

Investigation of association of biomarkers of iron metabolism and insulin resistance in Egyptian patients with impaired glucose metabolism and type 2 diabetes

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Introduction

Type 2 diabetes is an expanding overall medical issue. A large portion of the enthusiasm for the job of supplements in diabetes is fixated on macronutrients, yet a micronutrient, iron, is additionally closely connected with diabetes.

Aim

To study biomarkers of iron metabolism, including serum ferritin, transferrin saturation, iron, and insulin resistance, in diabetic and prediabetes patients.

Patients and methods

This is a cross-sectional study directed on a cohort of 50 patients, comprising 25 patients with impaired glucose tolerance and 25 patients recently discovered to have type 2 diabetes mellitus (T2DM), as well as 20 healthy controls of matched age and both sexes. All patients enrolled in the study were subjected to full history taking, full examination, laboratory investigations including iron, total iron-binding capacity, ferritin, insulin, lipid profile, fasting blood glucose, 2-h postprandial glucose, urea, creatinine, complete blood count, alanine aminotransferase, and aspartate aminotransferase.

Results

We found that patients with T2DM have significant higher body weight and BMI than prediabetes patients and controls, and also statistically significant difference in serum iron between the studied groups, but no statistical significance in serum ferritin between the studied groups. In addition, we found a positive correlation of serum iron and insulin resistance in T2DM, a significant positive correlation of serum ferritin with low-density lipoprotein and negative correlation with high-density lipoprotein in T2DM, positive correlation of ferritin with cholesterol and triglycerides in impaired glucose tolerance group. Moreover, transferrin saturation was negatively correlated with glycated hemoglobin, BMI, and total iron-binding capacity and is positively correlated with iron and creatinine and hemoglobin among the studied groups.

Conclusion

The distinguished relationship of several markers of iron metabolism with hyperglycemia and insulin resistance recommends that iron stores add to the pathogenesis of IGM and T2DM.

Keywords:

ferritin, impaired glucose tolerance, iron, type 2 diabetes mellitus, transferrin saturation

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Introduction

Type 2 diabetes is a typical and increasing overall medical issue. It is very much acknowledged that the most solid indicator for the disease is obesity; therefore, consideration has additionally been paid to the commitment of supplements and supplement-detecting pathways in circumstances of endless caloric abundance. A large portion of the enthusiasm for the job of supplements in diabetes is focused on macronutrients, yet a micronutrient, iron, is likewise intently connected with diabetes [1].

Inherited hemochromatosis, a hereditary issue portrayed by fundamental iron overburden, is accounted for to be related with diabetes [2].

Moreover, an overrepresentation of diabetes cases has likewise been identified among people with states of obtained iron overburden, for example, thalassemia major [3]. This could clarify that abnormal amounts of body iron are a hazard factor for type 2 diabetes in the community, and this may have suggestions for the prevention and treatment of type 2 diabetes. Cross-sectional and prospective studies report a positive relationship between ferritin and type 2 diabetes [4,5]. Ferritin is additionally an acute phase

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reactant and is increased in the presence of inflammation, liver disease, and insulin resistance, which are likewise connected with type 2 diabetes [6,7]. Different biomarkers of iron metabolism may give additional data on the role of iron in the pathogenesis of type 2 diabetes. Transferrin is the iron-binding protein available for use, and its levels increase with growing iron prerequisites. Serum iron is hard to interpret in separation, as it has a diurnal variety and consequently differs altogether without changes in all out-body iron [8]. Transferrin saturation (TSAT) is the extent of transferrin bound to serum iron and is to some extent a marker of iron absorption; it mirrors the extent of coursing iron in the milieu of iron prerequisites. TSAT is raised within the sight of nontransferrin-bound iron, which thus is in charge of iron-related oxidative harm [9,10].

We research the independent relationship of the markers of iron processing, serum ferritin, transferrin, TSAT, and iron with hyperglycemia and insulin resistance in Egyptian population with impaired glucose tolerance (IGT) and type 2 diabetes mellitus (T2DM), attempting to affirm the role of ferritin in insulin resistance and T2DM.

Patients and methods

The present study included three groups of patients aged 30–45 years. Participants were recruited from Internal Medicine Department and Clinic of Tertiary Care Hospital and were divided into group A, which included 25 patients newly diagnosed with T2DM; group B, which included 25 patients with IGT; and group C, which included 20 healthy controls. The exclusion criteria were (a) type I DM, (b) pregnancy-induced DM, (c) drug-induced diabetes, (d) liver and kidney diseases, and (e) hereditary hemochromatosis.

In all patients, thorough clinical evaluation was performed including fasting blood sugar, 2-h postprandial (2HPP) sugar, glycosylated hemoglobin (HbA1C), serum triglycerides (TGs), total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, serum aspartate aminotransferase, alanine aminotransferase, serum urea, creatinine, serum insulin measured by radioimmunoassay used in homeostatic measurement assessment insulin resistance (HOMA-IR), serum iron, total iron-binding capacity (TIBC), TSAT, serum ferritin, and albumin-creatinine ratio in urine.

This cross-sectional study was conducted from April 2017 till April 2018 after approval of the institutional ethical

committee. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients for being included in the study. All participants signed a written informed consent form before enrollment in the study to complete the requirement of the ethical committee of the faculty of physical therapy, Cairo University, and to ensure complete satisfaction. All patients of the study were informed about the study, its aim, and all risks and expected benefits, and they were also assured of their anonymity and confidentiality of data obtained before signing the informed consent.

Quantitation of ferritin in serum

The serum level of ferritin was measured using an ELISA kit provided by Immunospec Corporation (Canoga Park, California, USA).

Principle of the test

The Immunospec Ferritin Quantitative Test Kit depends on a solid-phase compound-connected immunosorbent examination. The measured framework uses charge interaction of ferritin to the wells of microtiter plate for solid phase immobilization, and then a mouse monoclonal antibody for ferritin conjugated with a neutralizer catalyst (horseradish peroxidase) is added. Ferritin is allowed to interact all the while with the antibodies, bringing about the ferritin atoms being sandwiched between the strong stage and protein-connected antibodies. Following a 30-min incubation at room temperature, the wells are washed to expel unbound antibodies. Then TMB (chromogenic substrate used in staining procedures in immunohistochemistry as well as being a visualizing reagent used in enzyme-linked immunosorbent assays) is included and incubated for 15 min; it interacts with enzyme and brings about the advancement of a blue shading. The shading improvement is ceased with the expansion of stop arrangement, and the shading is changed to yellow and estimated by ELISA peruse at 450 nm. The centralization of ferritin is straightforwardly corresponding to the shading power of the test.

Materials provided with the test kits include the following:

- (1) Antibody-covered microtiter plate with 96 wells.
- (2) Reference standard set, which contains 0, 15, 50, 200, 400, and 800 ng/ml (fluid, prepared to utilize).
- (3) Enzyme conjugate reagent, 12 ml.

- (4) TMB substrate, 12 ml.
- (5) Stop solution, 12 ml.
- (6) Wash buffer concentrate (100×), 15 ml.

Materials required but not provided were as follows:

- (1) Precision pipettes: 0.5, 50 µl, 0.05, 0.2 ml, and 1.0 ml.
- (2) Disposable pipette tips.
- (3) Distilled water.
- (4) Vortex blender or identical.
- (5) Absorbent paper or paper towel.
- (6) Graph paper.
- (7) Microtiter well peruser.

Reagent preparation:

- (1) All reagents were brought to room temperature (18–22°C) before use.
- (2) One volume of wash buffer (100×) was weakened with 99 volumes of refined water.

Assay procedures:

- (1) 10 µl of standard, examples, and controls was administered into suitable wells.
- (2) 100 µl of enzyme conjugate reagent was administered into each well.
- (3) Thoroughly blend for 30 s was finished. It is essential to have total blending in this arrangement.
- (4) Incubation was done at room temperature (18–22°C) for 30 min.
- (5) The hatching blend was evacuated by flicking plate content into a waste holder.
- (6) The microtiter wells were washed multiple times with washing cushion (1×).
- (7) The wells were stroked pointedly onto permeable paper or paper towels to evacuate all lingering water beads.
- (8) 100 µl of TMB substrate was apportioned into each well and blended delicately for 5 s.
- (9) Incubation was done at room temperature in obscurity for 15 min.
- (10) The response was ceased by including 50 µl of stop solution to each well.
- (11) Gently blend was accomplished for 30 s. It is essential to ensure that all the blue shading changes to yellow shading totally.
- (12) The optical thickness was perused at 450 nm with a microtiter peruser inside 15 min.

Calculation of results

The mean absorbance esteem (A₄₅₀) for each arrangement of reference models, examples, controls,

and patient examples was determined. A standard bend was made by plotting the mean absorbance acquired from each reference standard against its focus in ng/ml on diagram paper, with absorbance esteems on the vertical or Y hub and fixations on the level or X pivot. The mean absorbance esteems for every example was resolved, and the ferritin fixation was resolved in ng/ml from the standard bend.

Sensitivity of the kit

- (1) The minimal sensitivity of the test was 5.0 ng/ml.

Quantitative determination of glycosylated hemoglobin

Principle of the test

Glycosylated hemoglobin (GHb) has been defined operationally as the fast fraction hemoglobins HbA1 (Hb A1a, A1b, and A1c), which elute first during column chromatography. The non-GHb, which consists of the bulk of hemoglobin, has been designated HbAo. A hemolyzed preparation of whole blood is mixed continuously for 5 min with a weakly binding cation-exchange resin. The labile fraction is eliminated during the hemolyzate preparation and during the binding. During this mixing, HbAo binds to the ion exchange resin leaving GHb free in the supernatant. After the maxing period, a filter separator is used to remove the resin from the supernatant. The percent GHb is determined by measuring absorbances of the GHb fraction and the total hemoglobin fraction. The ratio of the absorbances of the GHb and the total hemoglobin fraction of the control and the test is used to calculate the percent GHb of the sample

Contents	10 tests
Ion exchange resin	10×3 ml
Predispensed tubes	
Lysing reagent	5 ml
Resin separators	10 nos

Sample material

Whole blood preferably fresh is collected in EDTA tube. GHb in the whole blood is reported to be stable for 1 week at 2–8°C.

Procedure

Wavelength	415 nm (Hg 405 nm)
Temperature	RT
Light path	1 cm

Hemolysate preparation

- (1) 0.5 ml lysing reagent was dispensed into tubes labeled as test (T).
- (2) 0.1 ml of the reconstituted well mixed blood sample was added into the appropriately labeled tubes; in mixed unit, complete lysis was evident.

- (3) The mixture was allowed to stand for 5 min.

Glycosylated hemoglobin separation

- (1) Top was expelled from the ion-exchange resin tubes and named as test.
- (2) 0.1 ml of the hemolysate from stage A was included into the properly marked ion exchange resin tubes.
- (3) A gum separator was embedded into each cylinder so the elastic sleeve was around 1 cm over the fluid degree of the sap suspension.
- (4) The cylinders were blended on a rocker, rotator, or a vortex blender ceaselessly for 5 min.
- (5) The tar was permitted to settle then the sap separator was pushed into the cylinders until the sap was immovably pressed.
- (6) Every supernatant was poured or suctioned legitimately into a cuvette, and every absorbance was estimated against refined water.

Total hemoglobin fraction

- (1) 5.0 ml of distilled water was dispensed into tubes labeled as test.
- (2) 0.02 ml of hemolysate from step A was added to it into the appropriately labeled tube and mixed well.
- (3) Each absorbance was read against distilled water.

Calculations:.

$$\text{Ratio of Test(RT)} = \frac{\text{Abs test GHb}}{\text{Abs test THb}}$$

$$\text{GHbA in\%} = \text{Ratio of Test(RT)} \times 10$$

Insulin resistance (homeostatic measurement assessment insulin resistance)

$$\text{HOMA - IR} = \frac{\text{Glucose} \times \text{Insulin}}{405}$$

(Glucose in mass units mg/dl) [11]

Category	HOMA score
Normal insulin resistance	<3
Moderate insulin resistance	Between 3 and 5
Severe insulin resistance	>5

Statistical analysis

Numerical variables were described as mean±SD. Categorical variables were described as percentages. Comparisons were done using Student *t* test for numerical variables, and χ^2 test for categorical variables. Unpaired *t* test was used to compare quantitative variables, in parametric data (SD<50% mean). Mann-Whitney test was used instead of

unpaired *t*-test in non-parametric data (SD>50% mean). One-way analysis of variance test was used to compare more than two groups regarding quantitative variables. Spearman correlation coefficient test was used to rank variables versus each other positively or inversely. Correlations were plotted and *r* values (correlation coefficients) were stated. *P* value was considered significant if less than or equal to 0.05. Univariate and multivariate regression analyses were run to predict potential determinants of insulin resistance. Statistics were calculated using SPSS 21 package. Software package that originated at what formerly was the National Opinion Research Center (NORC), at the University of Chicago.

Results

Demographic, clinical, and laboratory characteristics of participants

Our study was done on an age group ranging from 30 to 45 years old. There were statistically significant differences between the studied groups regarding sex ($P<0.001$) with more females than males in group A and group B than group C ($P=0.002$), and there was no significant difference in age among the three groups. Moreover, we found a statistically highly significant difference between the studied groups regarding BMI ($P<0.001$). Serum glucose level (fasting, 2HPP, and HbA1C) was higher in group A compared with group B and group C ($P<0.001$); however, group B had higher levels of both fasting blood sugar and 2HPP blood sugar and HbA1C than group C. Albumin-creatinine ratio was higher in group A and group B compared with controls ($P<0.001$). There was a statistically highly significant difference between the studied groups regarding fasting insulin, which was higher in group A than group B and group C ($P<0.001$). The cholesterol level was significantly higher in group A and group B than in control group ($P=0.019$). LDL level was statistically significantly higher in group A than in group B and control group ($P<0.001$). HDL level was significantly lower in group A compared with group B and control group ($P<0.001$), and there was no significant difference between the studied groups regarding TGs level ($P=0.087$). We found a statistically significance difference in Hb level among the studied groups, with higher Hb level in control group than group A and group B ($P=0.012$). Moreover, there was a statistically significant difference in serum iron among the studied groups, with high serum iron in control group than group A and group B ($P=0.033$). No statistically significant differences were found between the studied groups regarding serum TIBC, TSAT, and serum ferritin;

Table 1 Demographic, clinical, and laboratory characteristics of participants

Variables	Newly diagnosed DM2 (N=25) (group A)	IGT (N=25) (group B)	Control (N=25) (group C)	F	P value
Age	40.68±4.58	37.2±4.11	34.5±3.96	1.8	0.171
Sex				12.8	0.002
Male	7 (28)	7 (28)	15 (75)		
Female	18 (72)	18 (72)	5 (25)		
Weight (kg)	88.4±10.4	82.3±12.3	73.2±9.4	10.9	<0.001
Height (m)	172.3±6.9	171.7±7.6	175±6.2	1.7	0.192
BMI (kg/m ²)	29.9±3.9	28±4.2	23.8±3	15.0	<0.001
FBS (mg/dl)	179±64	109.4±7.2	82.7±8.1	38.1	<0.001
2HPP (mg/dl)	271±79.7	161.8±10	122.4±9.6	58.8	<0.001
HbA1C (%)	9.9±2	6.3±1.1	4.8±0.3	86.3	<0.001
A/C ratio	218.9±205	147.8±112.5	43.5±19.7	8.7	<0.001
Insulin (μU/ml)	21±16.9	10±2	9.3±1.8	9.8	<0.001
TGs (mg/dl)	162.3±72.5	142.2±102.6	111.4±16.5	2.5	0.087
Cholesterol (mg/dl)	195.8±40.5	196.5±45.3	167±20.8	4.2	0.019
HDL (mg/dl)	35.6±10	41±8.2	45.9±4	9.2	<0.001
LDL (mg/dl)	90±16.7	83.4±18.5	73.5±9.4	6.8	0.002
Hemoglobin (g/dl)	12±1.2	12.9±1.3	13.7±1.2	4.8	0.012
Iron (μg/dl)	65.7±20.1	72.5±21.1	80.4±10.4	3.6	0.033
TIBC (μg/dl)	382.1±55.3	393.4±61.9	376.4±28.8	0.6	0.533
TSAT	17.5±5.9	18.8±5.9	21.5±3.5	3.2	0.480
Ferritin (ng/dl)	92.7±178.3	65.5±147.7	50.4±37.2	0.5	0.587
Urea (mg/dl)	23.6±9.7	24.2±10	25.6±5.6	3.7	0.129
Creatinine (mg/dl)	0.7±0.2	0.7±0.2	0.8±0.1	2.2	0.120
HOMA-IR Index	8.4±5.8	2.7±0.6	1.9±0.4	24.4	<0.001
ALT (U/l)	36.9±12.6	42.6±27.5	31.5±7.6	2.0	0.143
AST (U/l)	30.5±13.5	32.1±13.6	25.9±6.2	1.6	0.208

Data are presented as mean±SD and n (%). 2HPP, 2 h postprandial; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DM, diabetes mellitus; F, result of the equation of analysis of variance test; FBS, fasting blood sugar; HbA1C, glycated hemoglobin; HDL, high-density lipoprotein; HOMA-IR, homeostatic measurement assessment insulin resistance; IGT, impaired glucose tolerance; LDL, low-density lipoprotein; TG, triglycerides; TIBC, total iron binding capacity; TSAT, transferrin saturation.

however, serum ferritin was higher in group A and group B than control group. Moreover, we found that insulin resistance (HOMA-IR) was significantly higher in group A than in group B and group C ($P<0.001$). Lastly, we found no significant difference of liver and kidney function between the studied groups (Table 1).

Correlations between insulin resistance (homeostatic measurement assessment insulin resistance) with ferritin, fasting blood sugar, 2 h postprandial, lipid profile, and age in each group separately

There was no significant correlation between HOMA-IR index and different laboratory data in the studied groups except positive correlation between ferritin and FBG in prediabetes group (Table 2).

Correlation between serum iron and insulin resistance (homeostatic measurement assessment insulin resistance) in newly diagnosed type 2 diabetes mellitus (group A)

There was a significant positive correlation between serum iron and insulin resistance represented by HOMA-IR index in newly diagnosed T2DM (Fig. 1).

Correlation between ferritin versus different variables among patients with impaired glucose tolerance

We found a significant positive correlation between (HbA1C, cholesterol, and TGs) and ferritin level in IGT group (Figs 2 and 3).

Correlation between ferritin versus different variables among newly type 2 diabetes mellitus group

There was a significant positive correlation between ferritin level and LDL and negative correlation with HDL levels in newly diagnosed diabetic patients.

Correlation between ferritin versus different variables among controls

There was no significant correlation between ferritin and other variables in the control group.

Comparison between males and females regarding ferritin among the studied groups

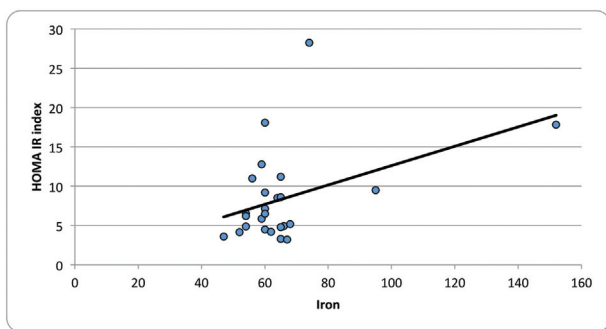
There was a significant positive correlation between males and females in the newly diagnosed T2DM (group A) regarding ferritin, which was significantly higher in males than in females, but there was no statistically significant correlation between males and females in IGT group or control group (Fig. 4).

Table 2 Correlations between insulin resistance (homeostatic measurement assessment insulin resistance) with ferritin, fasting blood sugar, 2 h postprandial, lipid profile and age in each group separately

	New T2DM	IGT group	Control
Ferritin			
<i>R</i>	-0.135	-0.095	-0.235
<i>P</i>	0.521	0.653	0.319
FBG			
<i>R</i>	0.063	0.491*	0.290
<i>P</i>	0.766	0.013	0.214
2HPP			
<i>R</i>	0.101	-0.034	-0.017
<i>P</i>	0.631	0.873	0.942
Triglycerides			
<i>R</i>	-0.168	0.295	-0.006
<i>P</i>	0.423	0.153	0.979
Total cholesterol			
<i>R</i>	0.124	0.195	0.286
<i>P</i>	0.554	0.350	0.222
HDL			
<i>R</i>	-0.010	-0.194	-0.172
<i>P</i>	0.964	0.354	0.468
LDL			
<i>R</i>	-0.012	0.131	0.233
<i>P</i>	0.955	0.533	0.322
Age			
<i>R</i>	0.243	0.157	0.309
<i>P</i>	0.242	0.453	0.186

2HPP, 2 h postprandial; HDL, high-density lipoprotein; IGT, impaired glucose tolerance; LDL, low-density lipoprotein; *r*=Spearman correlation coefficient; T2DM, type 2 diabetes mellitus.

Figure 1



Correlation between serum iron and insulin resistance (HOMA-IR) in newly diagnosed type 2 diabetes mellitus group. HOMA-IR, homeostatic measurement assessment insulin resistance.

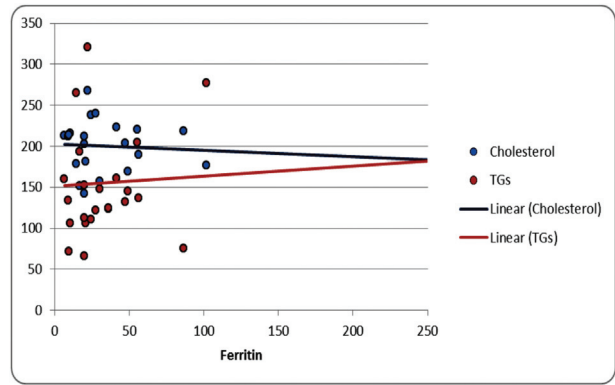
Correlation between transferrin saturation and other variables between the studied groups

TSAT was negatively correlated with HbA1C, BMI, and TIBC and is positively correlated with iron and creatinine and hemoglobin (Figs 5–7).

Validity of ferritin in prediction of insulin resistance

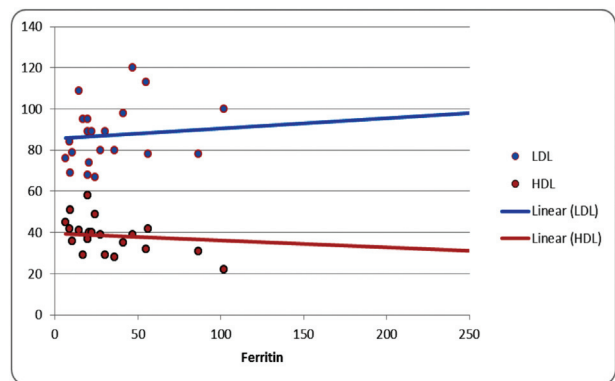
We detected that serum ferritin is better positive than negative in prediction of insulin resistance (Table 3).

Figure 2



Positive correlation between ferritin level and TGs and cholesterol in IGT group. IGT, impaired glucose tolerance; TG, triglyceride.

Figure 3



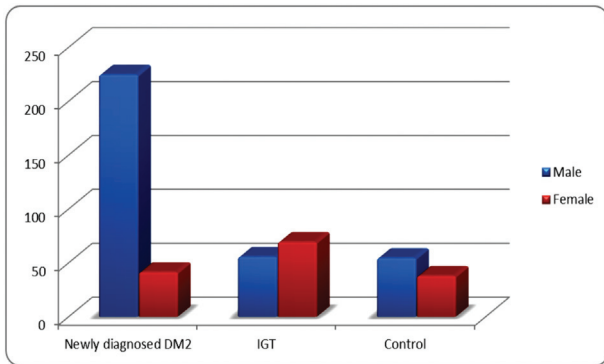
Correlation between ferritin level and HDL and LDL levels in newly diagnosed diabetic patients. HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Discussion

Our study showed a very high statistically significant difference in weight and BMI among study groups ($P < 0.001$), with increased values in patients with type 2 diabetes and IGT than controls. Moreover, fasting plasma glucose, fasting insulin, HbA1C, and insulin resistance were significantly higher in T2DM compared with patients with prediabetes and controls. In agreement with our results, Kim *et al.* [12] discovered altogether higher BMI, fasting plasma glucose, fasting insulin, and insulin opposition in T2DM in contrast with controls. Likewise, Hu *et al.* [13] revealed that insulin resistance and risk of T2DM increased with increment in body fat.

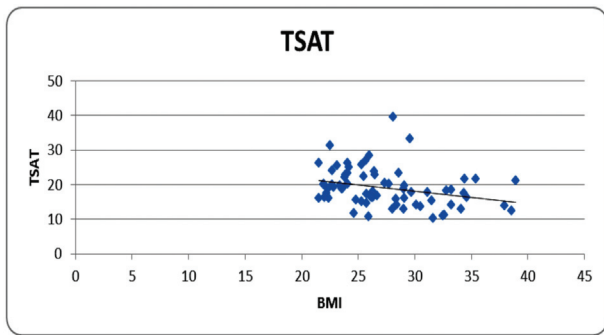
Our study showed a statistically significant difference in serum iron between the studied groups, with higher serum iron present in the controls than patients with type 2 diabetes and prediabetes. Additionally, we found no statistically significant correlation between serum

Figure 4



Correlation between males and females in the studied groups regarding ferritin.

Figure 6



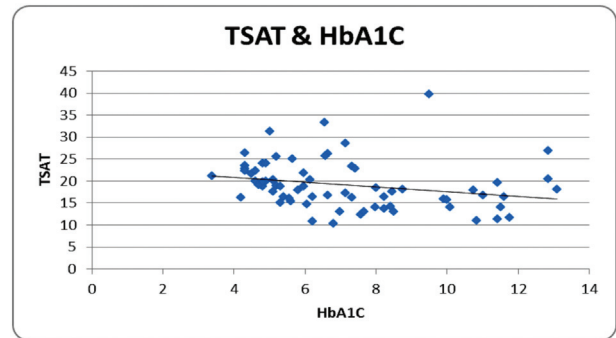
Negative correlation between TSAT and BMI. TSAT, transferrin saturation.

ferritin among the studied groups; however, serum ferritin is higher in diabetic patients than prediabetes and control groups. This comes in accordance with an examination done by Podmore *et al.* [14] who found that higher ferritin level was related with an increased risk of type 2 diabetes among people.

The relationship of ferritin with type 2 diabetes [4] has recently been mentioned in most recent meta-analysis of forthcoming examination done by Kunutsor and colleagues. Additionally, the aftereffects of our examination were bolstered by an investigation done by Fumeron *et al.* [15], who found that ferritin and transferrin are both prescient of the beginning of hyperglycemia in people over more than 3 years.

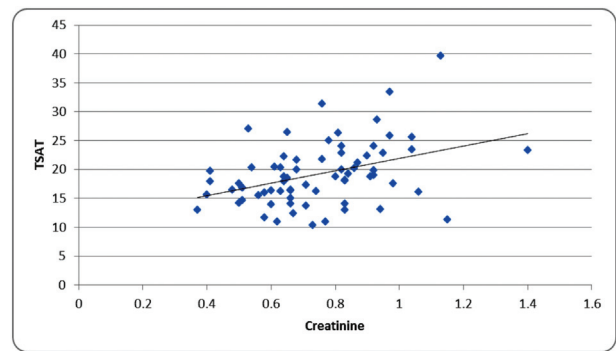
As opposed to our outcomes, Kim *et al.* [12] found no elevation in serum ferritin in patient with T2DM compared with controls. Likewise, Dinneen *et al.* [16] revealed that type 2 diabetes was not related with a considerable degree of iron overburden. We found no measurably statistically significant difference in the level of TSAT between the studied groups;

Figure 5



Negative correlation between TSAT and HbA1C. HbA1C, glycated hemoglobin; TSAT, transferrin saturation.

Figure 7



Positive correlation between TSAT and creatinine. TSAT, transferrin saturation.

Table 3 Validity of ferritin in prediction of insulin resistance

Variables	%
Best cut off	250
Area under the curve	40.9
Sensitivity	8.6
Specificity	97.1
Positive predictive value	75.0
Negative predictive value	50.0
Accuracy	51.4

however, patients with diabetes and prediabetes have lower TSAT than control and could contribute to the risk of development of insulin resistance and type 2 diabetes. Moreover, we found that the level of TSAT was negatively correlated with HbA1C, BMI, and TIBC and is positively correlated with iron and creatinine and hemoglobin among the studied groups. This comes in accordance with Podmore *et al.* [14] who found that high level of TSAT was related with a lower risk of type 2 diabetes in women, when a cutoff of 45% was utilized in light of the fact that higher TSAT could reflect increasingly fruitful searching of non-transferrin-bound iron and in this

way be protective against type 2 diabetes. Moreover, Misra *et al.* [17] discovered that increases in the degrees of serum free iron and serum TSAT levels with poor glycemic control show a significant role of free iron in the advancement of diabetic complications.

Results from prospective studies of TSAT with type 2 diabetes are clashing. An investigation utilizing information from the National Health and Nutrition Examination Survey did not discover any relationship among TSAT and type 2 diabetes utilizing distinctive cutoffs for TSAT [18].

Conversely, a meta-examination of three Danish investigations found that TSATs more than or equal to 50% was related with a higher danger of type 2 diabetes [19]. In any case, these were small studies, with less than 1500 cases in each.

Huth *et al.* [20] found through cross-sectional studies comparable relationship of high ferritin and low TSAT among people with prediabetes.

TSAT is a helpful biomarker of iron metabolism notwithstanding ferritin [21] in light of the fact that TSAT levels are less influenced by inflammation than ferritin [22] and are thought to reflect levels of nontransferrin-bound iron [9].

In patients with hemochromatosis, which is described by high iron absorption, TSAT is raised first, trailed by ferritin once iron accumulates in tissues [2].

Nontransferrin-bound iron is believed to be a significant wellspring of organ iron deposition and toxicity, as it is ardently taken up by tissues, independent of transferrin receptor [10], and deposition have been demonstrated to be higher in patients with type 2 diabetes contrasted and control [23].

We found a significant positive correlation between serum iron and insulin resistance in patients with type 2 diabetes, and no correlation between serum iron and insulin resistance in prediabetic and control groups. In addition, there is proof that high body iron may affect both insulin secretion and sensitivity [24], and numerous past investigations have uncovered the connection of high serum ferritin levels with an assortment of conditions that add to the metabolic disorder and T2DM. These studies have reflected high ferritin as an impression of iron overburden [25]. Our result disagrees with a study done by Fumeron *et al.* [15] who discovered absence of relationship among serum iron and type 2 diabetes.

Our study demonstrated a positive correlation between ferritin and each of HbA1C ($P < 0.001$), cholesterol ($P < 0.023$), and TGs level ($P < 0.001$) in IGT group. This in agreement with a study done by Sharifi *et al.* [26] who discovered a positive relationship among ferritin and each of HgA1c, cholesterol, and TG levels in patient with IGT and inferring that hyperferritinemia happens before elevation of plasma glucose over 126 mg/dl. Hepatic iron over-burden disorder, unrelated to genetic hemochromatosis, has been portrayed and is described by hyperferritinemia, normal TSAT, and expanded predominance of glucose intolerance and diabetes [27].

Mendler *et al.* [28] announced that patients with unexplained hepatic iron over-burden are portrayed by mild to moderate iron load and a positive relationship of insulin resistance regardless of liver harm. The metabolic syndrome is carefully connected to insulin resistance, and numerous investigations demonstrate a connection to hepatic iron overburden. Increased serum ferritin, reflecting hepatic iron over-burden is regularly connected with insulin resistance [29]. Bozzini *et al.* [30] announced expanded pervasiveness of body iron overabundance in patients with the metabolic syndrome.

In our study, there is a significant positive correlation between serum ferritin and LDL and negative correlation with HDL in patients with type 2 diabetes. This is in agreement with Raghavani and Sirajwala [31], who found higher ferritin in patients with type2 diabetes with positive relationship with LDL and negative connection with HDL, and it may add to cardiovascular ailment in type 2 diabetic patients.

In our study, no correlation was found between serum ferritin and blood sugar, TSAT, or insulin resistance in type 2 diabetic patients. In agreement with our result, Kim *et al.* [12] did not find a relationship between ferritin and insulin resistance. Jehn *et al.* [32] also revealed no unfaltering relationship between serum ferritin and insulin opposition in premenopausal women, and see a pattern of increasing insulin resistance with increment serum ferritin in postmenopausal ladies and men. As opposed to this outcome, an investigation done by Haap *et al.* [6] found that ferritin was related with 2-h glucose level and contrarily with insulin sensitivity in people without type 2 diabetes. Sultan *et al.* [33] found a statistically positive relationship in diabetic patients between serum ferritin and insulin resistance. Additionally, ongoing investigation demonstrated

that ferritin was linked with hepatic, muscular, and adipocyte insulin resistance [34].

There was a significant correlation between males and females in the newly diagnosed T2DM regarding ferritin, which was significantly positively correlated in males than in females. In contrast to our result, Podmore *et al.* [14] found that the relationship of iron bio-markers incorporating ferritin with type 2 diabetes were more grounded in women than in men. This likely uncovers physiological changes in iron metabolism and absorption among people, causing the overall danger of supreme biomarker contrasts to be more prominent in women.

Ultimately ferritin can be viewed as a better positive indicator of insulin resistance, and this concurs with an examination done by Kim *et al.* [35] who found that increased serum ferritin levels were related with an increased risk of insulin resistance in postmenopausal women. Jung *et al.* [36] uncovered that higher degree of serum ferritin as a pattern was related with episode type 2 diabetes in an Asian population. Likewise, Podmore *et al.* [14] uncovered a direct relation among ferritin and the danger of type 2 diabetes.

Iron is a transitional metal that can be effectively turned out to be oxidized, and in this manner, goes about as an oxidant. The general effect of synergist iron is to change over ineffectively responsive free radicals, for example, hydrogen peroxide, into exceptionally receptive radicals, for example, the hydroxyl radical. Increased accumulation of iron influences insulin release in the pancreas [37] and interferes with the insulin extrication limit of the liver [38], and iron overload in the muscles diminishes glucose take-up in view of muscle damage [39]. In contrast, insulin animates cell iron take-up through increased transferrin receptor externalization [40]. Iron accumulation in the liver may likewise cause insulin resistance by meddling with the capacity of insulin to stifle hepatic glucose generation [12]. The causes and outcomes of the insulin resistance-hepatic iron overload are obscure. Treatment of insulin resistance-hepatic iron overload is centered around metabolic disorder, and phlebotomies are flawed on the grounds that the over-burden is unobtrusive and intestinal retention of iron has all the earmarks of being low [41]. Overall, 50% of transfusion-treated patients with thalassemia have an unusual glucose resistance [42], and up to 65% of inherited hemochromatosis patients are affected by DM [43], and the connection between high iron intake exceedingly body stores outside the setting of hereditary iron over-burden and type 2 diabetes is notable [44]. Loma Lind

University's Adventist Health Study was the first to report the relationship between meat intake and type 2 diabetes hazard [45]. Many investigations have affirmed that this connection is identified with the high heme substance of meat and expanded dietary heme consumption [46]. High body iron stores have been related to insulin resistance [47] and metabolic disorder [31]. Treatment with an iron-chelating operator prompted an expansion in the control of diabetes in a gathering of patients with ineffectively controlled T2DM [48]. Increased iron stores anticipated the advancement of diabetes in epidemiological investigations [44]. It is fascinating that a lower event of diabetes was noted among successive blood givers [49]. Reactive oxygen species influence insulin signaling at different levels, declining insulin take-up through an immediate effect on insulin receptor work [50] and hindering the translocation of GLUT4 in the plasma membrane [51].

Conclusion

The example of relationship of these markers of iron metabolism and type 2 diabetes proposes an increasingly perplexing affiliation and being a hazard factor for type 2 diabetes. It stays to be enlightened whether the relationship of higher ferritin and transferrin with type 2 diabetes is because of the role of iron in the pathogenesis of type 2 diabetes or whether it mirrors the basic progression of insulin resistance. The hereditary qualities of iron metabolism in general and explicitly of various issue of iron metabolism dependent on their mechanism might be important in tending to these inquiries.

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Conflicts of interest

There are no conflicts of interest.

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