Influence Of Iron Deficiency Anemia Over Hemoglobin A1c Levels

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Abstract

Background: Iron deficiency anemia is commonest form of anemia worldwide.Hemoglobin A1c (HbA1c) or glycated hemoglobin is used as gold standard method for assessment of glycemic control.It is used as an indicator to reflect glucose levels of the previous 3 months. HbA1c can be affected by other non glycemic parameters like hemoglobin variants, anemia, uremia, pregnancy , acute blood loss etc. Reports on the effects of iron deficiency anemia on HbA1c levels were inconsistent.We conducted a study to ascertain the effects of iron deficiency anemia on HbA1c levels and to assess if the treatment of iron deficiency anemia affects HbA1c levels.

Methods: Fifty patients who were confirmed cases of iron deficiency anemia were enrolled in this study.Complete blood count, anemia profile including serum ferritin and HbA1c levels were measured at baseline and after three months of treatment of iron deficiency anemia. These values were compared with those in the control population.

Results: Among 50 patients with iron deficiency anemia, 70% were females and 30% males. The mean baseline HbA1c in anemic patients (6.3) was significantly higher than controls (5.2).After treatment with iron supplement, significant decline from 6.3 to 5.4 was noted in the HbA1c levels.

Conclusion: HbA1c levels can be falsely high in patients with iron deficiency anemia and decreases significantly after correction with iron supplementation. Iron deficiency anemia, which is very common among the Indian subjects ,should be ruled out, if HbA1c levels are high and should be corrected to achieve true HbA1c levels.

Introduction

Iron deficiency anemia is the most common form of anemia observed in the Indian settings[1]. Hemoglobin A1c (HbA1c) is a glycated hemoglobin that is used as an indicator of a patient's glycemic status over the previous three months [2]. According to the recent American Diabetes Association guidelines, HbA1c levels should be maintained below 7% in all diabetic patients in order to prevent the development of microvascular complications[3].The achievement of glycemic control requires individualized therapy and a comprehensive approach that incorporates lifestyle and pharmacological interventions . In conditions associated with increased red blood cell turnover, such as sickle cell disease, pregnancy (second and third trimesters), glucose-6-phosphate dehydrogenase deficiency , hemodialysis, recent blood loss or transfusion, or erythropoietin therapy[4,5], only plasma blood glucose criteria should be used to diagnose diabetes [6].HbA1c is less reliable than blood glucose measurement in other conditions such as the postpartum state[7-9], HIV treated with certain protease inhibitors (PIs) or nucleoside reverse transcriptase inhibitors (NRTIs)[10], and iron deficient anemia .

Initial studies done on the association of Iron deficiency anemia with HbA1c levels by Horton and Huisman [11] ,Brooks et al[12]. Sluiter et al.[13], and Mitchell et al[14]. revealed a relationship existing between them and attempted to explain the alteration in HbA1c levels in iron deficiency anemia on the basis of both -modifications to the structure of hemoglobin and levels of HbA1c in old and new red blood cells. Later, Heyningen et al.[15] and Hansen et al.[16] reported that there were no differences between the HbA1c levels of anemic patients and controls. Rai et al.[17] investigated different methods to assay HbA1c levels and found no differences in HbA1c levels detected when using calorimetric assays, ion exchange chromatography, and affinity chromatography. An Indian study done by Nitin et al.[18] came out with the results that iron deficiency anemia and HbA1c levels were directly proportional and both of them increased or decreased in the same direction.

The results of studies done previously on this topic are conflicting, and the exact mechanism and relationship between iron deficiency anemia on HbA1c levels is not yet definitely known. So due to lack of corresponding evidence and since inconclusive studies existing on this topic, we carried out the current study to investigate the effects of Iron Deficiency Anemia on HbA1c levels in Indian patients.

Methods

It was a prospective Observational study done over a span of 1 year in 50 randomly selected non-diabetic individuals of age 18-60 years with iron deficiency anemia with Hemoglobin <12 mg/dl in females and <13 mg/dl in males .Their iron deficiency anemia was confirmed using iron profile values and peripheral smear with picture of microcytic hypochromic anemia, Serum Ferritin levels <30 ng/dl and serum Iron levels<65 µg/dl in males and <50 µg/dl in females. Patients with history of acute blood loss, hemolytic anemia, hemoglobinopathies, chronic kidney disease, chronic liver disease, pregnancy, established diabetes, impaired fasting glucose or impaired glucose tolerance, chronic alcoholics and known cases of malignancy were excluded from the study. Women of child bearing age who have amenorrhoea and those with reticulocyte count more than 2.5 or blood urea greater than 40 were also excluded from the study. 50 healthy and matched controls were included for comparison. However, exclusion criteria for controls was same as that for patients. All the patients enrolled in the study were from outdoor and indoor departments of DMCH,Laheriasarai and a written informed consent was taken from all before the enrolment.

All patients were subjected to a detailed history and physical examination. Splenomegaly was graded as mild (<2 cm), moderate (2 cm to 7 cm) and severe (>7 cm). Hemoglobin, MCV, MCH, MCHC, hematocrit, platelet count, TLC, DLC, ESR and peripheral smears were examined at the time of enrolment (baseline) and at the end of three months the second time.

Based on hemoglobin values, patients were termed as anemic with cut off of <13 mg/dl in men and <12 mg/dl in women. Reticulocyte count was measured at baseline and reticulocyte production index was calculated. If patients are found to have reticulocyte production index >2.5 at baseline in the absence of overt bleeding were considered to be having hemolytic anemia and were then excluded. Those with predominantly microcytic (MCV<80 femtolitre) and hypochromic (MCH<26 picogram/cell) indices were considered to be having iron deficiency anemia which was confirmed by iron profile.

Serum ferritin was measured by an ELISA test kit (Biochek Inc. India). HbA1c was measured for the study group patients at the time of enrolment (baseline) and after 3 months of iron correction therapy. HbA1c levels were measured using the glycohemoglobin reagent kit (TECO Diagnostics India) which works on the principle of exchange column chromatography. Fasting blood sugar levels were measured at the beginning and then at 3 months of treatment to exclude diabetes or impaired glucose tolerance. Those with fasting blood sugar less than 100 mg/dl were included in the study. Also done was urine pregnancy test in females to rule out pregnancy. Liver function tests and kidney function tests were done at baseline to rule out any liver or renal failure.

All patients were treated with oral ferrous sulphate (195 mg in each tablet) in adequate doses as per the severity of anaemia. Those in control group were subjected to Hemoglobin, MCV, MCH, MCHC, TLC, DLC, ESR, platelet count, hematocrit, fasting blood glucose levels,serum iron, serum ferritin and HbA1c measurements along with peripheral smear examination at the time of enrolment only once in the research period. The data was presented as mean (SD) for continuous variables. A student's test was applied for comparison of group means. Pearson's coefficient of correlation was calculated to find correlation between two variables. A $p<0.05$ was considered statistically significant.

Results

Among the 50 patients in study group, there were 35 females (70%) as compared 20 (40%) in the controls. This states that iron deficiency is commoner among females. The mean age of patients in study group was 34.6 years as compared to 35.7 years in controls. The minimum age in patients in study group was 18 years and maximum age was 60 years. The minimum age in controls was 18 years and maximum was 60 years.

Of all the symptoms in patients of study group, weakness was seen in 45 (90%), malaise in 40 (80%), disinterest in work in 30 (60%), dyspnea was present in 20 (40%), pica in 2(4%) and 4 (8%) patients gave history of passage of worms in stools. Asking questions on overt bleeding, it was revealed that 11 (22%) out of 35 female patients had menstrual complaints out of which menorrhagia was the most prevalent, being present in 10 (91%) out of 11 patients. 11 (22%) patients had bleed from other sites of which piles was the most prevalent cause, being present in 10 (91%) patients. Dietary pattern in iron deficiency anemia patients revealed 36 (72%) patients to be vegetarians and the remaining 14 (28%) to be non-vegetarians. This observation goes on to suggest that iron deficiency anemia is seen more commonly among vegetarians.Pallor was present in all patients. Nail changes were seen in 5(10%) cases .Mild Splenomegaly (<2cm) was seen in 8(16%) patients.

The blood parameters of cases before and after 3 months of iron supplementation are listed in Table 2. As can be seen there was a significant improvement in hemoglobin and RBC indices in cases from 0 month to 3 months after iron supplementation. The mean hemoglobin of patients at baseline was 7.12 (SD=2.16) which was significantly lower than mean hemoglobin value of controls 13.16 (SD=0.60) ($p<0.01$).The mean hemoglobin in patients rose to 12.54 (SD =1.10) after 3 months of treatment but was still lower than the control value which was 13.16 and this difference was still significant .

The mean serum iron levels at baseline in study group was found to be 16.65 mcg/dl (SD=5.89) while at 3 months after treatment these levels rose to 110.65mcg/dl (SD=17.46). This signifies that with treatment of anaemia in these 3 months, the iron levels had increased markedly. Serum iron levels in controls was found to be 98.78 mcg/dl (SD=27.07).

The mean serum ferritin values in patients of study group at baseline was 8.54 ng/ml (SD=3.76) and that in controls at the baseline was 220.85ng/ml(SD=86.67). This difference between patients and controls was highly significant ($p<0.01$).The mean ferritin value in patients rose to 278.52 (SD=83.75) which was highly significant ($p<0.01$). The mean serum ferritin value of patients at 3 months period was higher than that of controls and this difference was also significant ($p<0.01$). The rise in serum ferritin on treatment was as expected indicating increased availability of iron and increasing iron stores.

The mean HbA1c value (%) in patients of study group at baseline was 6.3 (SD=0.12) which was significantly higher than that of controls, 5.2 (SD=0.76) at the baseline (p<0.0l). However, at 3 months, the mean HbA1c (%) value of 5.64 in patients was significantly higher than that of controls ($p<0.01$). There was a significant decline in the mean HbA1c (%) value,5.64 after 3 months from the baseline value after the treatment ($p<0.01$). These observations suggest that the HbA1c values decreased along with the corresponding increase in hemoglobin values in the 3 months of treatment which was significantly correlated with each other. On comparing the two groups of the study , it was found that the variables of reticulocyte count, ESR, HbA1c and FBS were higher in group (I) of cases which were iron deficiency anemic patients. Other variables of hemoglobin, hematocrit, MCV, MCH, MCHC, TLC, platelet count, S. ferritin and S. iron were found to be comparatively higher in group II of controls which consisted of normal subjects (Table1).Difference between the two groups was found to be statistically significant for most of the variables .

Table 1 Comparison of hematological and biochemical variables between cases and controls at baseline

	Group I (n=50) cases		**Group II (n=50) controls**		**Statistical Significance**	
	Mean	SD	Mean	SD	't'	'P'
Hemoglobin (g/dl)	7.12	2.16	13.16	0.60	-18.574	<0.001
Hematocrit(%)	20.34	5.72	39.18	3.05	-15.632	<0.001
RBC counts (millions/cumm)	3.02	1.8	4.56	1.04	-10.531	<0.001
MCV(fl)	68.98	6.65	94.45	3.54	-21.894	<0.001
MCH(pg)	20.12	3.16	34.37	2.01	-24.565	<0.001
MCHC(g/dl)	30.34	3.56	35.87	1.78	-7.098	<0.001
Total leucocyte count (cells/cumm)	7894.00	2387.13	7805.00	2208.23	-0.065	0.879
Platelet count(lakhs/mm^3)	2.67	0.87	2.75	0.65	-0.967	0.367
ESR(mm/hr)	15.76	4.7	11.07	3.64	2.653	0.145
Reticulocyte count(%)	2.12	0.34	1.7	0.45	10.546	<0.001
Serum Iron(mcg/dl)	16.65	5.89	98.78	27.07	-25.768	<0.001
Serum Ferritin(ng/ml)	8.54	3.76	220.85	86.67	-19.879	<0.001
FBS(mg/dl)	78.67	5.77	80.45	5.56	1.567	0.178
HbA1C(%)	6.3	0.12	5.2	0.76	12.185	<0.001

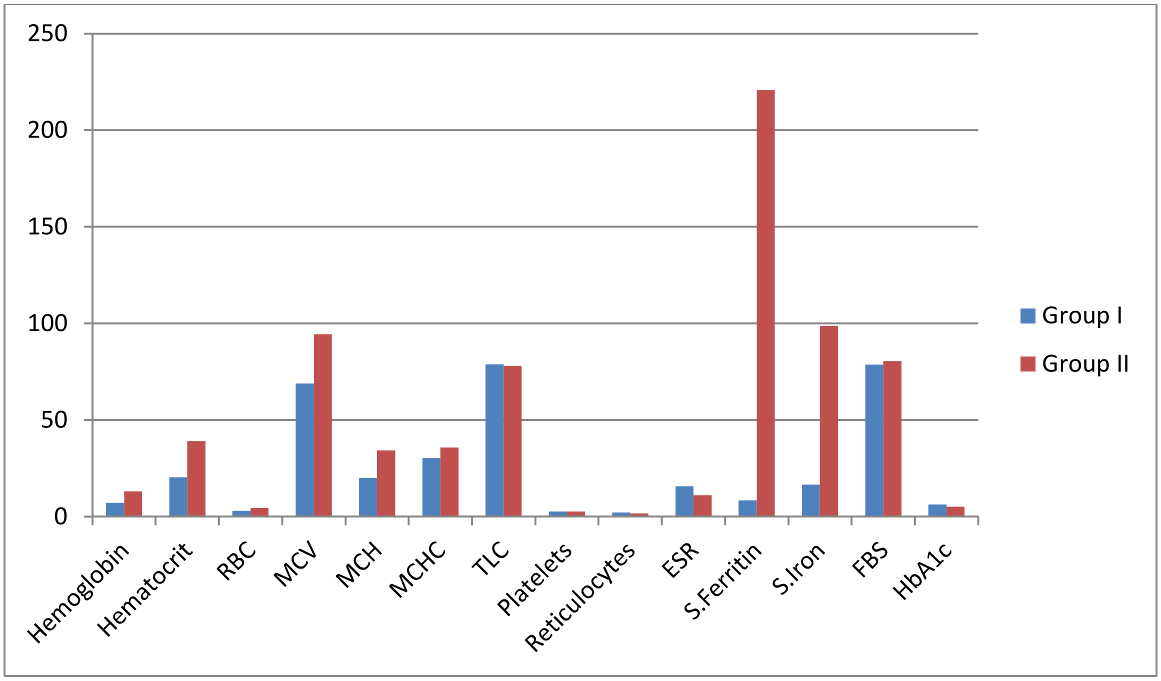


Figure 1- Comparison of case group with control group suggesting that in iron deficient patients there is low level of hemoglobin,hematocrit,MCV,MCH,MCHC,S.Iron,S.Ferritin.However,there is higher level of Reticulocyte,ESR and HbA1c and this was statistically significant.

Table 2 Comparison of hematological and biochemical variables before and after 3 months of treatment

	Before treatment Group I (n=50) cases		**After treament Group I (n=50) cases**		**Statistical Significance**	
	Mean	SD	Mean	SD	‘t’	‘P’
Hemoglobin (g/dl)	7.12	2.16	12.54	1.10	20.344	<0.001
Hematocrit(%)	20.34	5.72	36.16	3.74	13.784	<0.001
RBC counts (millions/cumm)	3.02	1.8	4.56	1.60	16.735	<0.001
MCV(fl)	68.98	6.65	96.55	4.76	30.754	<0.001
MCH(pg)	20.12	3.16	31.12	2.57	23.402	<0.001
MCHC(g/dl)	30.34	3.56	33.56	1.34	3.676	<0.001
Total leucocyte count (cells/cumm)	7894.00	2387.13	7504.00	1189.85	2.012	0.085
Platelet count(lakhs/mm^3)	2.67	0.87	1.76	0.74	2.324	0.056
ESR(mm/hr)	15.76	4.7	10.23	2.67	4.057	<0.001
Reticulocyte count(%)	2.12	0.34	1.5	0.36	8.675	<0.001
Serum Iron(mcg/dl)	16.65	5.89	110.65	17.46	36.034	<0.001
Serum Ferritin(ng/ml)	8.54	3.76	278.52	83.75	22.713	<0.001
FBS(mg/dl)	78.67	5.77	85.65	5.67	2.961	0.345
HbA1C(%)	6.3	0.12	5.64	0.34	17.435	<0.001

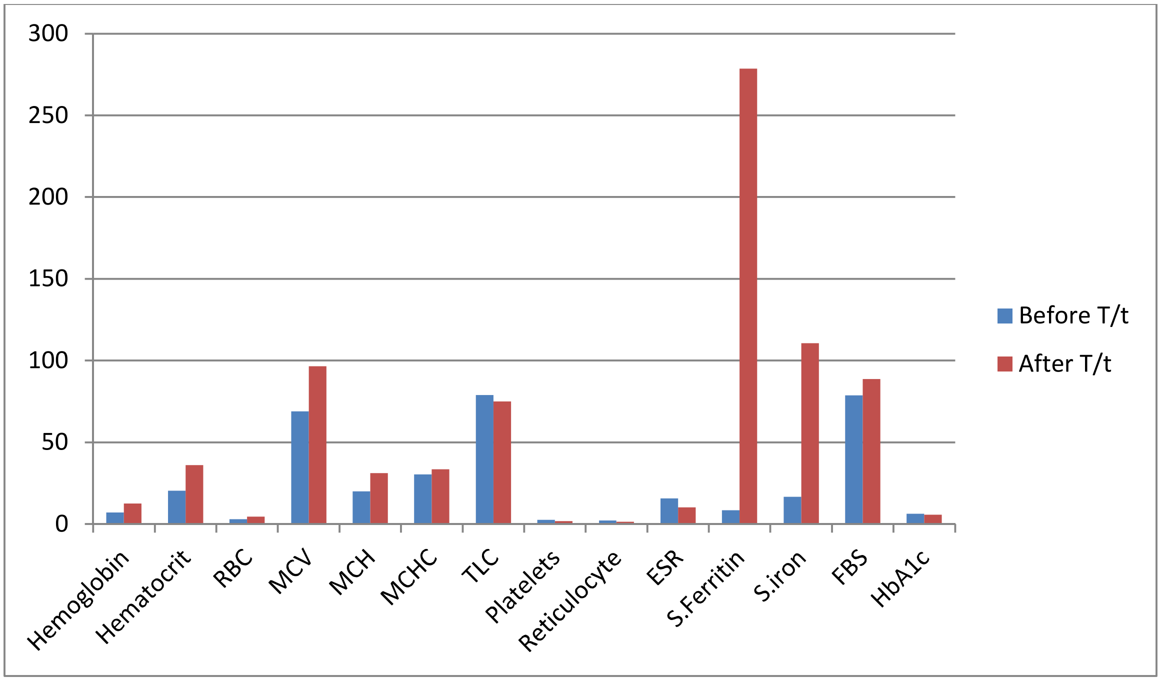


Figure 2 Comparison of cases group before and after treating Iron deficiency showing that hemoglobin,MCV,MCH,MCHC,S.ferritin,S.Iron levels improved with iron therapy .Moreover,HbA1c levels declined correspondingly.

At baseline (r^2=-0.764; $p<0.001$) in group I cases

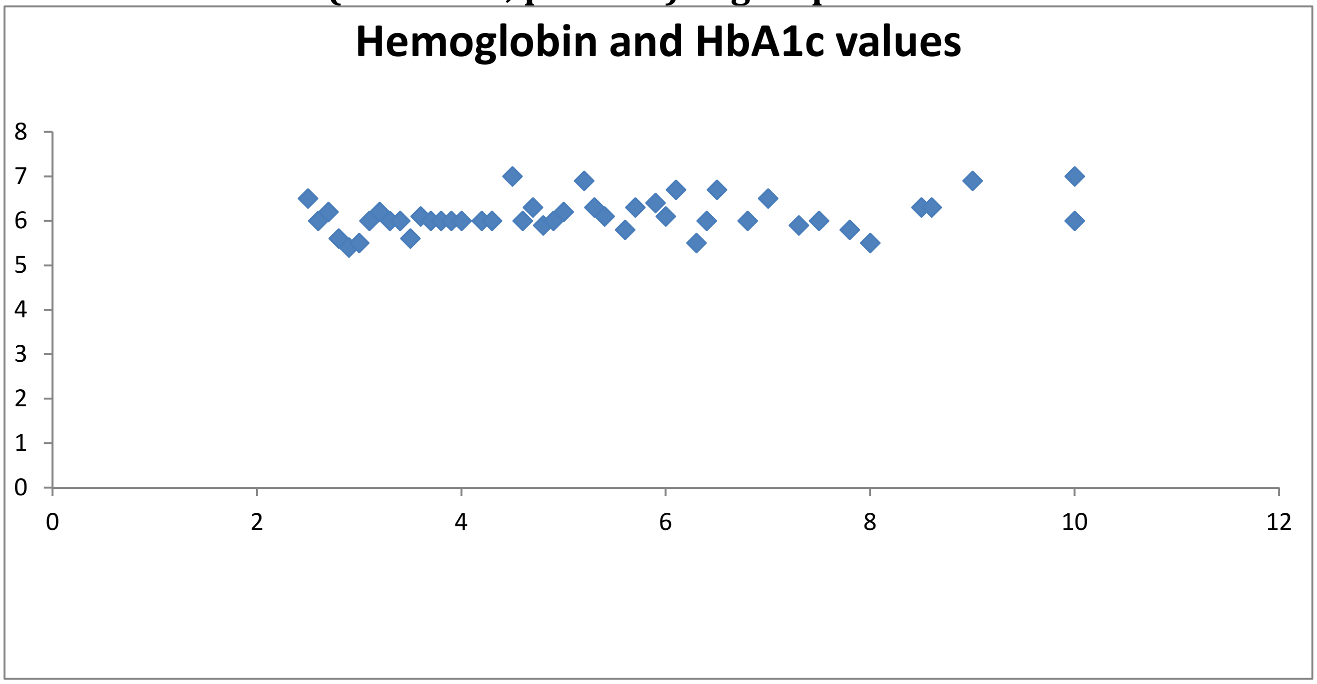
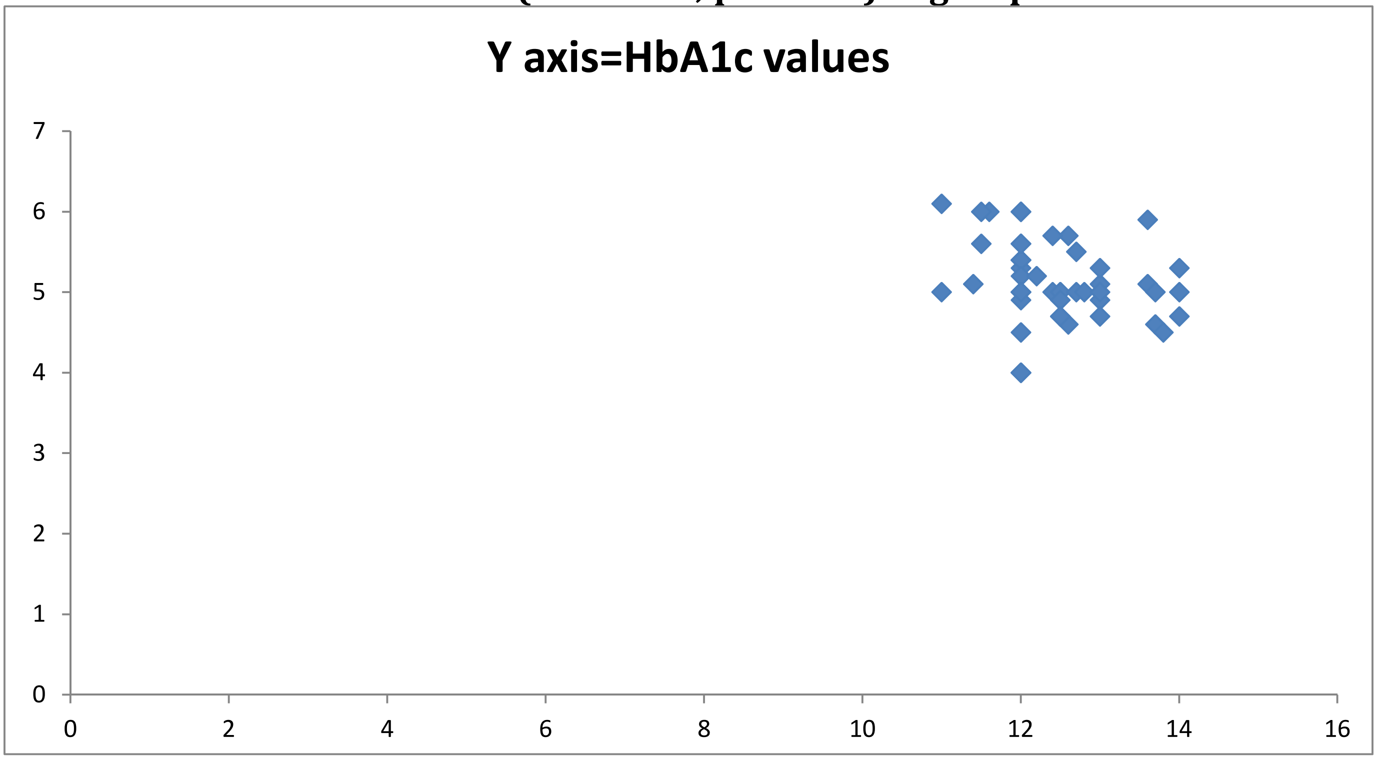


Figure 3 **X axis= Hemoglobin (g/dl)---→**

After 3 months of treatment (r^2=-0.275; p=0.001) in group I cases



X axis= Hemoglobin (g/dl)---→

Figure 4 :Correlation between hemoglobin and HbA1c values at baseline and after 3 months of treatment.

Discussion

Iron deficiency anemia is the most common form of anemia observed in India. HbA1c is glycated hemoglobin which is used to assess the glycemic status of an individual over last 2-3 months and is mostly being used in diabetics and in those with impaired glucose tolerance. Besides high blood glucose, other conditions such as hypothyroidism, hemolytic anemia, hemoglobinopathies, acute and chronic blood loss, pregnancy, and uremia have been shown to affect HbA1c levels. In one of these studies done by Horton and Huisman[11] they showed that HbA1c is decreased due to the reason that the life span of the RBC's is reduced. So, from these studies it became evident that HbA1c should be taken as a measure of glycemic control only if such disorders are ruled out. However, interest further arose as to what happens to HbA1c levels in more commonly encountered anemias like iron deficiency anemia. Brooks et al.[12] showed that HbA1c levels were higher in patients of iron deficiency anemia at baseline and decreased on treatment. The reason speculated by them was that the quaternary structure of hemoglobin gets altered and that, glycation of beta globin chain occurs more readily in the relative absence of iron. Sluiter et al.[13] later gave a different reason to explain the findings of Brooks et al. They were of the view that the formation of glycated hemoglobin is an irreversible process and hence, the concentration of HbA1c in one erythrocyte will increase linearly with the cell's age. In patients with normal blood glucose values but with red cells that are younger than usual, as after treatment of iron deficiency anaemia, HbA1c concentration falls. However, if the iron deficiency has been persisting for a long time, the red cell production rate falls, leading not only to anemia but also to a higher than normal average age of circulating erythrocytes and, therefore, of increased HbA1c. Mitchell et al.[14] commented on the study done by Brooks et al. and also on the reasoning of Sluiter et al. From the values available in Brooks et al. study, they rather than taking HbA1c (%), calculated mean corpuscular hemoglobin HbA1c i.e. amount of HbAlc per cell and observed that though the percentage of HbA1c decreased during treatment, MCHbA1c remained relatively constant and there was no significant difference noted between baseline and post treatment values. They were also of the view that red cell age as proposed by Sluiter et al, was unlikely to explain the changes observed in HbA1c (%) in the Brooks et al.study. Heyningen et al.[15] demonstrated that there was no difference seen in HbA1c (%) values at baseline and after treatment in iron deficiency anemia patients and speculated that the differences observed previously could be due to different methods used in calculating HbA1c. Hansen et al.[16] showed that there was no difference in HbA1c values at baseline between iron deficiency anaemia patients and controls but demonstrated a fall in HbA1c levels after treatment which they explained by stating that it was due to increase in the number of immature erythrocytes. . But Rai et al.[17]reported that there was no significant difference in HbA1c values calculated by colorimetry, ion exchange chromatography or affinity chromatography. Further studies also demonstrated a baseline higher HbA1c in patients and fall after treatment but the reason speculated was different from the one given by Brooks et al. and Sluiter et al. In these studies, the probable explanation of elevated HbAlc in iron deficiency-anaemia at baseline is that, if serum glucose is accepted to remain constant, a decrease in the hemoglobin concentration might lead to an increase in the glycated fraction but the exact mechanism still remained elusive. Nitin et al.[18] showed drastically different results, with values of HbA1c decreasing with fall in hemoglobin values and with treatment these values increased in the next 2-3 months. The probable reason for these observations given was that the population in study was generally from a lower socio economic strata, being quite poor.

Among the 50 patients studied, 35 were female, suggesting that iron deficiency anemia is more common in women. As expected, the mean hemoglobin and mean serum ferritin levels increased in anemic patients over 3 months with iron treatment. None of our patients had non responsive iron deficiency anemia. Our observation of increased HbAlc levels at baseline and its subsequent fall on iron supplementation was in accordance with most of the studies done in the past. There are a number of variable explanations available to explain these findings. We used ion- exchange chromatography in estimating HbA1c and the analysis was validated in our laboratory. A strict quality control was ensured and samples were tested in assorted manner i.e. the controls and the tests were not analyzed in separate batches but in mixed batches during the period of study.

Iron deficiency anemia has correlation with HbA1c levels and the relationship is inverse between them. This means as the level of hemoglobin drops with increasing severity of iron deficiency in anemic subjects, at the same time HbA1c levels increase correspondingly. Moreover, with correction of iron deficiency in the anemic subjects, the HbA1c levels decline . This concludes that whenever HbA1c is calculated to detect past three months glycemic status, factors other than glucose also play a part in its calculated value. These other factors should be kept in mind before doing any treatment modification. Iron deficiency anemia being extremely common in Indian settings should always be ruled out when high HbA1c levels are detected and should be corrected to achieve true levels of HbA1c. India being the diabetic capital of the world and HbA1c being such a common investigation in day to day medical practice, should always be interpreted carefully keeping in mind all the factors affecting its value, including some very common ones like iron deficiency anemia. The reason behind this correlation between iron deficiency anemia and HbA1c is still not clear and various theories exist which are not fully explanatory. Therefore more large scale studies are required to find out the proper mechanism underlying this correlation.

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