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Effects of Iron Deficiency Anemia on A1c levels in Non-Diabetic patients

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Abstract:
***Background*:** *Iron deficiency anemia(IDA) is the most common form of hypoproliferative anemia encountered in clinics. The ADA and WHO have agreed that HbA1c may be used to diagnose diabetes. Glycated hemoglobin A1c comprises approximately 5% of the total hemoglobin in non-diabetic individuals. IDA can increase the red blood cell turnover which can increase glycation of Hb leading to higher A1c. Early diagnosis of diabetes can help in preventing its complications but A1c levels can give a false picture of glycemic status in patients of IDA. The present study was conducted to evaluate the Effects of Iron Deficiency Anemia on A1c levels in Non-Diabetic patients and changes in A1c level after the correction of iron deficiency anemia.*
Materials and Methods: *200 Non-diabetic patients were included in the Study out of which 100 were having IDA and 100 were non anemic. All participants were evaluated by history, examination and laboratory investigations. A1c values were compared of anemic and non anemic group. Patients with IDA were given Iron therapy for 3months and A1c was compared in the anemic patients before and after treatment. Statistical analysis has been done by using statistical software SPSS (version 22).*
Results: *In this study, 30.0% of the participants in IDA group were Male and 70.0% were Female. 64.0% of the participants in non anemic group were Male and 36.0% were Female. The mean (SD) of Hemoglobin (g/dL) (Baseline) in Case group was 6.68 (1.78) and in the Control group was 13.23 (0.87). The mean (SD) of HbA1c (%) in the IDA group was 5.30 (0.49) and in the non anemic group was 5.11 (0.38). There was a significant difference between the 2 groups in terms of HbA1c (%) (Baseline) (W = 6379.500, p = 0.001),. The mean HbA1c (%) decreased from a maximum of 5.30(0.49) at the Baseline time point to a minimum of 5.28(0.44) at the 3-Months time point. This change was not statistically significant (Wilcoxon Test: V = 1152.5, p = 0.214)..*
Conclusions: *Hemoglobin and A1c showed statistically significant negative correlation in patients with iron deficiency anemia. A1c was higher in IDA patients as compared with non anemic group. After correction of anemia, Hemoglobin and A1c showed statistically insignificant negative correlation. Hence IDA should be kept in mind while using A1c to diagnose diabetes.*
Key Word: *Iron deficiency anemia, A1c ,iron therapy.*

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I. Introduction

Anemia affects one-third of the world population with IDA being the most common cause.[(1,2)] IDA is more prevalent in women and children of developing countries where meat intake is low, food is not fortified with iron, malaria, intestinal infections and parasitic worm infestation are common due to poverty and malnutrition.[(3,4)]. India has the highest total prevalence of Anemia at 39.86% in the world. According to NFHS-5 as many as 68.4 per cent children and 66.4 per cent women suffered from anemia in 2019. With socio-economic development and lifestyle changes in India, the epidemic of DM is increasing. Prevalence of DM ranged from 3% to 12% across different rural areas of India with an expected rate of increase of 2.0 per 1000 population per year.[(5)] The WHO, ADA and an International Expert Committee have now recommended the use of HbA1c to diagnose diabetes and recommended the A1c level of more than 6.5% as a cut-off point to diagnose diabetes and considering the diabetes preventive measures at A1c level between 6.0 to 6.5% and less.[(6,10)] Glycated hemoglobin A1c is a major part of HbA1 and comprises approximately 5% of the total hemoglobin in non-diabetic individuals.. The Glycation reaction is mostly irreversible, so that the concentration of A1c is a function of the concentration of glucose to which the erythrocytes are exposed over their lifespan 3 moths (120 days on

average). IDA can increase the red blood cell turnover which can increase glycation of Hb leading to higher A1c values as observed in blood loss, hemolysis, hemoglobinopathies, red cell disorders and myelodysplastic disease.[7] A bidirectional relationship between iron metabolism and glucose homeostasis is seen, higher iron levels modulate both the action and secretion of insulin and lower the iron levels, higher is the glycation of A1c leading to its false-high values in diabetic as well as non-diabetic individuals.[8,9]As IDA and DM are two very prevalent health problems in India, our study aimed to assess the Effects of Iron Deficiency Anemia on A1c levels in Non-Diabetic patients and changes in A1c level after the correction of iron deficiency anemia so that better diagnosis and monitoring of diabetic and prediabetic patients can be done.

II. Material and Methods

This prospective case controlstudy study was carried out on patients coming to the department of General Medicine, SRN Hospital, MLN Medical College, Allahabad, U.P. India from June 2020 to june 2021. A total of 200 subjects (both male and female) participated in the study.

Study Design : Prospective Case Control study

Study Location : Department of General Medicine, SRN Hospital, MLN Medical College, Allahabad, U.P. India

Study Duration : June 2020 to June 2021.

Sample size : 200 patients

Subjects and selection method : The study population was drawn from consecutive non diabetic patients who presented to SRN Hospital, Prayagraj with and without IDA. Two groups were made- Case and Control, with 100 patients in each group. Baseline Hb, S.Ferritin, MCV, Recticulocyte count,RDW, HbA1c, FBS, PPBS were measured of both groups. Case group was supplemented oral iron and was followed for 3 months after which again same investigations were performed.

Case(n=100)- Non diabetic patients with IDA

Control(n=100)-Non diabetic patients without IDA

Inclusion criteria :

Patients who have been diagnosed to have iron deficiency anemia of any age group and both the sex (male and female) with Hb<11mg/dl in women and Hb<12mg/dl in men will be included in the study.

Exclusion criteria :

1. Patients with diabetes/IFG/IGT
2. Patients with chronic renal failure/ chronic liver disease
3. Patients with haemolytic anaemia and hemoglobinopathies
4. Chronic alcoholism
5. Known case of malignancy

Procedure methodology : After written informed consent was obtained, a well- designed questionnaire was used to collect the data of the recruited patients. A detailed history was recorded along with complete clinical examination as in the proforma. Samples were collected from all the participants to estimate complete blood count, blood urea, serum creatinine, serum electrolytes, blood sugar- FBS/PPBS, HbA1c level, anemia profile including serum ferritin, Serum Iron, TIBC levels, based on standard tests available in our hospital. In addition, ECG, chest X-ray and ultrasonogram abdomen were done in necessary cases. The final data was entered onto Microsoft excel sheet 2007 version**.** Patient will be investigated for-

- CBC (by five part method through autoanalyzer).
- Reticulocyte Count (by Supra vital Staining & Manual Counting).
- A1c (by Ion Exchange High Performance Liquid Chromatography, BioRad).
- Total Iron Binding Capacity (by Chemiluminescent Immunoassay)
- Serum Iron (Spectrophotometry)
- Serum Ferritin (by Chemiluminescent Immunoassay).

Patients in the case group were supplemented with oral iron according to their individual need and tolerance to oral iron for a period of 3 months and again required investigations were done at the end of three months. Patients having severe anemia were transfused PRBC after assessing their sign and symptoms. All the above investigations were repeated after 3 months of the case group.

Statistical analysis :

Data was analysed using SPSS (version 22). Non-parametric tests (Wilcoxon-Mann-Whitney U Test) were used to ascertain the significance of difference between the mean values of two continuous variables. Chi-squared test was used to explore the association between 'Group' and 'Gender' and 'Group' and 'HbA1c (Baseline)'. Stuart-Maxwell test was used to assess the change in Anemia between the two time points. A

significant result denotes that the distribution of patients in terms of Anemia changed significantly over time. Paired Wilcoxon test was used to explore the difference in Hb, S.Ferritin, MCV, Recticulocyte count, RDW, HbA1c at the two time points. McNemar's test was used to assess the change in HbA1c between the two time points.

III. Result

Total 200 Non-diabetic participants enrolled for the study. Out of 200 patients 100 had Iron deficiency anemia and formed the case group and rest 100 patients had no anemia and formed the control group for the study. All the participitants in the case group were treated with oral iron or blood transfusion according to the sign and symptoms of the patients and were followed for 3 months after which again required investigations were done.

Table 1: Association between baseline characteristics of Case and Control

Parameters	Group		p value
	Case(Anemic) (n = 100)	Control(Non- Anemic) (n = 100)	
Age (Years)* **	28.12 ± 9.80	30.82 ± 8.25	0.003[1]
Gender* **			<0.001[2]
Male	30 (30.0%)	64 (64.0%)	
Female	70 (70.0%)	36 (36.0%)	
Hemoglobin (g/dL) (Baseline)* **	6.68 ± 1.78	13.20 ± 0.87	<0.001[1]
Anemia (Baseline)			
Absent (>11g/dl)	0 (0.0%)	100 (100.0%)	
Mild (9-11)g/dl	9 (9.0%)	0 (0.0%)	
Moderate (7-8.9)g/dl	37 (37.0%)	0 (0.0%)	
Severe (<7)g/dl	54 (54.0%)	0 (0.0%)	
MCV (fL) (Baseline)* **	69.38 ± 4.34	85.85 ± 4.12	<0.001[1]
S.Ferritin (ng/dL) (Baseline)* **	48.08 ± 22.99	327.55 ± 106.70	<0.001[1]
Retic Count (%) (Baseline)	1.28 ± 0.57	1.27 ± 0.34	0.424[1]
RDW (%) (Baseline)* **	15.34 ± 1.35	13.47 ± 1.18	<0.001[1]
S. Iron (µg/dL) (Baseline)	57.77 ± 17.83	-	-
TIBC (µg/dL) (Baseline)	422.60 ± 59.87	-	-
HbA1c (%) (Baseline)* **	5.30 ± 0.49	5.11 ± 0.38	0.001[1]
HbA1c (Baseline)* **			0.003[2]
<5.7%	78 (78.0%)	93 (93.0%)	
≥5.7%	22 (22.0%)	7 (7.0%)	
FBS (mg/dL) (Baseline)	90.99 ± 7.53	91.97 ± 8.79	0.535[1]
PPBS (mg/dL) (Baseline)	143.04 ± 8.62	143.98 ± 8.49	0.561[1]

****Significant at p<0.05, 1: Wilcoxon-Mann-Whitney U Test, 2: Chi-Squared Test*

Table 2: Degree of Anemia in case and control group (Baseline) (n = 200)

The 0.0% of the participants in the Case had Anemia (Baseline) absent and 9.0% of the participants in the Case had mild anemia (Baseline), 37.0% of the participants in the group Case had moderate anemia (Baseline), 54.0% of the participants in the group Case had severe anemia (Baseline). 100.0% of the participants in the Control group had no Anemia (Baseline)

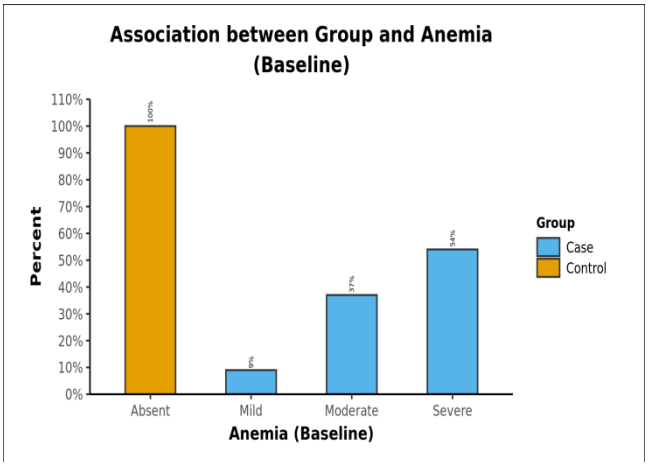

Table 3: Comparison of the Case and Control Group in Terms of HbA1c (%) (Baseline) (n = 200)

The mean (SD) of HbA1c (%) (Baseline) in the Case group was 5.30 (0.49). The mean (SD) of HbA1c (%) (Baseline) in the Control group was 5.11 (0.38). The HbA1c (%) (Baseline) in the Group: Case ranged from 3.7 - 6.3. The HbA1c (%) (Baseline) in the Group: Control ranged from 4.2 - 5.9.
There was a significant difference between the 2 groups in terms of HbA1c (%) (Baseline) (W = 6379.500, p = 0.001), with the median HbA1c (%) (Baseline) being highest in the Case group

Table 4: Comparision between Case and Follow up

Parameters	**Group**		**p value**
	Case (n = 100)	**3 month follow up (n = 100)**	
Hemoglobin (g/dL)* **	6.68 ± 1.78	8.86 ± 1.32	<0.001[1]
Anemia * **			<0.001[3]
Absent(>11g/dl)	0 (0.0%)	8 (8.0%)	
Mild(9-11)g/dl	9 (9.0%)	33 (33.0%)	
Moderate(7-8.9)g/dl	37 (37.0%)	56 (56.0%)	
Severe(<7g/dl)	54 (54.0%)	3 (3.0%)	
MCV (fL)* **	69.38 (4.34)	74.10 (3.42)	<0.001[1]
S.Ferritin (ng/dL)* **	48.08 (22.99)	138.28 (31.59)	<0.001[1]
Reticulocyte Count (%)	1.28 (0.57)	1.43 (0.30)	<0.001[1]
RDW (%) * **	15.34 (1.35)	13.94 (0.83)	<0.001[1]
A1c (%) * **	5.30 (0.49)	5.28 (0.44)	0.214[1]
A1c* **			0.003[2]

<5.7 %	78 (78.0%)	87 (87.0%)	
≥5.7 %	22 (22.0%)	13 (13.0%)	

****Significant at p<0.05, 1: Wilcoxon-Mann-Whitney U Test, 2: McNemar's Chi-Squared Test, 3:* Stuart-Maxwell Chi-Squared test

Table 5: Assessment of change in Hemoglobin (g/dL) over time

Timepoint	Hemoglobin (g/dL)			Wilcoxon Test	
	Mean (SD)	Median (IQR)	Range	V	P Value
Baseline	6.68 (1.78)	6.60 (2.52)	2.80 - 10.50	0.0	<0.001
3-Months	8.86 (1.32)	8.60 (1.60)	5.50 - 12.00		
Absolute Change	2.18 (1.20)	2.00 (1.50)	0.00 - 6.00		
Percent Change	40.3% (37.8)	29.8% (29.7)	0% - 180%		

The mean Hemoglobin (g/dL) increased from a minimum of 6.68 at the Baseline time point to a maximum of 8.86 at the 3-Months time point. This change was statistically significant (Wilcoxon Test: V = 0.0, p = <0.001).

Table 6: Change in Anemia Over 3 months in case group

Anemia		Baseline					Stuart-Maxwell test	
		Absent	Mild	Moderate	Severe	Total	$\chi2$	P Value
3-Months	Absent	0 (0.0%)	4 (4.0%)	4 (4.0%)	0 (0.0%)	8 (8.0%)	78.403	<0.001
	Mild	0 (0.0%)	5 (5.0%)	24 (24.0%)	4 (4.0%)	33 (33.0%)		
	Moderate	0 (0.0%)	0 (0.0%)	9 (9.0%)	47 (47.0%)	56 (56.0%)		
	Severe	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (3.0%)	3 (3.0%)		
	Total	0 (0.0%)	9 (9.0%)	37 (37.0%)	54 (54.0%)	200 (100.0%)		

The uncolored cells on the diagonal represent patients whose category did not change. The red shaded cells represent patients who moved to a lower category. The green shaded cells represent patients who moved to a higher category.

Stuart-Maxwell test was used to assess the change in Anemia between the two time points. A significant result denotes that the distribution of patients in terms of Anemia changed significantly over time.
The changes observed in Anemia over time were as follows:
The 4 (4.0%) patients moved from the category mild anemia to the category Absent. 4 (4.0%) patients moved from the category moderate anemia to the category Absent. 24 (24.0%) patients moved from the category moderate anemia to the category Mild anemia. 4 (4.0%) patients moved from the category severe anemia to the category Mild. 47 (47.0%) patients moved from the category severe anemia to the category Moderate anemia.
The overall change in Anemia was statistically significant (Stuart-Maxwell test: $\chi2 = 78.403$, p = <0.001).

Table 7: Assessment of change in MCV (fL) over time

The mean MCV (fL) increased from a minimum of 69.38 at the Baseline timepoint to a maximum of 74.10 at the 3-Months timepoint. This change was statistically significant (Wilcoxon Test: V = 1.5, p = <0.001).

Table 8: Assessment of change in S.Ferritin (ng/dL) over time

The mean S.Ferritin (ng/dL) increased from a minimum of 48.08 at the Baseline timepoint to a maximum of 138.28 at the 3-Months timepoint. This change was statistically significant (Wilcoxon Test: V = 1.0, p = <0.001).

Table 9: Assessment of change in Reticulocyte Count (%) over time

The mean Reticulocyte Count (%) increased from a minimum of 1.28 at the Baseline time point to a maximum of 1.43 at the 3-Months time point. This change was statistically significant (Wilcoxon Test: V = 923.5, p = <0.001).

Table 10: Assessment of change in RDW (%) over time

The mean RDW (%) decreased from a maximum of 15.34 at the Baseline time point to a minimum of 13.94 at the 3-Months time point. This change was statistically significant (Wilcoxon Test: V = 4532.0, p = <0.001).

Table 11: Assessment of change in A1c (%) over time

The mean HbA1c (%) decreased from a maximum of 5.30 at the Baseline time point to a minimum of 5.28 at the 3-Months time point. This change was not statistically significant (Wilcoxon Test: V = 1152.5, p = 0.214).

Table 12: Change in HbA1c Over Time

HbA1c		Baseline			McNemar's test	
		<5.7%	≥5.7%	Total	$\chi 2$	P Value
3-Months	<5.7%	78 (78.0%)	9 (9.0%)	87 (87.0%)	9.000	0.003
	≥5.7%	0 (0.0%)	13 (13.0%)	13 (13.0%)		
	Total	78 (78.0%)	22 (22.0%)	100 (100.0%)		

The uncolored cells on the diagonal represent patients whose category did not change. The red shaded cells represent patients who moved to a lower category. The green shaded cells represent patients who moved to a higher category.

The changes observed in HbA1c over time were as follows:

9 (9.0%) patients moved from the category HbA1c: ≥5.7% to the category <5.7%.

The overall change in HbA1c was statistically significant (McNemar's test: $\chi 2 = 9.000$, p = 0.003).

IV. Discussion

Epidemic of Diabetes is rising in world. Many indiviuals with type 2 Diabetes Mellitus have one or more diabetes specific complications at time of their diagnosis, so an early diagnosis can help in prevention of long term complications of diabetes mellitus by timely intervertion. According to International Expert Committee A1c can be used for screening and diagnosis of Diabetes Mellitus. The WHO also agreed that HbA1c may be used to diagnose diabetes, with appropriate measures i.e. standardized assay, calibration against IFCC standards and low coefficient of variability as it provides a better estimate of average glycemic control than routine determinations of blood glucose concentration and is the most widely used index of chronic glycaemia. A1c level of more than 6.5% is used as a cut-off point to diagnose diabetes and A1c level of 5.7% to 6.4% is considered impaired glucose tolerance.

Many studies have showed that A1c levels are altered by structural hemoglobinopathies, mean age of RBCs in the circulation and thus, A1c can give a wrong estimate of glycemic state of patient that can effect the diagnosis. IDA can increase the red blood cell turnover which can increase glycation of Hb leading to higher A1c values. Higher iron levels modulate both the action and secretion of insulin and lower the iron levels, higher is the glycation of A1c leading to its false-high values in diabetic as well as non-diabetic individuals. Iron

deficiency anemia is the most common form of hypoproliferative anemia encountered in clinics all over the world. Many studies have concluded that HbA1c levels can be falsely high in patients with IDA and decreased significantly after iron supplementation. AK Varshney et al[(11)] studied on effect of Iron Supplementation on Glycosylated Haemoglobin in Non-Diabetic Individuals with Iron Deficiency Anaemia on 100 patients and concluded 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.

To further strengthen the correlation, an observational study was carried out that included a total of 200 non diabetic patients. Cases were 100 with IDA and 100 were control without IDA. My study showed that anemia is more common in females as 70.0% of the participants in case group were female and only 36.0% of the participants in control group were female. The mean (SD) of Hemoglobin (g/dL) (Baseline) in Case group was 6.68 (1.78) and in the Control group was 13.23 (0.87). 9.0% of the participants in Case had Mild Anemia, 37.0% had Moderate Anemia and 54.0% had Severe Anemia. Baseline mean(SD) of MCV, S.ferritin, Reticulocyte count and RDW in case group was (69.38 (4.34) ; 48.08 (22.99) ; 1.28 (0.57) ; 15.34 (1.35)) and in control was (85.85 (4.12) ; 327.55 (106.70) ; 1.27 (0.34) ; 13.47 (1.18)). Study showed that the mean Hb, MCV, S.ferritin, were significantly lower in IDA group compared to the control group ($P < 0.05$), RDW was significantly higher ($p<0.001$) wheras mean of Reticulocyte count was insignificantly higher in case than in control group ($p= 0.424$). The mean (SD) of A1c (%) in the Case group was 5.30 (0.49) and in the Control group was 5.11 (0.38). There was a significant difference between the 2 groups in terms of A1c (%) (Baseline) ($W=6379.500$, $p = 0.001$).

After 3 months of follow up of Case group with iron supplementation, the mean Hb, MCV, S.Ferritin, Reticulocyte count and RDW were (8.86(1.32) ; 74.10(3.42) ; 138.28(31.59) ; 1.43(0.30) ; 13.94(0.83)) and this change from baseline mean was significant($P < 0.05$). The mean HbA1c (%) decreased from a maximum of 5.30(0.49) at the Baseline time point to a minimum of 5.28(0.44) at the 3-Months time point. This change was not statistically significant ($V= 1152.5$, $p = 0.214$). The changes in HbA1c over time were 9 (9.0%) patients moved from the category HbA1c: ≥5.7% to the category <5.7%. My study favoured many past studies and concluded that Iron deficiency anemia has to be kept in mind before using the A1c to diagnose diabetes.

V. Conclusion

Hemoglobin and A1c showed statistically significant negative correlation in patients with iron deficiency anemia. A1c was higher in IDA patients as compared with non anemic group. After correction of anemia, Hemoglobin and A1c showed statistically insignificant negative correlation. Hence IDA should be kept in mind while using A1c to diagnose diabetes.

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