Original Research Article

Observational study of the level of HbA1c in iron deficiency anaemia cases: Before and after treatment

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Abstract

Background: Our aim is to study the levels of HbA1c in iron deficiency anemia patients and the changes in HbA1c level after the correction of iron deficiency anemia. **Methods:** It was prospective interventional study, all patients coming to the department of general medicine, Patna medical college and Hospital during the period of November 2017 to December 2019, and fulfilling the inclusion and exclusion criteria were enrolled in the study. 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. All the biochemical studies were carried out with Auto biochemistry analyzer (Selectra Pro-M) in Department of Biochemistry, PMCH. **Results:** Mean HbA1c of patients with iron deficiency anemia (7.12 ± 0.608) was higher, compared to healthy control group (5.85 ± 0.681) with p value less than 0.001(significant).In iron deficiency anemia patients mean HbA1c decreased from 7.12 ± 0.608 % to 6.32 ± 0.681% after iron treatment with p value less than 0.001 which is statistically significant. Among patients with iron deficiency anemia, hemoglobin and HbA1c showed negative correlation (r = -0.26) which was statistically very significant (p= 0.005). A possible explanation is increased level of Malondialdehyde in cases of iron deficiency anemia which increses glycation which needs to be studied. **Conclusion:** Hemoglobin and HbA1c showed statistically significant negative correlation in patients with iron deficiency anemia. Iron deficiency anemia has to be kept in mind before using the HbA1c as diagnostic tool for diabetes especially in 2nd decade of life. HbA1c is preferably avoided in gestational diabetes as a diagnostic tool

Key Word: anemia, malondialdehyde, HbA1c.

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INTRODUCTION

Iron deficiency is the state in which the iron content of the body is less than normal. The earliest stage of iron deficiency is depletion of iron stores, in which the serum iron, transferrin saturation and hemoglobin levels will be normal but the storage iron is decreased or absent. Further advanced stage is iron deficiency without anemia, characterized by depleted iron stores, low serum iron and transferrin saturation but without anemia.[1] Iron deficiency anemia is the far most advanced stage of iron deficiency. It is characterized by absent iron stores, low serum iron levels, low transferrin saturation with low hemoglobin levels. Iron deficiency anemia is most prevalent in women and children in regions where meat intake is low, food is not fortified with iron, malaria, intestinal infections and parasitic worms are common. Serum ferritin concentration represents the total body iron stores. In iron deficiency state the serum ferritin level will be as low as 10mcg/L. Ferritin concentration is elevated in inflammatory disorders like rheumatoid arthritis, chronic kidney disease and malignancies. The normal serum ferritin value differs according to the age and gender.[2] Glycated hemoglobin (HbA1c) is a form of hemoglobin, modified with a stable

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adduct of glucose linked covalently to the N-terminal valine of the β-chain. In adults normal hemoglobin consists of HbA (α2β2), HbA2 (α2δ2) and HbF (α2γ2) in 97%, 2.5% and 0.5% respectively. Among the total HbA, about 6% is termed as HbA1. HbA1 consists of HbA1a1, HbA1a2, HbA1b and HbA1c. These fractions are characterized by their individual electrophoretic and chromatographic properties. Despite the identical amino acid sequences of HbA1 and HbA0, these fractions differ slightly in their electrophoretic and chromatographic properties from those of the major component HbA1c.[3] HbA1c is the predominant HbA1 fraction. In healthy people it constitutes approximately 5% of the total HbA fraction. There is no known physiological role for HbA1c. The hallmark of diabetes is chronic hyperglycemia resulting in diabetes specific complications. So the HbA1c which represents the longterm glucose exposure predicts the diabetes specific complications better than single glucose measurement. Studies proved a consistent and significant correlation between retinopathy and HbA1c levels than with the fasting glucose levels[4]. Multiple controlled clinical trials and large volume of data from different populations have provided a strong evidence for assigning an HbA1c cut off point of more than 6.5% for diagnosing diabetes as this HbA1c level is associated with an increased prevalence of diabetes specific complications especially retinopathy[5]. This cut off point is not an absolute demarcation between diabetes and normal glycemic status. But this level is sensitive and specific to detect the patients at risk for developing retinopathy. An International Expert Committee has recommended the HbA1c level of more than 6.5% to diagnose diabetes[6]. The same has been affirmed by American Diabetes association[7]. But this diagnostic HbA1c test should be done by a standard method certified by the NGSP- National Glycohemoglobin Standardization Program and traceable or standardized to the Diabetes Control and Complications Trial (DCCT) reference assay.[8]

MATERIAL AND METHODS

Study population

All patients coming to the department of general medicine, Patna medical college and Hospital, fulfilling the inclusion and exclusion criteria were enrolled in the study. An informed written consent was obtained from the patients.

Data collection

A detailed history was recorded along with complete clinical examination as in the pro forma. Provisional diagnosis was made and this was subsequently revised after completion of the investigations.

Laboratory investigations

Samples were collected from all the participants to estimate complete blood count, blood urea, serum creatinine, serum electrolytes, blood sugar-FBS/PPBS/GTT, urine R/E, HbA1c level, anemia profile including serum ferritin, vitamin B12 and folic acid levels, based on standard tests available in our hospital. In addition, ECG, chest x-ray and ultrasonography abdomen were done in necessary cases to rule out certain diseases. The final data was entered onto Microsoft excel sheet 2007 version**.**

Study protocol

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. All the biochemical studies were carried out with Auto biochemistry analyzer (Selectra Pro-M) in Department of Biochemistry, PMCH.

Statistical analysis

The clinical parameters were compared and analyzed using Pearson chi square method. The diagnostic accuracy of all the parameters was then compared and interpreted with reference to clinical data. In the present study, the statistical methods for quantitative data, descriptive statistics was presented by N, Mean, Standard Deviation and Range. For qualitative data, frequency count, N and percentage were put in a tabular manner. To analyze the data, appropriate statistical tests were applied. The significance of difference between means in two groups was calculated using student t test and the significance of difference in proportions using 65 chi-square test. 2 x 2 tables were constructed for each variable and chi square value for degree of freedom calculated. All the statistical analysis has been done by using statistical software SPSS (version 22). Other data, displayed by various tables and charts, by using Microsoft excel (windows 7).

Study is approved by Institutions Ethical Committee of Patna Medical College and Hospital.

RESULTS

Table 1: Age distribution

AGE (years)	CONTROL GROUP N (%)	STUDY GROUP N (%)
≤20	2(10)	5(4.2)
21-30	10(42.5)	21(33.3)
31-40	4(23.3)	20(34.2)
>40	4(24.2)	14(28.3)
TOTAL	20(100)	60(100)
Mean	32.06	34.067
SD	8.34	8.398
P value		0.064 Not Significant

Interpretation: About 67% of study subjects were in the age group of 21-40 years while 28% were aged 41-60 years. Minimum age: 18 years, maximum age: 59 years. Mean age (±SD): in control group was 32.1 (8.34) and in study group was 34.1 (8.398).P value was 0.064 which is not significant i.e. the age distribution among the control and study group were equal.

Table 2: Sex distribution

SEX	CONTROL GROUP N (%)	STUDY GROUP N (%)
MALE	8(40)	24(40)
FEMALE	12(60)	36(60)
TOTAL	20	120
P value		0.136 Not Significant

Majority of the study subjects were females (60%) while the remaining 40% were males. It confirms the fact that iron deficiency anemia is more common in females. P value was 0.136 which is not significant i.e. sex distribution among the control group and study group were equal.

Table.3: Distribution of hemoglobin in control group

HB (g/dl)	CONTROL GROUP N (%)
12-13	7(35)
>13	13(65)
N	20
Mean	13.408
SD	0.354

Mean (±SD): 13.408(0.354) gm/dl, minimum: 12gm/dl, maximum: 14gm/dl.

Table 4: distribution of hemoglobin in study group pre correction

HB (g/dl)	STUDY GROUP PRE N (%)
<8	47(78.3)
8.0-8.9	13(22.6)
≥9	0(0)
N	60
-Mean	6.778
SD	1.085

Mean (±SD): 6.778(1.085) gm/dl, minimum: 6.2gm/dl, maximum: 8.3gm/dl.

Table: 5: Distribution of hemoglobin in study group pre and post correction

HB (g/dl)	STUDY GROUP PRE	STUDY GROUP POST
≤13	60	41
>13	0	19
N	60	60
Mean	6.778	12.659
SD	1.085	0.446
P value		<0.001 Significant

P value was less than 0.001 which is highly significant i.e. mean hemoglobin level had increased significantly in study subjects after iron treatment as expected.

Table 6: Distribution of serum ferritin

SERUM FERRITIN (g/L)	CONTROL GROUP N(%)	STUDY GROUP PRE N(%)	STUDY GROUP POST N(%)
<15	0(0)	60(100)	0(0)
16-50	0(0)	0(0)	0(0)
51-150	0(0)	0(0)	0(0)
151-300	19(95)	0(0)	60(100)
>300	1(5)	0(0)	0(0)
N	20	60	60
Mean	232.264	6.871	237.239
SD	28.394	1.5	25.267
P value	<0.001 Significant		<0.001 Significant

P value between control group and study group before anemia correction was less than 0.001 which is highly significant. It conveys that serum ferritin was significantly lower in anemia group. P value between study group pre and post correction was less than 0.001 which is highly significant. It indicates that serum ferritin improved after iron treatment as expected.

Table 7: Mean Difference result in pre and post correction for Hb and HbA1c values

Parameters	Mean Difference
Hb (gm%)	5.88***
HbA1c (%)	0.8***

In this study HbA1c significantly decreased after correction of anemia.

Table 8: Pre correction result for correlation

Hb – Pre correction	HbA1C- pre correction	Interpretation
Pearson Correlation coefficient r	–0.26	negetive, correlation
P value	0.005**	very significant
Sample size (n)		60

In this study group, pre correction Hb and HbA1C showed negative, correlation (r = –0.26) which was statistically very significant (p= 0.005)

DISCUSSION

Iron deficiency is the commonest malnutrition. It is a major public health problem in both developing and developed countries. Iron deficiency contributes to 50 percentage of anemia worldwide. Annually, about 8,41,000 deaths were attributed to iron deficiency anemia. Parts of Asia and Africa are affected more. These countries bear approximately 71 percentage of the global mortality burden. In India, about 50% of anemia is attributed to iron deficiency. Children and women are the most vulnerable population. The factors contributing to iron deficiency anemia varies in different population. Physiologically, HbA undergoes glycosylation in a slow and non enzymatic manner. The degree of glycosylation depends on the concentration of glucose. HbA1c is the predominant form of glycated hemoglobin. Glucose gets attached to the NH2 group in the terminal valine of the β-globin chains irreversibly.

Glycosylation process occurs throughout the life span (120 days) of red cells. Hence the measured glycohemoglobin levels reflect the glycemic status of the preceding 3 months. HbA1c levels can be affected by multiple factors other than the plasma glucose level. Several conditions can result in falsely lower or higher values. Hemolytic anemia, hemoglobinopathies, uremia and chronic blood loss influence the HbA1c assays.

Age distribution of the study population

In this study about 60 patients were allotted to the study group. 20 age and sex matched controls were taken. The mean age group of the study population was 34.1±8.4 years. The minimum age was 18 years and the maximum was 59 years. About 67.5% of the study subjects were in the age group of 21-40 years while 23.3% were aged 41-60 years. Thus in our study, the prevalence of iron deficiency anemia is more common in 2nd to 4th decade of life.

Sex distribution of the study population:

In this study, out of 60 patients 68.3% were females and 31.7% were males. It confirms the fact that women are more vulnerable to iron deficiency than men. The age and gender distribution of population in both the study and control groups were equal and comparable.

Hemoglobin distribution of the study population:

The mean hemoglobin of the study population was 6.78 (±1.1) gm/dl. About 78.3 % of the study population had severe anemia i.e. less than 8 gm/dl. The minimum hemoglobin observed in the study population was 6.2 gm/dl and the maximum was 8.3 gm/dl. The p value of unpaired t test between the study group hemoglobin and control group was less than 0.001 which is highly

significant. It indicates that mean hemoglobin level in study group was significantly lower than the control group as expected.

The mean hemoglobin level in the study group increased from 6.78(±1.1)gm/dl to 12.7(±0.4)gm/dl after correction of anemia with iron. The minimum hemoglobin observed in the study population after iron treatment was 12gm/dl and the maximum was 14gm/dl. The p value of paired t test in the study group hemoglobin before and after iron treatment was less than 0.001 which is highly significant. It indicates that mean hemoglobin level had increased significantly in study subjects after iron treatment as expected.

HbA1c level of the study population

The mean HbA1c of the study population was 7.12(±0.608) %. About 91.5% of study subjects had HbA1c level >6.5% while 6.5% had HbA1c level between 6.1-6.5%. The mean HbA1c of the control group was 5.86(±0.681) %. The p value of unpaired t test between the study group HbA1c and control group was less than 0.001 which is highly significant. It indicates that mean HbA1c level in study group was significantly lower than the control group. The mean HbA1c level in the study group increased from 7.12(±0.608) % to 6.3(±0.623)% after correction of anemia with iron. After correction of anemia about 48.3% of study subjects had HbA1c level between 5.6-6.0% while 25.6% had HbA1c level between 6.1-6.5%. The p value of paired t test in the study group HbA1c before and after anemia correction was less than 0.001 which is highly significant. It indicates that mean HbA1c level had increased significantly in study subjects after anemia correction.

Correlation between Hb and HbA1C

In this study group, pre correction Hb and HbA1C showed negative, correlation (r =-0.26) which was statistically very significant (p= 0.111). That is, when the hemoglobin increases the HbA1c will decrease and vice versa. Similar to this study, in 2014 a study was conducted by Vishal Kalasker *et al.*[9] on the effect of iron deficiency anemia on glycosylated hemoglobin levels in non diabetic indian adults. They postulated that Hb concentrations are positively corrected with HbA1c concentration and that HbA1c concentration tended to be lower in the presence of iron deficiency anemia. But they concluded that iron deficiency anemia is unlikely to be a major concern in diagnosing diabetes using concentration of HbA1c according to the American Diabetes Association (ADA) guideline. In contrast to our study, a study done by Alap L. Christy *et al.*[10] concluded that iron deficiency anemia elevates HbA1c levels in diabetic individuals with controlled plasma glucose levels. They postulated that iron deficiency anemia has a positive correlation with increased HbA1c levels. A study done by Catherine Kim *et al.*[11] concluded that iron deficiency shifted the HbA1c slightly upwards independent of fasting glucose level. In non GDM mothers, Sasekala *et al.*[12] conducted a descriptive cross sectional study. They showed that in anemic non GDM mothers the HbA1c levels are higher. So they advised to be cautious in interpreting the HbA1c and plasma sugar levels. Alap L. Christy *et al.*[13] conducted a study to evaluate the relationship between HbA1c and anemia in hypothyroid patients. They concluded that Nondiabetic hypothyroid individuals with anemia shows elevate A1C levels in prediabetes range. Hence care should be exercised while using HbA1C as a diagnostic tool for diabetes in such patients. Study done by Van Heyningen *et al.*[14] found out that there was no significant influence of iron deficiency anemia over HbA1c concentrations. They suggested that differences observed in previous studies could be due to the various laboratory methods used in estimating the HbA1c. Hansen *et al.* also observed similar results. Contradicting the conclusion of Van Heyningen *et al.*, Rai *et al.* conducted a study using various assay methods to estimate HbA1c and found no significant alterations in HbA1c levels measured by those methods. El-Agouza *et al*[15] reported that iron deficiency anemia patients had higher HbA1c levels and it decreased after treatment. They believed that there was a balance between hemoglobin concentration and HbA1c level. That is if the plasma glucose was maintained, the lower hemoglobin concentration would lead to rise in HbA1c levels. In our study mean HbA1c of iron deficiency anemia patients (7.12 ± 0.608) was significantly lower than control population (5.9 ± 0.681) and it decreased (6.3 ± 0.423%) significantly after iron treatment.

CONCLUSION

The prevalence of iron deficiency anemia is more common in females during the second to fourth decades of life.

HbA1c was higher in patients with iron deficiency anemia compared to healthy control group.

After correction of anemia, HbA1c level decreased significantly in iron deficiency anemia patients.

Hemoglobin and HbA1c showed statistically significant negative correlation in patients with iron deficiency anemia.

Longer period of study and a larger sample size may be required to show a statistically significant negative correlation.

Iron deficiency anemia has to be kept in mind before using the HbA1c as diagnostic tool for diabetes especially in 2nd decade of life. HbA1c is preferably avoided in gestational diabetes as a diagnostic tool**.** Increased malondialdehyde in Iron Deficiency Cases leading to increased glycation is a possible explanation.

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