Relation of Iron, TIBC and Oxidative Stress with Glycosylated Haemoglobin in Diabetes Mellitus

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Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

ABSTRACT

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism. Scientific evidences suggest that high iron storage may play a role in pathogenesis of type 2 diabetes. Excess iron accumulation induces organ damage due to the overproduction of ROS through Fenton reaction. Thus, the aim of this study was to find out the relation between serum iron, total iron binding capacity (TIBC) and oxidative stress (OS) with glycoylated haemoglobin (HbA1c) in type 2 diabetes mellitus patients. The study consisted of 90 subjects, which were divided into 3 different groups; Group 1 comprised of 30 healthy individuals, Group 2 included 30 T2DM patients with normal glycemic control and Group 3 included 30 T2DM patients with poor glycemic control. Blood samples were collected from the three groups and fasting plasma glucose (FPG), post-prandial plasma glucose (PPPG), HbA1c, Iron, TIBC, Hemoglobin (HB), Malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT) levels were analysed.

We found, that mean levels of FPG, PPPG, HbA1c, Iron and MDA were significantly higher (p < 0.05) and mean levels of TIBC, SOD and CAT were significantly decreased (p < 0.05) in group 3 as compared to group 2 and group 1. There was no significant difference (p > 0.05) observed in iron.
TIBC and Hb levels between group 1 and group 2. We found a significant positive correlation of iron and MDA with HbA1c and significant negative correlation of TIBC, SOD and CAT with HbA1c in group 3.

In our study we found significant positive correlation of HbA1c with MDA and iron that indicates poor glycemic control leads to increased glycation of haemoglobin and other heme containing proteins. It causes more release of iron that leading to production of oxidative stress and thereby it might plays significant role in early appearance of diabetic complications.

Keywords: Reactive oxygen species; glycosylated hemoglobin; total iron binding capacity; iron.

1. INTRODUCTION

Diabetes mellitus (DM) is a combination of heterogeneous disorders commonly presenting with episodes of hyperglycaemia and glucose intolerance, as a result of lack of insulin, defective insulin action, or both [1] Diabetes is a growing public health problem world-wide and especially in India which pronounced as the capital of diabetes by WHO. In 2000, India (31.7 million) topped the world with highest number of people with DM followed by China (17.7 million). This number can be double globally from 171 million in 2000 to 366 million in 2030 with the maximum increase in India [2].

Iron is a very important transition metal for the cells in the body, and its abnormal homeostasis is associated with the pathogenesis of various chronic diseases, including diabetes [3]. Iron in its free form i.e, in non-transferrin form is known to induce oxidation of biomolecules through Haber–Weiss and Fenton reactions by producing harmful hydroxyl radicals [4]. Iron deposition in the liver may cause insulin resistance by interfering with the ability of insulin to suppress hepatic glucose production. Catalytic iron converts poorly reactive free radicals like H₂O₂ into highly reactive ones like hydroxyl radical (OH’) and superoxide anion (SO’) that can initiate and propagate the cascades leading to oxidative damage [5].

Few studies have reported the relation of HbA1c and iron in diabetes mellitus; however relation of iron with oxidative stress and HbA1c is rarely addressed in Diabetic patients with normal and poor glycemic control, which may be helpful to predict the diabetic complications. Therefore the present study was aimed to assess relation of iron and oxidative stress markers with glycosylated haemoglobin in T2DM patients with poor glycaemic control, T2DM patients with normal glycaemic control and healthy controls.

2. MATERIALS AND METHODS

2.1 Study Design and Population

The present study is prospective which was carried out in the Department of Biochemistry and Department of Medicine, MGM Medical College and Hospital, Kamothe, Navi Mumbai from August 2017 to February 2018. The total number of subjects included were 90 having age group 41-70 years which were divided into three groups: Group 1: Healthy individual (n=30). Group 2: Type 2 Diabetes Mellitus patients with normal glycemic controls (n=30), Group 3: Type 2 Diabetes Mellitus patients with poor glycemic controls (n=30). The Institutional Ethical Clearance was obtained from Ethical Committee, MGM Medical College, Kamothe, and Navi Mumbai. Diagnosed Type -2 Diabetes Mellitus patients (as per WHO criteria) were enrolled in the study from Medicine department and apparently healthy individuals were enrolled from general population.

2.2 Biochemical Analysis

Blood samples were collected from three groups. Plasma fasting glucose and post-prandial glucose was estimated by Glucose oxidase peroxidase (GOD-POD) method. HbA1c was estimated by HPLC method. Haemoglobin was estimated by cyanmethemoglobin method. Serum Iron and TIBC [6] was estimated by Ferrozine /MgCO₃ method. Serum MDA [7] was estimated by KEI Sathos method. SOD [8] was estimated by Marklund and Marklund method CAT [9] was estimated by Sinha Method. Here SOD and CAT was estimated by RBC hemolysate procedure [10].

3. OBSERVATIONS AND RESULTS

The comparison of biochemical parameters between group 1 (healthy controls), group 2 (T2DM with normal glycemic control) and group 3
(T2DM with poor glycemic control) is shown in Tables 1 and 2. Correlation analysis between HbA1c and other variables in group 2 and group 3 is shown in Table 3.

4. DISCUSSION

In present study mean levels of FPG, PPPG, HB and HbA1c were significantly higher in group 3 (T2DM with poor glycemic controls) as compared to group 2 (T2DM with normal glycemic controls) and group 1 (Healthy controls). Mean levels of FPG, PPPG and HbA1c were significantly higher in group 2 (T2DM with normal glycemic control) as compared to Group 1 (Healthy controls). In present study we found that the mean levels of Iron in Group 3 (T2DM with poor glycemic control) were significantly higher and mean levels of TIBC significantly decreased as compared to Group 2 (T2DM with normal glycemic controls) and Group 1(Healthy controls). We found a significant positive correlation of HbA1c with Iron (r = 0.636; p= P < 0.001) and negative correlation with TIBC (r = -0.301, p < 0.05) in Group 3. There was no significant difference observed in PPPG, iron, TIBC & Hb levels between group 1 & group 2.

Our results are in accordance with Kamalam, et al. [3], Shetty et al. [4], Saha et al. [11], Kapoor et al. [12] and Misra et al. [13] Kamalam et al. [3] found a significant increase in serum iron in poor glycemic controls as in comparison with

Table 1. Shows comparison of Hb, FPG, PPPG, HbA1c, Iron and TIBC of Group 1, Group 2 and Group 3

| Sr. no. | Parameters     | Group 1 healthy controls | Group 2 T2DM with normal glycemic control | Group 3 T2DM with poor glycemic control |
|---------|----------------|--------------------------|------------------------------------------|----------------------------------------|
| 1       | Hb (%)         | 11.91±1.76               | 11.67±1.63<sup>g</sup>                   | 11.05±1.53<sup>g</sup>                 |
| 2       | FPG (mg/dl)    | 83.37±13.73              | 100.83±21.65<sup>g</sup>                 | 177.96±75.81<sup>g</sup>               |
| 3       | PPPG (mg/dl)   | 116.17±14.05             | 130.12±36.40<sup>g</sup>                 | 279.33±118.59<sup>g</sup>             |
| 4       | HbA1c (%)      | 5.44±0.475               | 5.81±0.410<sup>*</sup>                   | 8.86±2.15<sup>g</sup>                  |
| 5       | Iron (µg/dl)   | 97.53±36.93              | 104.00±32.29<sup>g</sup>                 | 216.87±30.33<sup>g</sup>              |
| 6       | TIBC (µg/dl)   | 376.67±67.18             | 346.53±74.10<sup>g</sup>                 | 315.63±41.24<sup>g</sup>              |

Table 2. Shows comparisons of parameters of MDA, SOD and CAT in Group 1, Group 2 and Group 3

| Sr. no. | Parameters     | Group 1 healthy controls | Group 2 T2DM with normal glycemic control | Group 3 T2DM with poor glycemic control |
|---------|----------------|--------------------------|------------------------------------------|----------------------------------------|
| 1       | MDA nmoles/ml  | 0.535±0.360              | 1.12±0.337<sup>g</sup>                   | 1.91±0.138<sup>g</sup>                 |
| 2       | SOD U/g Hb     | 12.72±2.13               | 11.41±2.44<sup>g</sup>                   | 9.34±2.08<sup>g</sup>                  |
| 3       | CAT U/g Hb     | 14.91±2.45               | 7.37±1.68<sup>g</sup>                    | 5.93±1.68<sup>g</sup>                  |

Table 3. Correlation between HbA1c with Iron, TIBC, MDA, SOD and CAT in Group 2 and Group 3

| Sr. No | Parameters | Group 2 (r value) | Group 3 (r value) |
|--------|------------|------------------|------------------|
| 01     | HbA1c      | 0.077            | 0.636            |
| 02     | TIBC       | -0.009           | -0.301           |
| 03     | MDA        | 0.149            | 0.730            |
| 04     | SOD        | -0.179           | -0.243           |
| 05     | CAT        | -0.256           | -0.392           |

<sup>*p ≤ 0.05 significant, **p ≤ 0.001 highly significant and 1p ≥ 0.05 non-significant; Group I vs II -- p ≤ 0.05 significant, **p ≤ 0.001 highly significant and 1p ≥ 0.05 non-significant; Group II vs III -- p ≤ 0.05 significant, **p ≤ 0.001 highly significant and 1p ≥ 0.05 non-significant</sup>
good glycemic controls. They showed positive correlation between iron and HbA1c ($r=0.5968; \ p < 0.0001$). Shetty et al. [4] reported that increased level of free iron in group 3 (Type-2 DM in poor glycemic control) as compared to group 2 (Type-2 DM in good glycemic control ($p < 0.001$)). They also found a significant correlation in iron and HbA1c ($r = 0.46; \ p < 0.01$).

Graph 1. Shows comparisons of Iron and TIBC of Group 1, Group 2 and Group 3

Graph 2. Mean value of MDA, SOD and CAT of Group1, Group 2 and Group 3

Graph 3. Correlation between HbA1c and Iron of Group 3 ($r=0.636; \ p \leq 0.001$)
Graph 4. Correlation between HbA1c and MDA of Group 3 (r = 0.730; \( p \leq 0.001 \))

Graph 5. Correlation between HbA1c and Catalase of Group 3 (r = -0.392 \( p \leq 0.05 \))

Graph 6. Correlation between MDA and Iron of Group 3 (r = 0.548; \( p \leq 0.01 \))

Saha et al. [11] reported significantly higher levels of Serum ferritin, Serum iron & serum transferrin saturation in patients with T2DM compared to control group. Kapoor et al. [12]
found lower levels of TIBC and UIBC in diabetic patients as compared to the Healthy individuals. Misra et al. [13] reported, significant difference between mean serum free iron and TIBC concentration between Group I (Healthy individuals) and Group III (T2DM patients with suboptimal glycemic control) (p < 0.05).

Increase concentration of iron can be explained in various ways. Firstly, iron stores in the pancreas may lead to defective synthesis and secretion of insulin [14]. Secondly, excess iron deposition culminates in hyperinsulinemia due to obstruction in the insulin withdrawing ability of the liver [15]. Such deposits hinder insulin action, resulting in insulin resistance, which suppresses the yield of glucose in the liver [16].

Iron is an indispensable mineral for Hb synthesis of erythrocytes, myoglobin, cytochromes, oxidation – reduction reaction and cellular proliferation whereas excess iron accumulation causes organ dysfunction through the production of reactive oxygen species (ROS) [17].

In our study we found positive correlation of HbA1c with iron which indicates that Poor glycemic control leads to increased glycation of haemoglobin which causes more release of iron from Hb. However, there was no significant difference in iron, TIBC and Hb levels between group 1 & group II which indicates that normal glycemic control could reduce iron release by decreasing glycation of proteins and may control iron overload, and related diabetic complications.

Mean levels of MDA in Group 3 (T2DM with poor glycemic control) were significantly higher and mean levels of SOD and CAT were significantly decreased as compared to Group 2 (T2DM with normal glycemic controls) and Group 1 (Healthy controls). There was significant difference observed in the mean levels of MDA, SOD and CAT between group 2 and 1. There was strong positive correlation observed between HbA1c and MDA (r= 0.73, p <0.001) and moderate positive correlation observed between Iron and MDA (r= 0.54, p <0.01) in Group III. We also noted negative correlation of HbA1c with SOD (r = -0.243, p <0.05) and CAT (r = -0.392, p < 0.05).

Our results are concurrent with Sayeda et al. [18] Verma et al. [19] & Kundu et al. [20] for MDA. Sayeda et al. [18] found significantly higher mean MDA levels in diabetic patients with poor glycemic controls as compared to diabetic patients with normal glycemic controls and healthy controls. Verma et al. [19] showed that the serum MDA levels were significantly higher in T2DM subjects as compared to healthy controls.

Kundu et al. [20] reported, serum iron, serum ferritin, HbA1c and MDA levels were significantly higher in type 2 diabetics compared with apparently healthy controls. Elevations in serum iron, ferritin and HbA1c are accompanied by a parallel increase in blood glucose.

Oxidative stress represents one of the mechanisms involved in the pathogenesis and progression of T2DM and its complications, suggesting that it may be considered as an additional target for pharmacotherapy [21].

MDA is the end product of lipid peroxidation which act as a marker of balance between pro-oxidant and antioxidants. In DM patients there is an imbalance between pro-oxidant and antioxidant and pro-oxidant is adversely predominant [22]. MDA levels in diabetic patients may be increased due to the action of iron producing free radicals through Fenton reaction ultimately initiating the chain reaction to cause lipid peroxidation [23].

Superoxide dismutase plays an important protective role against cellular and histological damages that are produced by ROS. Catalase acts as a main regulator of hydrogen peroxide metabolism. The deficiency of this enzymes in the beta cells, leads to an increase in oxidative stress and ultimately failure of this cell type [24].

Our study was correlated with Merzouk et al. [25] Bhatia et al. [26] and Mukhopadhyay et al. [27] Merzouk et al. [25] reported that the mean erythrocytes SOD activities in 2 diabetic patients were significantly lower as compare to controls. They suggested that diabetes mellitus may be associated with altered antioxidant status regardless to various complications. Bhatia, et al [26] also reported that the erythrocytes SOD levels are decreased in the patients with diabetes mellitus. They suggested that low SOD activity could be resulted in diabetes with glycation of the enzymes, which has been reported to occur with Poor glycemic controls.

Mukhopadhyay et al. [27] reported, that the mean serum CAT level in diabetic patient was significantly lower (p < 0.05) as compared to
the control. They concluded that free radical mediates the cellular damage in type-2 diabetic patients.

In present study significant low levels of antioxidants and significant high levels of MDA indicate that increased oxidative stress leads to increased consumption of antioxidants (SOD & Catalase) to scavenge free radicals. Secondly glycation of enzymes might cause low antioxidants. In addition to that significantly decreased (p < 0.001) catalase activity indicates that glycation of catalase can also be responsible for release of iron since catalase is a heme containing enzyme.

We have not observed significant correlation of HbA1c with Iron, TIBC, MDA, SOD & Catalase in group 2, this might be due to normal glycemic control. There was significant difference observed in HbA1c, MDA, SOD & catalase levels between group 1 & group 2, it shows that slight increase of HbA1c levels, though it is normal, could increase oxidative stress but may not propagate harmful glycation sequelae which are a root cause of diabetic complications.

5. RECOMMENDATIONS

This study has some limitations. Firstly, small sample size; Secondly, ferritin is considered a good measure of body iron stores, which is not included in present study. Therefore further research is needed with large sample size, on the associations of serum ferritin, iron, TIBC and HbA1C concentration with oxidative stress in diabetes mellitus with and without complications.

6. CONCLUSION

In our study we found significant positive correlation of HbA1c with MDA and iron that indicates poor glycemic control leads to increased glycation of haemoglobin and other heme containing proteins. It causes more release of iron that leading to production of oxidative stress and thereby it might plays significant role in early appearance of diabetic complications. Hence, estimation of iron and oxidative stress markers along with HbA1c can be used as valuable markers for the prediction of T2DM complications. Thus periodical monitoring of these markers is essential to prevent diabetic complications.

CONSENT

All authors declare that written informed consent was obtained from the patient

ETHICAL APPROVAL

All authors hereby declared that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/54364