Original Article

DOI: 10.21276/APALM.1276

A comparison of Effect of Iron Deficiency Anemia on HbA1c Levels in Controlled Diabetics and Non-diabetics: A Cross Sectional Analysis of 300 Cases

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ABSTRACT

Background: Diabetes Mellitus (DM) has become a major health problem worldwide. American Diabetes Association has considered HbA1C levels ≤ 6.5 % as the prime target for glycemic control and as a diagnostic criterion for DM. Anemia is common in DM (8-66%). Studies on alteration of HbA1C in IDA have conflicting results.

Objectives: To identify and compare the effect of IDA on HbA1C levels among controlled diabetics (Fasting plasma glucose (FPG) <126mg/dl since last 6 months) and non-diabetics and its variation according to the degree of anemia.

Methods: This cross-sectional study done in SRM Medical College Hospital and Research Centre, Chennai includes 150 Controlled diabetics (75 with IDA and 75 without IDA) and 150 non diabetics (75 with IDA and 75 without IDA). HbA1C, complete hemogram, iron profile and FPG were tested. Medical history was recorded.

Results: The mean HbA1C in controlled diabetics with and without IDA were 8.81±0.13 & 5.79±0.01 respectively (P<0.05) and in non-diabetics with and without IDA were 6.84±0.07 & 5.12±0.04 respectively (P<0.05). The difference between no, mild, moderate and severe anemia in both diabetics and non-diabetics was statistically significant (p< 0.05). Mean HbA1C% was highest in groups with severe anemia.

Conclusion: IDA falsely elevates HbA1C level independent of blood glucose concentration in both controlled diabetics and non-diabetics. Hence prior to alteration of treatment regimen based on HbA1C for diabetes, IDA should be diagnosed and corrected. Concurrent evaluation for anemia is critical to correctly interpret glycemic status in Indian population with prevalent IDA.

Keywords: *HbA_1C, Iron deficiency Anemia, Diabetics, Non-diabetics*

Introduction

Diabetes has become a major health problem worldwide with an estimated 300 million people to be diagnosed with the disease in the next 10 years and 370 million by 2030.[1] In 2010, American Diabetes Association (ADA) has considered HbA_1C levels as the prime target for glycemic control and as a diagnostic criterion for Diabetes Mellitus (DM). $HbA_1C \geq 6.5\%$ has been established for the diagnosis of DM for its high specificity and certified by the World Health Organization (WHO) in 2011.[1,2,3] Anemia is common among DM and its incidence ranges from 8-66%.[4] Several mechanisms have been discussed about the association of anemia in DM and its complications like nephropathy.[5,6]

Initially it was believed that HbA_1C was affected only by blood glucose levels.[4,5] However certain study results have proven that HbA1C levels are altered by various other co-existing factors, along with DM, especially that of IDA which is a major public health problem in developing countries like India. Other factors interfering with HbA_1C levels are hemoglobinopathies, renal impairment, pregnancy, hypothyroidism, hemolytic anemia etc. Christy AL et al reported that controlled plasma glucose levels for last 3 months correlates well with controlled HbA_1C (< 6.5%).[7]

One of the well-known pathological ill effects of IDA in the biological system is the glycosylation of proteins.[8] Although different studies have been carried out to analyze the influence of IDA on HbA1C levels in both diabetic and non-diabetic population individually, only very few studies have been conducted comparing HbA_1C variation in both these groups. Due to the variation in the results of multiple studies, we decided to investigate and compare the effects of IDA on HbA_1C levels in both controlled diabetic and non-diabetic Indian adults.

Materials and Methods

In this cross-sectional study, we collected data of 1360 individuals aged >18years who consulted our SRM

Medical College Hospital and Research Centre, Chennai, Tamil Nadu between February 2016 to November 2016.

Of the 1360 persons enrolled in this study, 230 were diabetics and 1130 were non-diabetics. After exclusion of individuals with hypothyroidism, renal insufficiency (elevated serum urea, creatinine), hemoglobinopathies, Pregnancy, Fasting plasma glucose (FPG) >126 mg/dl, hemolytic anemia and those who completed laboratory investigations, 170 controlled diabetics (FPG level is <126 mg/dl since last 6 months) and 365 non diabetics were finalized for our study.

Among 170 controlled diabetics, 95 were anemic (56%) out of which 75 were diagnosed as IDA and others were excluded. Among 365 non-diabetics, 90 were anemic. Out of 90 nondiabetics with anemia, 75 had IDA and others were excluded. Patients with IDA were chosen by their hemoglobin level (Hb <13gm% in males and <12gm% in females) based on definition of WHO and with predominantly microcytic indices [Mean Corpuscular Volume (MCV) < 76 fl], hypochromic indices [Mean corpuscular Hemoglobin (MCH) < 27 pg/cell] and [Mean Corpuscular Hemoglobin Concentration (MCHC) < 32 g/dl], and microcytic hypochromic picture in peripheral smear. This is later confirmed by low serum iron (< 59 µg/dl in males and < 37 µg/dl in females) & low serum ferritin (< 15 ng/ml in males and <9 ng/ml in females).[9]

Age, Sex and FPG matched controls were selected for each group and the data results were analyzed. Blood samples from both groups were tested for FPG, HbA_1C, Hb, RBC count, Hct, MCV, MCH, MCHC, Serum iron and Ferritin. Medical history was recorded

On the basis of hemoglobin level, anemic patients were further categorized as Mild anemia (male 12-12.9 gm/dl and female 11-11.9gm/dl), Moderate anemia (male 9-11.9 gm/dl and female 8-10.9 gm/dl) and Severe anemia (male <9 gm/dl and female <8 gm/dl).

Measurements: Hb, MCV, MCH, MHC were estimated by SYSMEX XT-1800i analyzer. HbA_1C estimated by HPLC method in Bio-Rad D10 analyzer. Glucose oxidase / peroxidase method for plasma glucose and serum iron (TPTZ method), serum ferritin (Bio-Rad Quanimune Ferrin IRMA, Bio-Rad lab)

Statistical Analysis: The data are presented as mean ±S.D for continuous variables. Group means were compared by student t-test. Pearson's co-efficient of correlation was used to determine the correlation between two variables. p value < 0.05 was considered significant.

Results

The mean HbA_1C in controlled diabetics with and without IDA were 8.81±0.13 & 5.79±0.01 respectively and in non-diabetics with and without IDA were 6.84±0.07 & 5.12±0.04 respectively. This result show that the mean HbA_1C was higher in those with IDA than in those without IDA in both diabetics and non-diabetics which was statistically significant ($p < 0.05$) in both groups. [Table1/ Fig 1]. The mean Hb, Hct, MCV, MCH & MCHC in controlled diabetics with and without IDA were statistically significant p (<0.05). [Table 2]

Among controlled diabetics, 11 had mild anemia with mean HbA_1C % of 7.40±0.70, 39 had moderate anemia with mean HbA_1C % of 8.50±0.11 and 25 had severe anemia with mean HbA_1C % of 9.15±0.17. Similarly in non-diabetics, 40 had mild anemia with mean HbA_1C% of 6.57±0.09%, 28 had moderate anemia with mean HbA_1C % of 7.19±0.11 and 7 had severe anemia with mean HbA_1C% of 8.31±0.01%.

The mean serum iron and ferritin levels in controlled diabetics with and without IDA were 32.68±0.71 µg/dl,10.06±0.79 µg/l and 75.26±0.79 µg/dl, 45.19±1.11 µg/l respectively which was statistically significant ($p < 0.05$). The mean serum iron and ferritin levels in non-diabetics with and without IDA were 42±0.61 µg/dl, 12.09±1.21 µg/l and 74±0.32 µg/dl, 41.06±0.43 µg/l respectively which was statistically significant ($p < 0.05$).

The difference was statistically significant ($p< 0.05$) between no, mild, moderate and severe anemia in both controlled diabetics and non-diabetics and moreover mean HbA_1C% was higher in groups with severe anemia in relation to HbA_1C levels in patients without IDA. [Table 3/Fig 2]

TABLE 1: COMPARISON OF HbA1C% BETWEEN ANEMIC AND NOT ANEMIC IN CONTROLLED DIABETICS & NONDIABETICS.

	Parameters	IDA	Not anemic	T test	P value
Controlled diabetics	HbA_1C %	8.81 ± 0.13	5.79 ± 0.01	23.974	< 0.05
Non-diabetics	HbA_1C %	6.84 ± 0.07	5.12 ± 0.04	22.219	< 0.05

TABLE 2: Comparison of Red Cell Indices Between Anemic and Not Anemic in Controlled Diabetics & Nondiabetics

PARAMETERS	SUBJECTS	IDA	NOT ANAEMIC	T TEST	P VALUE
Hb (g/dl)	Controlled diabetics	9.22 ± 0.23	13.83 ± 0.13	-17.483	< 0.05
	Non diabetics	11.46 ± 0.08	14.31 ± 0.16	-16.078	< 0.05
Hct (%)	Controlled diabetics	31.35 ± 0.71	41.61 ± 0.45	-12.221	< 0.05
	Non diabetics	37.07 ± 0.29	42.20 ± 0.47	-9.328	< 0.05
MCV (fl)	Controlled diabetics	66.79 ± 1.53	87.40 ± 0.51	-12.791	< 0.05
	Non diabetics	78.56 ± 0.22	86.19 ± 0.59	-3.425	< 0.05
MCH (pg/cell)	Controlled diabetics	23.88 ± 0.50	29.04 ± 0.14	-9.910	< 0.05
	Non diabetics	27.17 ± 0.33	29.75 ± 0.21	-6.511	< 0.05
MCHC	Controlled diabetics	29.29 ± 0.29	32.72 ± 0.08	-11.409	< 0.05
	Non diabetics	29.29 ± 0.29	32.72 ± 0.08	-11.409	< 0.05

TABLE 3: HbA_1C Variation According to The Degree of Anemia

DEGREE OF ANEMIA	CONTROLLED DIABETICS		NONDIABETICS	
	NUMBER	MEAN HBA_1C%	NUMBER	MEAN HBA_1C%
NO	75	5.79 ± 0.01	75	5.16 ± 0.04
MILD	10	7.40 ± 0.70	40	6.57 ± 0.09
MODERATE	40	8.50 ± 0.11	30	7.19 ± 0.11
SEVERE	25	9.15 ± 0.17	5	8.01±0.01

CONTROLLED DIABETICS

ANOVA – 202.613, p – 0.0001
No anemia to mild anemia, T test: -7.591, p – 0.0001
Mild anemia to Moderate anemia, T test: -3.306, p – 0.001
Moderate anemia to Severe anemia, T test: -5.805, p – 0.0001

NON DIABETICS

ANOVA - 229.815, p – 0.0001.
No anemia to mild anemia, T test: -14.323, p – 0.0001
Mild anemia to Moderate anemia, T test: -4.483, p – 0.0001
Moderate anemia to Severe anemia, T test: -3.397, p – 0.0001

TABLE 4: Comparison of Present Study Hba_1c Levels with Previous Studies Having Similar Results [Diabetics]

Study	Year	Glycemic status	Number screened	IDA	Not-anemic	Significance
Tarim et al[11]	1999	DM	37	10.1±2.7	8.2±3.1	p < 0.05
Ng et al[13]	2010	DM	15	7.4±0.2	6.9±0.1	p < 0.05
Christy et al[15]	2014	DM	120	6.8±1.4	5.6±0.6	p < 0.05
Present study	2016	DM	150	8.8±0.1	5.7±0.01	p < 0.05

TABLE 5: Comparison Of Present Study Hba_1c Levels with Previous Studies Having Similar Results [Non-Diabetics]

Study	Year	Number screened	IDA	Not-anemic	Significance
El-Agouza et al[6]	2002	81	6.1±0.6	5.2±0.4	p < 0.05
Coban et al[12]	2004	100	7.4±0.8	5.9±0.5	p < 0.05
Shanthi et al[11]	2013	100	7.6±0.5	5.5±0.8	p < 0.05
Silva et al[14]	2015	122	5.6±0.4	5.3±0.4	p < 0.05
Present study	2016	150	6.8± 0.07	5.1± 0.04	p < 0.05

TABLE 6: Previous Studies with Contradicting Hba$_1$c Results from The Present Study [Non-Diabetics]

Study	Year	Glycemic status	Number screened	IDA	Not-anemic	Significance
Ford ES et al[17]	2011	NDM	8296	5.5±0.1	5.4±0.2	p > 0.05
Sinha et al[18]	2012	NDM	100	4.6±0.6	5.5±0.6	p > 0.05
Kalasker et al[19]	2014	NDM	80	5.9±0.4	6.5±0.3	p > 0.05

TABLE 7: Comparison of Study Results Showing Hba$_1$c Variation According to The Degree of Anemia

Study	Year	Number screened	Degree of Anemia				Significance
			No	Mild	Moderate	Severe	
Silva et al[20]	2015	122	5.3±0.40	5.5±0.40	5.6±0.40	5.7±0.40	P < 0.05
Present study	2016	150	5.1±0.04	6.5±0.09	7.1±0.11	8.0±0.01	P < 0.05

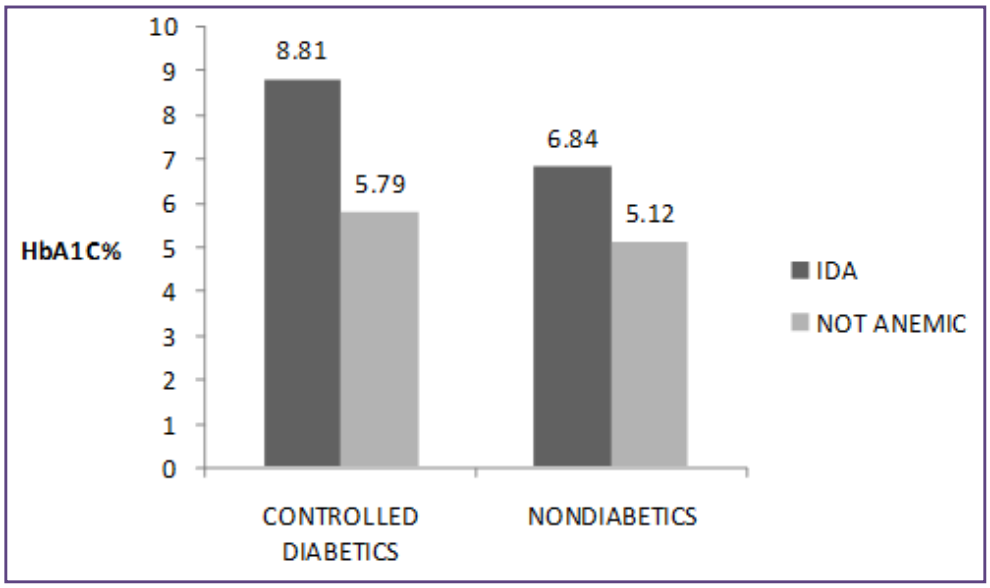

Fig. 1: Comparison of Hba1c% in Study Groups with and Without Anemia.

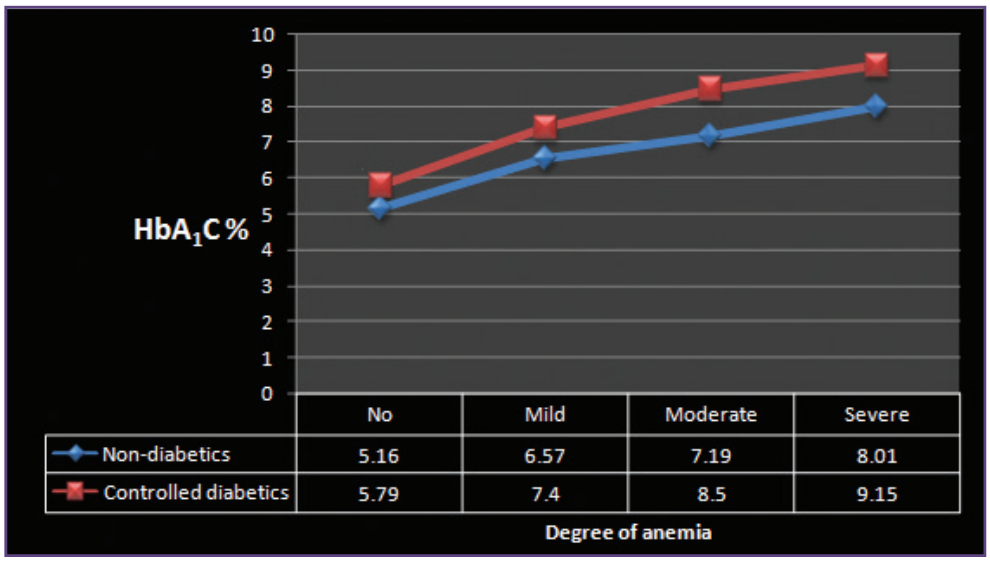

Fig 2:HbA^{1}C Variation in Mild, Moderate And Severe Anemia.

Discussion

Glycated hemoglobin reflects the glycemic status when monitored over 3 months and predicts the risk of long term complications in diabetics. Glycation of the NH2 terminal valine residue of the β-chain of hemoglobin results in the formation of HbA_1C. It also identifies individuals who are at high risk for developing diabetes.[10] According to many study results, anemia is almost twice common in diabetics than non-diabetics.[4,11,12] Apart from blood glucose, HbA1c levels can be affected by factors unrelated to diabetes like IDA.[13]

Increased HbA_1C levels in IDA were explained by a) Quaternary structure of hemoglobin is altered leading to rapid glycation of globin chain .[14,15] b) Increase in the glycated fraction of hemoglobin due to decrease in total hemoglobin at a constant glucose level occurs because HbA_1C is measured as a percentage of total Hemoglobin A.[16] c) higher average age of circulating erythrocytes noticed in IDA due to reduced red cell production lead to increased HbA_1C levels.[14]

Anemia is a major risk factor for cardiovascular complications and diabetic retinopathy in diabetes. But only very few studies has investigated whether IDA alter the value of HbA_1C till now inspite being widely used as a diagnostic tool for DM . Thus leading to over or under diagnosis of DM when diagnosed based on the cutoff value < 6.5% of HbA1C as approved by ADA.[4,5,17]

The results of this study show that HbA_1C levels are spuriously elevated in the presence of IDA independent of blood glucose concentration in both controlled diabetics and non-diabetics. Our finding confirms the study results of Tarim et al, who reported HbA_1C level is elevated in diabetics with IDA than with iron-sufficient controls. This may be explained by iron deficiency related changes in the quaternary structure of hemoglobin molecule increasing the glycation of globin chain.[18] This result also coincides with the study results of Christy et al who also observed that HbA_1C levels were significantly higher in IDA patients and decreased after treatment with iron.[7] Ng et al concluded that iron and Erythropoietin stimulating agents in diabetic patients cause a significant fall in HbA1C values without a change in glycemic control.[19] [Table 4]

Our results contradicts with the study results of Sharifi et al, who reported that there was no correlation between serum iron , serum ferritin and HbA1c in diabetic patients of either sex. Ferritin levels in patients with DM is high, but not related to levels of HbA1c and blood glucose control.[20] Ford ES et al concluded that there was no evidence of difference in the relationship between fasting glucose and HbA1c when groups of anemic and non-anemic diabetic individuals with and without iron deficiency when examined individually.[21]

Our study results are also consistent with the study done by El-Agouza et al in non-diabetics who reported that a decline in the Hb level might lead to increase in the glycated fraction at a fixed glucose level, because HbA_1C is measured as a percentage of total Hb.[16] Our results were also in concordance with the study results of Shanthi et al, Coban et al, and Silva et al.[15,22,23] Coban et al showed a very large difference between HbA_1C levels in non-diabetic patients with and without IDA.[22] Shanthi et al conducted study in non –diabetics and reported that iron deficiency was associated with higher proportions of HbA1C and suggested that iron status must be considered during the interpretation of the HbA1c concentrations in Diabetes mellitus.[15] Silva et al reported that IDA affects HbA1c levels and causes spurious increase in their results. Although these upward changes in HbA1c values are statistically significant, they may be not clinically relevant when the overall variability of the HbA1c test is considered. This effect is dependent on anaemia degree and the presence of mild anaemia is likely to have a minor effect on HbA1c levels.[23] [Table 5]

Studies by Ford ES et al reported no significant difference in mean HbA1C concentration according to the IDA status as well as before and after iron treatment.[21] Sinha et al and Kalaskar et al contradicts with our results reporting that HbA_1C levels are lowered in IDA.[13] [Table 6]

Also Saudek et al suggested that red cell age was unlikely to be a significant factor in explaining the changes in HbA_1C levels during the treatment of IDA and believed that the reported differences in HbA_1C concentrations before and after iron supplementation were due to differences in the laboratory methods used for measuring HbA_1C.[24] Ferritin is a storage form of iron, and it reflects the true iron status [WHO]. In our study, serum ferritin as well as serum iron level was indirectly proportional to HbA_1C. As explained previously, in IDA, ferritin is decreased with increase in the red cell life span which is associated with increased HbA_1C. This goes in hand with other study results of Shanthi et al and Raj et al.[15,25]

We also analyzed HbA_1c results in different degrees of anemia and found that HbA_1C level increases as severity

of anemia worsens. This result of ours was in accordance with the results of Silva et al.[23] [Table 7]

Because the above studies were performed mostly in subjects mostly in individuals without diabetes, they could not conclude whether the presence of IDA affect HbA_1C level at the cutoff point of <6.5% vs ≥6.5% the newly recommended diagnostic cutoff point for diabetes by the ADA. This study has few limitations. They are small sample size and the results were obtained from a single centre and with a cross-sectional design, we couldn't follow up after iron therapy.

Conclusion

Presence of IDA spuriously elevates HbA_1C level independent of blood glucose concentration in both diabetics and non-diabetics. Hence prior to alteration of treatment regimen based on HbA_1C for diabetes, IDA should be diagnosed and corrected. Also this study suggests concurrent measurement of iron, Hb, HbA_1C is critical to correctly interpret glycemic status in Indian population where IDA is highly prevalent.

Acknowledgement

I would like to thank our Dean Dr. A. Sundaram, SRM Medical College Hospital and Research Centre for his support throughout the study. Am thankful to all the participating people for their cooperation.

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Financial or other Competing Interests: None.

Date of Submission : 10.01.2017
Date of Acceptance : 30.01.2017
Date of Publication : 15.04.2017