*Ibrahim Card Med J 2023; 13 (1&2): 32-39*


ORIGINAL ARTICLE

# Iron Status in Male Type 2 Diabetic Patients and Its Association with Their Glycemic Status

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## ABSTRACT

***Background & objective:*** Alteration in iron metabolism may occur in diabetic patients especially those who have poor glycemic control. Serum iron is involved in free radical formation associated with hyperglycemia and causes diabetic complications. The present study was undertaken to observe the iron status and its relation with glycemic control in male type 2 diabetic patients.

***Methods:*** This cross-sectional analytical study was carried out in the Department of Physiology, Sir Salimullah Medical College (SSMC), Dhaka from July 2017 to June 2018. A total of 48 diagnosed male type 2 diabetic patients (cases) were recruited from the Out-patient Department (OPD) of Endocrinology based on predefined eligibility criteria. Of them, 24 had good glycemic control (HbA1c level < 7%) (Group B1) and 24 had poor glycemic control (HbA1c level ≥ 7%) (Group B2). To compare the outcome (serum iron status) of these diabetic patients, another 48 age- and BMI-matched healthy non-diabetic male subjects were selected from personal contact as control. While diabetes and glycemic status (fasting blood glucose level, and HbA1c) of the diabetic patients were exposure variables, serum iron, serum total iron binding capacity (TIBC), transferrin saturation, and serum ferritin were the outcome variables.

***Results:*** The mean age of the subjects of either study group was around 43 and there was no significant difference between the groups concerning age ($p = 0.610$). The distribution of BMI and serum creatinine between groups was also similar ($p = 0.135$ and $p = 0.134$ respectively). The cases had a significantly higher level of serum iron, transferrin saturation, and an insignificantly lower total iron binding capacity (TIBC) than their non-diabetic peers ($p = 0.045$, $p = 0.036$, and $p = 0.061$ respectively). A comparison of outcome variables among the three groups shows that the groups were significantly heterogeneous in terms of serum iron, TIBC, transferrin saturation, haemoglobin level ($p = 0.032$, $p = 0.012$, $p = 0.008$ and $p = 0.018$ respectively). The associations were more conspicuous in patients with poor glycemic status. Correlation analyses revealed that while there was a significant linear correlation between serum iron and HbA1c ($r = + 0.376$, $p < 0.01$), it was negatively correlated with serum TIBC ($r = -0.478$, $p < 0.001$). The glycemic status (HbA1c) was also found to be linearly correlated with serum ferritin and transferrin saturation ($r = + 0.354$, $p = 0.013$ and $r = + 0.462$, $p < 0.001$ respectively).

***Conclusion:*** The study concluded that type 2 diabetic patients have a significantly higher level of serum iron, transferrin saturation, and a lower total iron binding capacity (TIBC) than the non-diabetic healthy subjects, all of which may lead to reduced synthesis of hemoglobin. The compromised iron metabolism is even more evident as the hyperglycemia aggravates.

***Keywords:*** Type 2 diabetes, glycemic control, serum iron, total iron binding capacity (TIBC), serum ferritin, transferrin saturation, etc.

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## INTRODUCTION:

Diabetes mellitus is a syndrome of impaired carbohydrate, fat, and protein metabolism caused by either a lack of insulin secretion or decreased sensitivity of the tissue to insulin. The majority of diabetes is type 2 diabetes, caused by decreased sensitivity of insulin to target cells (insulin resistance).[1] Long-term diabetes mellitus is associated with vascular complications like retinopathy, nephropathy, peripheral and autonomic neuropathy, and cardiovascular and cerebrovascular diseases.[2] Glycated hemoglobin (HbA1c), provides an accurate and objective measure of glycemic status over a period of months. An HbA1c of 6.5% is recommended as the cut-off point, above which the diabetes is diagnosed as uncontrolled diabetes.[3]

Iron in the blood prevails as a part of the heme prosthetic group in proteins such as hemoglobin, myoglobin, and cytochromes. Free iron is toxic because it can cause the production of hydroxyl radical, a reactive oxygen species (–ROS).[4] Poor glycemic control causes increased glycation of proteins, especially of hemoglobin, which releases iron in the free state.[5,6] Free iron is involved in the formation of free radicals like hydroxide and superoxide anions which cause lipid peroxidation.[7] The pancreatic beta cells are particularly susceptible to oxidative damage because of their weak antioxidant effect.[8] Excess iron impairs pancreatic β-cell function and causes β-cell apoptosis.[9] Increased accumulation of iron affects insulin synthesis and secretion from pancreatic β-cells and interferes with hepatic insulin extraction by the liver.[10]

Serum ferritin is a reliable marker of body iron stores. Normally there is little ferritin in human plasma proportionate to the total body iron stores. Ferritin is one of the key points that play an important role in iron regulating homeostasis.[11] Hyperferritinemia has been linked to the glycemic status of individuals and is also known to be associated with diabetic complications.[12] Iron deposition in the liver may cause insulin resistance by interfering with the ability of insulin to suppress hepatic glucose metabolism. Iron is auto-oxidized to form iron-oxygen complexes. These free radicals can change membrane properties and result in tissue damage.[7] Iron overload occurs in skeletal muscles, the main effector organ of insulin action. Increased muscle iron may enhance free fatty acid oxidation, and decrease glucose oxidation thereby interfering with glucose disposal.[13] Kamalam and associates.[14] in a study found that there is a positive correlation of serum ferritin with FBG and HbA1c with the higher value of serum ferritin the higher the HbA1c in diabetic subjects. Serum-free iron and ferritin levels were found higher in diabetic patients with poor glycemic control than those in healthy control.[15,16] In another study, Misra and associates[6] observed higher serum iron levels, transferrin saturation, and lower total iron binding capacity (TIBC) in patients with uncontrolled diabetes than those in healthy subjects.

From the above relationships of serum ferritin, serum iron levels, transferrin saturation, and TIBC with the glycemic status of diabetic individuals, it seems important to screen iron status in type 2 diabetes mellitus.[17] Several studies have been done in this regard with conflicting results. Besides, there is a paucity of studies done in our country. So, the present study was undertaken to observe the associations and correlations of iron status with glycemic status in male type-II diabetic patients. It is expected that the findings derived from the study would guide physicians in better management of type 2 diabetic patients.

## METHODS:

This cross-sectional analytical study was conducted in the Department of Physiology, Sir Salimullah Medical College & Mitford Hospital (SSMC & MH), Dhaka over a period of one year from July 2017 to June 2018 after obtaining permission from the Institutional Ethics Committee (IEC) of SSMC. A total of 48 diagnosed male type 2 diabetic patients (cases) were recruited from the Out-patient Department (OPD) of Endocrinology based on predefined eligibility criteria. Of them, 24 were with good glycemic control (HbA1c level < 7%) (Group B1) and 24 with poor glycemic control (HbA1c level ≥ 7%) (Group B2). To compare the outcome of these groups, 48 age- & BMI-matched healthy non-diabetic male subjects were selected from personal contact as control (Group A). The inclusion criteria of both

groups were male subjects ranging from 35-60 years of age, and BMI within the normal range (18.5-24.9 kg/m2). Type 2 diabetes was diagnosed if fasting plasma glucose (FPG) ≥ 126 mg/dL (7.0 mmol/L), or 2 hours after 75 gm glucose load ≥ 200 mg/dL (11.1 mmol/L) or HbA1C ≥ 7% or patients under anti-diabetic medications. Patients with a history of per rectal bleeding or bleeding from the GI tract, iron supplementation, hepatic disease, or a history of recent blood donation, major operation, & trauma, or a history of chronic infection like tuberculosis, inflammation like rheumatoid arthritis, or presence of other hormonal abnormalities and malignancy were excluded from the study.

Pertinent personal, family, and medical history were collected. Estimation of fasting blood glucose and HbA1c levels was done to observe their glycemic status. While Fasting blood glucose level was estimated by glucose oxidase (GOD) method[18] in the Department of Physiology, Sir Salimullah Medical College (SSMC) Dhaka, HbA1c level was estimated by ion exchange high-performance liquid chromatography (HPLC) method[19] in the Department of Biochemistry, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka. The serum iron and serum total iron binding capacity (TIBC) were measured at BSMMU by the Backmen Coulter method, and serum ferritin was estimated by the Chemiluminescent microparticle immunoassay (CMIA) method.[20] However, the transferrin saturation level was determined by dividing the serum iron value by the total iron binding capacity and expressing the result as a percentage.[21] Blood hemoglobin level was measured by the cyanmethemoglobin method[22] and serum creatinine level was measured at SSMC by the fixed kinetic method in a semi-autoanalyzer.[23]

Statistical analysis was done using the statistical package of social sciences (SPSS) for Windows, version 22. Data were presented as mean and SD (standard deviation) from the mean. While ANOVA and Unpaired t-tests were performed to compare quantitative data among the study groups, Pearson's correlation test was done to see the nature of relationships between two continuous variables. The level of significance was set at 5% and a p-value <0.05 was considered significant.

## RESULTS:

The mean age of the subjects was around 43 and there was no significant difference between the study groups concerning age (p = 0.610). The distribution of BMI and serum creatinine between the study groups was also similar (p = 0.135 and p = 0.134 respectively). The FBG and HbA1c were significantly higher in Group B than in Group A (p < 0.001 and p<0.001 respectively). The distribution of serum creatinine was almost similar between groups (Table I). Multiple comparisons with the Post-hoc Hochberg test revealed a significant difference between A vs. B2 and B1 vs. B2 concerning FBG and HbA1c (p<0.001 in each case). However, no significant difference was observed between A vs. B1 in terms of the same variables (p = 0.702 and p = 0.145 respectively) (Table III).

The case group (Group B) had a significantly higher level of serum iron and an insignificantly lower total iron binding capacity (TIBC) than their control comparator (p = 0.045, and p = 0.061 respectively). While transferrin saturation was significantly higher in cases than in controls (p = 0.036), the level of hemoglobin was significantly lower in the former group than in the latter group (p = 0.007) (Table IV). A comparison of outcome variables among the three groups shows that the groups were significantly heterogeneous in terms of serum iron, TIBC, transferrin saturation, haemoglobin level (p = 0.032, p = 0.012, p = 0.008 and p = 0.018 respectively) (Table V). Multiple comparisons by the post hoc Hochberg test revealed significant differences between groups A vs B2 in terms of serum iron, TIBC, transferrin saturation, and haemoglobin level (p=0.027, p = 0.012, p = 0.007, p = 0.021 respectively); however, no significant difference was found between Group A and B1 (controlled diabetes) in terms of the same variables (Table VI).

Correlation of glycemic status with different parameters of serum iron status, where glycemic status was measured in terms of HbA1c (independent variable) and iron status was measured in terms of different iron parameters (serum iron, TIBC, serum ferritin, transferrin saturation, haemoglobin level). Analyses revealed that while there was a significant linear correlation between serum iron and HbA1c

(r=+ 0.376, p < 0.01), it was negatively correlated with serum TIBC (r = - 0.478, p < 0.001). The glycemic status (HbA1c) was also found to be positively correlated with serum ferritin and transferrin saturation (r = + 0.354, p = 0.013 and r=+0.462, p < 0.001 respectively). The level of haemoglobin tends to be negatively correlated with HbA1c (r = - 0.100, p = 0.497) (Table VII & fig. 1–5).

**Table I. Comparison of baseline characteristics between case and control groups**

| Baseline characteristics | Group | | *p-value |
|---|---|---|---|
| | Case (n =100) | Control (n =100) | |
| Age (years) | 42.8 ± 6.9 | 43.5 ± 7.0 | 0.610 |
| BMI (kg/m2) | 23.8 ± 1.5 | 23.2 ± 2.2 | 0.135 |
| FBG (mmol/L) | 5.6 ± 1.2 | 7.5 ± 2.4 | < 0.001 |
| HbA1c (%) | 5.3 ± 0.4 | 7.8 ± 2.5 | < 0.001 |
| S. Creatinine (mg/dL) | 1.0 ± 0.3 | 0.9 ± 0.2 | 0.134 |

*Data were analyzed using an **Unpaired t-Test** and were presented as **mean ± SD.**

**Table II. Comparison of glycemic status among the study groups**

| Groups | Group | | | #p-value |
|---|---|---|---|---|
| | Group A (n = 48) | Group B1 (n = 24) | Group B2 (n = 24) | |
| FBG (mmol/L) | 5.6 ± 1.2 | 6.1 ± 1.4 | 8.9 ± 2.4 | < 0.001 |
| HbA1c (%) | 5.3 ± 0.4 | 6.0 ± 0.6 | 9.6 ± 2.4 | < 0.001 |

#Data were analyzed using **ANOVA statistics** and were presented as **mean ± SD.**

**Table III. Multiple comparisons by Post-hoc Hochberg test**

| Groups | Significance value (p-value) | |
|---|---|---|
| | FBG | HbA1c |
| A vs. $B_1$ | 0.702 | 0.145 |
| A vs. $B_2$ | < 0.001 | < 0.001 |
| $B_1$ vs. $B_2$ | < 0.001 | < 0.001 |

**Table IV. Comparison of outcome variables between case and control groups**

| Outcome variables | Group | | #p-value |
|---|---|---|---|
| | Group A (n = 48) | Group B (n = 48) | |
| S. Iron (µg/dL) | 86.7 ± 14.4 | 94.3 ± 21.8 | 0.045 |
| TIBC (µg/dL) | 374.8 ± 51.0 | 353.8 ± 57.1 | 0.061 |
| Ferritin (µg/L) | 96.6 ± 59.3 | 113.9 ± 70.7 | 0.195 |
| Transferrin saturation (%) | 23.8 ± 6.2 | 27.7 ± 11.1 | 0.036 |
| Hemoglobin (g/dL) | 14.9 ± 2.3 | 13.5 ± 2.7 | 0.007 |

#Data were analyzed using an **Unpaired t-test** and were presented as **mean ± SD.**

**Table V. Comparison of outcome variables between case and control groups**

| Outcome variables | Group | | | p-value |
|---|---|---|---|---|
| | Group A (n = 48) | Group B1 (n = 24) | Group B2 (n = 24) | |
| S. Iron (µg/dL) | 86.7 ± 14.4 | 89.8 ± 18.3 | 98.8 ± 24.4 | 0.032 |
| TIBC (µg/dL) | 374.8 ± 51.0 | 371.8 ± 51.7 | 335.8 ± 57.7 | 0.012 |
| Ferritin (µg/L) | 96.6 ± 59.3 | 99.4 ± 75.5 | 128.5 ± 63.8 | 0.132 |
| Transferrin saturation (%) | 23.8 ± 6.2 | 24.7 ± 6.4 | 30.7 ± 13.9 | 0.008 |
| Hemoglobin (g/dL) | 14.9 ± 2.3 | 13.8 ± 2.3 | 13.2 ± 2.9 | 0.018 |

#Data were analyzed using **ANOVA statistics** and were presented as **mean ± SD.**

**Table VI. Multiple comparisons by Post-hoc Hochberg test**

| Groups | Significance value (p-value) | | | | |
|---|---|---|---|---|---|
| | S. Iron (µg/dL) | TIBC (µg/dL) | Ferritin (µg/L) | Transferrin saturation (%) | Haemoglobin (g/dl) |
| A vs $B_1$ | 0.988 | 0.955 | 0.973 | 0.976 | 0.235 |
| A vs $B_2$ | 0.027 | 0.012 | 0.155 | 0.007 | 0.021 |
| $B_1$ vs $B_2$ | 0.271 | 0.062 | 0.371 | 0.065 | 0.999 |

**Table VII. Correlation of HbA1c with iron status**

| Parameters | Correlation coefficient (r) | p-value |
|---|---|---|
| S. Iron (µg/dL) | +0.376 | 0.009 |
| TIBC (µg/dL) | -0.478 | < 0.001 |
| S. Ferritin (µg/dL) | +0.354 | 0.013 |
| Transferrin Saturation (%) | +0.462 | < 0.001 |
| Level of Hb (g/dl) | -0.100 | 0.497 |

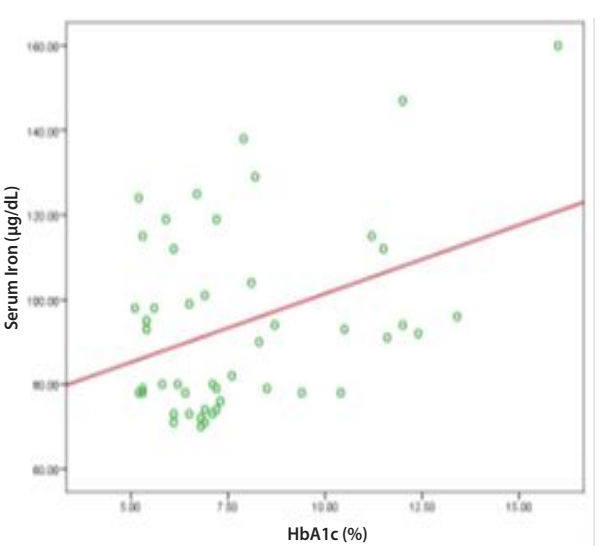


**Fig. 1. Correlation of HbA1c with serum iron level**

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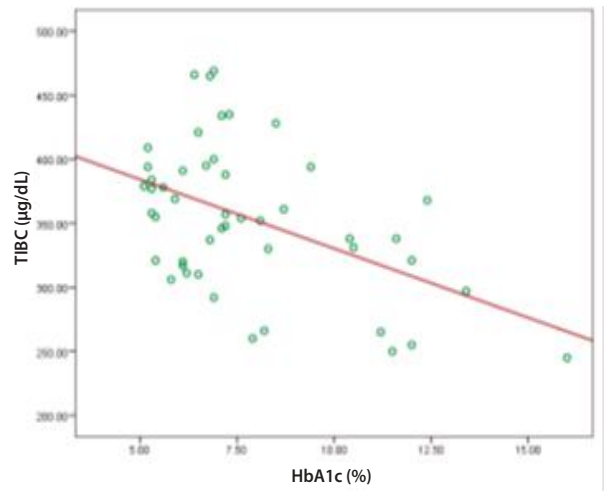


Fig. 2. Correlation between HbA1c and serum TIBC

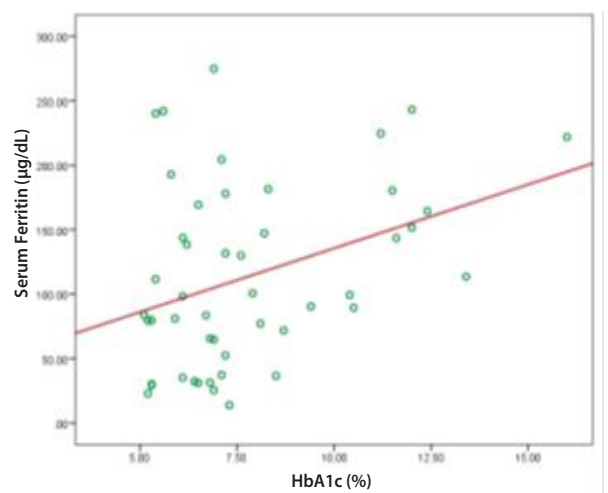


Fig. 3. Correlation of HbA1c with serum ferritin

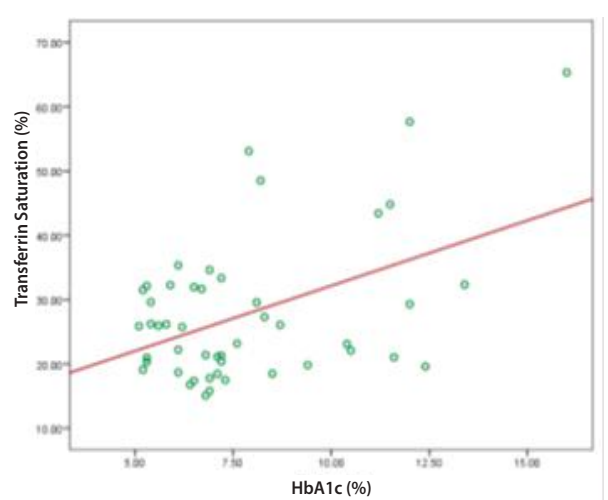


Fig. 4. Correlation of transferrin saturation with HbA1c

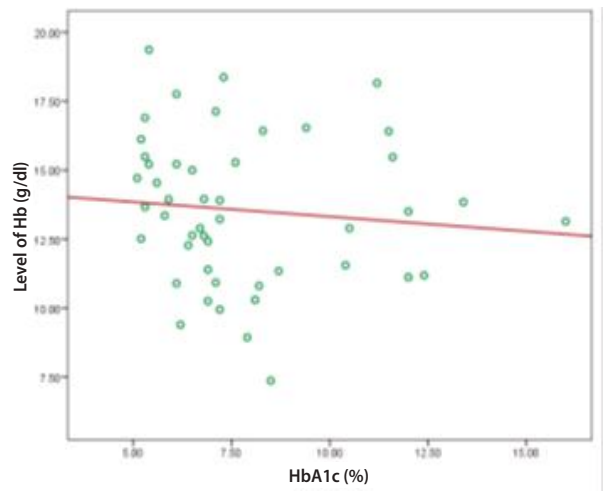


Fig. 5. Correlation of Hemoglobin level with HbA1c

## DISCUSSION:

In the present study, the mean serum iron level was significantly higher in diabetic patients compared to that in non-diabetic subjects, which is in agreement with several other studies.[24-26] Whereas, Sindhu and Ramkrishnan[27] found insignificantly higher serum iron levels in diabetic patients than that in non-diabetic subjects. On the contrary, Wolide et. al.[28] found significantly lower serum iron levels in diabetic patients than that in non-diabetic controls. This discrepancy in findings between studies might be due the inadequate dietary intake, asymptomatic illness, and poor absorption rate. Meanwhile, the mean serum iron level was significantly higher in diabetic patients with poor glycemic control compared to that in their counterparts with good glycemic control (p=0.027). Consistent with these findings, several researchers also found significantly higher serum iron levels in diabetic patients with poor glycemic control than in patients with good glycemic control.[5,6]

In our study, the mean serum TIBC was lower in diabetic patients than that in non-diabetic subjects but the difference was not statistically significant. However, sub-group analysis shows that the difference between the uncontrolled diabetics and healthy subjects concerning the same variable was significant. A similar finding was reported by some other researchers in patients with type 2 diabetes.[6,29-31] On the contrary, Okonkwo and Okoye[9] found significantly higher serum TIBC levels in diabetic

patients in comparison to those of non-diabetic subjects. Again, the mean serum TIBC level was lower in poor glycemic control diabetic patients than in patients with good glycemic control, although the difference was not statistically significant. This discrepancy might be due to the presence of anemia.

The mean serum ferritin level was considerably higher in diabetic patients (either controlled or uncontrolled diabetics) than in non-diabetic subjects, although the difference did not turn significant. A similar finding was reported by some other researchers.[32] Whereas, some researchers found significantly higher serum ferritin levels in diabetic patients than in non-diabetic subjects.[10,17] On the contrary, Wolide and associates found significantly lower serum ferritin levels in diabetic patients than in the non-diabetic group.[28] This discrepancy might be due the inadequate dietary intake, asymptomatic illness, and poor absorption rate. The mean transferrin saturation (%) was significantly higher in diabetic patients than that of non-diabetic subjects ($p=0.036$). The difference is even more pronounced when the comparison is made between uncontrolled diabetics and healthy controls ($p = 0.008$) and no difference was noted when the comparison was made between controlled diabetics and healthy subjects. A consistent finding was reported by several other investigators as well.[6,9,31] Whereas, Dulal in a similar study reported an orthodox finding.[29]

The serum hemoglobin level was significantly lower in diabetic patients compared to that in non-diabetic subjects, which is consistent with the findings of other similar studies.[33,34] Sharply contrasting with these findings, Ardekhani and Rashdi[35] observed significantly higher hemoglobin levels in patients with gestational diabetes mellitus (GDM) than in non-diabetic pregnant subjects. This discrepancy might be due to maternal nutritional status, iron supplementation, and dietary behaviour. The hemoglobin level was observed to be even lower in type 2 diabetics with poor glycemic status than in those with good glycemic control, although the difference was not statistically significant between controlled diabetics and healthy control subjects. While the study demonstrated a significantly linear correlation of HbA1c with serum iron, serum ferritin, and transferrin saturation, it showed a negative correlation between HbA1c and TIBC. This finding was in agreement with the findings of several studies.[5,15,34,36,37] In contrast, some researchers found no correlation between serum iron level and HbA1c[6] and between transferrin saturation (%) and HbA1c. Surprisingly, Lee[38] found a significant positive correlation between serum TIBC and HbA1c.

## CONCLUSION:

From the findings of the study, it appears that type 2 diabetic patients have a significantly higher level of serum iron, transferrin saturation, and a lower total iron binding capacity (TIBC) than their non-diabetic counterparts. The associations are even more pronounced as the glycemic status of the patients is worsened. The study also demonstrated a significantly linear correlation of HbA1c with serum iron, serum ferritin, and transferrin saturation, and a negative correlation between HbA1c and TIBC. All these findings suggest that iron metabolism is somehow comprised in type 2 diabetic subjects leading to decreased synthesis of haemoglobin. The abnormal iron metabolism is even more noticeable as the hyperglycemia heightens.

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