Section: General Medicine

Original Research Article

TO STUDY THE EFFECT OF IRON DEFICIENCY ANAEMIA ON HBA1C LEVELS IN NON DIABETICS AT TERTIARY CARE CENTRE OF CENTRAL INDIA A CROSS SECTIONAL STUDY

Received : 10/02/2023
Received in revised form : 08/03/2023
Accepted : 07/04/2023

Keywords:
Iron Deficiency Anaemia, HbA1C Levels, Non-Diabetics.

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DOI: 10.47009/jamp.2023.5.2.302

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2023; 5(2); 1446-1450

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Abstract

Background: Iron deficiency is the state in which the content of iron in the body is less than normal. Iron depletion is the earliest stage of iron deficiency, in which storage iron is decreased or absent but serum iron concentration, transferrin saturation, and blood hemoglobin levels are normal. The objective is to determine whether the HbA1c levels will increase among the patients suffering from iron deficiency anemia without diabetes. **Materials and Methods:** This Hospital based cross sectional study was conducted in OPDs & Wards of Tertiary Care Centre of Central India. **Result:** Out of 112 participants 56(50%) were non anaemic, 42(37.5%) had severe anaemia and 14(12.5%) had moderate anaemia. Parameter which was found to be significantly different are mean serum iron value (p-value <0.0000001), mean serum ferritin value (p-value <0.0000001), mean serum TIBC value (p-value <0.0000001). The mean HbA1c value for the anaemic group was 6.04 ± 0.74 % and for the non- anaemic group, it was 4.91 ± 0.65 %.The difference is statistically significant. 21.4% have HbA1c levels ≤5.5%, 71.5% subjects between 5.6-6.5% and only one has HbA1c levels >6.5%. Out of the 42 subjects having severe anaemia, 16.7% have HbA1c ≤5.5%, 40.5% between 5.6-6.5% and 42.8% have their HbA1c between 6.6-7.5%. **Conclusion:** Iron deficiency anaemia is an independent factor affecting HbA1C level in non-diabetic patients and it should be interpreted carefully in all deficiency anaemia patients.

INTRODUCTION

Iron deficiency without anemia is a somewhat more advanced stage of iron deficiency, characterized by absent storage iron, usually low serum iron concentration and transferrin saturation, but without frank anemia.
Iron-deficiency anemia, the most advanced stage of iron deficiency, is characterized by absent iron stores, low serum iron concentration, low transferrin saturation, and low blood hemoglobin concentration. Iron-deficiency anemia is the most common anemia worldwide, and is especially prevalent in women and children in regions where meat intake is low, food is not fortified with iron, and malaria, intestinal infections, and parasitic worms are common.[1]Women with frequent pregnancies may be particularly susceptible. Iron deficiency is most common in children 1 to 4 years old and in adolescent, reproductive age, or pregnant women & it develops over time if the body does not have enough iron to manufacture red blood cells.[2]
Glycated hemoglobin A1c (HbA1c) is a major part of HbA1 and comprises approximately 5% of the total hemoglobin in non-diabetic individuals.
The types of hemoglobin found in adults include hemoglobin A (HbA) (95–98%), hemoglobin A2 (HbA2) (2–3%), and fetal hemoglobin (HbF) (1%).[3] Also, HbA0, HbA1a1, HbA1a2, HbA1b, and HbA1c are subtypes of HbA that can be identified by electrophoresis. HbA1c represents 70–90% of HbA1, and is the glycated form of HbA1. 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.
Glycated hemoglobin has been defined as the fast fraction hemoglobin (HbA1a, A1c) which elute first during column chromatography with cation exchange resin. It is formed continuously by

addition of glucose to the N– terminal of the hemoglobin beta–chain throughout the circulatory life. The mechanism of glycation involves the non-enzymatic binding of glucose to the N-terminal valine and internal lysine amino groups of hemoglobin. The glycation reaction is mostly irreversible, so that the concentration of HbA1c is a function of the concentration of glucose to which the erythrocytes are exposed over their lifespan of 3 months (120 days on average). HbA1c therefore represents a marker of average blood glucose concentration over the previous 2 to 3 months.[3]

HbA1c level is affected by not only blood glucose levels alone but also conditions that affect the lifespan of red blood cells. They are acute or chronic blood loss, thalassemia, sickle cell anemia, hemolytic anemia, hemoglobinopathies, uremia, aplastic anemia, splenectomy, pregnancy, vitamin-B12 and folate deficiency anemia. Falsely elevated HbA1c concentration can be encountered when there is increased circulating erythrocyte life span. They are alcoholism, hyperbilirubinemia, and iron deficiency anemia.

According to WHO, iron deficiency is the commonest of all deficiency diseases worldwide. Iron deficiency anemia is also the most common in India. One key factor thought to be a confounder in the use of HbA1c is an altered erythrocyte lifespan, in particular due to anemia. The WHO defines anemia in adults as 120 g/l Hb in non- pregnant women and 130 g/l in men. It is widely purported that haemolytic anemia can lead to decreased HbA1c values due to reduced erythrocyte lifespan, and iron deficiency anemia (IDA) may result in increased HbA1c values due to an elongation of the erythrocyte lifespan. However, it is not known to what degree alterations in erythrocyte indices affect HbA1c values especially around the diagnostic cut point of 6.5% (48 mmol/mol) or the degree of severity required to result in a significant change.

Some studies show that HbA1c levels are increased in iron deficiency anemia and attempted to explain based on both modifications to the structure of hemoglobin and levels of HbA1c in old and new red blood cells.[4]

According to some studies, there were no differences between HbA1c levels of anemia patients and controls. Study done by El–Agouza L et al showed that HbA1c level were higher in patients with iron deficiency anemia and decreased significantly upon treatment with iron.[5]

Recently Sinha et al showed that HbA1c levels and absolute HbA1c levels increased with treatment of iron deficiency anemia.[6]

The results of all these studies are conflicting; hence decision to study the effects of Iron deficiency Anemia on HbA1c levels in Indian non-diabetic adults was taken. Iron deficiency produces a hypochromic microcytic anemia. At the outset of chronic blood loss or other states of negative iron balance, reserves in the form of ferritin and hemosiderin may be adequate to maintain normal hemoglobin and hematocrit levels as well as normal serum iron and transferrin saturation. Progressive depletion of these reserves first lowers serum iron and transferrin saturation levels without producing anemia. In this early stage there is increased erythroid activity in the bone marrow. Anemia appears only when iron stores are completely depleted and is accompanied by low serum iron, ferritin, and transferrin.[7]

Thus, the objective of the present study is to determine whether the HbA1c levels will increase among the patients suffering from iron deficiency anemia without diabetes.

MATERIALS AND METHODS

This Hospital based cross sectional study was conducted in OPDs & Wards of Tertiary Care Centre of Central India. The study was conducted from Dec 2020 to Dec 2022. Approval from the Institutional Ethics Committee (IEC) was sought. Permission was sought from Head of the Department and other unit Chiefs, Department of General Medicine for carrying out the study. Informed written consent in Subject's vernacular language was taken before enrolment for study. 112 study subjects were included in study based on inclusion and exclusion criteria.

Inclusion Criteria

All patients aged >18 years who are non-diabetic with clinical features and lab findings of iron deficiency anemia [Hb<13 g/dl in males, <12g/dl in females] will be taken up for the study.

For controls- Age and sex matched individuals having no evidence of iron deficiency anemia and non-diabetics.

Exclusion Criteria

Patient or caretaker not willing to give consent for study, Diabetic patients

Non anemic patients

Patients with sickle cell disease, Thalassemia

Patients with chronic renal failure

Pregnant female Malignancy

Patients with history of acute or chronic blood loss

Anemia due to causes other than other than iron deficiency

Methodology

After obtaining ethical approval from the institutional Ethics review board, a written informed consent was taken and eligible participants were enrolled in the study. The following data were collected from each patient which includes sociodemographic information like name, age, address, occupation, sex, present illness. Examination of patients also included general examination and system examination along with recording of vitals. Metabolic parameters like fasting blood sugar, postprandial blood sugar, glycated haemoglobin, serum ferritin, serum iron, TIBC were done. Other investigations as deemed necessary by the treating physician were also conducted and their findings were noted.

Data Collection Tool / Instrument:

- Questionnaire of the study is provided to study subjects.
- GlycatedHaemoglobin(HbA1c)wasmeasuredbyLatexenhanced Immunoturbidimetric method using RandoxImolaanalyser.
- Complete blood count : by Mindray and Erma machines,
- Blood sugar : by Glucspark glucometer
- Following blood investigations by Beckman Coulter au5800 chemistry analyser with principles of spectrophotometry and potentiometry.
  a) Liver function test
  b) Renal function test
  c) Iron studies

Statistical Analysis

The data was entered in Microsoft Excel and analyzed using SPSS ver 26 (2017), STATA version 16 (College Station, Texas, USA: StataCorp LLC) and Epi Info software. Means +/- SD or proportions were calculated. Continuous data variables were tested for statistical significance by using the Students'' test and Mann- Whitney test as applicable. Qualitative and categorical variables were tested using Chi-square test. Independent associations were predicted using binomial logistic regression analysis and p values < 0.05 were considered as statistically significant.

RESULTS

Out of the total 112 subjects, 74 (66.1%) are females and the remaining 38 (33.9%) are males.

Of the total 112 study subjects, 8 (7.2%) age less than 25 years, 39 (34.8%) have their ages between 25-35 years, 24 (21.4%) in the age group 36-45 years, 24 (21.4%) belong to the age group 46-55 years, 15 (13.3%) in age group 56-65 years and there are 2 (1.8%) study subjects who are above 65 years. Out of the total 56 anaemic patients, 42(75%) had severe anaemia and 14 (25%) had moderate anaemia.

Out of the total 56 anaemic subjects, all except one i.e. 55(98.2%) have pallor, 21 (37.5%) have pedal edema and 19(33.9%) have koilonychia. Many of the study subjects have more than one clinical sign present.

In the study subjects having iron deficiency anaemia, 20 (35.7%) were males and 35 (64.3%) were females. In the non- anaemic study group, 18(32.1%) were males and 38 (67.9%) were females.

In the subjects having Iron deficiency Anaemia, 5(8.9%) have age <25 years, 19 (33.9%) have ages between 25- 35 years, 14 (25%) in age group 36-45 years, 12 (21.4%) are in the age group 46-55 years, 4 (7.2%) belong to 56-65 years age group and only 2 (3.6%) have age >65 years. Among the non-anaemic study subjects, 3(5.3%) have ages <25 years, 18 (32.2%) belong to 25-35 years age group, 9(16.2%) have age between 3-45 years, 15(26.8%) between 45-55 years, 10(17.8%) are in the age group 55-65 years and only one has age >65 years.

Independent student t-test was applied to the mean values to compare. The serum Iron values for both the groups were found to be significantly different with P-value <0.0000001. Also, while comparing mean serum Ferritin values, it was seen that the mean values for both the groups were different which was found to be statistically significant (P-value<0.0000001). Similarly, the Total Iron Binding Capacity (TIBC) mean values were significantly different in both the groups with P- value <0.0000001. [Table 1].

Table 1: Comparison between Iron Indices in Anaemic and Non-anaemic groups

Indices	Non-anaemic	IronDeficiencyanaemia	T-test	P value
SERUM IRON(MCG/DL)	110.37 ± 24.28	58.85 ± 32.97	9.415	<0.0000001,Significant
SERUM FERRITIN(NG/ML)	138.34 ± 42.11	56.28 ± 64.26	7.992	<0.0000001, Significant
TIBC(MCG/DL)	218.04 ± 85.72	398.81 ± 83.42	-11.309	<0.0000001,Significant

Here also, independent student t-test was applied to determine significant difference between the two groups. With P-value <0.0000001, mean Haemoglobin values are different for the group not having anaemia and those suffering from Iron Deficiency Anaemia. [Table 2]

Table 2: Comparison between Mean Haemoglobin levels in Anaemic and Non- anaemic groups

Haemoglobin	Non-anaemic	Iron Deficiency Anaemia	Independent student t-test value=10.83, dF=110, P<0.0000001, Significant
MEAN ± SD	13.74 ± 3.09	6.74 ± 3.72	

The mean HbA1c value for Anaemic group is 6.04 ± 0.74 % and for the non-Anaemic group, it is 4.91 ± 0.65 %. On applying independent student t-test it was seen that these values significantly different with P-value <0.0000001.[Table 3]

Table 3: Comparison between Mean HbA1c values in Anaemic and Non-anaemic groups

HbA1c	Iron Deficiency Anaemia	Non-Anaemic	Independent student t-test value=8.58, dF=110, P<0.0000001, Significant
MEAN ± SD	6.04 ± 0.74	4.91 ± 0.65	

Out of 14 subjects having moderate anaemia, 3(21.4%) have HbA1c levels ≤5.5%, 10(71.5%) subjects between 5.6-6.5% and only one has HbA1c levels>6.5%. Out of the 42 severely anaemic subjects, 7(16.7%) have HbA1c ≤5.5%, 17(40.5%) between 5.6-6.5% and 18(42.8%) have their HbA1c between 6.6-7.5%.[Table 4].

Table 4: Severity of anaemia and HbA1c levels

Severity of anemia	HbA1CLEVELS			
	≤5.5	5.6-6.5	6.6-7.5	ChiSq=6.167, P<0.05, significant
Moderate(14)	3 (21.4%)	10 (71.5%)	1 (7.1%)	
Severe (42)	7 (16.7%)	17 (40.5%)	18 (42.8%)	
Total	10	27	19	

DISCUSSION

Anemia is characterized by a shorter erythrocyte life span, reduced hemoglobin concentration, and compensatory hyperplasia.[8] All these factors exert significant changes in the production of HbA1c. Iron deficiency has been reported to be independently associated with increased HbA1c, regardless of plasma glucose levels and the degree of anemia.[9]

The present study has 33.9% males and 66.9% females in the total 112 study subjects. Similar ratio was seen in the study conducted by Sinha N et al., 34 (68%) were female and 16 (32%) were male, Sinha N et al,[10] these results are suggestive that iron deficiency anaemia is more common in females.

In the present study, we had 56 anaemic study subjects, out of which 25% were moderately anaemic, 75% were severely anaemic but there were none who had mild anemia. In a study conducted in Rewa, Rajasthan, out of 500 cases 107 (21.4%), 315 (63%) and 78 (15.6%) have mild, moderate and severe anaemia respectively Manjhvar SK et al,[11] in another study conducted in Delhi, India, severe anemia was seen in 38 (76%) patients, and moderate anemia, in 12 (24%) patients and no cases of mild anemia.[10]

In the current study, the most common signs in anaemic subjects were pallor (98.2%) and pedal edema (37.5%). Manjhvar SK et al,[11] also reported all patients having pallor, 26.8% bald tongue and 27.2% koilonychia to be the most common signs. The study done by Sinha N et al,[10] on examination revealed pallor in all patients (50/50, 100%), nail changes in 6 patients, mild splenomegaly (<2 cm) was noted in 12 patients (24%) and Ejection systolic murmur in the aortic area was observed in 40 patients (80%).

Of the anaemic study subjects, males (35.7%) were less than females (64.3%) in our present study. The population studied by Coban E et al,[12] also showed more number of women (30) than men (20) having IDA with mean age 35.7 +/- 11.9 years. The Iron indices (serum iron, serum ferritin, TIBC) were all seen to be reduced in subjects with iron deficiency anaemia as compared to the non-anaemic study subjects and these values were statistically significant. Sinha N et al,[10] also reported the mean baseline serum ferritin levels as significantly lower in patients than in controls (p<0.01).

The mean Hemoglobin value was 6.74 ± 3.72 mg/dl in anaemic study group and 13.74 ± 3.09 mg/dl in non-anaemic study group which shows a statistically significant difference in the results. The data from the study done in Delhi, India also provided evidence that hemoglobin was indeed lower in anemic patients than in healthy controls, and the observed difference was statistically significant (p<0.01).[10]

The results of a study conducted in the US showed that Hb concentrations are positively correlated with HbA1c concentrations, that HbA1c concentrations tended to be higher in the presence of iron deficiency, and that participants with IDA had similar HbA1c concentrations as participants with normal concentrations of Hb and a normal iron status.[13]

Attard et al,[14] studied the levels of HbA1c and fasting blood glucose as diagnostic criteria to evaluate the effect of iron deficiency and IDA on diabetes. The results showed that iron deficiency and IDA caused changes in HbA1c levels, which were not consistent with the actual disease state. The mean HbA1c values were also significantly different for the study subjects suffering from iron deficiency anaemia from the ones not having anaemia. Similar results were seen in the study done by Rajagopal L et al,[15] where, non-diabetics with IDA had a mean HbA1C % (6.84±0.07) which was significantly higher (p < 0.05) than the non-anaemic group (5.12±0.04).

The same was reported by Sinha N et al,[10] in their study, showing the mean baseline HbA1c level in anaemic patients (4.6%) as significantly lower than that in the control group (5.5%, P<0.05). In another study conducted at the Cukurova University, also the mean HbA1c level was significantly lower in the group with IDA (5.4%) than in the healthy control group (5.9%; p < 0.05).[16]

It was proposed that, in iron deficiency, 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. Brooks AP et al,[17]Sluiter et al,[18]tried to provide an explanation for the above findings. They proposed that the formation of glycated hemoglobin is an irreversible process and hence, the concentration of HbA1 in 1 erythrocyte will increase linearly with the cell's age.

The results from a nationwide US study suggested that IDA has little population effect on concentrations of HbA1c or on diabetes prevalence. Ford ES et al.[13] Another study from India that included 15 non-diabetic patients with IDA and 12 controls also failed to find a difference in mean concentrations of HbA1c. Rai KB et al.[19]

In a report from Denmark, there were no differences found in HbA1c concentrations between 10 non-diabetic patients with IDA and 10 healthy controls.[20]

The present study results showed a significant association between the severity of anaemia and HbA1c levels in the study subjects. Similar to this, Manjhvar SK et al,[11] also reported glycosylated haemoglobin (HbA1c) values to be significantly higher in non-diabetic IDA patients implying that iron deficiency anaemia leads to a rise in the glycosylated haemoglobin values.

Sinha N et al,[10] also observed a significant correlation between hemoglobin and HbA1c levels in patients at baseline (coefficient of correlation=0.593; p<0.01) and after 1 month of treatment (coefficient of correlation=0.490; p<0.01. The studies by El- Agouza et al,[21] and Coban E et al,[12] also showed that HbA1c levels were higher in patients with iron deficiency anemia and decreased significantly upon treatment with iron. Rajagopal L et al,[15] in their study, also observed a positive correlation between IDA and elevated

HbA1C level in the non-diabetic population. HbA1C increased with severity of anaemia. In the Ford ES et al,[11] study reported no significant difference in mean HbA1C concentration according to the IDA status as well as before and after iron treatment.

CONCLUSION

Mean HbA1C level is more in iron deficiency anaemic non diabetic patients in comparison to non anaemic non diabetic patients. It is 4.91 ± 0.65 in the control group, while the mean HbA1c level in the case group is 6.04 ± 0.74.

The difference is statistically significant (p-value <0.0000001), HbA1c level is shifted to higher range in iron deficiency anaemic non diabetic patients in comparison to the non-anaemic non diabetic patients. In iron deficient anaemic non diabetic patients HbA1C level is increased in comparison to the non-anaemic patients independent of blood sugar level.HbA1c level is found to be increase with severity of anaemia. Hence it is concluded that iron deficiency anaemia is an independent factor affecting HbA1C level in non-diabetic patients and it should be interpreted carefully in all deficiency anaemia patients.

REFERENCES

1. Stoltzfus RJ: Iron interventions for women and children in low-income countries. J Nutr 141(4):756S–762S, 2011.
2. McLean E, Cogswell M, Egli I, et al: Worldwide prevalence of anaemia, WHO Vitamin and Mineral Nutrition Information System, 1993–2005. Public Health Nutr 12(4):444–454, 2009.
3. Telen MJ, Kaufman RE. The mature erythrocyte. In: Greer JP, Forester J, et al., editors. Wintrobe's clinical hematology. 11th ed. Lippincot: Williams and Wilkins; 2004. p. 230.
4. Bernstein RE. Glycosylated hemoglobin's: hematologic considerations determine which assay for glycohemoglobin is advisable. Clin Chem. 1980;26:174175.
5. Starkman HS, Wacks M, Soeldner JS, Kim A. Effect of acute blood loss on glycosylated hemoglobin determinations in normal subjects. Diabetes Care. 1983;6:291–294.
6. Horton BF, Huisman TH. Studies on the heterogeneity of hemoglobin. VII. Minor hemoglobin components in hematological diseases. Br J Haematol. 1965;11:296–304.
7. Robbins &Cotran Pathologic Basis Of Diseases 8th edition; page:1232.
8. Gallagher EJ, Bloomgarden ZT, Le Roith D. Review of hemoglobin A1c in the management of diabetes. Journal of Diabetes, 2009, 1:9-17.
9. Kim C, Bullard KM, Herman WH, Beckles GL. Association between iron deficiency and A1C Levels among adults without diabetes in the National Health and Nutrition Examination Survey, 1999–2006. Diabetes Care. 2010;33:780–85. ; Ford ES, Cowie CC, Li C, et al. Iron-deficiency anemia, non- iron-deficiency anemia and HbA1c among adults in the US. J Diabetes. 2011;3:67–73.
10. Sinha N, Mishra TK , Singh T, Gupta N. Effect of iron deficiency anemia on hemoglobin A1c levels. Ann Lab Med. 2012 Jan;32(1):17-22 doi:10.3343/alm.2012.32.1.17. Epub 2011 Dec 20. PMID: 22259774; PMCID: PMC3255499.
11. Manjhvar SK, Singh B, Niranjan AS. To study the effect of iron deficiency anaemia on HbA1c in non-diabetic. J. Evolution Med. Dent. Sci. 2017;6(94):6863-6867, DOI: 10.14260/jemds/2017.
12. Coban E, Ozdogan M, Timuragaoglu A. Effect of iron deficiency anemia on the levels of hemoglobin A1c in nondiabetic patients. Acta Haematol. 2004;112:126–128.]
13. Ford ES, Cowie CC, Li C, Handelsman Y, Bloomgarden ZT. Iron-deficiency anemia, non-iron-deficiency anemia and HbA1c among adults in the US. J Diabetes. 2011;3:67–73
14. Attard SM, Herring AH, Wang H, et al. Implications of iron deficiency/anemia on the classification of diabetes using HbA1c. Nutr Diabetes. 2015;5:e166
15. Rajagopal, Lavanya&Ganapathy, Shivashekar&Arunachalam, Sundaram& Raja, Veena&Ramraj, Balaji. (2017). Does Iron Deficiency Anemia and its Severity Influence HbA1C Level in Non-Diabetics? An Analysis of 150 Cases. Journal of Clinical and Diagnostic Research JCDR.11. EC13-EC15. 10.7860/JCDR/2017/25183.9464.
16. ÇetinkayaAltuntaş, S., Evran, M., Gürkan, E. et al. HbA1c level decreases in iron deficiency anemia. Wien KlinWochenschr 133, 102–106 (2021). https://doi.org/10.1007/s00508-020-01661-6.
17. Brooks AP, Metcalfe J, Day JL, Edwards MS. Iron deficiency and glycosylated haemoglobin A. Lancet. 1980;2:141.
18. Sluiter WJ, van Essen LH, Reitsma WD, Doorenbos H. Glycosylated haemoglobin and iron deficiency. Lancet. 1980;2:531–532.
19. Rai KB, Pattabiraman TN. Glycosylated haemoglobin levels in iron deficiency anaemia. Indian J Med Res. 1986; 83: 234– 6.
20. Gram-Hansen P, Eriksen J, Mourits-Andersen T, Olesen L. Glycosylated haemoglobin (HbA1c) in iron- and vitamin B12 deficiency. J Intern Med. 1990; 227: 133– 6.
21. El-Agouza I, Abu Shohla A, Sirdah M. The effect of iron deficiency anaemia on the levels of haemoglobin subtypes: possible consequences for clinical diagnosis. Clin Lab Haematol. 2002;24:285–289.

ISSN(O): 2687-5365; ISSN(P): 2753-6556