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ORIGINAL ARTICLE

Iron deficiency anemia and its effect on HbA1c levels in patients above 30 years

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ABSTRACT

Background: Iron deficiency contributes 50% of the anemic burden worldwide. Glycated hemoglobin (HbA1c) is an irreversible product formed due to slow non-enzymatic catalysis of the β-chain of matured hemoglobin (Hb). The glycemic status of the patients with diabetes is monitored through HbA1c for the three months period. IDA is associated with blood loss, hemolysis, hemoglobinopathies, red cell abnormalities, and myelodysplastic disease. IDA also can elevate the turnover of red blood cells, which can increase the glycation of hemoglobin and lead to higher HbA1c concentrations.
Aim: To establish a relationship between the levels of HbA1c And Iron Deficiency Anemia.
Methods: Total 5ml of Venous blood was collected from patients above 30 years under aseptic conditions and distributed in EDTA and plain vial for serum. Blood samples from participants were sampled in 10ml Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) containing EDTA and plain tubes (Becton Dickinson) for hematological analysis and serum separation respectively. The plain vial serum was used for estimation of serum ferritin levels. Complete Blood Count (CBC) and HbA1c estimation were performed using the EDTA samples. Biochemical analyses were performed by Sysmex-350 five-part auto analyzer. Results were systematically entered into a master chart in which were also cross referenced to clinical and other pertinent patient data. The data was then analyzed statistically for interpretation.
Results: There was a statistically significant difference when comparing hemoglobin (Hb) levels and mean corpuscular volume (MCV) between the control and study groups. Before treatment, every subject in the study group had Hb ≤13.0 g/dL (p13.0 g/dL. In this study, by the end of treatment in the study group, hemoglobin values showed a statistically significant increase where the percent of patients with hemoglobin >13.0 g/dL was found in 10% and basal mean hemoglobin value of patients was detected to be improved from 6.82±0.32g/dL to 12.66±0.42 (p90%, comparable to controls, indicating adequate resolution of iron deficiency anemia (p<0.001).
Conclusion: The evidence shows that iron supplementation has a strong beneficial effect on hemoglobin levels and reverses microcytic anemia in patients with anemia. All subjects in the study group showed low level of Hb and microcytic hypochromic mild anemia suggesting iron deficiency anemia before treatment. Significant uptick of Hb and MCV values on intervention —a quintessential hematological response. This emphasizes the need for early detection and proper treatment of anemia to bring back normal hematological parameters to improve general health.
Keywords: Anemia, IDA, Glycated Hemoglobin, microcytic, iron deficiency

INTRODUCTION

Glycated hemoglobin called as hemoglobin A1c (HbA1c) is an irreversible product formed due to slow non-enzymatic catalysis of the β-chain of matured hemoglobin (Hb)[1,2]. Considering the life span of a red blood cells, to monitor the glycemic status of the patients with diabetes for the previous three months period, HbA1c is being used as the "Gold Standard"[3].

Determination of HbA1c level of a patient could provide an integrated measure of the glycemic status is helpful in two ways such as determination of the short term modulation of blood glucose and also in tracking the diabetic medication given to the patients. Determination of the level of HbA1c for the diagnosis of type 2 diabetes have been approved by both the World Health Organization (WHO) and the American Diabetes Association (ADA[4,5] where 4 to 6% has been stated as the normal range for a healthy person[6].

However, levels between 5.7% to 6.4% is termed as pre-diabetes, whereas levels >6.5% is considered as diagnostic for diabetes[7]. Abnormalities in hemoglobin can theoretically falsely influence the HbA1c with regard to the HbA1c measures the sugar that is linked to the β-chain of matured hemoglobin[8]. However, the major clinical factors on which HbA1c levels are dependent are reticulocytes released from bone marrow containing HbA1Cand the rate of hemoglobin glycation.

In both industrialized and developing nations, anemia is the most common kind of dietary insufficiency. Only iron deficiency contributes 50% of the anemic burden worldwide[9,10]. Iron, which plays a role in the majority of critical metabolic activities, including oxygen transport, control of cell development and differentiation, production of deoxyribonucleic acid (DNA), and electron transport, regulates the clinical profile of many systemic disorders[11-13]. IDA is still the most common cause of anemia, affecting over 2 billion people globally[14].

An association between IDA and atypical results of glycosylated hemoglobin (HbA1c) has been published previously. To elucidate the differences in HbA1c levels amongst patients with IDA, the researchers examined glycated hemoglobin from newly developed and older red blood cells. They also considered structural changes in hemoglobin molecules as a possible contributing factor. HbA1c reductions due to iron deficiency may not be justified as reflecting improved glycemic control resulting from standard diabetes management practices since the half-lifeis affected by red blood cell turnover and hemoglobin structure[25].

The main focus of this study is to establish a relation between HbA1c levels And Iron Deficiency Anemia.

MATERIAL AND METHODS

Study Design and Setting: A cross-sectional study was performed over one year period from January 2023 to January 2024, in Hematology section of the Department of Pathology of a tertiary care center located in Western Uttar Pradesh, India. Ethics approval and consent to participate Ethical clearance obtained from the Institutional Ethics Committee (IEC), reference number: SU/2022/3108[38]
Study Population: This study included a total of hundred subjects, fifty cases with Iron Deficiency Anemia (IDA). Participants were selected to be above 30 years of age and non-diabetic. Participants provided written informed consent before the study.

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Inclusion Criteria

- Male and female patients aged above 30 years.
- The Diagnosis of Iron Deficiency Anemia

Exclusion Criteria

- Pregnant females.
- Diabetes mellitus, known cases.
- Patients with hemolytic anemia, hemoglobinopathies, uremia, HIV, tuberculosis, or cancer
- Chronic alcoholics.

Sample Collection and Processing:

- Under aseptic conditions, 5mL of venous blood was taken from each of the participants. The samples were split into two vials:
- EDTA vial: for sending Complete Blood Count (CBC) and HbA1c.
- Vial without additive: for separation of serum and estimation of serum ferritin.
- Biochemical analysis: Sysmex-350 Five-Part Auto Analyzer, Department of Biochemistry, Santosh Medical College & Hospital.

Data Collection:

- Alongside lab parameters, pertinent clinical data and questionnaire-based data (such as anthropometric measurements and medical history, e.g., antibiotics use, diabetes history) were documented.
- Parameters Assessed
- Complete Blood Count (CBC)
- Serum Ferritin
- Glycated Hemoglobin (HbA1c)

Statistical Analysis: All data were compiled and entered in Microsoft Excel 2021. Statistical analysis was performed by SPSS version 29 (trial version). Continuous data were analyzed using ANOVA. Correlation analysis was performed to determine the association of quantitative variables. p-value 0.05 was considered non-significant. Results were shown in suitable tables and graphical conventions for ease of interpretation.

RESULTS

Table 1: Comparison of hemoglobin range between the control and study group before treatment

HB (g/dl)	Control Group	Study Group Before Treatment
≤13	9(18%)	50(100%)
>13	41(82%)	0(0)
N	50	50
Mean	13.36	6.82
SD	0.32	0.32
p Value	<0.001	

Table 1 depicts the range of Hb in the control and study group before treatment, which showed that maximum number of participants in the control group (82%) has >13.0 and only 18% has to ≤13. However in the study group before treatment values of Hb showed all the 100% participants has ≤13.0 g/dL of Hb. The results between the two groups showed a significant difference (p<0.001).

Table 2:Comparison of Hemoglobin Range between the values observed in Study Group before Treatment and Post Treatment

Hb (g/dL)	Study Group Before Treatment	Study Group After Treatment
≤13	50(100%)	45(90%)
>13	0(0%)	5(10%)
N	50(100%)	50(100%)
Mean±SD	6.82±0.32	12.66±0.42
p Value	<0.001	

Table 2: Distribution of Hemoglobin (Hb) level in the study group before and after treatment for iron deficiency anemia. At baseline, all participants (n=16) had Hb levels ≥13g/dL (Hb=6.82±0.32 g/dL), indicative of severe anemia. Ninety percent post-treatment had maintained Hb ≤13 g/dL and 10% had improved with Hb >13 g/dL. The mean Hb level rose significantly to 12.66±0.42 g/dL. The p-value (<0.001) suggests that this rise in Hb levels after treatment was statistically significant, providing evidence that iron therapy was effective in correcting the anemia.

Table 3: Percentage of MCV Distribution in the control and Study Groups

MCV	Control	Treatment	N(%)
<80%	0(0)	50(100)	0(0)
80% – 90%	18(36)	0(0)	14(28)
>90%	32(72)	0(0)	36(72)
N	50(100)	50(100)	50(100)
Mean±SD	91.49±2.71	65.21±7.39	92.07±2.9
p Value	<0.001		<0.001

Table 3 showed the percentage of MCV in the control and study groups. The results of our study showed a significant difference (p<0.001) between the control and the study group before treatment, however, it was also very clear the after iron supplementation to the study group, the MCV percentage was significantly increased and shows a significant difference (p<0.001) between the values observed before and after treatment.

Table 4: MCV Levels in the control and Study Groups

MCH (pg/cell)	Control N(%)	Treatment N(%)	Study Group Post Treatment; N(%)
<26	0(0)	50(80)	0(0)
26-28	17(34)	0(0)	13(26)
>28	33(66)	1(0.8)	37(74)
N	50	50	50
Mean	28.47	19.85	28.66
SD	0.88	0.88	0.93
P value		<0.001 Significant	<0.001 Significant

Table 4 showed the MCH in the control and study groups. The results of our study showed a significant difference (p<0.001) between the control and the study group before treatment, however, it was also very clear the after iron supplementation to the study group, the MCH was significantly increased and shows a significant difference (p<0.001) between the values observed before and after treatment.

Table 5: Levels of Serum Iron in Control and Study Groups

Serum Iron	Control N(%)	Treatment N(%)	N(5)
<30	0(0)	50(100)	0(0)
31 to 60	0(0)	0(0)	0(0)
61 to 90	3(2.5)	0(0)	1(2)
90 to 120	21(44.2)	0(0)	27(54)
>120	26(53.3)	0(0)	22(44)
N	500(100)	50(100)	50(100)
Mean±SD	119.32±SD	21.96±5.05	115.42±11.8
p Value	<0.001		<0.001

Table 5 showed the serum iron level in the control and study groups. The results of our study showed a significant difference (p<0.001) between the control and the study group before treatment; however, it was also very clear that after iron supplementation to the study group, the serum iron level was significantly increased and shows a significant difference (p<0.001) between the values observed before and after treatment.

Table 6: Comparison of Hb1Ac between the Control and Study Group before Treatment

HbA1c (%)	Control N (%)	Treatment N(%)
≤5	3(6)	0(0)
5.1-5.5	28(56)	0(0)
5.6-6.0	17(34)	3(6)
6.1-6.5	2(2)	13(26)
>6.5	0(0)	34(68)
N	50(100)	50(100)
Mean±SD	5.49±0.27	7.16±0.88
P value	<0.001	

Table 6 depict the range of HbA1c in the control and study group before treatment, which showed that maximum number of participants in the control group (56%) have shown the range of 5.1% to 5.5% and 34% has 5.6% to 6.0%. However, in the study group before treatment values of Hb1Ac showed a maximum number of participants (68) has values >6.5% g/dL and 26% showed values ranging 6.1% to 6.5% whereas only 6% has values between 5.6% to 6.0% of Hb. Then mean value of control group was 5.49% and in study group before treatment it was 7.16%. The results between the two groups showed a significant difference (p<0.001).

Table 7: Comparison of Hb1Ac between the Study Group Before Treatment and Post Treatment

HbA1c(%)	Study Group before treatment	Study Group Post Treatment
≤5	0(0)	3(6%)
5,1-5.5	0(0)	22(44%)
5.6-6.0	3(6%)	23(46%)
6.1-6.5	13(26%)	2(4%)
>6.5	34(68%)	0(0)
N	50(100%)	N(100%)
P value	<0.001	5.48±0.31

Table 7 depicts the range of HbA1c in the study group before treatment and post treatment, which showed that maximum number of participants (668%) in the before treatment group has values >6.5% g/dL and 26% showed values ranging 6.1% to 6.5% whereas only 6% has values between 5.6% to 6.0% of Hb. However, after iron supplementation to the study group the values were significant increased. The results between the two groups showed a significant difference (p<0.001).

Table 8: Mean Difference of Hb and HbA1c Values observed in the Study Group before treatment and post treatment

Parameter	Mean Difference
Hb (g/dL)	5.84***
HbA1c (%)	1.68***

***Highly Significant (p<0.0001)

Table 8 shows that in the current study, there was an increase in the Hb and decrease in the HbA1c observed on comparison of the mean values obtained before treatment and post treatment of the study group. The mean difference showed a highly significant difference (p<0.0001). As expected, Hb1Ac was significantly decreased after the correction of anemia through iron supplementation.

Table 9: Correlation Analysis of Hb and HbA1c values of Study Group Before Treatment

Hb before treatment	HbA1c before treatment	Interpretation
r	-0.26	Negative correlation
P value	<0.005	
Sample size	50	

Table 10: Correlation Analysis of Hb and HbA1c values of Study Group Post Treatment

Hb post treatment	HbA1c post treatment	Interpretation
r	-0.15	Negative correlation
P value	<0.005	
Sample size	50	

Table 9 HbA1c levels prior to treatment in iron-deficient patients demonstrated a weak inverse correlation with Hb level (r = -0.26, p<0.005). A consequence of this may be that HbA1c levels are falsely elevated by iron deficiency itself, regardless of glycemic status.

Table 10 The association was still in the negative, but had become less strong (r=-015, p<0.005), highlighting that the relationship between Hb and HbA1c continued post treatment, but the strength of the association was decreased. This decrease may indicate partial correction of HbA1c distortion after recovery of an iron status.

These findings corroborate the hypothesis associating an impact of iron deficiency on the accuracy of the HbA1c glycomarker, with potential improvements on the reliability of the HbA1c marker following the correction of anemia.

DISCUSSION

In the present study, it was observed that the levels of Hb & HbA1c in the study group showed a negative correlation before treatment with a statistical significance of p<.005.

Our finding is in agreement with several of the earlier findings, where it was reported that decrease in Hb and an increase HbA1c in iron deficiency anemia would show a negative correlation, upon correction the values would show vice versa[16-19]. Whereas values of Hb & HbA1c after post-treatment in the present study also showed a significant change in the correlation values as well.

Similarly, few other studies have also reported elevated levels of HbA1c due to iron deficiency anemia in patients with diabetes mellitus even with controlled plasma glucose levels. Studies have also reported that iron deficiency without the elevated plasma glucose could shift the HbA1c slightly upwards. [(20-22)] An earlier study has showed that HbA1c levels could increase in anemia with non-gestational diabetes mellitus as well[23].

But in the ongoing study we found that average HbA1c of iron deficiency anemia patients before treatment (shown in the form of iron supplementation) was significantly higher than the values after treatment not only of the study group (5.49±0.27) but also the control group (5.48±0.31).

A recent research reported the rise in the levels of HbA1c in iron deficiency anemia(patients): Alzharni et al. Iron supplementation to the patient has significantly reduced the HbA1c percent and return to the normal,(24) which is in accordance with our findings.

In this systematic review summarizing the impact of anemia and the aim of optimal HbA1c control in the time frame of 2010–2020 it was concluded that iron deficiency anemia plays a major role in the rise in hemoglobin glycosylation which supports our finding[8]. (95) Other studies have also shown that the Iron deficiency in Women have a significant role in raising the glycosylated hemoglobin[26-30]. Our findings are also consistent with those of the above studies.

In contrast to our results and other supportive evidence indicating that iron deficiency anemia was associated with significantly higher HbA1c levels, very few studies have reported an association between iron deficiency anemia and low HbA1c levels[31,32].

CONCLUSION

The findings or our study implies that iron deficiency anemia has a significant impact on the hemoglobin and glycosylated hemoglobin levels on comparison to the healthy control subjects. More interestingly, it was also noticed that the iron deficiency is more prone and prevalent in female population aged during their 3rd and 4th decade of life.

It was also quite prominent that the average levels of HbA1c in patients affected with iron deficiency was 7.16±0.88, which was significantly higher (P<0.001) than that of the health control subjects involved in the present study, where the average levels was determined to be 5.49±0.27. However, upon supplementation of iron to the patients suffering from iron deficiency anemia, the level of Hb1Ac was found to be significant decreased (p<0.001) on compared to the values obtained from the same group of patients before treatment.

Quite interestingly, it was also noticed that the levels after treatment period showed almost near to normal and there was no

significant difference when compared with the health control subjects.

It can be concluded that Hb and HbA1c has a negative correlation in iron deficiency anemia patients.

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1. Conception and design of or acquisition of data or analysis and interpretation of data.
2. Drafting the manuscript or revising it critically for important intellectual content.
3. Final approval of the version for publication.

All authors agree to be responsible for all aspects of their research work.

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