

## Effects of iron on blood glucose levels in type 2 diabetes mellitus – A study in tertiary care centre.

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### Abstract:

**Introduction:** Iron is an important mineral whose metabolism is tightly regulated in our body. Iron is capable of initiating oxidative stress when in excess. Type 2 Diabetes mellitus is a chronic disorder of glucose metabolism with multiple causes. Ferritin is a protein that stores iron in the body. Iron can affect the metabolism of glucose by influencing insulin's release and actions.

**Aim:** We aimed at determining and comparing the serum levels of Iron, Total iron binding capacity(TIBC) and Ferritin in T2DM and healthy subjects and to correlate these with blood glucose levels.

**Materials and methodology:** It was a case control study, which involved 50 T2DM patients and 50 controls. The study was undertaken in Subbaiah Institute of Medical Sciences. Serum blood glucose, Iron, TIBC were estimated in autoanalyser and serum ferritin was estimated by chemiluminescence method.

**Statistical analysis:** Mann Whitney U test and Median,interquartile range was applied. Spearman's rho correlation was applied for correlations.  $p < 0.05$  was considered as statistically significant.

**Results:** Blood sugar levels and serum Ferritin levels were increased significantly in cases. Whereas serum iron and TIBC were not significantly elevated in cases. Only Ferritin showed a significant positive correlation with blood sugar levels.

**Conclusions:** Increased levels of iron can lead to oxidative damage and lead to complications in diabetes mellitus patients. Hence frequent monitoring of serum iron and ferritin levels is advisable in T2DM.

**Keywords:** Blood glucose, Ferritin, Insulin, Iron, Reactive oxygen species

### Introduction:

Type 2 diabetes mellitus is a metabolic disease which is chronic in onset. Beta cells of islet of Langerhans of the pancreas secrete less insulin in T2DM. complete absence or relative decrease in the levels of insulin leading to chronic hyperglycemia is the main observation in T2DM. (1,2)

It is worth mentioning that insulin resistance, dyslipidemia and low grade chronic inflammation are the key features of T2DM.(3)

International diabetes federation had estimated in 2015 that globally 1 in 11 adults between 20 – 79 years had DM. According to experts prevalence of DM is going to increase from 415 to 642 million by 2040. Low to middle-income population is expected to have a significant rise in prevalence of DM. Since there is insulin resistance, glucagon levels increase leading to hyperglycemia in DM.(4)

Iron is a crucial element to all cells. Iron can donate and accept electrons. Iron is an active redox cofactor for many important biochemical reactions. This redox property of iron makes it toxic element as it generates free radicals, when it is in excess. That's why iron metabolism and absorption is tightly regulated by various mechanisms. Iron is a one way element. Excess iron can be toxic as it generates reactive oxygen species. The most important reaction which generates ROS is the Fenton reaction.(5)

The human body has various detoxification processes to neutralize ROS. If the ROS are not neutralized effectively then they can cause damage to cellular components. Excess iron and ROS cause cell membrane damage and lead to pathological processes. (6) ROS induced by iron overload interferes with insulin signaling and causes impairment in translocation of Glucose transporter 4 to the cell membrane and impairs insulin uptake.(5)

Insulin is shown to have its action on iron metabolism by exerting its action on glucose transport. Insulin helps in redistribution of transferrin receptors from inside the cells to cell surface and helping in uptake of iron by adipocytes. There are also studies showing that iron interferes with insulin action by inhibiting insulin's action on hepatic glucose production. Increased iron also causes reduction in production of insulin and metabolism causing increased levels of insulin.(7,8)

Iron is stored in the body mainly in the form of protein, ferritin. Ferritin is a spherical shaped protein, present inside the cell, which can store up to 4000 iron atoms. Ferritin is secreted into plasma in small quantity. This plasma concentration of ferritin usually depends on body iron stores, when there is no inflammation.(9)

Objectives:

1. To determine and compare the serum levels of Iron, Total iron binding capacity and ferritin in T2DM and healthy subjects.
2. To correlate levels of blood glucose with Iron, Total iron binding capacity and ferritin.

Materials and methods:

Study was designed as case control study. Study setting was in medicine outpatient department of Subbaiah Institute of Medical Sciences, Shimoga. We included 100 subjects in the study. Among 100, 50 were cases of T2DM, and 50 were age and sex matched healthy individuals as controls. Simple random sampling technique was chosen. Sample size was calculated by convenient sampling. Study duration was from October 2018 to March 2019. Institutional ethical clearance was taken before commencing the study.

Inclusion criteria:

Cases : 50 subjects were cases. T2DM patients , diagnosed by physicians using American diabetes association criteria(10), age group of 30 -65 years, who were taking oral anti hyperglycemic drugs were included in study. They gave consent for the study.

Controls : age and sex matched 50 apparently healthy individuals .

Exclusion criterias: Patients of diabetes having complications, Severe inflammatory diseases, infections, hepatic or renal diseases and persons on drugs that would affect blood glucose levels, Blood loss, blood transfusions and blood donation in last 6 months duration, subjects on Iron supplements, chronic alcoholics, thyroid dysfunction, pregnant and lactating women.

Methodology: Information was obtained by preformed questionnaire. Basic investigations were using standard protocol . Informed and written consent was taken from all subjects. A sample of 5 ml venous blood was collected under aseptic conditions to study the following parameters

- Blood sugar by Glucose oxidase - peroxidase method.(11) - kits supplied by Robonik.
- Serum Iron and Total iron binding capacity by Ferrozine method.(12) - kits supplied by Coral crest diagnostics.
- Serum Ferritin by chemiluminiscence method.(13) – kits supplied by Minividas.

Blood sugar was measured in autoanalyzerAurora, serum Iron and TIBC were measured in semiautoanalyserRobonik and serum Ferritin was measured in Hormone analyser Minividas.

Statistical analysis:

Data was expressed as Mean  $\pm$  SD and was analysed in SPSS software version 20. Mann whitney U test and Median,inter quartile range was applied as values were in extreme range.Spearman's rho correlation was applied for correlations.  $p < 0.05$  was considered as statistically significant.

Results:

Table/Fig1 :Comparison of FBS,PPBS, Serum Iron, TIBC and Serum Ferritin in cases and controls.

		Median	Percentile 25	Percentile 75	p value
FBS mg/dl	Cases	137.8	121.7	178.15	< 0.0005**
	Controls	85.5	74	96	
PPBS mg/dl	Cases	199.3	174.4	246.05	< 0.0005**
	Controls	117	110.5	124	
Serum Iron microgm/dl	Cases	99.55	71.75	132.05	0.428
	Controls	98.1	78.9	104.35	
TIBC microgm/dl	Cases	278.4	255.15	321.25	0.07
	Controls	298.15	265.35	349.9	
Serum Ferritin ng/ml	Cases	233.3	146.9	256.3	< 0.0005**
	Controls	47.55	36.3	64.25	

< 0.0005\*\* Highly statistically significant.

Table/Fig 2 : Spearman's rho correlation between blood sugar levels and Iron, TIBC, Ferritin in cases.

	Iron	TIBC	Ferritin
FBS	0.201	- 0.42	0.773**
PPBS	0.192	- 0.51	0.710**

\*\*correlation coefficient at 0.01 level.

Table/Fig 1 is showing comparison of FBS,PPBS, Serum Iron, TIBC and Serum Ferritin in cases and controls. Levels of FBS, PPBS and serum Ferritin were elevated in cases compared to controls and showed statistically high significance.

Table/Fig 2 is showing Spearman's rho correlations between FBS,PPBS and Serum Iron, TIBC, Serum Ferritin levels in cases. Ferritin showed highly statistically significance with FBS,PPBS levels.

### **Discussion :**

We are seeing awareness among urban population about nutritional benefits of trace elements, nowadays. Researchers and even clinicians are interested in knowing the association of trace elements with T2DM because the disease has multi factorial causes in onset, progression and development of complications.(14). Insulin resistance is a well known finding in T2DM and there are many evidences to show a link to elevated iron stores.(15).Ferritin is a storage protein for body iron stores and also a known inflammatory marker.(16,17). Ferritin stores iron in ferric state and protects cells from oxidative damage. (2,18,19)At normal concentrations also iron influences glucose metabolism. Elevated levels of iron and thus ferritin cause insulin resistance by deposition in liver. Insulin is supposed to act in a duplex way. insulin helps in synthesis of ferritin and facilitates uptake of iron by intestinal cells and adipocytes.(20) On the other hand iron influences insulin's actions on hepatic glucose output. Excess Iron is also found to affect skeletal muscles, which is the chief regulator of insulin actions.(21). Iron is a strong oxidant and can accelerate the synthesis of ROS. These ROS interfere with functions of beta cells of pancreas and affect insulin secretion and actions.(2) Excess iron can also affect mitochondria and thus production of ATP leading to glucose metabolism disorders.(3,22–24).

Hepcidin is a peptide which is involved in iron regulation.it is secreted when insulin is secreted from beta cells. So it can be considered that iron concentration in blood is regulated by serum glucose levels, because when glucose level increases insulin is secreted and hepcidin is also released and it regulates iron release into blood.(2)

In this study we have tried to find out the association of hyperglycemia with iron profile parameters. In our study it was seen that serum iron in cases and controls showed no statistical significance. Same result was obtained in other studies.(3,20,25)

In our study we found elevated ferritin levels in cases. Reasons for the elevation could be decreased clearance of glycosylated ferritin from blood in T2DM patients. (26). Similar finding was found in other studies.(27–30)

In our study we found normal iron stores and increased ferritin levels in cases, which suggests probably ferritin acts as an antioxidant sequestering all free iron into the cells and protecting cells from oxidative damage. (30)

We found significant spearman's rho correlation between blood sugars and serum ferritin levels in T2DM.

**Conclusion:**

Serum ferritin was positively correlated to blood glucose levels indicating its association in development of disease. So it can be used as an indicator in predicting the risk of future complications. Measures can be taken to reduce the levels of iron if it is elevated in diabetes mellitus patients. Inadvertent use of iron prophylaxis without checking blood glucose levels should be discouraged in health centres.

Limitations: A further study with more sample size could show better correlation. Glycemic control and transferrin saturation were not estimated. Prospective studies can be undertaken to study role of ferritin in development of complications of DM.

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