Effects of sitagliptin on hematological parameters, erythropoietin levels, and renal and liver functions in patients with type 2 diabetes

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Abstract. The present study aimed to reveal the prospective interplay between sitagliptin and hematological parameters in patients with type 2 diabetes mellitus (Ty2-DM) in order to provide insightful modalities for optimized patient care. For this purpose, a retrospective cohort study was conducted in private clinics and a total of 135 participants were enrolled. The study was designed to adhere to the World Medical Association's Declaration of Helsinki on the moral conduct of trials on humans or animals. The patients were divided into three groups as follows: Group 1 (the control group) included 45 healthy individuals, group 2 consisted of 45 patients with Ty2-DM treated with metformin daily as a monotherapy, and group 3 included 45 patients with Ty2-DM treated with a combination of sitagliptin plus metformin daily. Blood samples were collected from all participants to estimate the values of the glycemic status, renal and liver functions, complete blood count, ferritin and erythropoietin (EPO). As demonstrated by the results, the renal and liver function parameters were comparable between groups 2 and 3. Group 3 had higher hemoglobin (Hb) (P=0.006) and ferritin (P=0.02) levels, and a greater number of red blood cells (RBCs) (P=0.004), in comparison to group 2. Additionally, in group 3, the correlation analysis revealed a significant inverse correlation between ferritin and EPO, with a significant direct correlation between ferritin and Hb, RBCs, hematocrit and mean corpuscular volume, respectively. On the whole, the present study highlights the potential of sitagliptin to alter hematological parameters in patients with Ty2-DM, which is closely associated with ferritin levels.

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Introduction

Type 2 diabetes mellitus (Ty2-DM) is associated with marked alterations in several parameters, including cellular, metabolic, immunological and hematological abnormalities, which eventually lead to vascular complications (1,2). The most commonly encountered hematological abnormalities experienced by patients with Ty2-DM are changes in the function, structure and metabolism of red blood cells (RBCs), white blood cells (WBCs), platelet counts and indices, and relevant hematological parameters (3). Anemia is a widespread blood-related condition which affects patients with diabetes (4,5). It has been shown to be linked to various diabetes complications, the main one being diabetic nephropathy (6,7). The deterioration in kidney functions leads to a decrease in erythropoietin (EPO) production, a hormone responsible for the regulation of RBC production (8). Moreover, diabetic anemia can also occur in the absence of nephropathy and may be related to other factors, such as hypo-responsiveness to EPO, diabetic neuropathy, uncontrol hyperglycemia, chronic inflammation, increased oxidative stress, high levels of advanced glycation end products, deficiency in vitamin B12, iron and folate, and antidiabetic agents (9). Some studies have provided evidence of an association between antidiabetic agents and diabetic anemia in certain cases; some antidiabetic agents can cause anemia as a side-effect, some may increase the likelihood of developing anemia, while others may not affect anemia (10,11).

Dipeptidyl peptidase 4 (DPP-4) inhibitors (sitagliptin, linagliptin, saxagliptin, and alogliptin) are examples of antidiabetic agents that are expected to produce a lower incidence of anemia among patients with Ty2-DM (12). In general, DPP-4 decreases EPO activity by breaking and negatively affecting colony-stimulating factor potential and stress hematopoiesis. Based on these actions, it is possible that DPP-4 inhibitors can increase EPO hypo-responsiveness, which in turn controls hematopoietic stem cells and progenitor cells, increasing EPO production and erythroid colony formation (13). Despite this promising effect of DPP-4 inhibitors on EPO and RBC production, limited studies are available assessing these effects on hematological parameters among patients with Ty2-DM. Therefore, the principal aim of the present study

was to investigate the possible alterations in hematological parameters and EPO levels in patients with Ty2-DM treated with sitagliptin and to estimate the renal and liver functions in order to provide valuable insight into its potential to protect patients with diabetes against hematological, renal and liver abnormalities. The correlation between the investigated parameters, if any, is also explored.

Materials and methods

Study design, study period and ethical considerations. The present study is a retrospective cohort study that was designed to investigate the hematological parameters, in addition to renal and hepatic functions in patients with Ty2-DM treated with sitagliptin. The patients were gathered from private clinics, and the study was carried out during the period between December, 2023 and April, 2024. The study was designed to adhere to the World Medical Association's Declaration of Helsinki on the moral conduct of trials on humans or animals. Ethical approval for the conduct of the study was obtained from the Collegiate Committee for Medical Research Ethics at the University of Mosul, Mosul, Iraq (Code: CCMRE-phA-23-16). All participants had a clear explanation about the purpose of the study and an informed consent was requested to be signed by them. A study questionnaire was also distributed to all participants, which included information about age, weight, height, body mass index (BMI), the duration of diabetes, the co-existence of diseases and co-existence medications, and the duration of the sitagliptin and metformin usage; this information facilitating the recognition of the inclusion and exclusion criteria of the study before performing the laboratory tests.

Study participants. The present study included 135 participants, who were divided into three groups after being fully examined by the specialists to detect their eligibility for inclusion in the study and to exclude any abnormalities based on their medical, family and bleeding disorders history. A control group of 22 males and 23 females with a mean age of 52.82±7.94 years, apparently healthy individuals, who were not taking any medicine for any chronic conditions was included as the first group (group 1). The participants in the second group (group 2) had a mean age of 53.52±8.65 years, consisting of 23 male and 22 female patients with Ty2-DM taking metformin at 1,000 mg daily. The third group (group 3) consisted of 22 male and 23 female patients with Ty2-DM who took 50 mg of sitagliptin and 1,000 mg of metformin daily; their mean age was 54.64±8.66 years. Patients with type 1 diabetes mellitus, those taking antidiabetic agents instead of sitagliptin and metformin, those whose duration of medication use under investigation was <3 months, pregnant females and lactating mothers were not included in the patient groups. Individuals who had chronic renal, liver diseases, or hematological diseases were also excluded, as were those who took medications that affected renal and liver functions, or hematological parameters. The healthy control group establishes baselines for measured variables, allowing researchers to assess how diabetic patients deviate from normal physiological functions. This comparison helps to evaluate the effectiveness of treatments, such as sitagliptin and metformin and any adverse effects on hematological parameters, EPO levels

and renal and liver functions. Including a healthy group also controls for confounding factors related to ethnicity, ensuring the groups are comparable demographically. Moreover, having a healthy reference helps to rule out influences from laboratory assessment devices, enhancing the validity and reliability of the results. Overall, the presence of a healthy control group is crucial to attributing outcomes directly to the investigated drugs rather than other confounding factors. The patients with Ty2-DM in group 2 who were treated with metformin only served as the control group for group 3, and the patients with Ty2-DM in group 3 who were treated with sitagliptin plus metformin comprised the group which included the drug under investigation.

Laboratory methods. A total of 7 ml venous blood were collected and 2 ml were transferred into an anticoagulant EDTA tube to prevent clotting before the blood tests were performed. Whole blood was used to measure hemoglobin A1c (HbA1c) levels and complete blood count (CBC). The turbidimetric inhibition immunoassay (TINIA), which used a Cobas c 111 auto-analyzer and kit from Roche Diagnostics was the basis for determining HbA1c levels (14). The CBC test was performed using the Swelab Alpha Plus hematological analyzer; Sweden; Boule Medical AB company; Swelab Alpha Plus is an automated hematological analyzer whose measurement principles are based on the electric resistance (impedance) of the cell counts and spectrophotometry of hemoglobin. Hemoglobin, WBCs, RBCs, mean corpuscular volume (MCV), hematocrit (Hct), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were all estimated. Moreover, 5 ml blood were transferred into a gel tube with a clotting activator to obtain a serum after clotting and centrifugation at a speed of 3,000 rpm for 10 min at 20°C. Serum ferritin levels were estimated using a Cobas e 411 immunoassay analyzer (Roche Diagnostics); this device depends on an electrochemiluminescence immunoassay (ECLIA) method using kits supplied by Roche Diagnostics. Serum EPO was measured following the manufacturer's instructions, using a Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) with a ChroMate@ Microplate Reader manufactured by Awareness Technology, Inc. and a kit supplied by ELK Biotechnology. Serum glucose, renal function tests (including urea, creatinine and uric acid) and liver function tests [including aspartate aminotransferase (AST), alanine aminotransferase (ALT) and total bilirubin] were estimated photometrically using a Cobas c 111 auto-analyzer and kits supplied by Roche Diagnostics (15-17).

Statistical analysis. All data are presented as the mean values with standard deviation (SD). Using one-way ANOVA followed by Tukey's post hoc test, the statistically significant differences among the studied groups were investigated. Pearson's correlation coefficients, linear regression and 95% confidence intervals were employed to examine the correlation between the variables being studied. The studied groups were validated for the normal distribution of data before any statistics, using normality tests (Kolmogorov-Smirnov and Shapiro-Wilk tests). GraphPad Prism 8 software (Dotmatics) was used to perform the statistical analyses. A P-value <0.05 was considered to indicate a statistically significant difference.

Table I. Baseline characteristics of the study groups.

Variables	Group 1 (n=45)	Group 2 (n=45)	Group 3 (n=45)
Age (years)	52.82±7.9	53.52±8.65	54.64±8.66
Number (M/F)	45 (22/23)	45 (23/22)	45 (22/23)
BMI (kg m^{-2})	24.05±0.8	29.71±4.65[a]	29.33±3.58[a]
Duration of diabetes (years)	-	4.22±3.10	5.54±2.63

The results are expressed as the mean ± standard deviation and significant differences where indicated ([a]P<0.001, in comparison to group 1) were determined using one-way ANOVA followed by Tukey's post hoc test. n, number; group 1, control; group 2, patients with diabetes treated with metformin; group 3, patients with diabetes treated with sitagliptin plus metformin; M, male; F, female; BMI, body mass index.

Table II. Glycemic status of the study groups.

Variables	Group 1 (n=45)	Group 2 (n=45)	Group 3 (n=45)
FSG (mg/l)	856.7±125	1,309±417.6[a]	1,515±477.4[a,b]
HbA1c (%)	5.44±0.35	6.71±1.41[a]	7.10±1.16[a]

The results are expressed as the mean ± standard deviation and significant differences where indicated ([a]P<0.001, in comparison to group 1; and [b]P<0.01, in comparison to group 2) were determined using one-way ANOVA followed by Tukey's post hoc test. n, number; group 1, control; group 2, patients with diabetes treated with metformin; group 3, patients with diabetes treated with sitagliptin plus metformin; FSG, fasting serum glucose; HbA1c, hemoglobin A1c.

Table III. Liver functions of the study groups.

Variables	Group1 (n=45)	Group 2 (n=45)	Group 3 (n=45)
AST (U/l)	18.37±4.69	20.59±5.01	19.57±6.97
ALT (U/l)	18.96±7.46	21.66±5.28	21.88±10.53
Total bilirubin (mg/l)	4.5±2.0	4.9±2.3	4.8±3.2

The results are expressed as the mean ± standard deviation; data were analyzed using one-way ANOVA followed by Tukey's post hoc test. No significant differences were found between the groups. n, number; group 1, control; group 2, patients with diabetes treated with metformin; group 3, patients with diabetes treated with sitagliptin plus metformin; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Results

Baseline characteristics of the study participants. A significantly higher BMI value (P<0.001) was observed in group 3 and group 2 compared with the control group, however; however, there were no significant differences in BMI values and in the duration of diabetes between groups 3 and 2. The baseline characteristics of the study population are presented in Table I.

Glycemic status. The levels of fasting serum glucose (FSG) exhibited a significant difference (P<0.001) in group 3 group and group 2 compared with group 1. A significant decrease in FSG levels was detected in group 2 compared with group 3 (P<0.005). Similarly, the HbA1c levels in groups 3 and 2 exhibited a significant difference (P<0.001) compared with those in group 1 (Table II).

Liver functions. The levels of liver function parameters, including AST, ALT and total bilirubin, were within the normal range in all of the studied groups. Liver function parameters in the patients using sitagliptin were comparable to those of other groups, with no significant differences observed (Table III). The assessment of liver function parameters is crucial for excluding liver issues that may affect EPO secretion and directly identify any blood abnormalities linked to the examined drug.

Renal functions. The levels of renal function parameters exhibited variable values among the study groups, as demonstrated in Table IV. The urea levels were significantly increased in group 3 (P<0.001) and group 2 (P=0.014), when compared with those in group 1. The creatinine levels were elevated in both group 3 (P<0.03) and group 2 (P<0.02) compared with group 1, although they remained within the normal range. The uric acid levels were significantly higher (P<0.003) in group 3 compared with both groups 1 and 2. However, creatinine clearance (CrCl) showed no significant differences among the study groups. This observation of renal parameters is critical as it aids in ruling out potential renal abnormalities that could

affect EPO secretion, thereby establishing a clear connection between any observed blood irregularities and the drug being studied.

Hematological parameters. Assays of hematological parameters in the present study revealed notable changes and associations between the studied groups, as summarized in Table V. Hemoglobin levels were within the normal range for all groups; however, they were significantly higher (P=0.006) in group 3 compared to group 2, and considerably lower (P=0.02) in group 2 compared to group 1. As regards serum ferritin levels, although all participant groups had values within the normal range, a significant increase (P=0.02) was found in patients on sitagliptin plus metformin (group 3) compared to both the control and metformin groups. Furthermore, the RBCs exhibited a significant increase (P=0.004) in group 3 compared to group 2. Despite lower levels of WBCs observed in group 2, a relatively significant increase in WBCs (P=0.012) was observed in group 3 compared to group 2.

There were no statistically significant differences in Hct levels in groups 3 and 2. Moreover, the MCV of group 3 was significantly lower (P=0.03) than that of group 1 and group 2 (P<0.001). Likewise, MCH was significantly lower (P=0.009)

Table IV. Renal functions of the study groups.

Variables	Group 1 (n=45)	Group 2 (n=45)	Group 3 (n=45)
Urea (mg/l)	234.2±49	276.7±41.6[a]	305.6±100[c]
Creatinine (mg/l)	7.1±1.2	8.4±2.1[a]	8.5±2.2[a]
Uric acid (mg/l)	43.7±10.8	46.6±9.3	51.7±12.5[b]
CrCl (ml/min)	103.60±23.18	112.70±29.57	110.30±29.08

The results are expressed as the mean ± standard deviation and significant differences where indicated ([a]$P<0.05$, [b]$P<0.01$ and [c]$P<0.001$ in both metformin or sitagliptin plus metformin groups compared with the control group) were determined using one-way ANOVA followed by Tukey's post hoc test. n, number; group 1, control; group 2, patients with diabetes treated with metformin; group 3, patients with diabetes treated with sitagliptin plus metformin; CrCl, creatinine clearance.

Table V. Hematological parameters of the study groups.

Variables	Group 1 (n=45)	Group 2 (n=45)	Group 3 (n=45)
Hb (g/dl)	138.9±13.3	128.7±12.9[a]	139.9±16[e]
Ferritin (ng/ml)	100.50±49.79	99.45±55.62	150.90±99.98[a,d]
RBCs (x10^6/μl)	4.85±0.38	4.74±0.36	5.15±0.66[e]
WBCs (x10^3/μl)	7.50±1.66	6.80±1.40	8.06±2.11[d]
Hct (%)	42.78±3.45	43.43±3.95	42.47±8.32
MCV (fl)	88.45±7.65	91.61±5.09	84.62±4.77[a,f]
MCH (pg)	28.99±2.95	29.81±195	27.14±2.15[b,f]
MCHC (g/l)	327.4±13.9	317.8±38.7	320.2±19.9
EPO (pg/ml)	17.28±3.12	18.37±1.37	17.90±1.98

The results are expressed as the mean ± standard deviation and significant differences where indicated ([a]$P<0.05$ and [b]$P<0.01$ in comparison to group 1; and [d]$P<0.05$, [e]$P<0.01$ and [f]$P<0.001$ in comparison to group 2) were determined using one-way ANOVA followed by Tukey's post hoc test. n, number; group 1, control; group 2, patients with diabetes treated with metformin; group 3, patients with diabetes treated with sitagliptin plus metformin; Hb, hemoglobin; RBCs, red blood cells; WBCs, white blood cells; Hct, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; Plts, platelets; EPO, erythropoietin.

in group 3 when compared with group 1 and a highly significant reduction ($P<0.001$) was observed compared to group 2. When comparing the two patient groups to the control group, there were no statistically significant variations in MCHC or EPO.
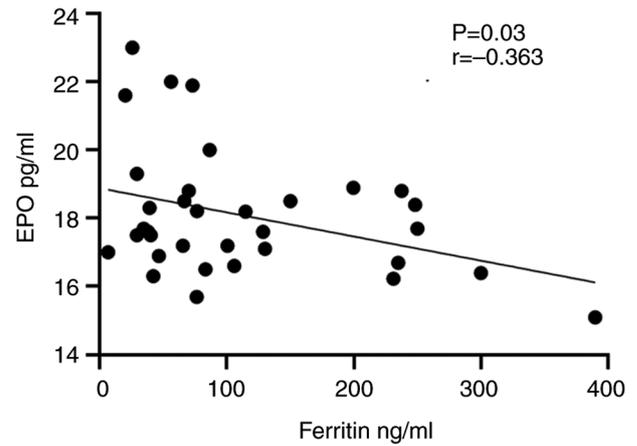

Figure 1. Impact of the combination of sitagliptin plus metformin agents on EPO as related to the ferritin levels. An inverse correlation was observed between the EPO levels and ferritin. EPO, erythropoietin.

Correlation between ferritin, and EPO, Hb, RBCs, Hct and MCV. The results of the correlation analysis revealed a strong inverse correlation between ferritin and EPO in group 3 who took sitagliptin with metformin at $P=0.03$, as shown in Fig. 1. Conversely, in the same group, a significant direct correlation was found between ferritin and Hb, RBCs, Hct and MCV at ($P=0.001$, $P=0.012$, $P=0.009$ and $P=0.03$), respectively as illustrated in Fig. 2.

Discussion

Since hematological, liver and kidney abnormalities are increasingly common as a side-effect of antidiabetic agents, it is critical to establish a strategy that treats diabetes, without potentially aggravating any concomitant conditions. Despite the abundance of existing studies about the action of DPP-4 inhibitors on different body organs, there is limited information available of how these inhibitors affect hematological parameters in patients with diabetes. This gap inciates the importance of investigating the potential of DPP-4 inhibitors in modulating hematological parameters and EPO levels, as well as their effect on renal and liver function in individuals with Ty2-DM. In the present study, sitagliptin was used as a representative of DPP-4 inhibitors to attain the desired aim.

The baseline characteristics of the study groups were matched as regards age and sex. In the two patient groups, the BMI values and the duration of diabetes were similar, eliminating the impact of these factors on the assessed parameters. The duration of the treatment in the two patient groups was over a period of 6 months, and there was no significant difference in the duration of the investigated drug among the patient groups. Thus, any confounding factor regarding the duration that could affect the examined parameters was ruled out.

The significant differences in FSG observed between the metformin and sitagliptin plus metformin groups may be due to the primary effects of sitagliptin on postprandial blood glucose levels. This effect has been demonstrated by the addition of sitagliptin to insulin plus metformin therapy, which provides additional postprandial glycemic control (18). However, the non-significant differences in HbA1c observed
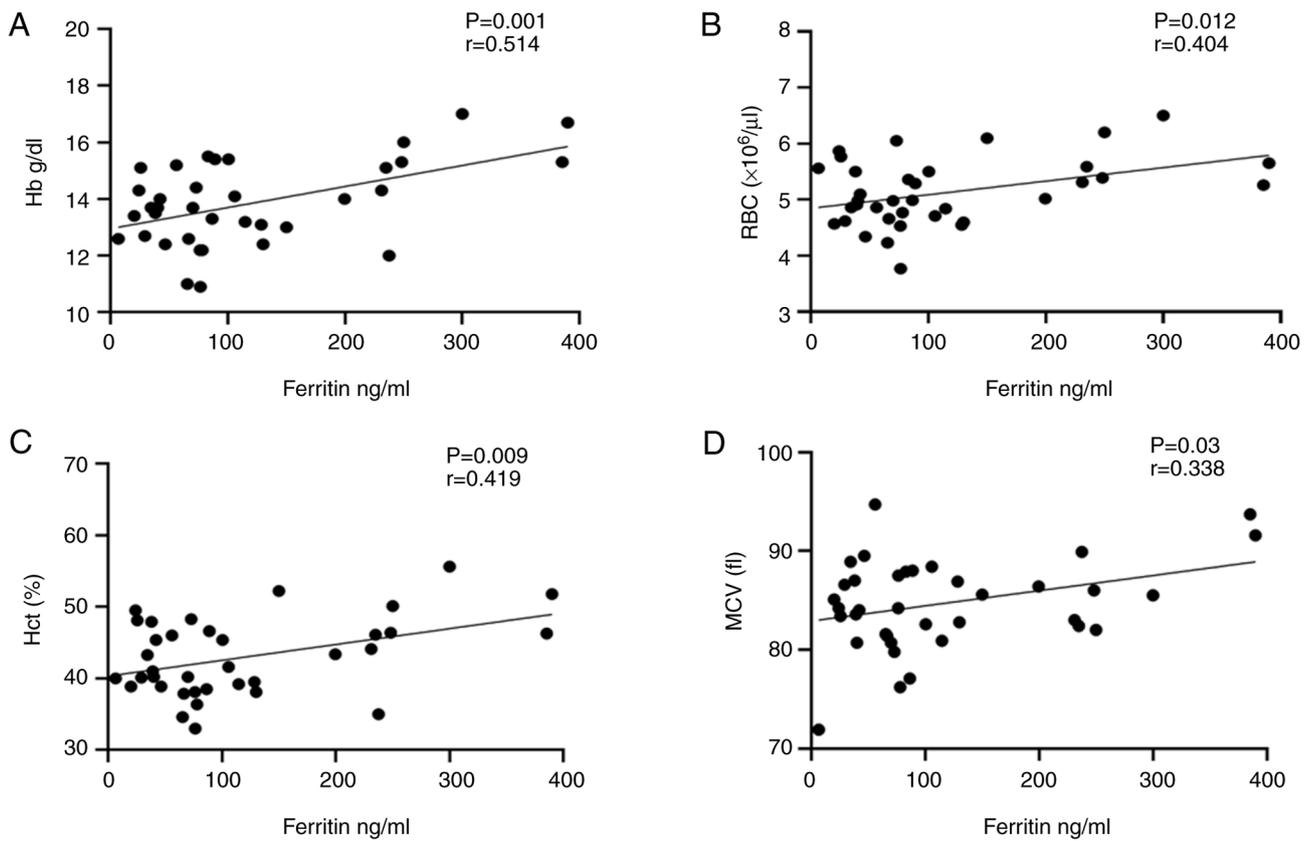
Figure 2. Impact of the combination of sitagliptin plus metformin agents on hematological parameters in relation to the ferritin levels. (A) A positive correlation was observed between blood Hb levels and ferritin levels in the patients with diabetes treated with sitagliptin plus metformin. (B) A positive correlation was observed between red blood cells and ferritin levels in the same group. (C) A positive correlation was observed between Hct and ferritin in the same group. (D) A positive correlation was observed between the level of MCV and ferritin in the same group. Hb, hemoglobin; RBC, red blood cell; Hct, hematocrit; MCV, mean corpuscular volume.

between the two patient groups may be due to the metabolic memory phenomena, which refer to good or poor blood glucose control that has a long-lasting effect on the metabolic process of the body even after the blood glucose is normalized (19).

Both metformin and sitagliptin clearly exhibited their hepatoprotective effect, which is consistent with the findings of previous studies (20,21), thus, ruling out any liver issues that could lead to anemia and directly ascertaining any blood abnormalities found with the examined drug. Sitagliptin demonstrates its hepatic protection through the suppression of NF-κB, a key mediator in the stimulation of proinflammatory genes, thus suppressing inflammation (22). In addition, sitagliptin acts as a scavenger for reactive oxygen species and has been shown to improve diabetic liver inflammation in mice (23). Taken together, the abovementioned results display the potential positive effects of sitagliptin when combined with metformin on liver function (24).

Linked closely to the liver in maintaining hematological balance, the kidney is another vital organ handling blood abnormalities (25). Notably, in the present study, renal function parameters in the examined patients were notable for being higher than those in the control group. These findings in renal parameters following sitagliptin therapy are in accordance with those of previous reports, where it was suggested that DDP-4 inhibitors, by activating the glucagon-like peptide-1 (GLP-1) receptor, suppress the type 3 transporter of

the Na^+/H^+ exchanger, thereby promoting sodium excretion. Hence, the elevation in creatinine levels, which is enhanced by sitagliptin, is linked to the natriuretic effect of GLP-1. A slight increase in serum creatinine levels was observed in a 2-year study of individuals taking sitagliptin. A correlation was also observed between the increase in creatinine and the decrease in HbA1c levels. These findings suggest that the higher creatinine may be associated with incretin activation, possibly due to the induction in GLP-1 diuretic activity, upregulation and subsequent dehydration (26). Likewise, in a previous study, the administration of GLP-1 agonist increased sodium excretion, decreased urea excretion and increased urine pH levels, which may be mediated by GLP-1 agonist-induced natriuresis in the proximal tubule (27). Previously, sitagliptin has been reported to increase serum uric acid levels (28); however, the precise mechanisms involved remain unknown. However, the imbalance between uric acid production and excretion or indirect natriuresis activity are suggested as possible mechanisms (28). Thus, such mechanisms are concisely linked to the results in the present study regarding the elevated serum urea, Cr and uric acid levels, compared to healthy individuals. Notably, the non-significant results of creatinine clearance were inexorably linked to normal kidney function and health. However, the abnormalities of renal function can frequently cause alterations in the hematological parameters. Notable findings were obtained in the present study regarding hematological

parameters in the sitagliptin group compared to the control and metformin groups. The significant reduction in the Hb level in the metformin group was associated with the reduced levels of ferritin due to reduced intestinal iron absorption or effects on iron metabolism (29). In contrast to the metformin group, sitagliptin users exhibited a significant increase in Hb levels compared to the control group. Consequently, the RBC counts were increased in this group compared to metformin-only group. This is in line with the action of DPP-4 inhibitors in revealing the Hb decline in diabetic kidney disease (12). Moreover, DPP-4 inhibitors have not exhibited a direct linkage to hematological abnormalities in Ty2-DM (30).

Although Hb and ferritin levels are associated with iron status, the association between them can vary and depends on a number of factors. Generally, elevated levels of serum ferritin are typically caused by prolonged alcohol intake, cancer, metabolic syndrome, renal or liver illness, and acute or chronic inflammation rather than iron overload (31,32). Herein, the strict inclusion criteria of the involved participants excluded the possible pathological conditions that could elevate serum ferritin levels. Moreover, a number of factors, including iron, cytokines, oxidative stress and hormones, tightly control the expression levels of ferritin. Surprisingly, the incretin hormone can play a key role in the regulation of ferritin levels. Furthermore, the nuclear factor-erythroid 2 related factor (Nrf2) is regarded as a chief regulator of ferritin synthesis and degradation to preserve iron homeostasis (33). Of note, the incretin hormone upregulates the expression of Nrf2, which turns on the activation of genes that code for antioxidant enzymes, including heme oxygenase-1 (HO-1) which is a cyto-protective antioxidant protein (34). Following the induction of HO-1, this enzyme catalyzes heme oxygenation to produce a physiologically active molecule, such as iron, resulting in the high expression of ferritin for iron sequestration (35). Notably, sitagliptin has hepatoprotective activity partly through the modulation of the Nrf2/ HO-1 signaling pathway (36). Additionally, the result in the study by Genc *et al* (37) was consistent with the result of the present study; in their study, the serum ferritin levels were higher before and after 1 year of treatment in both the metformin and gliptin plus metformin groups. In the present study, serum ferritin levels were higher in patients with Ty2-DM who were on sitagliptin plus metformin than those on metformin alone. Thus, the aforementioned signaling pathways were inevitably linked to the indirect effect of sitagliptin on the elevation of serum ferritin levels through the action of the incretin hormone, which is in line with the significantly higher results of ferritin.

Additionally, due to the stored iron, higher ferritin levels resulted in a greater number of RBCs with sufficient Hb. Ferritin and EPO may be inversely associated due to sufficient blood indices, balanced erythropoiesis and RBC formation, by providing negative feedback to EPO induction. The direct correlation between ferritin and blood indices in the sitagliptin plus metformin group indicates that ferritin may be a potential target to combat DM complications through the incretin mimetic action of sitagliptin. Serum ferritin and WBCs are not directly associated; however, inflammation may be the cause of an indirect link. High ferritin levels during infection protect the immune cells of the body, prevent bacteria from using iron, reduce harmful free radicals, regulate the immune system and indicate inflammation. Medical professionals use these values as a signpost for therapeutic interventions (38). However, the statistically significant results concerning WBCs in sitagliptin users elucidate the capability of sitagliptin in directly affecting the colony-stimulating factors (CSF) in the bone marrow, thus promoting the production of blood cells regardless of its effect on erythropoiesis. This is consistent with the captivating function of DPP-4 in the regulation of proteins that govern different cell types, such as more mature blood cells and hematopoietic stem cells. It has been shown that a reduced DPP-4 activity is associated with the increased ability of hematopoietic stem cells to engraft (39). Furthermore, DPP-4 truncates growth factors including granulocyte-CSF, granulocyte macrophage-CSF and interleukin-3, which can drastically lower the capacity of cells stimulated with them to form colonies in comparison to the full-length proteins (40). Collectively, DPP-4 modifies proteins and peptides that regulate hematopoietic cells; thus, by targeting these proteins, a better understanding of blood cell regulation and treatment of hematological-related diseases could be attained (41). This precise matching procedure enabled the immediate correlation of the obtained results to the positive impact of sitagliptin on the hematological parameters in patients with Ty2-DM.

Despite the significant findings on Hb, RBCs and MCV counts, there were no significant changes in EPO levels in the sitagliptin group. This was contradicted by the ability of another member of DPP4 inhibitors, linagliptin, in reducing the dose of the erythropoiesis-stimulating agent (ESA) darbepoetin alpha and decreasing ESA resistance in patients undergoing hemodialysis (42). Additionally, DPP-4 inhibitors have been reported to enhance the ESA-resistance index in patients without iron deficiency undergoing hemodialysis (43). Moreover, sitagliptin may reduce oxidative stress in hematopoietic cells through its therapeutic effect on hematopoietic injury (23). The appropriate explanation for the unaltered EPO level in the present study was related to the ferritin level in the sitagliptin plus metformin group compared to metformin-only users. This speculation was based on the concept that a high level of ferritin would activate the factor inhibiting hypoxia-inducible factor 1 under normoxic and hypoxic conditions. This factor is a co-regulator of the HIF gene, which is a master manager of erythropoiesis. Thus, the high level of ferritin may activate this factor and suppress the HIF gene, which will restrict the production of EPO and erythropoiesis (44).

Even with the obtained result concerning EPO, both serum ferritin and RBCs exhibited a statistically direct correlation in sitagliptin users. Consequently, there was a direct correlation between ferritin, Hb, Hct and MCV in this group of patients. Alongside the inverse correlation between ferritin and EPO, the results indicated the potential role of sitagliptin in improving erythropoiesis through the direct inhibition of the DPP-4 enzyme, thereby reducing the degradation of EPO. Simultaneously, sitagliptin may regulate the overproduction of EPO through indirect incretin action on ferritin levels and may maintain EPO levels within the normal range. Notably, correlation analyses were performed among all the studied groups and on all the study parameters in groups 2 and 3, and no significant associations were found (data not shown). This indicates that the correlations observed are related solely to groups 3 (patients on sitagliptin only).

Nonetheless, it is essential to address the limitations of the current study, such as the relatively small sample size, which needs to be expanded in future studies. Additionally, long-term prospective trials are recommended to validate the results of this study. Even with these limitations, the present study provided valuable insight into the potential of sitagliptin on significant modulation of hematological indices, while keeping normal renal and liver functions. This effect is closely correlated with ferritin level rather than the impact of the kidneys or the liver.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

FAA and MNA conceived and designed the study. RIA conducted the experiments. FAA and MNA analyzed data. RIA, FAA and MNA drafted the manuscript. FAA and MNA confirm the authenticity of all the raw data. All the authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was designed to adhere to the World Medical Association's Declaration of Helsinki on the moral conduct of trials on humans or animals. Ethical approval for the conduction of the study was obtained from the Collegiate Committee for Medical Research Ethics at the University of Mosul, Mosul, Iraq (Code: CCMRE-phA-23-16). All participants had a clear explanation about the purpose of the study and an informed written consent was requested to be signed by them.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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