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Effect of Iron Supplementation on Glycosylated Haemoglobin in Non-Diabetic Individuals with Iron Deficiency Anaemia

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Abstract

Introduction: *Iron deficiency anaemia (IDA) is a common disorder especially in developing countries. People with IDA can have many metabolic and laboratory abnormalities and treatment for IDA causes reversal or significant improvement in many of these. Studies have shown a reduction of HbA1c in diabetics after treatment of IDA. However, the same has not been studied well in non diabetics and hence the present study was planned.*

Methods: *It was a prospective observational study done at PGIMER Dr RML Hospital, New Delhi, India. 100 confirmed non diabetic individuals with HbA1c < 5.7% with definite IDA along with 50 age and sex matched healthy controls were recruited for this study. After overnight fasting, routine blood and laboratory parameters along with serum iron studies and serum ferritin were done. Appropriate statistical tests were applied.*

Results: *Amongst 100 cases with IDA, 54% were females and 46% were males. Pure vegetarian diet followed by bleeding from any site and overzealous use of proton pump inhibitors and NSAID's were the major probable reasons for anaemia. The mean baseline HbA1c at 0 month was 5.49 ± 0.8% which reduced to 4.88 ± 0.43% (P < 0.001) after 2 months of iron supplementation. There was a concomitant improvement in erythrocyte indices, levels of serum ferritin, and markers of iron profile after 2 months of treatment. The reduction in HbA1c was seen to have a significant inverse association with an increase in haemoglobin, S. ferritin, S. transferrin and direct association with reduction in total iron binding capacity (TIBC). There was a concomitant statistically significant reduction of triglycerides (P < 0.01) and an increase in serum creatinine levels (P < 0.03) and albumin: globulin ratio (P < 0.02) with iron supplementation.*

Conclusion: *HbA1c levels can be falsely high in patients with IDA and dropped significantly after iron supplementation. In patients with IDA, even with normal serum glycaemia, HbA1c can be misleadingly high and hence caution is warranted before labeling these patients as diabetic or prediabetic.*

Introduction

Iron deficiency is one of the most prevalent forms of malnutrition. Globally 50% of anaemia is attributed to iron deficiency.Various laboratory parameters like haemoglobin with peripheral smear, serum iron levels, transferrin saturation, total iron binding capacity and serum ferritin levels can objectively estimate iron deficiency[1]. Ferritin is the storage form of iron and it reflects the iron status accurately.Earlier studies have shown that reduced ferritin levels have a link with increased glycosylated haemoglobin (HbA1c) leading to false-high values of HbA1c in diabetic individuals[2]. HbA1c is a marker of glycaemic index which can be performed at any point of time and can be used as an objective measure of glycaemic control. It reflects the average plasma glucose over the previous eight to twelve weeks. It can be performed at any time of the day and does not require any special preparation such as fasting[3]. HbA1c is majorly affected by the blood glucose levels alone however, certain studies have found that HbA1c levels are altered by various other coexisting factors especially iron deficiency anaemia which is a major public health problem in developing countries like india. Evidence has accumulated which supports the hypothesis that the glycation reaction, can be modulated by the iron status of the patient[4]. Many studies have been done regarding the effect of iron supplementation on HbA1c in diabetic and prediabetic individuals, however the same has not been studied well in individuals with HbA1c < 5.7% (i.e., neither diabetic nor prediabetic) especially in this part of the world and hence the present study is was planned.

Methods

It was a prospective observational study done over a span of 1 year in 100 randomly selected non diabetic individuals of age 18 - 40 years with HbA1c < 5.7% with confirmed iron deficiency anaemia (cases) who were recruited from outpatient department of medicine at Post-graduate institute of medical education and research (PGIMER) Dr

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RML Hospital, New Delhi. 50 age and sex-matched adult healthy volunteers without iron deficiency anaemia and HbA1c < 5.7% were also recruited as controls. All cases with haemoglobin less than 12 gm% in females or 13 gm% in males with either peripheral blood picture of microcytic hypochromic anaemia or iron profile suggestive of iron deficiency anaemia, S. iron < 60 mg/dl, total Iron binding capacity < 240 mcg/dl and S. transferrin saturation < 15% (males) and < 12% (females) or serum ferritin levels less than 15 microgram/l were included in this study. Females with present or recent pregnancy within last 1 year, haemolytic anaemia, haemoglobinopathies, chronic alcoholism, chronic liver or kidney diseases or cases with dimorphic or megaloblastic anaemia or thyroid dysfunction were excluded. Similarly cases with past history of stroke or coronary artery disease on treatment with aspirin, clopidogrel, heparin or steroids or individuals with history of acute blood loss were excluded. Individuals on iron/folate/vitamin-D/vitamin-B_{12}/multivitamin supplementation (presently or within last 6 months) were also excluded. A bilingual informed consent was taken and the cases were subjected to detailed history and physical examination through preset performa at the time of recruitment in the study (0 month). A detailed complete blood count including haemoglobin, total and differenctial leucocyte counts, platelet counts, packed cell volume (PCV), red blood cell count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), liver function tests, kidney function tests, blood sugar fasting and post-prandial were done in a 10 ml fasting venous sample. HbA1c and iron profile including serum ferritin, total iron, total iron binding capacity (TIBC) and transferrin saturation were also done after a 12 hours overnight fasting. All cases were then subjected to 100 mg of elemental iron twice a day for two months and then re-evaluation including clinical and haematological profile was done after two months (2 months).

Estimation of HbA1c:

The per cent determination of HbA1c in whole blood samples was done by using D-10™ Dual Programme (Biorad, USA) based upon ion exchange high performance liquid chromatography (HPLC). The reportable range/linearity of the extended program was 3.7 - 18.4%. The total CV% for the HbA1c levels 5.7% and 5.9% was 1.2% and 1.8%, respectively. Serum iron, cholesterol, HDL, TG and TIBC were measured by fully automated analyser vitros-5,600, (Orthoclinical diagnostics USA). Serum iron levels were estimated by using dry slide technology based on two point rate with reportable range 2.0 - 600 µl/dl and intra-assay CV% 1.9. Serum total cholesterol HDL and triglyceride levels were estimated by using dry slide base on colorimetric method. TIBC levels in serum were measured by using dTIBC reagent based on two point rate with reportable range and CV% 60 - 650 µl/dl and 1.8 respectively. All the data was analysed using IBM SPSS software for window version 20. Quantitative variables were reported as mean ± standard deviation. Chi square tests was used for test of association. $P < 0.05$ was considered as statistically significant.

Results

A total of 100 cases and 50 controls were recruited for this study. The mean age of cases was 32.14 ± 3.11 years (range 18 - 40 years) whereas the mean age of controls was 35.60 ± 4.57 years. 46% of the cases were male whereas 54% were females. The anthropometric parameters were normal and comparable in both the groups.

The predominant symptoms in majority of the cases were weakness, malaise, disinterest in job, easy fatiguability, myalgias and bodyaches. 50% of the males with iron deficiency anaemia complained of lack of energy and loss of sexual drive. 31% of females with iron deficiency anaemia had menstrual complaints including irregular cycles, decreased flow, dysmenorrhoea and menorrhagia. However, after 2 months of treatment with iron, most of these symptoms were reduced to insignificant levels. The most common attributable cause for IDA was pure vegetarian diet (36%) followed by bleeding from any site (32%), overzealous use of NSAID's and proton pump inhibitors (20%), worm infestation (8%) and history of pica (4%).

The blood parameters of cases before and after 2 months of iron supplementation are listed in Table I. As can be seen there was a significant improvement in haemoglobin and RBC indices in cases from 0 month to 2 months after iron supplementation. There was a significant reduction of HbA1c from 5.49 ± 0.8 % to 4.88 ± 0.43 % ($P < 0.001$, CI 1.81 - 3.11) and the levels of triglycerides (TG) reduced from 212.84 mg% to 183.76 mg% ($P < 0.01$, CI 1.14 - 1.36). There was also a statistically significant increase in the level of serum creatinine ($P < 0.03$, CI 1.17 - 1.46) and albumin to globulin ratio, i.e., A:G ratio ($P < 0.02$, CI 1.23 - 1.55). All other laboratory parameters remained more or less unchanged. The mean HbA1c amongst controls was 4.68%. The mean fasting and post-prandial (PP) glucose in cases at 0 month were 86.22 ± 10.72 gm% and 136.28 ± 16.4 gm% respectively whereas the same after 2 months of iron supplementation were 84.30 ± 8.69 gm% ($P < 0.76$) and 134.21 ± 14.71 gm% ($P < 0.74$) respectively. The mean fasting and post-prandial (PP) glucose in controls were 84.52 ± 8.74 gm% and 130.68 ± 10.10 gm% respectively. It can be seen that in spite of no change in serum fasting as well

Table 1: Haematological profile of cases (0 and 2 months) and controls.

	Cases		P value	Controls
	0 month (at the time of recruitment)	2 months of (after 2 months of iron supplementation)		
Haemoglobin (gm%)	9.73 ± 0.83	12.98 ± 4.01	0.001	14.00 ± 0.72
PCV (%)	29.08 ± 2.10	38.66 ± 6.24	0.01	41.84 ± 3.22
RBC counts (x10^6 cells/µl)	3.85 ± 0.48	4.56 ± 0.36	0.04	4.98 ± 0.37
MCV (fl)	70.9 ± 9.27	86.86 ± 6.82	0.007	94.2 ± 6.61
MCH (pg)	22.2 ± 3.73	30.2 ± 4.16	0.02	31.74 ± 3.22
MCHC (g/dl)	30.4 ± 3.38	33.2 ± 3.76	0.05	34.28 ± 2.44
S. ferritin (ng/ml)	7.50 ± 2.38	108.0 ± 12.24	0.001	131.91 ± 17.88
S.Total Iron (mcg/dl)	34.24 ± 4.98	111.50 ± 12.86	0.005	123.38 ± 20.13
Total iron binding capacity (mcg/dl)	552.50 ± 43.12	336.28 ± 56.42	0.005	304.12 ± 45.78
Transferrin saturation (%)	10.0 ± 2.1	34.24 ± 4.98	0.001	40.63 ± 4.34
RBC distribution width (%)	9.1 ± 1.62	11.4 ± 1.31	0.07	13.4 ± 1.9
TLC (cells/mm^3)	7600 ± 340	7440 ± 422	0.12	7180 ± 464
DLC (P/L/M/E)	77/19/2/2	70/23/2/5	0.10	70/26/2/2
Platelet counts (lakhs/mm^3)	1.78 ± 0.41	1.65 ± 0.44	0.09	1.74 ± 0.32
ESR (mm/hour)	15 ± 2	16 ± 2	0.12	22 ± 3
Retic counts (%)	3.0 ± 0.2	40.0 ± 0.4	0.18	2.8 ± 0.22
Fasting blood sugar (mg/dl)	86.22 ± 10.72	84.30 ± 8.69	0.76	84.52 ± 8.74
Postprandial blood sugar (mg/dl)	136.28 ± 16.4	134.21 ± 14.71	0.74	130.68 ± 10.10
HbA1c (%)	5.49 ± 0.8	4.88 ± 0.43	0.001	4.68 ± 0.28
Blood urea (mg/dl)	36.3 ± 3.44	34.7 ± 3.21	0.27	30 ± 0.38
S. creatinine (mg/dl)	0.62 ± 0.07	0.73 ± 0.06	0.03	0.74 ± 0.08
Bilirubin (mg%) (T/D/I)	0.5/0.4/0.1	0.3/0.2/0.1	0.15	0.4/0.3/0.1
SGOT (AST) (IU/l)	39 ± 3.47	35 ± 4.43	0.16	34 ± 3.66
SGPT (ALP) (IU/l)	40 ± 3.84	38 ± 2.36	0.21	36 ± 2.55
ALP (IU/l)	136 ± 8.0	110 ± 8.0	0.14	104 ± 6.0
S. protein (gm/dl)	6.3 ± 1.1	6.2 ± 1.4	0.21	6.6 ± 1.6
Albumin (gm/dl)	3.3 ± 0.62	3.6 ± 0.44	0.08	3.7 ± 0.53
Globulin (gm/dl)	3.0 ± 0.48	2.6 ± 0.53	0.10	3.0 ± 0.34
A : G ratio	1.1 ± 0.11	1.5 ± 0.14	0.02	1.2 ± 0.16
Total cholesterol (gm/dl)	218.42 ± 34.26	210.36 ± 30.44	0.14	208.20 ± 32.18
S.Triglycerides (gm/dl)	212.84 ± 20.30	183.76 ± 18.31	0.01	174.31 ± 14.78
Low density lipoprotein (gm/dl)	118.39 ± 10.76	114.94 ± 11.18	0.11	110.21 ± 13 ± 53

as post-prandial sugar levels after iron supplementation there was an improvement in iron profile and a significant drop in HbA1c levels.

A significant inverse correlation was seen between the mean haemoglobin levels and HbA1c ($P < 0.001$, CI 0.37 - 0.81) and a HbA1c reduction of 0.6% was achieved against

a rise of 3.25 gm% of haemoglobin without any concomitant reduction in blood glucose levels. On applying multivariate regression analysis a statistically significant inverse correlation was seen between HbA1c and serum ferritin ($P < 0.001$), serum iron levels ($P < 0.007$) and transferrin saturation ($P < 0.003$) respectively. A strong significant direct relationship was seen between HbA1c and total iron binding capacity (TIBC) ($P < 0.01$, CI 1.24 - 1.73). Similarly a significant inverse correlation was seen between HbA1c and the erythrocyte indices including PCV ($P < 0.02$), MCV ($P < 0.01$) and MCH ($P < 0.05$) respectively. No correlation was found between HbA1c and other laboratory parameters.

Discussion

HbA1c is the glycated haemoglobin used to assess the glycaemic status of diabetics during previous 3 to 4 months. Besides high blood glucose, other conditions such as hypothyroidism, haemolytic anaemia, haemoglobinopathies, acute and chronic blood loss, pregnancy, and uraemia have been shown to affect HbA1c levels[5-8]; however, the effect of these parameters is not of great extent. Recently, researchers have become interested in studying HbA1c levels in more commonly encountered anaemias like iron deficiency anaemia.

Shanthi *et al*[9] found that the mean HbA1c levels in patients with IDA (7.6% ± 0.5%) was higher than in the control group (5.5% ± 0.8%) ($p < 0.001$). There were no differences in the levels of fasting and post-prandial glucose between the IDA and the control groups. They concluded that HbA1c is not affected by blood sugar levels alone and there are various confounding factors specially IDA when HbA1c is measured. Aggarwal *et al*[10] in 2011 studied the effect of IDA on HbA1c and found that the mean baseline HbA1c levels in anaemic patients (6.1% ± 0.2%) was significantly higher than in control group (4.1% ± 0.3%) ($P < 0.0001$) and a significant positive correlation of HbA1c was observed with total iron binding capacity. Similar results were seen in our study where a similar absolute reduction of 0.6% (11% reduction from baseline) of HbA1c (from 5.49% to 4.88%) after iron therapy was seen. The reason behind this reduction of HbA1c after iron supplementation in anaemic individuals is complex. The formation of glycated haemoglobin is an irreversible process and hence, the concentration of HbA1c in erythrocyte will increase linearly with the cell's age. In patients with normal blood glucose levels, but with very young red cells, as would be found after treatment of iron deficiency anaemia, HbA1c concentration is reduced. However, if iron deficiency has persisted for a long time, the red cell production rate would fall, leading not only to anaemia but also to a higher-than-normal average age of circulating erythrocytes and therefore increased HbA1c. In our study strict vegetarian diet and bleeding from any site were the most common probable reasons for IDA but 20% of cases reported abuse of NSAIDs and proton pump inhibitors. Hence causes of iron deficiency are not just bleeding, worm infestation, malabsorption or nutritional deficiency but overzealous use of over the counter and sometimes iatrogenic NSAID's and PPI abuse which may play an important role in the causation of iron deficiency by altering the PH of gastric mucosa and hence interfering with the absorption of dietary iron.

HbA1c reduction with iron supplementation in our study was only 11% as compared to other studies where more than 15% reduction of HbA1c was seen. El-Agouza *et al*[11] in their study on 730 university students concluded that HbA1c levels were higher in patients with iron deficiency anaemia and decreased from 6.15% to 5.25% after iron supplementation (a decrease of 17%). Tarim *et al*[12] demonstrated higher HbA1c concentrations associated with IDA among diabetic patients with similar level of metabolic control with HbA1c reducing to 8.2% from baseline value of 10.1% in diabetics and 6.2% from baseline of 7.6% in non diabetics. Coban *et al*[13] in their study found that the mean HbA1c concentrations decreased in patients with IDA from 7.4% to 6.2% after iron supplementation of 100 mg/day for 3 months. The decrease in HbA1c in above studies was much higher than our study because the cases in their study were either diabetics or prediabetics with much higher baseline HbA1c. However, since our cases by inclusion criteria were non diabetic and not even prediabetic (i.e., with HbA1c < 5.7%) so the HbA1c reduction was not that robust. Nevertheless there was a significant HbA1c reduction of almost 0.6% (similar to that cuased by many oral hypoglycaemic agents for treatment of diabetes including metformin) even in spite of no changes in blood sugar levels. These results again re-emphasize the fact that caution should be used while diagnosing diabetes or prediabetes (with HbA1c alone) amongst people with anaemia because the change in age of erythrocytes may alter the test result.

However, on the contrary Sinha *et al*[14] in 2010 found a significant increase (rather than decrease) in the absolute HbA1c levels at 2 months after treatment of IDA (from 4.6% to 4.9%). They found that the mean baseline HbA1c

level in anaemic patients (4.6%) was significantly lower than that in the control group (5.5%, $P < 0.05$) and a significant increase was observed in the patients' absolute HbA1c levels at 2 months after treatment (0.29 g/dl vs 0.73 g/dl, $P < 0.01$). This difference was because the baseline haemoglobin level in their cases was very low (severely anaemic study population) as compared to our cases (mild-to-moderately anaemic) and hence treatment with iron supplementation in their cases led to a robust improvement in haemoglobin leading to better overall general well being and the author suggested that this may have resulted into increase in appetite and suppression of inflammation and catabolism and hence an increase in HbA1c which was also accompanied by an increase in serum glucose levels in their cases.

Our results also showed that iron supplementation in cases with IDA leads to an increase in serum creatinine and albumin to globulin (A:G) ratio.This can be explained by the fact that patients with anaemia remain lethargic, with less appetite and poor physical activities and hence had lower serum creatinine values. Also constant low grade inflammation leads to reversal of A:G ratio and so with improvement in haemoglobin there is an increase in basal metabolic rate (BMR) leading to an increase in serum creatinine and a concomitant suppression of inflammation leads to an improvement in A:G ratio in these patients. Similarly the reduction of triglycerides in our cases can also be explained by the same reason.

In our study, HbA1c reduction was seen to be associated with an increase in the levels of serum ferritin, transferrin saturation, serum iron levels and routine erythrocyte indices and a decrease in TIBC. Majority of our cases had mild-to-moderate anaemia but treatment with iron supplementation led to a significant and robust reduction in HbA1c. Whether these results may be extrapolated to diabetic and prediabetic individuals needs to be tested in further studies.This study highlights the fact that anaemia secondary to iron deficiency may be associated with spuriously high HbA1c levels but normal glycaemic status and before diagnosing any patient as prediabetic or diabetic on the basis of HbA1c, a total haemoglobin with red cell indices should be ordered and values interpreted accordingly.If a diabetic has iron deficiency anaemia then he/she should be managed according to blood glucose levels and not HbA1c levels alone.

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