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Effect of Iron Deficiency Anemia and iron supplementation on HbA1c Levels- Implications for diagnosis of prediabetes and diabetes mellitus in Asian Indians

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ABSTRACT

Background: We investigated the effect of iron deficiency anemia (IDA) on levels of glycated hemoglobin (HbA1c) and to compare its levels before and after iron supplementations.

Methods: Age and sex matched subjects were enrolled and clustered in 2 groups: IDA (n=62) and healthy controls (HC; n=60). HbA1c levels were estimated by HPLC. Hemogram were estimated by hematology analyser. Serum ferritin (ELISA) and other parameters of iron profile were measured by standard guidelines of ICSH. HbA1c values and iron studies were repeated after 3 months of iron supplementation to determine the effect of iron therapy on HbA1c levels.

Results: Significantly higher HbA1c levels were observed in IDA subjects compared to HC (5.51 ± 0.696 v/s 4.85 ± 0.461 %, $p < 0.001$). A significant negative correlation was observed between HbA1c and hemoglobin, hematocrit, RBC count, MCH, MCHC and serum ferritin in IDA subjects (r= -0.632, -0.652, -0.384, -0.236, -0.192 and -0.441). Significant decline was noticed in HbA1c levels in IDA subjects after iron supplementation (5.51 ± 0.696 before treatment v/s 5.044 ± 0.603 post-treatment; $p< 0.001$). Post treatment, 70% subjects (14/20) with HbA1c in pre-diabetes range normalised to normal glucose tolerance (NGT) range and out of 6 patients with pre-treatment HbA1c in diabetes range, 5 reverted to pre-diabetes range while 1 of them reverted to the NGT range.

Conclusions: Caution must be exercised in interpreting the results of HbA1c in patients of IDA and iron deficiency must be corrected before diagnosing diabetes and pre-diabetes solely on the basis of HbA1c criteria.

Keywords: Iron deficiency anemia; diabetes mellitus; HbA1c

1. Introduction

Anemia affects 1.62 billion people, accounting for 24.8% of the global population [1]. India continues to be one of the countries with very high prevalence of Iron Deficiency Anaemia (IDA). National Family Health Survey (NFHS) 3 reveals the prevalence of anemia to be 70-80% in children, 70% in pregnant women and 24% in adult men [2]. The prevalence of Diabetes Mellitus (DM) has been increasing globally and the increase has been more dramatic in developing countries like India. There are an estimated 63 million people with diabetes in India presently and this number is predicted to rise to almost 87 million people by 2030 [3,4]. In Metropolitan cities of India such as Delhi, the prevalence of diabetes is over 11% with a nearly equal number of prediabetic individuals [5].

Considering the large prevalence of the 2 health problems viz. iron deficiency anemia and diabetes mellitus in India, it is important to study the relationship between iron deficiency anemia and glycated hemoglobin (HbA1c). The recent recommendations of American Diabetes Association (ADA) have included HbA1c as a sole diagnostic tool for diabetes mellitus, thereby making this study particularly important [6]. A value of ≥6.5% by a method traceable to Diabetes Control and Complication Trial (DCCT) has been recommended as the cut-off to diagnose DM. This has also been accepted by World Health Organization (WHO) to diagnose DM. ADA further defines persons with HbA1c levels ≥5.7% and <6.5% as prediabetic. Previous studies have documented that iron deficiency anemia (IDA) can affect the levels of HbA1c [7,8,9,10]. However, these were either inconclusive, measured HbA1c by methods other than the ones certified by National Glycohemoglobin Standardization Program (NGSP) or were retrospective analysis of pre-existing data.

2. Materials and Methods

2.1 Subjects

The present case-control study included age and sex matched 122 subjects attending Medicine OPD/Ward at University College of Medical Sciences and Guru Teg Bahadur Hospital, Delhi between November 2011 and April 2013. The study was approved by the institutional ethical committee for human research and subjects were enrolled into the study after taking informed written consent. The subjects were divided into 2 groups; Group I (n=62): subjects of Iron deficiency anemia with hemoglobin (Hb) levels were ≥50.0 gm/l and <100.0 gm/l, Group II (n=60): Healthy controls without iron deficiency anemia Diagnosis of IDA was made by WHO guidelines i.e. iron deficiency anemia was defined by a) microcytic hypochromic peripheral smear picture with MCV < 80 fl.,serum iron ≤7.17 µmol/l, b) total iron binding capacity ≥71.6 µmol/l, c) transferrin saturation ≤16 % and d) serum ferritin ≤33.7 pmol/l. Subjects with Mentzer index is >13 were included into the study. Mentzer index defined by the ratio of mean corpuscular volume and red blood cell count which is used to distinguish between thalassemia and iron-deficiency anemia. [11].

To avoid any potential confounding factors, patients with known diabetes mellitus (refers to a group of common metabolic disorders that share the phenotype of hyperglycemia, ADA 2015), chronic inflammatory illnesses (Inflammation is the body's attempt at self-protection; the aim being to remove harmful stimuli, including damaged cells, irritants, or pathogens for ≥3 months duration, CRP values >0.6 mg/dL), hemolytic anemia (anemia due to hemolysis, reticulocyte count > 1.5% and increases unconjugated bilirubin, presence of urine urobilinogen and free

hemoglobin) hemoglobinopathies (group of inherited blood disorders that result from variations in the structure and/or synthesis of hemoglobin , past history of multiple transfusions, family history and Mentzer index <13), kidney dysfunction (serum creatinine ≥132.6 µmol/l) or known chronic kidney disease (glomerular filtration rate (GFR) <60 ml/min/1.73 m^2 for 3 months are classified as having chronic kidney disease irrespective of the presence or absence of kidney damage, eGFR < 60ml/min/1.73 m^2) chronic alcohol ingestion (large amounts over a long time period, has difficulty cutting down, acquiring and drinking alcohol takes up a great deal of time, alcohol is strongly desired, usage results in not fulfilling responsibilities, usage results in social problems, usage results in health problems, positive CAGE questionnaire and elevated GGT) and malabsorption syndrome (chronic diarrhea $\geq$ 3 months) were excluded from the study. Subjects with Hb <50.0 gm/l who may require blood transfusions that can confound results were also excluded. In addition, patients with HbA1c ≥ 7.0% were subjected to oral glucose tolerance test (OGTT) and those detected to be diabetic were too excluded.

The patients with IDA were given 100 mg daily elemental oral iron supplementation daily for 3 months. During intervention period participants with IDA were on their routine diet and physical activity. The subjects were followed up once every 2 weeks to check for compliance and were motivated to take daily medicines. However, the subjects who did not attain the minimum required rise of Hb levels of 10 gm/l after 12 weeks of iron supplement were also excluded from the study. Therefore, a higher number of study subjects were selected initially (72 cases) to account for attrition and loss to follow up.

2.2 Methods

All patients were asked to provide a detailed history which included established diabetes mellitus, jaundice, repeated blood transfusion, acute blood loss, repeated pregnancies, amenorrhea, alcohol ingestion, diarrhea and urinary complaints. Physical examination was done to investigate for nutritional status, vitamin deficiency, Koilonychia, markers of chronic alcoholism, hepatosplenomegaly, thorough neurological & fundus examination for persistence peripheral neuropathy and optic atrophy. Fasting blood sample was withdrawn from ante-cubital vein under aseptic precautions and collected into plain and EDTA vacutainers for estimation of various biochemical and hematological parameters.

A complete blood count with RBC indices were measured by automated haematology cell counter (MS-9, Melit Schloesing Laboratries). Among iron studies, serum ferritin was estimated by sandwich ELISA (Calbiotech Inc, sensitivity < 5 ng/ml). However, rest of the parameters of iron studies i.e., serum iron [12], total iron binding capacity (TIBC) [13] were estimated using standard guidelines of International Committee for Standardisation in Hematology. However percentage transferrin saturation was determined using serum iron and TIBC (serum iron (µmol/l)/ TIBC (µmol/l) x 100). HbA1c estimations were carried out in all subjects using High Performance Liquid Chromatography method certified by NGSP using Bio-Rad D-10 Hemoglobin Analyser (inter-assay & intra-assay variation is 0.8 %CV and 1.4%CV) [14].

At the end of the therapy after 3 months, all the hematological parameters, RBC indices, iron studies and HbA1c were repeated to see the effects of iron supplementation.Response to iron therapy was assessed by improvement in the

repeat iron studies and a minimum rise in Hb value of 10 gm/l when compared with the pre-treatment values.

2.3 Statistical analysis

Data was expressed as mean ± SD. p-value <0.05 was considered statistically significant. The mean HbA1c values between iron-deficiency anaemia subjects and controls were compared using the student t-test. The mean HbA1c values of patients before and after iron therapy was compared using the paired t-test. A Pearson's correlation analysis was done between all the hematological and iron studies with HbA1c. All data analysis was done using SPSS ver 17.0.

3. Results

The baseline demographic, hematological, iron study parameters and HbA1c levels in the IDA and control subjects are shown in Table 1. The IDA and HC group were matched for age (p=0.572) and sex (p=0.710). The mean levels of hematological, RBC Indices and iron study parameters were significantly (p<0.001) in IDA group as compared to HC. The mean percent glycated hemoglobin levels in the pre-intervention arm in the IDA subjects were significantly higher by 0.66% (p<0.001) as compared to the HC. Six subjects of IDA had Diabetes (HbA1c 6.5% - 7.1%), 20 had prediabetes (HbA1c 5.7%-6.4%) and 36 were non-diabetics (HbA1c<5.7%) respectively according to ADA criteria [6].

A correlation analysis between various haematological, RBC indices, iron study parameters and HbA1c before iron supplementations are shown in Table 2.

There was a strong negative correlation of Hb (r = -0.632), Hct (r = -0.652), RBC Count (r = -0.384), MCH (r = -0.236), MCHC (r = -0.192) and serum ferritin (r = -0.441) with HbA1c levels while there was a negative but weak correlation of MCV (r = -0.156), TIBC (r = -0.035), % TS (r = -0.103), and serum iron (r = -0.173) with HbA1c levels.

The effect of iron supplementation on various hematological, RBC indices and iron study parameters are depicted in Table 3. We observed a significant ($p<0.001$) increase in the mean values of Hb, Hct, RBC count, MCV and MCH post iron supplementation in IDA subjects. Similarly, there was a significant increase in parameters of iron studies including serum ferritin, serum iron and % transferrin saturation ($p<0.001$), while there was a significant decline the TIBC values ($p<0.001$) post iron supplementation. There was also a significant decline in the values of HbA1c by 0.47% post iron supplementation compared to baseline ($p<0.05$).

Out of 62 patients treated with iron, the HbA1c values decreased in 54 patients, while it increased in 5 patients and it stayed the same in 3 patients. Out of the 20 pre-diabetic patients whose before treatment HbA1c levels were between 5.7 -6.4 %, 14 had HbA1c values < 5.7 % after treatment while in 4 out of 6 subjects HbA1c levels decreased but remained between 5.7% - 6.4% post-treatment (Table 4). One patient's HbA1c increased post-treatment while 1 patient didn't have any change in the HbA1c levels post treatment. Of the 6 subjects who had HbA1c levels in diabetic range, 5 of them reverted to the pre-diabetic range while 1 of them reverted to the non-diabetic range post iron supplementation (Table 4).

4. Discussion

The present study examined the effects of iron deficiency anemia on the levels of HbA1c. It also compared the effects of iron supplementation on HbA1c levels. We found significantly higher HbA1c levels in IDA subjects compared to controls. The hypothesis of our present study is that IDA leads to false high measurement of HbA1c and therefore should not be used as sole criterion for diagnosis of diabetes melitus.. This study's hypothesis is supported by the fact that HbA1c was significantly negativey correlated with hemoglobin, hematocrit, RBC count and serum ferritin in IDA subjects. The decline in HbA1c level after Iron supplementation wherein 70% of HbA1c based subject with prediabetes reverted to NGT range and all subjects with diabetes (HbA1C 6.5% -7.1%) reverted to either prediabetes or NGT post iron supplementation further supported our studies . Decreased hemoglobin, hematocrit, RBC count and serum ferritin is diagnostic of IDA. In IDA , the quaternary structure of the hemoglobin molecule becomes altered, therefore glycation of the globin chain occurred more readily in the relative absence of iron.[15]. It was also proposed that formation of glycated hemoglobin is an irreversible process and hence, the concentration of HbA1c in erythrocyte will increase linearly with the cell's age [16]. Clearly, HbA1c overestimated the diagnosis of diabetes in 100% and prediabetes in 70% of subjects of IDA when used for diagnosis in the HbA1c range of 5.7 – 7.1%.

Our study provides evidence against the use of HbA1c as the sole criteria for diagnosing diabetes and prediabetes particularly in communities with high prevalence of IDA such as India. Use of HbA1c alone would spuriously overestimate the prevalence of these two conditions in such population. Similarly, in subjects with coexisting IDA, interpretation of HbA1c values should be deferred till after complete correction of iron deficiency with iron supplementation. This would be important both

in the settings of diagnosing diabetes and monitoring glycemia in patients with diabetes. It may be better to rely on WHO criteria of 75 gm OGTT in such patients to label the patient diabetic or prediabetic [17].

The present study has a number of strengths. Firstly, we used standard HPLC method certified by NGSP traceable to DCCT trial (Bio-RAD) to measure HbA1c and we believe that this method allowed for the accurate and reliable estimation of HbA1c [14]. HPLC is a method for measuring HbA1c and the guidelines recommend the use of NGSP certified methods like HPLC for diagnosis of diabetes. Secondly, our subjects with IDA were well characterised for iron deficiency and underwent a battery of tests including serum iron, serum ferritin, % transferrin saturation and total iron binding capacity. Most of the previous studies on IDA and HbA1c either used ferritin alone or in combination with RBC indices to define iron deficiency. We also performed CRP levels to account for any confounding effect of chronic inflammation on ferritin levels and those found to have elevated CRP were excluded from the study. Similarly, we also excluded subjects having conditions which could affect the levels of HbA1c by appropriate history, examination and other battery of investigations. The patients of hemolytic anemia, hemoglobinopathies, kidney dysfunction or chronic kidney disease, chronic alcoholic were excluded by appropriate history, liver function tests, kidney function tests, calculation of Mentzer Index.

Thirdly, we excluded patients with known diabetes as well as those with HbA1c >7.0% if they were detected diabetic on OGTT. This was done as we were specifically evaluating the validity of using HbA1c in the diagnosis of prediabetes and borderline diabetes. The study also evaluated prospectively the effects of iron supplementation in subjects with IDA. This interventional study design provided an

opportunity to examine the relationship between IDA and HbA1c levels with greater strength and reliability. Finally, the subjects were followed up once every two weeks to ensure compliance of iron supplements.

The significantly higher baseline HbA1c levels in subjects of IDA compared to age and sex matched controls observed in our study are in agreement with some studies done previously by Shanthi et al. [9], Kim et al. [7] and Hardikar et al. [8] and confirm their applicability to the prediabetic and low diabetic range of values (5.7 – 7.1%). However, they are in contradiction to the studies done by Van et al. [18], Sinha et al. [10], and Ford et al. [19] who found either no difference in glycated hemoglobin levels in IDA and control subjects or decreased HbA1c values in IDA subjects compared to controls. The wide variability and differences in the results of these studies can be explained by differences in study design, method by which HbA1c was estimated, range of HbA1c level studied and differences in selection of subjects. Study done by Sinha et al. and Van et al. measured HbA1c by ion exchange mini column chromatography. Twenty percent of the HbA1c is not collected in the Alc peak and that the fraction collected for the HbA1c includes 12% of the HbA1a+ 1b.However, since it has been proposed that the quaternary structure gests disturbed in IDA and various of hemoglobin . It has ion exchange binding characteristics similar to HbA1c and will contribute to the HbA1c value if present and will produce lowered values [20]. Ford et al. estimated HbA1c by boronate affinity HPLC method in which *m*-aminophenylboronic acid reacts specifically with the *cis*-diol groups of glucose bound to Hb. This method measures total glycated GHB, including HbA1c and Hb glycated at other sites [21]. Therefore IDA leads to false lowered values. In our study we measured HbA1c by ion exchange HPLC. It was observed that the values were increased probably due overstimation to labile HbA1c

(Pre- HbA1c). But the interference is minimum and enabling the operator to identify the variants and derivatives. Ion exchange HPLC method for HbA1c estimations and studied very well characterised and carefully selected subjects to better validate our results.

Our study observed that there is a significant drop in HbA1c from a mean of 5.51% to 5.04% after 3 months of iron supplementation. This drop of 0.5% in HbA1c occurred despite a partial improvement in IDA observed post iron supplementation. It is possible that complete correction of IDA with normalisation of Hb levels and iron stores could have resulted in a further drop in HbA1c levels. This suggests that the effects of IDA on HbA1c levels were underestimated in the current study and we plan to follow up our subjects further to examine this issue. A few other studies done by Brooks et al., Coban et al., and Tarim et al. have also examined the effects of iron therapy in IDA subjects on the HbA1c levels [22-24]. All of these studies reported a significant decrease in HbA1c levels after iron supplements. It would thus appear that interpretation of HbA1c values in patients with IDA should be deferred till after adequate supplementation with Iron. This would be even more important if HbA1c is being used for diagnosis and categorisation of glucose intolerance.

The mechanism causing elevated HbA1c in patients of IDA is still unclear. It has been suggested that iron deficiency leads to prolongation of erythrocyte survival leading to elevated levels of HbA1c [25]. It has been hypothesized that iron deficiency may alter the quaternary structure of the hemoglobin molecule and facilitate glycation of the β-globin chain [22]. It is speculated that the emergence of young erythrocytes in the circulation with relatively less glycation of Hb after iron therapy could have led to dilution and lowering the concentration of previously

formed glycated hemoglobin [24]. Future studies are needed to address the mechanism of increased HbA1c levels in IDA.

HbA1c measurement is also important for monitoring of patients being treated for DM. HbA1c levels help the treating physician to guide therapy in them. Our study did not specifically examine the effects of IDA on HbA1c levels in this clinical setting. Nevertheless, if IDA affects the glycation of Hb independent of the glycemic status of the patients, then it can be hypothesised that in DM patients with similar levels of glycemia, the patients with IDA will have higher levels of HbA1c compared with patients with normal iron and Hb status. This can lead to a false interpretation of HbA1c as poor glycemic control leading to an overprescribing of anti-diabetic medications. IDA may also explain the discrepancies between self monitoring of blood glucose (SMBG) and HbA1c values in diabetes patients on treatment and needs to be ruled out when other factors can't explain this mismatch. Hence, SMBG and laboratory measurement of plasma glucose may be better indicators of glycemic control in this setting until IDA is fully corrected.

The limitations of the study are that the patients were followed up for only 3 months and many of the patients were only partially corrected for iron deficiency after 3 months of therapy. The measurement of HbA1c after complete correction of iron deficiency could have had more profound effects. Efforts were made to check for compliance of therapy; however, there was no reliable method of ensuring compliance as the patients were followed up on an outpatient basis. Another limitation was that we did not performed OGTT in all cases of IDA and controls. Diabetes were excluded by presence of history of diabetes mellitus and with HbA1c > 7% by an OGTT.

The levels of HbA1c are higher in patients of IDA and decrease after iron supplementation. HbA1c overestimates prediabetes and diabetes in these patients and caution must be exercised while diagnosing these conditions in the presence of IDA solely on the basis of HbA1c criteria.

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Table 1: Baseline Demographic, haematological, RBC indices, iron study Parameters and HbA1c levels between IDA and healthy subjects

Variables	Cases (n=62)	Control (n=60)	*p*-value
Age (y)	31.4 ± 11.3 (20-70)	32.5 ± 10.1 (20-56)	NS
Sex (M/F)	8/54	9/51	NS
Hb (gm/l)	73.9 ± 12.2 (44 – 100)	134.3 ± 13.2 (112 – 179)	<0.001
Hct (%)	24.9 ± 3.5 (16.0 – 34.1)	41.3 ± 4.7 (33.0 – 59.0)	<0.001
RBC Count (10^{12}/l)	3.7 ± 0.7 (2.5 – 3.2)	4.6 ± 0.6 (3.3 – 5.9)	<0.001
MCV (fL)	66.7 ± 7.3 (52.4 – 79.9)	89.7 ± 9.7 (80.1 – 98.0)	<0.001
MCH (pg)	20.1 ± 3.9 (13.7 – 33.0)	30.1 ± 2.9 (24.1 – 39.3)	<0.001
MCHC (g/dl)	29.3 ± 2.1 (26.1 – 33.1)	32.6 ± 1.3 (29.4 – 36.1)	<0.001
Iron (µmol/l)	5.4 ± 1.4 (1.8 – 7.2)	17.3 ± 4.5 (9.1 – 27.2)	<0.001
TIBC (µmol/l)	73.3 ± 1.7 (71.6 – 80.4)	57.9 ± 6.8 (47.1 – 82.3)	<0.001
TS (%)	8.1 ± 2.2 (4.4 – 14.4)	28.7 ± 8.2 (16.1 – 60.7)	<0.001
Ferritin (pmol/l)	17.3 ± 3.0 (11.7 – 26.7)	315.9 ± 63.8 (197.7 – 476.3)	<0.001
HbA1c (%)	5.5 ± 0.7 (4.4 – 7.1)	4.9 ± 0.5 (4.0 – 6.0)	<0.001
HbA1c (mmol/mol)	36.7 ± 4.6 (24.6 – 54.1)	29.5 ± 2.7 (20.2 – 42.1)	

Data represented in Mean±SD (upperlimit - lowerlimit) unless specified. A $p < 0.05$ considered significant. TS; Transferrin saturation.

Table 2: Correlation analysis of HbA1c level with haematological, RBC indices, iron study parameters before iron supplementation

Parameters	Pearson's Correlation coefficient (r)	p value
Hb (gm/l)	-0.632	<0.001
Hct (%)	-0.652	<0.001
RBC Count (10^{12}/l)	-0.384	<0.001
MCV (fl)	-0.156	NS
MCH (pg)	-0.236	<0.001
MCHC (g/dl)	-0.192	0.030
TIBC (µmol/l)	-0.035	NS
%TS (%)	-0.103	NS
Ferritin (pmol/l)	-0.441	<0.001
Iron (µmol/l)	-0.173	NS

p value < 0.05 considered significant. A P >0.05 considered as non significant.TS; Transferrin saturation.

Table 3: Comparison of haematological, RBC indices, iron study parameters and HbA1c levels before and after iron supplementation for 3 months

Variables	Before Treatment	After Treatment	*p*-value
Hb (gm/l)	73.9 ± 12.2 (44 – 100)	89.1 ± 12.1 (60 – 125)	<0.001
Hct (%)	24.9 ± 3.5 (16.0 – 34.1)	29.9 ± 3.1 (23.1 – 39.3)	<0.001
RBC Count (10^{12}/l)	3.7 ± 0.7 (2.5 – 3.2)	4.1 ± 0.6 (2.7 – 5.5)	<0.001
MCV (fl)	66.7 ± 7.3 (52.4 – 79.9)	74.3 ± 8.7 (58.7 – 94.7)	<0.001
MCH (pg)	20.1 ± 3.9 (13.7 – 33.0)	22.1 ± 3.3 (16.5 – 30.7)	<0.001
MCHC (g/dl)	29.3 ± 2.1 (26.1 – 33.1)	29.7 ± 1.9 (25.4 – 32.8)	NS
Iron (µmol/l)	5.4 ± 1.4 (1.8 – 7.2)	10.3 ± 2.7 (6.9 – 19.6)	<0.001
TIBC (µmol/l)	73.3 ± 1.7 (71.6 – 80.4)	64.4 ± 2.9 (57.1 – 71.7)	<0.001
TS (%)	8.1 ± 2.2 (4.4 – 14.4)	14.6 ± 2.6 (9.1 – 20.1)	<0.001
Ferritin (pmol/l)	17.3 ± 3.0 (11.7 – 26.7)	36.6 ± 10.6 (16.2 – 76.3)	<0.001
HbA1c (%)	5.5 ± 0.7 (4.4 – 7.1)	5.0 ± 0.6 (4.0 – 6.2)	<0.001
HbA1c (mmol/mol)	36.7 ± 4.6 (24.6 – 54.1)	31.6 ± 3.7 (20.2 – 44.3)	

Data represented in Mean±SD (upperlimit - lowerlimit) unless specified. A $p < 0.05$ considered significant. TS; Transferrin saturation.

Table 4: Change in glucose tolerance category based on HbA1c values after Iron supplementation in IDA subjects

	Number of subjects with change in category to non-diabetes (HbA1c < 5.7%) post-supplementation	Number of subjects with change in category to Prediabetes (HbA1c ≥5.7% and <6.5%) post-supplementation	Number of subjects with change in category to diabetes(HbA1c ≥6.5%) post-supplementation
Subjects pre-treatment classified as diabetes (n = 6)	1	5	0
Subjects pre-treatment classified as Pre-diabetes (n = 20)	14	6*	0
Subjects pre-treatment classified as Non-diabetes (n = 36)	36#	0	0

*4 of these had a decrease in HbA1c, 1 had an increase and 1 had similar HbA1c but all 6 remained in prediabetes category

30 of these had a decrease in HbA1c, 4 had an increase in HbA1c, 2 had similar HbA1c but all 36 remained in non diabetic category

Highlights

- Recently HbA1c has been recommended as a sole diagnostic criterion for prediabetes & diabetes mellitus.
- Iron deficiency anemia (IDA) was associated with a significant increase in baseline HbA1c measured by HPLC.
- Iron supplementation significantly decreases HbA1c levels.
- Iron deficiency must be corrected before any diagnostic decision is made solely on the basis of HbA1c in areas with a high prevalence of IDA.