

Correlation of iron deficiency anaemia with HAEMOGLOBIN A1C At A Tertiary Care Teaching Hospital In Uttar Pradesh

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Abstract

Background-Iron deficiency anemia (IDA) is the commonest form of nutritional anemia worldwide, posing wide public health risks. It can increase the red blood cell turnover, which can increase glycation of Hb leading to higher HbA1c values. **Aim**- The Aim of the study is to correlate the status of iron deficiency anaemia with glycosylated haemoglobin in adults belonging to the age group of 18- 60yrs. **Objectives**- To study iron deficiency anaemia for proper screening of patients for early prevention and management. To determine the effect of iron deficiency anaemia on the HbA1c levels. To determine whether the HbA1c levels were increased among anaemic patients. If so, iron deficiency has to be corrected before any diagnostic and therapeutic decision is made based on the HbA1c level. **Settings&Design**-The present study is a Prospective, Interventional, and Randomized study conducted in tertiary care rural teaching hospital in Uttar Pradesh. A total of 150 individuals (Patients-100, Control-50) were recruited in the study. Age Group of 15-60 years was studied. Proper Ethical approval from Institutional Ethical Committee was taken and all the participants. A written Informed Consent was obtained from each patient and control before starting the procedure. **Material&Methods**-150 participants of our study underwent clinical assessment, their history was taken, investigations including anemia profile and HbA1C levels were done. Anemia correction was done with iron supplementation and HbA1C level was measured post 2 month. Statistical analysis was performed using SPSS (Statistical Package for the Social Sciences) for Windows (version 16.0). **Results&Conclusion**-Our study shows that there is a decrease of HbA1c levels in patients of IDA as the mean value of HbA1c(%) in intervention group was at 7.66 at base line which was significantly lower than that of controls (baseline HbA1c = 4.92). Also there is an decrease in level HbA1c after 2 months of treatment as the mean value of HbA1c (%) in patients was 5.40 at 2 month and the mean HbA1c of controls over 2 months period was 5.5.

Keywords- Iron Deficiency Anemia, HbA1C, Hemoglobin

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Introduction

Iron deficiency anemia (IDA) is the commonest form of nutritional anemia worldwide, posing wide public health risks. World Health Organization (WHO) has reported that, globally there are 2.1 billion cases of iron deficiency anemia, which is approximately 30% of the world population. Hemoglobin A1c (HbA1c), or glycosylated hemoglobin is formed by an irreversible, slow non-enzymatic catalysis of the β chain of globin in mature hemoglobin (Hb)[1,2]. It is used as a gold standard for monitoring glycemic status for the previous three months (the life span of a red blood cell) in patients with diabetes [3]. Iron deficiency anemia (IDA) can increase the red blood cell turnover, which can increase glycation of Hb leading to higher HbA1c values as observed in blood loss, hemolysis, hemoglobinopathies, red cell disorders and myelodysplastic disease[4]. There are studies to support the idea that these levels are influenced by changes in the iron level in a body[5]. Lower levels of serum iron or serum ferritin have also been linked with increased levels of HbA1c [6] The results of various studies on relationship between HbA1c and

iron deficiency anemia were conflicting. Hence, the rationale of this study is to assess the levels of HbA1c in iron deficiency anemia patients and evaluate the changes in HbA1c levels after the correction of iron deficiency anemia.

Aim: The Aim of the study is to correlate the status of iron deficiency anaemia with glycosylated haemoglobin in adults belonging to the age group of 18- 60 yrs.

Objectives

1. To study iron deficiency anaemia for proper screening of patients for early prevention and management.
2. To determine the effect of iron deficiency anaemia on the HbA1c levels, so as to consider iron deficiency anaemia as an important factor which influenced the HbA1c levels.
3. To determine whether the HbA1c levels were increased among anaemic patients. If so, iron deficiency has to be corrected before any diagnostic and therapeutic decision is made based on the HbA1c level.

Materials and methods

The present study is a Prospective, Interventional, and Randomized study conducted in tertiary care rural teaching hospital. The study was conducted from August 2018 to September 2019. The study was carried out from the patients being referred to the Department of Pathology. A total of 150 individuals were recruited in the study. The participants were divided into two groups ie Patients-100, Control-

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50. In the present research, correlation of Iron Deficiency Anemia with Glycosylated Haemoglobin was done in the Age Group of 15-60 years with no further severe complications such as cancer. The **Inclusion Criteria** were Adult patients (aged 18 years to 60 years). Patients (both men and women) with confirmed iron deficiency type anemia, as defined by WHO. Patient agreeing with "signed and informed consent". The **Exclusion Criteria** were all patients lost to follow up, Haemolytic anaemias, Haemoglobinopathies, Acute and chronic blood loss, Chronic alcoholic ingestion, Infestation, Uraemia, Patients having severe co-morbidities i.e. failure of other organ systems, etc Diabetes or impaired glucose tolerance or fasting blood sugar greater than 100 miligram per deciliter at the beginning, during and after the completion of the study, Patient with bleeding disorders, Pregnant patients and Immuno-compromised patients.

Selection of Controls- When conducting the study, control group of people were randomly selected to be in this group. They closely resemble the participants who are in the experimental group or the individuals who receive the treatment. Controls were selected randomly from the same population from which the experimental group was selected.

Avoiding patient selection bias- Patient selection bias was avoided by random selection of patients and ensuring that patients selected under experimental and control groups were equivalent to the population at large, so that the characteristics of selected patients match with the population.

Double blinding was performed in this study. Both the patient and the investigator were unaware of the patient distribution among the experimental and control groups.

Determination of sample size: A sample size of 150 individuals was calculated based on the findings of a similar study using Open Epi v3.0. Sample selection was done using convenience sampling method (first come first serve basis) for the study.

Ethical Approval: Ethical Approval was taken from the Institutional Ethical Committee after explaining them the aims and objectives of the Study. The Institutional Ethical Committee issued a certificate stating that it has given the clearance to the submitted protocol to conduct the study.

Informed Consent: A written Informed Consent in bilingual language ie English/ Hindi was obtained from each patient and control before starting the procedure. The involvement of the subjects was voluntary. The consent form stated that the participant has been informed about the purpose, nature, duration of the study, instructions to be followed and various laboratory investigations to be carried out in the study. The consent form also stated that the subject was informed that the study is being conducted to understand the changes in glycosylated hemoglobin due to iron deficiency anemia which further would be helpful in taking therapeutic decisions.

Method of Data Collection: a total of 150 Patients aged between 18yr and 60 yrs with confirmed iron deficiency anaemia were included in the study to know the effect on glycosylated haemo-

globin. Detailed history taken and physical examination of the patients was conducted.

The levels of haemoglobin, mean corpuscular haemoglobin, hematocrit, mean corpuscular volume, mean corpuscular haemoglobin concentration, total leucocyte count, differential leucocyte count were measured by an automated counter at baseline and then again at 1 month and 2 months following treatment. Peripheral blood smear examination to define the anaemia type as well as all other investigations were also done in every subject. On the basis of haemoglobin levels patients were categorized as having mild, moderate or severe anaemia. Those with predominantly microcytic indices and hypochromic indices were taken as iron deficiency anaemia confirmed by low serum ferritin levels. Serum ferritin levels were measured at 1 week, 1 month and 2 months after treatment. HbA1c was measured at the time of enrollment and then again at 1 week, 1 and 2 months following the start of treatment. HbA1c was measured using glycohemoglobin ion exchange resin method on the basis of the principle that glycohemoglobin moves as a fast fraction and thus elutes during cation exchange column chromatography. Patients with iron deficiency anemia based on WHO criteria cut off point and age, sex matched control patients were assigned for study. History, clinical assessment and investigations including serum ferritin, HbA1c were done. **Statistical Analysis:** Statistical analysis was performed using SPSS (Statistical Package for the Social Sciences) for Windows (version 16.0). Categorical variables were compared between two or more groups using the Chi-square test. For all analyses, a two-tailed p-value of <0.05 was considered statistically significant.

P-value, degrees of freedom and confidence interval- p-value = less than 0.05, Degrees of freedom = 149 (150-1), Confidence interval = 95%.

Methods used for measuring various parameters: --1 Haemoglobin, TLC, DLC, Platelets count were done both manually and by an automated counter. MCV, MCH, MCHC and hematocrit were measured by automated counter only. Peripheral smear examination, reticulocyte count and ESR were done manually. 2. Reticulocyte count was done by supravital staining and ESR by Wintrobe's Method. 3. Serum ferritin: it is measured by an enzyme linked immune assay test kit (iOcheck, inc) This quantitative test was based on a solid phase enzyme linked immunosorbent assay (ELISA). 4. HbA1c- It was measured using Erba Reagent Kit. Kits were stored and used as per guidelines. Measurements are based on the principle that glycohemoglobin moves as a fast fraction (HbA1a, HbA1c) and elute first during column chromatography using cation exchange resins. 5. Hb electrophoresis was done on citrate agar electrophoresis. 6. KFT, serum electrophoresis, Lft, lipid profile and blood sugars were measured by an automated counter.

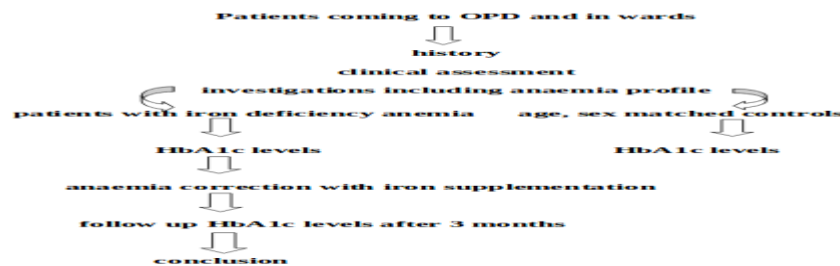


Fig 1: Whole procedure depiction

Observation and results

Table 1: Age wise distribution of study participants (n=150)

Age group (years)	Frequency		Percent (%)
	18-20	21-30	31-40
	18	10	12.0
	108	8	72.0
	6	8	4.0
	150	6	100.0

Table 2: Gender distribution of study participants

Gender (n)	Males	Females	Total
Intervention	40	60	100
Control	20	30	50
Total	60	90	150

Table 3: Anemia classification among study subjects

Valid	Frequency		Percent
	Mild Anemia	Moderate Anemia	Severe Anemia
	81	48	54.0
	21	21	32.0
	150	21	14.0
	150	21	100.0

Table 4: Type of anemia with respect to age, gender & socio-economic status

		Mild		Moderate		Severe		p-value
		N	%	N	%	N	%	
Age group	18-20 years	10	6.7%	5	3.3%	3	2.0%	0.983
	21-30 years	6	4.0%	3	2.0%	1	0.7%	
	31-40 years	58	38.7%	35	23.3%	15	10.0%	
	41-50 years	3	2.0%	3	2.0%	2	1.3%	
	51-60 years	2	1.3%	2	1.3%	2	1.3%	
Gender	Female	74	91.4%	39	81.3%	17	81.0%	0.187
	Male	7	8.6%	9	18.8%	4	19.0%	
Socioeconomic status	Lower class	2	2.5%	3	6.3%	1	4.8%	0.340
	Lower middle	8	9.9%	4	8.3%	5	23.8%	
	Upper middle	67	82.7%	37	77.1%	14	66.7%	
	Upper class	2	2.5%	4	8.3%	1	4.8%	

Table 5: Change in Hemoglobin levels over the study duration

Hemoglobin levels		N	Mean	SD	Min	Max	F-value	p-value
Control Group		50	14.332	1.099	14.1	16.2	10.782	0.001*
Intervention	At baseline	100	9.872	1.3578	7.7	12.1		
	One week treatment	100	10.031	1.3342	8.8	12.2		
	One month treatment	100	10.375	1.0085	9.0	12.2		
	Two month treatment	100	12.679	1.0085	10.3	13.5		

Table 6 shows the overall change in Glycated Hemoglobin levels (HbA1c) over the study period. The Hb1Ac estimation was done at baseline, and then at one week, one month and two months of iron therapy among study participants. The mean Hb1Ac levels decreased with iron therapy. The levels dropped down to mean value of 5.40 + 0.54 (SD), with a range of 4.5 to 6.6 (Figure1).

Table 6: Change in Hb1Ac levels over the study duration

HbA1c levels		N	Mean	SD	Min	Max	F-value	p-value
Control Group		50	4.92	.2318	4.5	5.3	12.235	0.001*
Intervention	At baseline	100	7.66	.6346	6.7	8.8		
	One week treatment	100	7.12	.6221	6.2	7.9		
	One month treatment	100	6.56	.6416	5.6	7.7		
	Two month treatment	100	5.40	.5411	4.5	6.6		

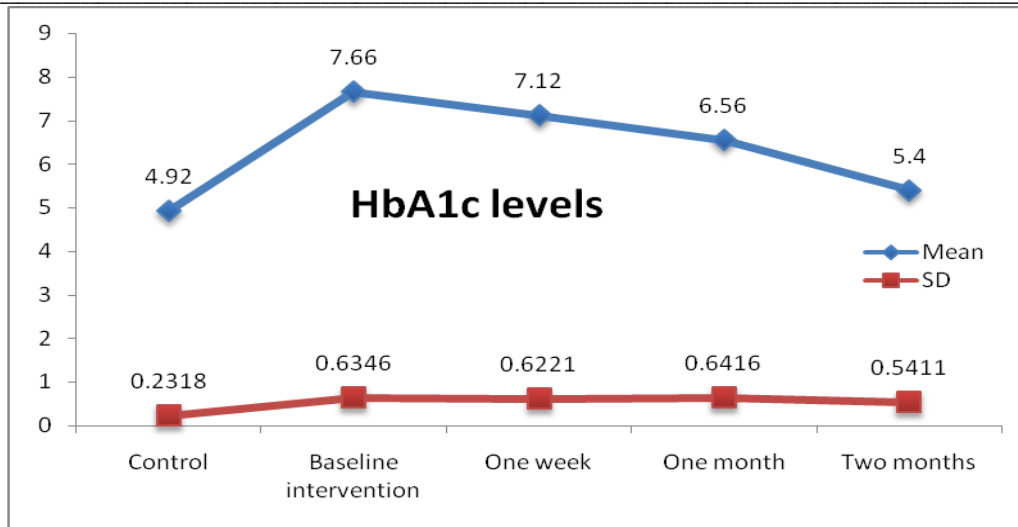


Fig 2: Change in Hb1Ac over the study duration

Table 7 shows the change in Hb1Ac levels, which was statistically significant using a one-way ANOVA test ($p < 0.05$). Upon conducting a post-hoc analysis, it was seen that significant difference was present among all comparisons at levels of observation.

Table 7: Post-hoc analysis for change in Hb1Ac values

Comparison	Mean Diff	SE	p-value	95% Confidence Interval	
				Lower Bound	Upper Bound
Control vs Baseline	-0.4492	.1242	0.001*	5.608	3.992
Baseline vs One week	-0.4544	.1158	0.023*	3.208	1.592
Baseline vs One month	1.1000*	.0733	0.001*	.924	1.276
Baseline vs Two month	2.2000*	.0733	0.001*	2.024	2.376
One month vs Two month	1.1000*	.0733	0.001*	.924	1.276

Table 8 shows the overall change in Absolute Glycated Hemoglobin levels (Abs Hb1Ac) over the study period. The Abs Hb1Ac estimation was done at baseline, and then at one week, one month and two months of iron therapy among study participants. The mean Abs Hb1Ac levels increased with iron therapy. The levels increased to mean value of $0.63 + 0.03$ (SD), with a range of 0.51 to 0.74 (Figure 2).

Table 8: Change in Abs Hb1Ac levels over the study duration

Absolute HbA1c levels		N	Mean	SD	Min	Max	F-value	p-value
Control Group		50	0.65	.02	0.58	0.76	8.481	0.001*
Intervention	At baseline	100	0.28	.03	0.17	0.35		
	One week treatment	100	0.41	.02	0.26	0.55		
	One month treatment	100	0.53	.04	0.45	0.71		
	Two month treatment	100	0.63	.03	0.51	0.74		

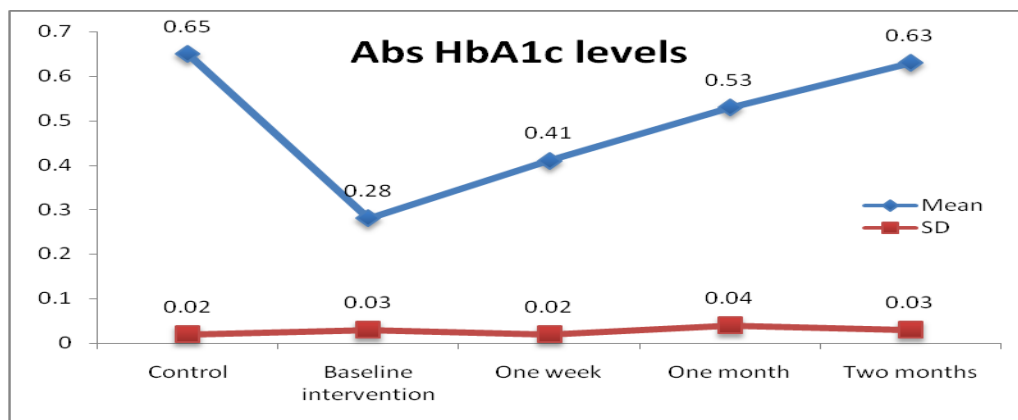


Fig 3: Change in Abs Hb1Ac over the study duration

Table 9 shows the change in Abs Hb1Ac levels, which was statistically significant using a one-way ANOVA test ($p < 0.05$). Upon conducting a post-hoc analysis, it was seen that significant difference was present among all comparisons at levels of observation.

Table 9: Post-hoc analysis for change in Absolute Hb1Ac values

Comparison	Mean Diff	SE	p-value	95% Confidence Interval	
				Lower Bound	Upper Bound
Control vs Baseline	-0.4492	.1242	0.001*	1.276	3.992
Baseline vs One week	-0.4544	.1158	0.512	3.208	1.276
Baseline vs One month	1.1000*	.0733	0.001*	.924	1.276
Baseline vs Two month	2.2000*	.0733	0.001*	2.024	2.376
One month vs Two month	1.1000*	.0733	0.001*	.924	1.276

Table 10 shows the overall change in Fasting Blood Sugar/Glucose levels (FBS) over the study period. The FBS estimation was done at baseline, and then at one week, one month and two months of iron therapy among study participants. The mean FBS levels decreased with iron therapy. The levels dropped down to mean value of 88.4 + 4.2 (SD), with a range of 81 to 93 (Figure 3).

Table 10: Change in FBS levels over the study duration

FBSlevels		N	Mean	SD	Min	Max	F-value	p-value
Control Group		50	84.6	3.8	81	90		
Intervention	At baseline	100	102.4	6.9	95	110		
	One week treatment	100	94.7	5.8	88	99		
	One month treatment	100	90.4	5.6	85	92		
	Two month treatment	100	88.4	4.2	81	93		

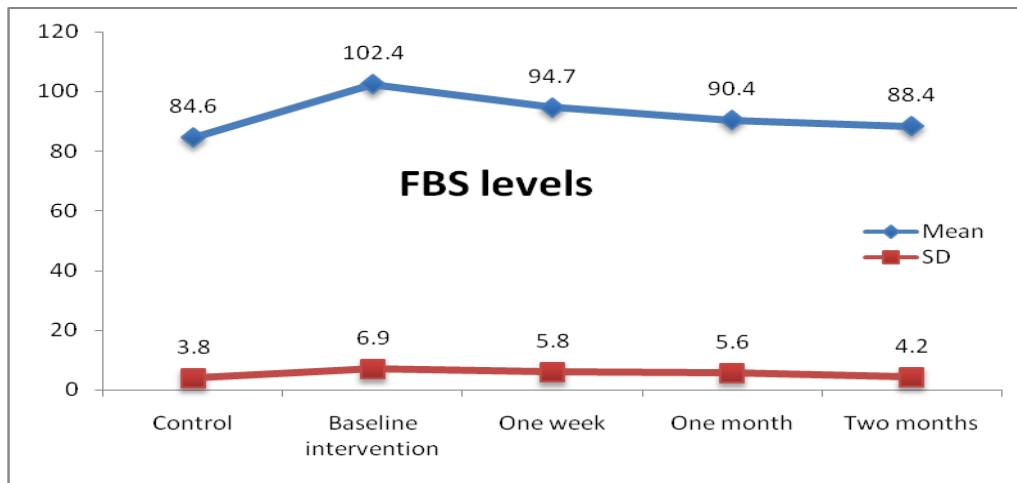


Fig 3: Change in FBS over the study duration

Table 11 shows the correlation of hemoglobin and glycated hemoglobin levels in the study population at the levels of observation. Hemoglobin levels were significantly correlated with HbA1c levels at various steps. Hemoglobin levels at one month of therapy were positively correlated with HbA1c levels at one month. Also, Hemoglobin levels at two months of therapy were positively correlated with HbA1c levels at one month and two months of therapy.

Table 11: Correlation of hemoglobin and glycated hemoglobin levels

		HbA1c (Baseline)	HbA1c (one month)	HbA1c (two month)
Hb (Baseline)	Pearson Correlation	-.065	-.065	-.065
	p-value	.431	.484	.470
Hb (one month)	Pearson Correlation	.013	1.000	.043
	p-value	.871	0.001*	.399
Hb (two month)	Pearson Correlation	.017	1.000	1.000
	p-value	.809	0.001*	0.001*

Table 12 shows the correlation of study parameters with HbA1c levels at Baseline. At baseline, it was noticed that none of the study parameter were significantly associated with HbA1c values.

Table 12: Correlation of study parameters with HbA1c values at BASELINE

Type of Anemia	Hb		PCV		MCHC		MCV	
	Correlation	p-value	Correlation	p-value	Correlation	p-value	Correlation	p-value
Control	-.048	0.902	-.072	0.567	-.051	0.223	-.022	0.376
Mild	-.072	0.567	-.051	0.677	-.095	.338	-.056	0.617
Moderate	-.022	0.376	-.091	.238	-.051	0.677	-.048	0.902
Severe	-.048	.518	-.048	0.902	-.095	.338	-.022	0.376

Table 13 shows the **correlation of study parameters with HbA1c** levels at One month of therapy. It was noticed that significant correlation was observed in Moderate anemic and severe anemic subjects at one month of therapy ion regards to Hemoglobin values and MCHC values. This implies that change in HbA1c values were associated with change in Hemoglobin and change in MCHC values at one month of therapy. No correlation was observed with control group (iron therapy not given).

Table 13: Correlation of study parameters with HbA1c values at ONE MONTH TREATMENT:

Type of Anemia	Hb		PCV		MCHC		MCV	
	Correlation	p-value	Correlation	p-value	Correlation	p-value	Correlation	p-value
Control	-.095	.338	-.048	.518	-.092	.902	-.051	0.677
Mild	.078	0.066	-.072	0.567	-.051	0.677	-.056	0.617
Moderate	.456	0.04*	-.056	0.617	-0.22	0.04*	-.091	.238
Severe	.823	0.03*	-0.22	0.376	0.911	0.02*	.456	0.051

Table 14 shows the **correlation of study parameters with HbA1c** levels at two months of therapy. It was noticed that significant correlation was observed in Hemoglobin, PCV, MCHC, and MCV values in all types of Anemia. This implies that change in HbA1c values was associated with change in Hemoglobin, PCV, MCHC, and MCV values at two months of therapy. No correlation was observed with control group (iron therapy not given).

Table 14: Correlation of study parameters with HbA1c values at TWO MONTH TREATMENT

Type of Anemia	Hb		PCV		MCHC		MCV	
	Correlation	p-value	Correlation	p-value	Correlation	p-value	Correlation	p-value
Control	-0.221	0.376	-.091	.238	-.051	0.677	-.048	0.902
Mild	0.911	0.02*	0.912	0.03*	.456	0.04*	.823	0.03*
Moderate	1.000	0.001*	1.000	0.001*	1.000	0.001*	1.000	0.001*
Severe	1.000	0.001*	1.000	0.001*	1.000	0.001*	1.000	0.001*

Discussion

Iron deficiency anemia is the most common form of anemia. HbA1c is a glycosylated hemoglobin which assesses the glycemic status of diabetic patient for the previous 3 months. It has been observed that researchers are now intrigued in analyzing and correlating HbA1c levels to commonly encountered anemias like iron deficiency anemia. Our observation of increased HbA1c 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. A study by Madhu et al[7] observed higher HbA1c levels in IDA subjects. Significant decline was noticed in HbA1c levels in IDA subjects after iron supplementation. Post treatment 70% subjects 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. Further studies by El-Agouza et al⁽⁸⁾ and Coban et al⁽⁹⁾ showed that HbA1c levels were higher in patients with iron deficiency anemia and decreased significantly upon treatment with iron. Christy, et al[10] conducted a study on Influence of Iron deficiency Anemia on Hemoglobin A1c levels in Diabetic individuals with controlled Plasma Glucose Levels and they found a positive correlation between iron deficiency anemia and increased HbA1c levels. Studies by Guo et al[11], Intra et al[12], Grossman et al[13], English et al[14], Silva et al[15], Musa et al[16], Veeramala et al[17] showed that HbA1c levels increased in diabetics with anemia compared to diabetics without anemia. Nitin Sinha et al[18] showed drastically different results with values of HbA1c decreasing with fall in haemoglobin values and with treatment these values increased in the next 2 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. A study by Hansen et al[19] showed there were no significant differences in HbA1c concentrations in iron-deficient patients, vitamin B12-deficient patients, and healthy controls. The probable reason for these observations given was that in iron deficiency anemia the erythrocyte survival rate is normal. They found that HbA1c levels decreased upon treatment of the

anemia, which was probably due to increased bone marrow erythropoiesis caused by the treatment, leading to production of new immature erythrocytes. A study by Rai et al⁽²⁰⁾ compared colorimetric assays, ion exchange chromatography, and affinity chromatography as methods for measuring HbA1c, and no significant differences were found in HbA1c values measured by any of these methods. A study by Heyningen et al⁽²¹⁾ showed no differences in HbA1c concentrations when comparing non-diabetic patients with iron deficiency anemia before and after iron treatment to healthy controls. They believed that the reported differences in other studies were because of different laboratory techniques used for measuring HbA1c. Bala Subramanian Shanthi, et al⁽²²⁾ conducted a study on Effect of Iron Deficiency on Glycation of Hemoglobin in Non diabetics in 75 patients. They concluded that HbA1c is not affected by the blood sugar levels alone, and there are various confounding factors when HbA1c is measured, especially that of iron deficiency, which is the commonest of the deficiency diseases worldwide. Our study showed that the mean fasting blood sugar levels decreased with iron therapy. Similar findings were observed by Ahmed et al⁽²³⁾. Our study also shows the positive correlation of hemoglobin and glycated hemoglobin levels. Similar findings were observed by El-Agouza et al [8], Coban et al[9] and Shanthi, et al[22]. However study by Narayan et al[24] documented no correlation between total Hemoglobin level and HbA1c. Our study also shows the correlation of study parameters with HbA1c levels at two months of therapy. It was noticed that significant correlation was observed in Hemoglobin, PCV, MCHC, and MCV values. This implies that change in HbA1c values was associated with change in Hemoglobin, PCV, MCHC, and MCV values at two months of therapy. No correlation was observed with control group (iron therapy not given). Similar findings were observed by Ahmed et al[23]. Thus it has been observed that correlation of IDA with HbA1c is dependant on multiple factors, therefore further research needs to be done to identify the factors that affect HbA1c levels. Currently, there are no clear guidelines or verified calibrated methods to measure HbA1c, and we suggest that other clinical factors are recognized to affect the interpretation of HbA1c diagnostically, including IDA. It is hence prudent to rule out

IDA before making a therapeutic decision, based on the HbA1c levels. Hence, before altering the treatment regimen for diabetic patient, presence of iron deficiency anemia should be considered.

Conclusion

1. Our study shows that there is a decrease of HbA1c levels in patients of iron deficiency anaemia as the mean value of HbA1c(%) in intervention group was at 7.66 at base line which was significantly lower than that of controls(baseline HbA1c = 4.92).
2. Our study also shows that there is an decrease in the levels of HbA1c after 2 months of treatment as the mean value of HbA1c (%)in patients was 5.40 at 2 month and the mean HbA1c of controls over 2 months period was 5.5
3. The interpretation of HbA1c based on information from hematological examination and iron metabolism indices may help to prevent misdiagnosis or under-diagnosis, and HbA1c should be evaluated carefully as a parameter of glycemic control in patients with IDA.

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