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Research Article

TO STUDY THE EFFECT OF IRON DEFICIENCY ON HbA1c IN NON DIABETICS IN BUNDELKHAND REGION

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ABSTRACT

Introduction: It is important to ascertain effect of iron deficiency on HbA1c levels since iron deficiency is the most common nutritional deficiency in world as well as in India. Its prevalence is especially higher in economically backward areas like Bundelkhand region, where, coexistence of iron deficiency with diabetes mellitus may limit the utility of HbA1c as the sole parameter for assessment of long term glycemic control in diabetics.
Aim: To study the effect of iron deficiency on HbA1c levels in non-diabetics.
Materials & Methods: We conducted this study on 100 randomly selected non-diabetic patients with confirmed iron deficiency anemia who were compared with 100 randomly selected age and sex matched healthy adults without diabetes mellitus and Iron deficiency anemia. This was a prospective case control observational study done during a period of 18 months (from March 2018 to September 2019).
Results: The mean±SD value of HbA1c among iron deficiency patients (study group) was found to be significantly higher (5.24±0.41) as compared to control group (4.76±0.60).
Conclusion: We concluded that iron deficiency must always be taken into accountbefore making any decision on glycemic control in patients of DM on the basis of HbA1c level alone.

INTRODUCTION

Deficiency of iron is the most common nutritional disorder in the world as well as in India[1] and results in clinical signs and symptoms that are mostly related to inadequate hemoglobin synthesis.

The term HBA1C refers to glycosylated haemoglobin. It develops when haemoglobin, a protein within red blood cells that carries oxygen throughout the body, joins with glucose non-enzymatically in the blood, becoming 'glycosylated'. Glycosylated haemoglobin is produced by a ketoamine reaction between glucose and the N-terminal valine of both β-chains of the haemoglobin molecule[2].

HbA1c is approved and widely used test for monitoring of long term (over past 2-3 months) glycemic control of diabetic patients[3] ,but it has long been debated as to what other factors apart from blood sugar affect its levels, one of which is Iron Deficiency ,also it is important to ascertain effect of iron deficiency on HbA1c levels since iron deficiencyanemia is the most common nutritional deficiencyanemia in the world as well as in India[1]. Its prevalence is especially higher in economically backward areas like Bundelkhand region, where, its coexistence with diabetes mellitus may limit the utility of HbA1c as the sole parameter for assessment of long term glycemic control in diabetics.

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Whatever its basis, iron deficiency produces a microcytic hypochromic anemia[4]. In peripheral bloodsmears[4],the red cells are small (microcytic) and pale (hypochromic).

Poikilocytosis in the form of small, elongated red cells (pencil cells) is also characteristically seen.

Aims & Objectives

- To study the effect of iron deficiency on HbA1c levels in non-diabetics.
- To study age and sex distribution of iron deficiency anemia in Bundelkhand region.

MATERIALS AND METHODS

We conducted this study in Department of Pathology, MLB Medical College, Jhansi with active collaboration from Department of Medicine, MLB Medical College, Jhansi on 100 randomly selected non-diabetic patients with confirmed iron deficiency anemia who were compared with 100 randomly selected age and sex matched healthy adults without diabetes mellitus and Iron deficiency anemia. This was a prospective case control observational study done during a period of 18 months (from March 2018 to September 2019).

The cases were included in the study on the basis of strict inclusion and exclusion criteria as mentioned below:

Inclusion Criteria

- Age > 18 yrs and ≤ 65 yrs.
- Presence of anemia as defined by WHO
  - Hb: <13.0 g/dl (adult males)
- <12 g/dl (non-pregnant women) [5]
- Microcytic, hypochromic picture in peripheral blood smear
- Serum ferritin:
  - <15 ng/ml (in both males and females) [5]
- Normal fasting and postprandial plasma glucose levels
- Normal blood urea, serum creatinine levels.

Exclusion Criteria

- Age < 18 yrs and > 65 yrs.
- Hemoglobinopathies
- Hemolyticanemia
- Chronic alcohol ingestion
- Chronic renal failure
- Pregnant females
- History of blood transfusion in the past 3 months.

All the patients enrolled in the study had normal FBS and PPBS levels, which confirmed their non-diabetic status. To exclude diabetes mellitus patients following criteria[6] were applied :-

HbA1C ≥ 6.5%
(even those patients who had HbA1c≥ 5.7% were excluded from our study to avoid confounding effect due to presence of Pre-Diabetes)
OR
FPG ≥126 mg/dl (7.0 mmol/I) (Fasting is defined as no calorie intake for at least 8 h).
OR
2-h plasma glucose ≥200 mg/dl (11.1 mmol/I) during an OGTT.
OR
In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥200 mg/dl (11.1 mmol/I).

An informed consent was taken from each patient. Detailed history and clinical examination of the patients were done. The study was conducted after approval from institutional thesis and ethical committee.

Medonic fully automated analyzer was used for the whole blood count; serum ferritin levels were measured by using Abbott hormone analyser (works on principle of chemiluminescence), and peripheral blood smears were examined in all the patients. The HbA1c levels were determined by Biorad D-10 analyser (works on principle of HPLC).

Patients with microcytic hypochromic pictures in peripheral smear, low hemoglobin levels of <13g/dl in males and <12g/dl in females, predominantly microcytic indices (MCV <83 fl or hypochromic indices (MCH < 27 pg) were considered to be suffering from iron deficiency anemia (IDA) and IDA was confirmed by serum ferritin levels <15 ng/ml in both males and females[5].

Statistical analysis: The clinical parameters were compared and analyzed using Pearson chi square method. The significance of difference between means in two groups was calculated using student's t-test and the significance of difference in proportions using chi-square test. All the statistical analysis has been done by using statistical software SPSS (version 22).

OBSERVATIONS AND RESULTS

The present study was conducted on 100 non-diabetic IDA patients, 18-65 yrs of age and 100 age and sex matched randomly selected normal healthy individuals were used as control. We recorded following observations:

Age distribution

The mean age distribution of the study population was found to be 28.97±9.32 yrs. Maximum patients were in 21-30 yrs. of age group (47%).

The age distribution of the control group was found to be matching that of the study group because the p-value was 0.2753, which is not significant i.e. the age distributions among the control and study groups were equal.

Table 1 Age wise distribution of control and study group

AGE (YEARS)	CONTROL GROUP		STUDY GROUP	
	NO	%	NO	%
≤20	13	13%	10	10%
21-30	47	47%	61	61%
31-40	16	16%	20	20%
41-50	13	13%	4	4%
51-60	11	11%	5	5%
> 60	0	0%	1	1%
Total	100		100	
Mean±SD	31.66±12.32		28.97±9.32	
p-value :	0.2753 (not significant)			

Sex distribution

Majority of the study subjects were females (88%) while the remaining 12% were males. It confirms the fact that iron deficiency anemia is more common in females. P-value was 1.000 which is not significant i.e. sex distributions among the control group and study group were equal.

Table 2 Sex distribution of control and study group

Sex	CONTROL GROUP		STUDY GROUP	
	No.	%	No.	%
Male	41	41%	12	12%
Female	59	59%	88	88%
Total	100	100%	100	100%
p-value	1.000 (not significant)			

Hb distribution

In study group, mean Hb was found to be 7.67±2.77 for males and 7.35±2.3 for females. The minimum haemoglobin level was found to be 2.0 g/dl and maximum was 11.8 g/dl.
We observed that 41.67% (5) males belonged to severe anemia category (Hb< 8 g/dl), same percentage (41.67%) were found

in moderate anemia category (Hb 8 to ≤10 g/dl) as well and 16.67% (2) males were found in the category of mild anemia (Hb 10 to < 13 g/dl).

Among females, majority (51.13%) of females (45) were found to be severely anaemic (Hb< 8 g/dl), followed by 35.22% (31) falling in moderate anaemia category i.e. (Hb 8 to ≤10 g/dl) and minimum 13.63% (12) females were found to be mildly anaemic (Hb 10 to < 12 g/dl).

Table 3 Hb distribution of study group(Males)

Hb (g/dl)	STUDY GROUP (Males)	
	NO.	%
< 8	3	25%
8 to <10	2	16.67
10 to < 13	7	58.38
Total	12	100%
Mean ±SD	9.4±3.69	

Table 4 Hb distribution of study group(Females)

Hb (g/dl)	STUDY GROUP (Females)	
	NO.	%
< 8	13	14.77%
8 to <10	44	50.00%
10 to < 12	31	35.23%
Total	88	100%
Mean ±SD	9.10±1.814	

HbA1c distribution

The mean±SD value of HbA1c among iron deficiency anemia patients (study group) was found to be 5.24±0.41 as compared to 4.76±0.60 among control group.

P value between control group and study group was found to be 0.0001 which is statistically highly significant.

Table 5 HbA1c distribution of control group and study group

HbA1c (%)	CONTROL GROUP		STUDY GROUP	
	NO.	%	NO.	%
< 3.5	4	4%	0	0%
3.5-4.5	27	27%	6	6%
4.6-5.6	69	69%	94	94%
> 5.6	0	0%	0	0%
Total	100	100%	100	100%
Mean ±SD	4.76±0.60		5.24±0.41	
p-value	0.0001 (highly significant)			

It indicates that HbA1c (%) was significantly higher among iron deficiency anemia patient (study group) as compared to control group.

DISCUSSION

Iron deficiency anemia is the most common nutritional deficiency anemia globally as well as in India. It is estimated that about 50% women and 25% men are affected by IDA in India[1].

HbA1c is approved and is widely used to assess glycemic control of past 2-3 months in diabetes mellitus patient. But many factors like IDA, macrocytic anemias, hemoglobinopathies, uremia etc. have been shown to affect HbA1c levels, resulting in falsely higher or lower values in DM patients[7]. IDA is especially concerning among these factors because of it being the commonest of all these factors in prevalence, especially in the diabetic patients , because India is said to be the diabetes capital of the world (Shendurnikaref, 2007).

Our study found that HbA1c (%) was significantly higher among iron deficiency anemia patients (study group)(5.24±0.41) as compared to control group(4.76±0.60). This could be possibly because of:

1. Alteration in the quaternary structure of the hemoglobin molecule in iron deficiency anemia [9]. This alteration results in increased level of glycosylation of the β-globin chain during iron deficient state.
2. Prolongation of life span of the red blood cells present in the circulation in IDA patients[7&17] resulting in higher HbA1c levels in non-diabetic iron deficiency anemia patients as compared to healthy control.

Comparison of Present Study with Previous Studies

Not many studies have been conducted on this topic, in fact, no study has been performed so far in Bundelkhand region. Whatever studies have been done, have yielded mostly variable and inconsistent results but majority of them have shown quite similar results to the present study, i.e. higher HbA1c levels in non-diabetic IDA patients as compared to healthy controls.

Few of these studies have used pregnant females and some have used controlled diabetics as their cases and majority of them found HbA1c levels to be higher in IDA patients. However, present study has excluded pregnant females and Diabetes mellitus patients.

In a similar study by **Brooks *et al.***[8],to assess HbA1c levels conducted on 35 non-diabetic patients having iron deficiency anemia both before and after treatment with iron, it was observed that HbA1c levels were significantly higher in iron deficiency anemia patients and decreased after treatment with iron. It was proposed that, in iron efficiency, the quaternary structure of the hemoglobin molecule was altered, and that glycation of the globin chain occurred more readily in the relative absence of iron. The findings were in agreement to the results of the present study.

Similar results were observed by **Sluiter*et al***[9]***and Coban et. al.***[10], and they postulated that this could be possibly because of decreased RBC production leading to a longer life span of RBCs present in the circulation in patients of iron deficiency anemia. After iron supplementation, HbA1c level decreased, attributable to shorter life span of RBCs post treatment.

Coban et. al.[10], alsofound that mean HbA1c levels decreased significantly after treatment with 100 mg/day iron for 3 months. They recommended that Iron deficiency must be corrected before making any diagnostic or therapeutic decision for diabetes mellitus based on HbA1c.

Rajendra Prasad Parlapally *et al.*[11], (2016)conducted a study on 63 non-diabetic, anemic patients, and 63 age-matched healthy subjects and their results were similar to the present study with the observation that iron deficiency anemia was associated with higher levels of HbA1c (6.13±0.6 %) compared to 5.12±0.5 % in controls, which could cause problems in the diagnosis of uncontrolled DM in iron-deficient patients.

Singh P *et al.,*[12] (2017)also hadsimilar observation to the present study with the conclusion that iron deficiency anemia is associated with higher level of HbA1c(5.87%) as copared to 5.03% in controls.

N.S Neki *et al.,*[13](2017) conducted a study to analyse change in HbA1c level with treatment of iron deficiency anaemia. 50 confirmed iron deficiency anaemia (IDA) patients who were non-diabetic were compared with 50 age-matched healthy subjects. The mean baseline HbA1c level in the patients with IDA (6.12+0.21%) was significantly higher than controls groups (6.12+0.21%).

In a study by VydehiVeeramalla[14]*et al.*conducted on diabetic patients, baseline HbA1c was found to be higher in patients with associated iron deficiency anemia (6.2%) and was found to reduce significantly after treatment with iron supplements for 2 months(5.1%)

Table 6 Comparison of present study with previous studies having similar results (using non diabetic IDA patients as cases)

Study	Year	Number of cases	HbA1c in study group(%)	Hba1c in control group(%)	p-value
El-Agouza *et al* [15]	2002	81	6.1±0.6	5.2±0.4	<0.05
Coban *et al* [10]	2004	100	7.4±0.8	5.9±0.5	<0.05
Shanthi *et al* [16]	2013	100	7.6±0.5	5.5±0.8	<0.05
Silva *et al* [17]	2015	122	5.6±0.4	5.3±0.4	<0.05
Rajendra Prasad Parlapally *et al* [11]	2016	63	6.13±0.6	5.12±0.5	<0.001
ManzuraRustumMulani *et al*[18]	2016	50	9.10±1.08	5.21±0.45	<0.001
Singh P *et al* [12]	2017	30	5.87	5.03	<0.01
N.S.Neki *et al* [13]	2017	50	6.12±0.21	5.21±0.16	<0.05
LavanyaRajagopal *et al* [19]	2017	300	6.84±0.07	5.12±0.04	<0.05
Present study	2020	100	5.24±0.41	4.76±0.60	0.0001

However, a few studies were in disagreement with the results of present study, some of which are as under:-

Table 7 Comparison of present study with previous studies having contradictory results (using non diabetic IDA patients as cases):

Study	Year	Number of cases	HbA1c in study group(%)	Hba1c in control group(%)	p- value
Ford ES etal[20]	2011	8296	5.5±0.1	5.4±0.2	>0.05
Sinha *et al* [21]	2012	100	4.6±0.6	5.5±0.6	>0.05
K.VijayaDurairaj[22]	2017	120	4.619±0.308	5.446± 0.281	<0.001

CONCLUSION

The present study found that Glycosylated Hb (HbA1c) levels in IDA patients are significantly higher than those of control group. We excluded all other causes which could increase HbA1c levels, thus meaning that Iron deficiency leads to observed rise in HbA1c levels despite the patients being non-diabetic. This may cause problem in assessing glycemic control in diabetic patients with co-existing IDA. Falsely higher value because of IDA can give an impression of poor glycemic control in diabetic patients despite the patients being well controlled, leading to unnecessary therapeutic changes. Hence, we recommend that iron deficiency anemia must always be taken into account before making any decision on glycemic control in patients of DM on the basis of HbA1c level alone. However, more studies on larger scale are required to further support these findings.

References

1. K. Park- Park's textbook of preventive and social medicine. 2017: EDI. 24th
2. John P. Greer MD, Daniel A. Arber MD, Bertil E. Glader, Alan F. List, Robert T. Means Jr., George M. Rodgers. Wintrobe's Clinical Hematology. LWW. Edi. 14th, 2018
3. Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson J, Loscalzo J. eds. Harrison's Principles of Internal Medicine, 19e. New York, NY: McGraw-Hill; 2015.
4. Vinay Kumar, Abul K. Abbas, and Jon C. Aster. Robbins &Cotran, Patologic Basis of Disease. South Asia Edition. 2018. Edi. 10th.
5. Barbara J. Bain FRACP FRCPath, Imelda Bates. Dacie and Lewis Practical Haematology Paperback. 12th Edition 2016
6. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2010;33(Suppl. 1):S62–S69
7. Praful B Godkar. Textbook of Medical Laboratory Technology 2 Volume Set 3rd Edition. Bhalani Publishing House. 2014Edition: 3rd.
8. Brooks AP, Metcalfe J, Day JL, Edwards MS. 1980. Iron deficiency and glycosylated haemoglobin A1. Lancet. Jul; 316(8186):141.
9. Sluiter WJ, van Essen LH, Reitsma WD, Doorenbos H. Glycosylated haemoglobin and iron deficiency. Lancet. 1980: 2: 531-532.
10. Coban E, Ozdogan M, Timuragaoglu A. The effect of iron deficiency anemia on the levels of haemoglobinAlc in nondiabetic patients. ActaHaematol. 2004; 112: 126-28.
11. Rajendra Prasad Parlapally, KatakamRajaniKumari, T Srujana: Effect of Iron Deficiency Anemia on Glycation of Hemoglobin in Non-diabetics. International Journal of Scientific Study. August 2016, Vol4; Issue 5 : 192-196.
12. Singh P, Singhal S, Virmani S.K.: The Effect of Iron Deficiency Anemia on Glycosylated Hemoglobin (HbA1c) in Non Diabetic Adults. Journal of Dental and Medical Sciences, Volume 16, Issue 2 Ver. V (February. 2017), PP 26¬31.
13. N.S Neki , Narendra Kumar Meena , Kuldip Singh , SatpalAloona , Amritpal Singh, Gagandeep Singh. Change in HbA1c Level with treatment of iron deficiency anaemia in non diabetic patients. Int. J. Curr. Res. Biol. Med. (2017). 2(11): 35-41.
14. VydehiVeeramalla and SwethaMadas : Correlation of Glycosylated Hemoglobin and Iron Deficiency Anemia among Diabetic and Non Diabetic Patients. Int. J. Curr. MicrobioI. App. Sci (2017) 6(12): 2669-2675.
15. EI Agouza I, Abu Shahla A, Sirdah M. The effect of iron deficiency vitamin B12 deficiency. J. Intern. anaemia on the levels of haemoglobin subtypes: possible consequences for clinical diagnosis. Clin Lab Haematol. 2002: 24(5): 285-289.

16. Shanthi B, Revathy C, Manjula Devi AJ, Subhashree. Effect of Iron Deficiency on Glycation of Haemoglobin in Nondiabetics. Journal of Clinical and Dignostic Research: JCDR, 2013; 7(1): 15-17. 17.
17. J.F. Silva, *et al*. Effect of iron deficiency anaemia on HbA1c levels is dependent on the degree of anaemia, ClinBiochem., 2015. http://dx.doi.org/10.1016/j.clinbiochem. 2015.09.004.
18. ManzuraRustumMulani,PandharinathShankarraoGawali : Effect of Iron deficiency on glycation of hemoglobin in non diabetics. ACCLM 2016; 2(2): 4-7.
19. LavanyaRajagopal, *et al*. A comparison of effect of Iron Deficiency Anemia on HbA1c levels in controlled diabetics and non-diabetics: A cross sectional analysis of 300 cases DOI:10.21276/apalm.1276 Vol 4 No.2(2017).
20. Ford ES, Cowie CC, Li C, Handelsman Y, Bloomgarden ZT. Irondeficiency anemia, non□iron□deficiency anemia and HbA1c among adults in the US. J Diabetes. 2011: 3: 67-73.
21. Sinha N, Mishra TK, Singh T, Gupta N. Effect of iron deficiency anemia on hemoglobin A1c levels. Ann Lab Med 2012: 32: 17-22.
22. A study on influence of Iron deficiency anemia over HbA1c levels- Dissertation by Dr. K.VijayaDurairaj

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