, S., Qin, X., Wu, T., Wu, Y., & Hu, Y. (2023). Metformin treatment and risk of diabetic peripheral neuropathy in patients with type 2 diabetes mellitus in Beijing, China. *Frontiers in Endocrinology*, *14*. https://doi.org/10.3389/fendo.2023.1082720
17. Wu, J.-H., Wu, Y., Wang, Z.-J., Wu, Y.-Q., Wu, T., Wang, M.-Y., Wang, X.-W., Wang, S.-Y., Wang, J.-T., Yu, H., & Hu, Y.-H. (2021). Healthcare Costs Associated with Complications in Patients with Type 2 Diabetes among 1.85 Million Adults in Beijing, China. *International Journal of Environmental Research and Public Health*, *18*(7), 3693. https://doi.org/10.3390/ijerph18073693
18. Yang, R., Yu, H., Wu, J., Chen, H., Wang, M., Wang, S., Qin, X., Wu, T., Wu, Y., & Hu, Y. (2023). Metformin treatment and risk of diabetic peripheral neuropathy in patients with type 2 diabetes mellitus in Beijing, China. *Frontiers in Endocrinology*, *14*. https://doi.org/10.3389/fendo.2023.1082720