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Evaluation Of The Effect Of Metformin On The Level Of Some Biochemical Parameters In Patients With Type$_2$ Diabetes Mellites

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Abstract

Introduction: Diabetes mellitus (DM) is a significant health disorder impacting individuals globally, characterized by a high prevalence rate. Diabetes mellitus (DM) is categorized by its etiology into type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM); several risk factors, including age, genetic predisposition, and obesity, contribute to the onset of T2DM. Metformin is an established first-line pharmacotherapy for individuals with type 2 diabetes mellitus, aimed at enhancing glycemic control. **Material and Methods**: This cross-sectional study examined 89 diabetes patients from September 2024 to June 2025, dividing them into two groups: a control group of 36 healthy individuals and a T_2DM group of 53 patients on metformin therapy. A pre-structured questionnaire was created to gather patient records, including age, gender, BMI, and metformin dosage. Serum parameter levels were evaluated to compare with the control group, assessing reduction or borderline values. **Result**: The study compared 53 type 2 diabetes patients and 36 control subjects, finding that females were more frequent in both groups. Age and body mass index showed no significant difference between the groups. Generally, the biochemical parameters in patients and controls show significant differences in ferritin, vitamin B12 levels, HbA1c, and adenosine triphosphate (ATP) levels. While RBC, Hb, GIF, and LA levels revealed that there is no significant difference between groups. Metformin (1000 mg)-induced biochemical parameter analysis revealed significant differences in ferritin, vitamin B12, HbA1c, and ATPS between control and T_2DM patients, while non-significant differences were observed in RBC, Hb, GIF, and LA. On the other hand, ATPS, RBC, GIF, LA, and vitamin B12 levels showed significant differences between control and T2DM patients by using 2000 mg of metformin per day, while RBC, Hb, GIF, and LA showed non-significant differences between the groups. **Conclusion**: The biochemical parameters showed significant differences in ferritin, vitamin B12, HbA1c, and ATP levels, but no significant differences in RBC, Hb, GIF, and LA levels. Metformin-induced biochemical parameters showed significant differences in ferritin, vitamin B12, HbA1c, and ATPS.

Keywords: T_2DM, Metformin, Vitamin B_{12}, ATPS

Introduction

Among the most challenging health problems of the (21st century) is diabetes mellitus (1, 2). It is a chronic condition characterized by high levels of glucose in blood, resulting in significant damage to multiple organs including the heart, kidney, liver, eyes and vessels (3, 4). Weak glycemic regulation is known to be correlated substantially with a longer period of diabetes. Diabetes is a progressive disease and as glucose levels increase, more medicines are required to regulate it (5).

Patients with T_2DM should have good glycaemic control. It is estimated by the marker HbA1c, which reflects the blood glucose of a patient over a 2–3-month duration (6). Researchers found that a patient who has poor glycaemic control may develop complications like microvascular defects, such as retinopathy, nephropathy and neuropathy (7). Therefore, early diagnosis and treatment of T_2DM can prevent micro and macrovascular complications, or at least, delay the symptom's progression. The treatments used will slow the disease

progression by reducing blood glucose levels, managing the patient's hypertension and dyslipidaemia, and lower in cardiovascular complications (8-10).

Metformin monotherapy is first treatment in diabetes type 2 patients which initiated when non-pharmacological therapy has failed to reach sufficient glycemic regulation (11, 12). Metformin inhibits vitamin B_{12} absorption via changing intestinal motility, causing bacterial overgrowth, and changing the vitamin B_{12}-IF complex (13). Publications have shown a relationship between serum ferritin levels, excessive iron absorption, and type 2 diabetes, but this relationship has not yet been proven(14). Although diets with low iron content are recommended, publications show that ferritin levels are high in patients whose glycemic control cannot be achieved (15, 16)**.**

Diabetes mellitus is associated with various pathological changes including metabolic, cellular, and blood disturbances resulting in vascular complications (17). It has been documented that several blood components including red blood cells (RBCs), white blood cells (WBCs), platelets, and the hemostasis systems are affected by diabetes (18). Light of evidence showed that qualitative and quantitative change of RBCs are common in diabetic patients. It has been documented that, hyperglycemia is responsible for the complications and adverse outcomes of diabetes (19). The persistent hyperglycemia in diabetes is associated with metabolic, structural, and functional changes in RBCs due to the glycation of hemoglobin (Hgb) and membrane proteins (20).

However, diabetes mellitus (DM) induces endothelial dysfunction and alters basal vascular tone in blood vessels through different mechanisms (21). The process of relaxation of the blood vessels partly includes the endothelial independent mechanism(s), such as hyperpolarization of the smooth muscle membrane, as a consequence of the activation of different potassium (K^+) channels. Among them, the smooth muscle adenosine triphosphate (ATP)-sensitive K^+ (KATP) channels have an important role in the control of the vascular tone (22, 23). Furthermore, metformin use is limited because of its potential adverse effects associated with lactic acidosis (LA), particularly in patients with reduced renal function. Scale and Harvey reported that LA was more common in patients with diabetes but was not more frequent in patients who had taken metformin (24). The aim of the study is to investigate whether metformin use was associated with ferritin, vitamin B_{12} and other parameters in patients with type 2 diabetes

Patients and Methods

From September 2024 to June 2025, diabetes patients who visited the Layla Qasim diabetic center, Hawler teaching hospital, and Rizgary teaching hospital, Galiwa center and private Kolab Laboratory as outpatients were studied in this cross-sectional study. A total of 89 cases were targeted for the study and divided into two groups. The control group comprised 36 apparently healthy individuals, and group T_2DM consists of 53 patients on metformin therapy. Patients with renal failure, Cushing syndrome or hepatic diseases were excluded from the study after the clinical evaluation. The samples were taken early in the morning between (8.30 and 11.00 A.M) while both patient subjects were relaxed and fasting for (12-14) hours. The blood sample was divided into two aliquots; 2 & 8 ml.

All patients' records were gathered, and a pre- structured questionnaire was created. The information obtained included the patient's age, gender, Body mass index (BMI), and metformin dosage (1000 mg and 2000 mg). The serum parameter levels (ferritin, vitamin B_{12}, HbA1c, RBC, Hb, GIF, ATPS, and LA) in patients undergoing treatment with metformin were evaluated to determine whether their levels were reduced or at border line values to compare them with the levels found in the control group.

The first aliquot blood was dispensed in a tube containing Ethylene Diamine Tetra acetic Acid (EDTA), this blood mixed gently, While the second aliquot was dispensed in a plain tube and left for around an (2-3min. at room temperature) to clot at room temperature (25°C), and then separated by centrifuge at (3000 rpm) for (10 min) to collect serum. The serum was divided into two Eppendorff tubes and stored in the deep Freeze (-20 ^{0}C) until the assay day.

The sample in the EDTA tube was used to determine glycated hemoglobin (HbA1c) using a Cobas c 111 automated analyzer and complete blood counts (CBC) using a Mythic 18 automated analyzer. To get the serum, Cobas e 411 was used to calculate serum vitamin B_{12} and ferritin levels. Serum GIF, ATPS and LA were determined by ELISA technique using the kit supplied by Spinreact, Spain.

Statistical Analysis:

The data was collected and tabulated in Microsoft Excel spreadsheets and analyzed with GraphPad Prism 8.0 for windows software. All values were expressed as Mean ±SE and P value of <0.05 was considered to be statistically significant and Paired t-test was used to compare the results of various parameters among the studied groups.

Result:

From 53 patients with type 2 diabetes patients and 36 control subjects. The female gender is more frequent in control and T_2DM groups than the males; the difference of gender between the groups is statistically non-significant (p=0.9961). When compare patient groups between themself showed the age among groups (control and T_2DM) was 68.89±2.961 and 63.04±1.496, respectively, was found increase but no significant (p>0.05). Furthermore, the body mass index results were measurement of the (means ± SE). Table (1) showed increase but no significant different (p>0.05) between the studied groups patients and control group (21.51±0.4926, 23.13±0.7721respectively).

Table 1: Demographic data of control and patients as test group with statistical analysis value

Parameter	Control	T_2DM	P Value
Sex (M/F)	(17/19)	(25/28)	0.9961
Age/ Year	68.89±2.961	63.04±1.496	0.1839
BMI (kg/m2)	21.51±0.4926	23.13±0.7721	0.0566

Biochemical parameters in patients and controls are listed in table 2 and showed that the Mean± SE value of Ferritin was 231±24.23 in control and 154.2±17.7 in T_2DM with p value 0.0104 (significant), the level of vitamin B_{12} in control and T_2DM patient was 353.1±27.13, 280.4±16.62, respectively and p value 0.0489 (significant), while the Mean± SE of HbA1c was 5.384±0.084 in control and 7.933±0.213 in T_2DM with p value <0.0001 (significant) and the level of RBC and Hb was 4.668±0.176, 13.03±0.373in control and 4.647±0.06, 13.03±0.195 in T_2DM with no significant difference between groups, respectively.

The present study showed statistically no significant differences for glycosylation inhibiting factor (GIF) and lactic acid (LA) in the control and T_2DM patients' groups. the mean value of GIF and LA (1477±34.49 and 227.2±11.28), respectively in the control and (1422±26.67 and 234.9±11.02), respectively in T_2DM group. On the other hand, Adenosine Triphosphate (ATPS) showed statistically significant differences between study groups (p=0.0161).

Table 2: Biochemical parameters mean level in the sera of control and patients as test group with statistical analysis value.

Parameter	Control (Mean± SE)	T2DM (Mean± SE)	P Value
Ferritin	231±24.23	154.2±17.7	**0.0104**

Vitamin B_{12}	353.1±27.13	286.8±16.05	**0.0437**
HbA1c	5.384±0.084	7.933±0.213	**<0.0001**
RBC	4.668±0.176	4.647±0.06	0.8978
Hb%	13.03±0.373	13.03±0.195	0.9989
GIF	1477±34.49	1422±26.67	0.2272
ATPS	69.30±1.675	83.33±4.028	**0.0161**
L.A.	227.2±11.28	234.9±11.02	0.8142

The biochemical parameters between control and T_2DM patients' groups was studied by using 1000mg of metformin. It showed statistically significant differences for Ferritin, Vitamin B_{12}, HbA1c and ATPS in the control and T_2DM patient's groups, whilst RBC, Hb, GIF and LA showed non-significant differences between study groups. The mean value showed in table (3).

Table 3: Biochemical parameters mean level in the sera of control and patients as test group (1000 mg Metformin) with statistical analysis value.

Parameter	Control (Mean± SE)	1000 mg	P value
Ferritin	231±24.23	150.2±21.76	**0.0008**
Vitamin B_{12}	353.1±27.13	280.4±16.62	**0.0489**
HbA1c	5.384±0.084	7.695±0.2373	**<0.0001**
RBC	4.668±0.176	4.658±0.06872	0.8998
Hb%	13.03±0.373	16.37±3.376	0.9844
GIF	1477±34.49	1433±32.14	0.5963
ATPS	69.30±1.675	84.4±4.937	**<0.0001**
L.A.	227.2±11.28	230.4±12.04	0.812

Table 4 represented biochemical parameters between control and T2DM patients' groups by using 2000mg of metformin. The result showed that, there was a significant difference for Ferritin, Vitamin B_{12}, HbA1c and ATPS in the control and T_2DM patient's groups, whilst RBC, Hb, GIF, and LA showed non-significant differences between the study groups.

Table 4: Biochemical parameters mean level in the sera of control and patients as test group (2000mg Metformin) with statistical analysis value.

Parameter	Control (Mean± SE)	2000mg Metformin	P Value
Ferritin	231±24.23	172±29.16	**0.0602**
Vitamin B_{12}	353.1±27.13	206.6±15.19	**<0.0001**
HbA1c	5.384±0.084	8.266±0.443	**<0.0001**
RBC	4.668±0.176	4.661±0.1178	0.9511
Hb%	13.03±0.373	13.24±0.359	0.7953
GIF	1477±34.49	1376±45.03	0.0734
ATPS	69.30±1.675	88.00±6.534	**0.0268**
L.A.	227.2±11.28	238.7±22.32	0.8987

Discussion

The gender distribution of diabetic cases in our study was 25 (47.17%) and 28 (52.83%) for males and females, respectively. This distribution is consistent with a study done in Ethiopia where 152 (39.6%) of the 384 participants were female and 232 (60.4%) were males. The higher prevalence of T_2DM in males might be related to central obesity associated with android obesity (25). According to our data, women had worse glycemic control than men. This result is in line with research from around the globe that showed women frequently have more trouble maintaining ideal glycemic control than males do (26). This may be because in many societies, women are often expected to take care of their families, which might cause them to neglect their own health. They may also have less time for physical activity, struggle to maintain healthy eating habits, and be less likely to seek medical care or adhere to treatment plans consistently (27). Furthermore, women are more likely to develop diabetes because they have less total muscle mass to absorb more glucose load and higher levels of progesterone and estrogen, which lower insulin sensitivity (26, 28).

In the present study, showed the age among groups (control and T_2DM) was 68.89±2.961 and 63.04±1.496, respectively. The result agreed with Raqib et al., which revealed that, the mean and SD of age for patients were 52.96±7.82 (13). Type 2 Diabetes in Iraq is most prevalent in older adults, with studies showing high numbers of patients over 60, and the disease can also affect younger age groups, especially with rising obesity rates. The International Diabetes Federation (IDF) projects a significant increase in adult diabetes in Iraq to 1.1 million by 2024, with numbers expected to rise further, indicating the need for ongoing management and awareness across all ages **(29).**

Biochemical parameters in patients and controls are listed in table 2 and showed that the mean ± SE value of ferritin was 231 ± 231±24.23 in the control and 150.2 ± 150.2±21.76 and 172±29.16 in T2DM (1000 mg and 2000 mg), respectively, with significant value in both cases of metformin intake. Metformin typically reduces serum ferritin levels in individuals with type 2 diabetes mellitus, although the underlying mechanism remains incompletely elucidated; it may relate to enhanced insulin sensitivity and diminished intracellular iron levels through the modulation of transferrin receptor internalization. Nonetheless, the administration of metformin is correlated with a heightened risk of iron deficiency anaemia, which may intensify oxidative stress, underscoring the necessity for monitoring iron levels in these individuals (15, 30). Furthermore, in our study, the patient group exhibited a significantly lower average serum vitamin B_{12} level (286.8±16.05and 280.4±16.62) in T_2DM patients receiving 1000 mg and 2000 mg of metformin, respectively, compared with the control group (353.1±27.13pg/mL, p=0.0437). These findings align with research conducted in Tripoli, Libya, where metformin-treated patients had significantly lower serum vitamin B_{12} levels compared with controls (216.6pg/mL vs. 555.1pg/mL) (31). Metformin is recognized for reducing serum vitamin B12 levels in individuals with type 2 diabetes mellitus (T2DM), resulting in an increased incidence of vitamin B12

insufficiency relative to non-users of the medication. This results from metformin's capacity to disrupt calcium availability in the ileum, hence hindering intrinsic factor-mediated absorption of vitamin B12. The likelihood of this insufficiency escalates with elevated doses and prolonged metformin treatment; thus, it is advisable for individuals on metformin to have their vitamin B12 levels regularly assessed, particularly if they possess risk factors for deficiency (32, 33). This significant reduction underscores the need for heightened awareness and proactive management of vitamin B12 deficiency in patients with T2DM on metformin therapy (8).

Our findings indicated dramatically reduced HbA1c levels in the case groups receiving 1000 mg and 2000 mg, underscoring metformin's effectiveness in enhancing glycaemic management. However, the significantly lower HbA1c levels in the M group ($p=0.004$) highlight metformin's efficacy in improving glycaemic control (8), as previously demonstrated in a randomized controlled study by González-Ortiz et al. which reported a marked reduction in HbA1c levels after 2 months of metformin therapy compared with the control group (34). Metformin remains the cornerstone of first-line pharmacologic treatment in T2DM due to its glucoselowering efficacy, safety profile, and low risk of hypoglycemia (35). The significant reduction in HbA1c in our study is consistent with previous research, which showed that Metformin can decrease HbA1c by 1.0% to 1.5% when used as monotherapy (36). Its primary mechanisms of action include suppression of hepatic gluconeogenesis, enhancement of insulin sensitivity, and promotion of peripheral glucose uptake (37, 38).

This investigation revealed no significant differences in the RBC and Hb values among the three tested groups. The result agreed with Qasim, 2013 which showed that there were no significant differences in all CBP parameters (except WBC and platelet) in the all tested groups (39). On the other hand, the result disagreed with Almuswie et al., which reported that, there was a significant decrease ($p<0.05$) in the level of hemoglobin in group II and group III compared with the control group, at the same time it was noted that there was a significant decrease in the concentration of hemoglobin in the group II compared to the group III (40). Metformin therapy in type 2 diabetes mellitus may result in diminished hemoglobin levels and reduced red blood cell (RBC) count, as well as an elevated risk of anaemia, probably attributable to impaired Vitamin B12 absorption and oxidative stress. Metformin enhances glycaemic management and diminishes oxidative stress; yet, it is independently linked to decreased haemoglobin levels and may lead to megaloblastic anaemia over time. Regular assessment of haemoglobin and Vitamin B12 concentrations is advised for individuals undergoing metformin therapy (41, 42). On the other hand, the glycosylation inhibiting factor (GIF) levels in patients and controls revealed a mean ± SE of 1477±34.49 in the control group, 1433±32.14 in the T2DM group receiving 1000 mg, with no significant difference ($p=0.5963$), and 1376±45.03 in the T2DM group receiving 2000 mg, with no significant differences observed between the 2000 mg group and the control group ($p=0.0734$). Metformin affects N-glycosylation intricately in individuals with type 2 diabetes, resulting in modified plasma N-glycome profiles characterized by reduced fucosylation and elevated galactosylation and sialylation. Although, metformin's comprehensive effects on N-glycosylation, including alterations in fucosylation, galactosylation, and sialylation, are regarded as components of its multifaceted mechanism in type 2 diabetes (43). Conversely, Advanced Glycosylation End Products (AGEs) result from sugar reactions and are detrimental; however, certain natural substances and vitamins, such as vitamin E, can impede these reactions and diminish protein glycosylation (44).

The parameters in patients and controls indicated that the Mean ± SE value of Adenosine Triphosphate was 69.30±1.675 in the control group, 84.4±4.937 in the T2DM group receiving 1000 mg with significant difference ($p=<0.0001$), and 88.00±6.534 in the T2DM group receiving 2000 mg, with significant differences seen among the groups 2000mg with the control group ($P= 0.0268$). Metformin diminishes intracellular adenosine triphosphate (ATP) levels by blocking mitochondrial complex I, resulting in a reduced cellular energy charge and the activation of the energy sensor adenosine monophosphate (AMP)-activated protein kinase (AMPK). The reduction in ATP availability impedes ATP-dependent processes, including hepatic gluconeogenesis, hence enhancing metformin's glucose-lowering benefits in type 2 diabetes. Metformin additionally restricts ATP release from cells, a mechanism that is associated with its efficacy in regulating elevated blood glucose levels (45, 46).

Biochemical parameters in patients and controls are showed that the Mean± SE value of lactic acid was 227.2±11.28 in control, 230.4±12.04 and 238.7±22.32 in T2DM (1000mg and 2000mg) with no significant difference between all groups. Metformin's impact on lactic acid is intricate; although it is fundamental for Type 2 Diabetes management, it may elevate plasma lactate levels by blocking hepatic mitochondrial respiration, potentially resulting in lactic acidosis (MALA). The risk increases with compromised kidney or liver function, hypoxaemia, or advanced age. MALA is an uncommon yet serious complication that is treated with supportive care and, in critical instances, dialysis (47, 48).

Conclusion

The study compared 53 type 2 diabetes patients and 36 control subjects, finding females more frequent. Biochemical parameters showed significant differences in ferritin, vitamin B12, HbA1c, and ATP levels, but no significant differences in RBC, Hb, GIF, and LA. Metformin-induced biochemical parameters showed significant differences in ferritin, vitamin B12, HbA1c, and ATPS, but no significant differences in LA.

Disclosure:

All authors declare that this research paper titled (Evaluation of the effect of metformin on the level of some biochemical parameters in patients with type 2 diabetes mellites) is entirely our work. We have cited all sources in this paper and have not plagiarised any material. Also, all data are available.

Conflict of Interest: None

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