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A Cross-Sectional study of effects of Iron Deficiency Anaemia on HBA1c levels among Type 2 Diabetic and Non-Diabetic patients

***Chuppala Bharath[1], Uma.M.Anand Kumar[2], K. Guru Ravi Tej[3]**

[1]Postgraduate Resident, Department of General Medicine, PESIMSR, Kuppam
[2]Professor and HOD, Department of General Medicine, PESIMSR, Kuppam
[3]Assistant Professor, Department of General Medicine, PESIMSR, Kuppam

***Corresponding Author: Chuppala Bharath**
Email: bharath.13994@gmail.com

Abstract

Background: Diabetes mellitus (DM) has become a major health problem worldwide. American Diabetes association has considered HBA1c levels ≤ 6.5 as prime target for glycemic control and as diagnostic criterion for DM.One of the variable influencing HBA1c levels is Iron Deficiency Anaemia.(IDA).

Objectives: To compare the effect of IDA on HBA1c levels among Diabetic and Non diabetic patients.

Methodology: After obtaining IEC clearance and informed consent from the study subjects, a cross-sectional study was conducted from October 2022 to October 2023. Data was collected from 200 patients(50 Diabetic patients and 150 non diabetics), at Department of general medicine PES Hospital, Kuppam, selected by purposive sampling, using a structured proforma, Data was collected and analysed by SPSS.

Results: Mean age of the patients in group of Non diabetic with Iron deficiencyanaemia patients was 45.92± 10.89yrs, while Mean age of the in group of Diabetic with Iron deficiency anaemia patients was 51.76 ± 9.59yrs. Among the two groups female patients(79.3%) were comparatively more than male patients(66.2%) in Non diabetics with IDA. HbA1c in Non diabetics with IDA is negatively correlated significantly with Body mass index, haemoglobin, PCV, MCHC and not correlated with age, MCV, MCH, Serum ferritin, FBS, PPBS. HbA1c in diabetics with IDA is negatively correlated significantly with age, haemoglobin, PCV, MCV, MCH, MCHC and Serum ferritin and not correlated with BMI, FBS, PPBS.

Conclusion: There is alteration of HBA1c levels with respect to Haemoglobin, PCV, MCHC, MCV,MCH and serum ferritin, in Diabetic and Non diabetic individuals with Iron Deficiency Anaemia.

Key words: HBA1c, Diabetes Mellitus, Iron Deficiency Anaemia

1. INTRODUCTION:

A significant portion of blood is made up of hemoglobin, which contains heme, whichisaniron-bindingporphyrin. Thereisarelationship between the iron concentration and pancreatic islet beta cells bloodglucose levels, suggesting that iron contributes to the development and course ofdiabetes mellitus (DM). [1–3] More precisely, iron-deficiency

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anemia (IDA) can result from disorders that impair an individual's ability to metabolize iron. This can then have an impact on an individual's glycemic status, specifically on their glycated hemoglobin (HbA1c) levels.[4-6] While HbA1c testing is more useful in clinical settings than other tests, the accuracy of its results might vary depending on a number of factors. [7-9]

One of the variables influencing HbA1c levels is IDA. Although the majority of these investigations discovered that IDA caused falsely high HbA1c levels,[10-15] several research showed the opposite—that IDA 2 caused falsely lowered HbA1c levels, or had no effect at all. Additionally, a numerous studies discovered relation between the IDA severity and its impact on HbA1c levels.

To enhance the patient's quality of life, lessen the blood transfusions need, and relieve symptoms, appropriate and prompt therapy of IDA is essential. Treatment options include both intravenous and oral iron replacement therapy (IRT); however, oral IRT is not successful in treating some disorders (e.g., gastrointestinal conditions).[16] Many studies have examined the relationship between HbA1c levels and IDA in individuals with IDA both before and after IRT. While the major studies revealed that IRT decreased HbA1c, a few studies reported increases in HbA1c levels following IRT. [17-20]

2. MATERIAL & METHODS:

After obtaining IEC clearance and informed consent from the study subjects, a cross-sectional study was conducted from October 2022 to October 2023. Data was collected from 200 patients(50 Diabetic patients and 150 non diabetics), at Department of general medicine PES Hospital, Kuppam, selected by purposive sampling, Sample size calculated on prevalence from previous study conducted by Lavanya Rajagopal, Sundaram Arunachalam et al[21] in march 2017. Age group of 18-60 years, Group A comprising of 50 Controlled diabetics (Fasting plasma glucose (FPG), Group B comprising of 150 non-diabetics with IDA are included in the study. Age more than 60 years, Hypothyroidism, renal insufficiency (elevated serum urea, creatinine), Hemoglobinopathies, Pregnancy, Fasting plasma glucose (FPG) >126 mg/dl, haemolytic anemia, VitaminB12 and folic acid deficiency and Anemia due to causes other than Iron Deficiency conditions which are excluded. Study tool using a structured proforma with details of demography, Presenting complaints, Clinical Examination, Complete hemogram , Fasting blood sugar ,HbA1c ,Serum ferritin ,A 12 lead ECG, data was entered into MS excel and analysed using SPSS version 20. Descriptive statistics will be analyzed as follows: The categorical data was analyzed using percentages and the continuous data was analyzed using mean and standard deviation. Inferential statistics was analyzed as follows: Chi-square test was used. A probability value of <0.05 was considered as statistically significant.

3. RESULTS:

Demographic profile:

Mean age of the patients in group of Non diabetic patients having IDA was 45.92± 10.89yrs, while Mean age of the in group of Diabetic with IDA patients was 51.76 ± 9.59yrs. When compared age group with the two groups, age gradually increased and >55years aged patients were more in Diabetics than Non diabetics. Age difference was statistically significant with the patients diabetes status. Among Non diabetics with IDA male were 28.7%, female 71.3%, while in Diabetics with IDA male were 44%, female 56%.

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Anaemia :
Mean Hb% in men and women was 8.79± 1.64g/dl, 8.35 ± 1.7g/dl respectively. 57% of them are moderately anaemic, 37.8% of them are severely anaemic , 5.2% of them are mild anaemic in women.55.4% are severely anaemic , 41.5% are moderately anaemic, 3.1% of them are mild anaemic in men

HBA1c:
Mean HbA1C in Non diabetics with IDA was 6.26 ± 0.41, while in Diabetic with IDA was 7.27± 0.63. Non diabetics had less HbA1C than Diabetics but compared with normal levels they were comparatively more. Significant difference was seen between 2 groups.

Complete Blood Count:
Mean Hematocrit in Non diabetics with IDA was 23.36 ± 5.42, while in Diabetic with IDA was 24.58± 5.05. Non diabetics had less Hematocrit than Diabetics. Non Significant difference was seen between 2 groups.Mean MCV in Non diabetics with IDA was 64.43 ± 7.85fl, while in Diabetic with IDA was 65.08± 8.09fl. Non diabetics had less MCV than Diabetics. Non Significant difference was seen between 2 groups. Mean MCH in Non diabetics with IDA was 19.28 ± 4.61pg, while in Diabetic with IDA was 19.05± 4.6pg. Non diabetics had less MCH than Diabetics. Non Significant difference was seen between 2 groups. Mean MCHC in Non diabetics with IDA was 27.45 ± 2.7g/dl, while in Diabetic with IDA was 23.15± 3.52g/dl. Non diabetics had more MCHC than Diabetics. Significant difference was seen between 2 groups. Mean Serum Ferritin in Non diabetics with IDA was 7.39 ± 8.97ng/ml , while in Diabetic with IDA was 6.43± 6.89. Non diabetics had more Serum Ferritin than Diabetics . non Significant difference was seen between 2 groups at $p<0.05$.

FBS and PPBS
Mean FBS in Non diabetics with IDA was 99.53 ± 11.03mg/dl, while in Diabetic with IDA was 114.04± 9.62mg/dl. Non diabetics had less HbA1C than Diabetics but compared with normal levels they were comparatively more. Highly significant difference was seen between 2 groups.Mean PPBS in Non diabetics with IDA was 6.26 ± 0.41(mg/dl), while in Diabetic with IDA was 7.27± 0.63 mg/dl. Non diabetics had less PPBS than Diabetics but compared with normal levels they were comparatively more. Significant difference was seen between 2 groups at $p <0.0001$.

Correlation between factors and HBA1c in non-diabetes with IDA patients
HbA1c in Non diabetics with IDA is negatively correlated significantly to Body mass index, haemoglobin,PCV, MCHC and not correlated with age, MCV, MCH, Serum ferritin, FBS, PPBS.

Correlation between factors and HBA1c diabetes with IDA patients
HbA1c in diabetics with IDA is negatively correlated significantly with age, haemoglobin, PCV, MCV, MCH, MCHC and Serum ferritin and not correlated with BMI, FBS, PPBS.

Logistic regression between Complete blood count and HbA1C in non Diabetes with IDA patients.
In non diabetic patients with IDA, by linear regression the predictors of HbA1c was found to be BMI, Hemoglobin estimation, PCV, MCHC and Serum Ferritin. As Hemoglobin values reduce the HbA1c values increase significantly in Non diabetics

Logistic regression between Complete blood count and HbA1C in Diabetes with IDA patients.
In diabetic patients with IDA, by linear regression the predictors of HbA1c was found to be MCH, MCHC and FBS. Hemoglobin values were not the significant predictor in diabetics as much seen in Non diabetics with IDA.

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Table 1.Correlation between other factors and HbA1C in non-diabetes with IDA patients.

Factors	Non Diabetic with IDA	
	R	p
Age	-0.036	0.665
BMI	-0.216	0.008**
Hb	-0.301	<0.0001**
PCV	-0.230	0.005**
MCV	-0.126	0.125
MCH	-0.058	0.477
MCHC	-0.204	0.012*
Serum Ferritin	-0.142	0.082
FBS	-0.09	0.274
PPBS	-0.012	0.884

**Correlation is significant at the0.01level (2-tailed),*Correlation is significantatthe0.05 level(2-tailed).

Table2: Correlation between n other factors and HbA1C in diabetes with IDA patients.

Factors	Diabetic with IDA	
	R	p
Age	-0.358	0.011*
BMI	-0.012	0.934
Hb	-0.612	<0.0001**
PCV	-0.613	<0.0001**
MCV	-0.450	0.001**
MCH	-0.780	<0.0001**
MCHC	-0.504	<0.0001**
SerumFerritin	-0.303	0.033*
FBS	0.240	0.094
PPBS	0.014	0.922

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**Correlation is significant at the 0.01level (2-tailed),*Correlation is significant at the 0.05 level (2-tailed).

Table 3. Logistic regression between Complete blood count and HbA1C in Diabetes with IDA patients.

Model	Standardized Coefficients	t	Sig.	95.0%ConfidenceIntervalforB	
	P value (Beta)			Lower Bound	Upper Bound
Age	.106	1.020	.314	-.007	.021
BMI	-.070	-.717	.478	-.060	.029
Hb	.138	.886	.381	-.063	.161
PCV	-.160	-1.444	.157	-.048	.008
MCV	-.195	-1.692	.099	-.033	.003
MCH	-.543	-3.816	.000	-.114	-.035
MCHC	-.296	-2.921	.006	-.089	-.016
Serumferritin	.016	.162	.872	-.017	.020
FBS	.175	2.046	.048	.000	.023
PPBS	.058	.678	.502	-.007	.014

Table 2: Logistic regression between Complete blood count and HbA1C in non-Diabetes with IDA patients.

Model	**Standardized Coefficients**	**t**	**Sig.**	**95% CI**	
	P value (Beta)			**Lower Bound**	**Upper Bound**
Age	-.035	-.446	.656	-.007	.005
BMI	-.177	-2.253	.026	-.065	-.004
Hb	-.263	-2.796	.006	-.111	-.019

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PCV	-.180	-2.076	.040	-.026	-.001
MCV	-.105	-1.286	.201	-.014	.003
MCH	.154	1.692	.093	-.002	.030
MCHC	-.218	-2.848	.005	-.055	-.010
Serumferritin	-.159	-2.068	.040	-.014	.000
FBS	-.072	-.915	.362	-.008	.003
PPBS	.008	.097	.923	-.004	.005

Figure 1: Box plot showing Association between HbA1C and diabetes status of participants

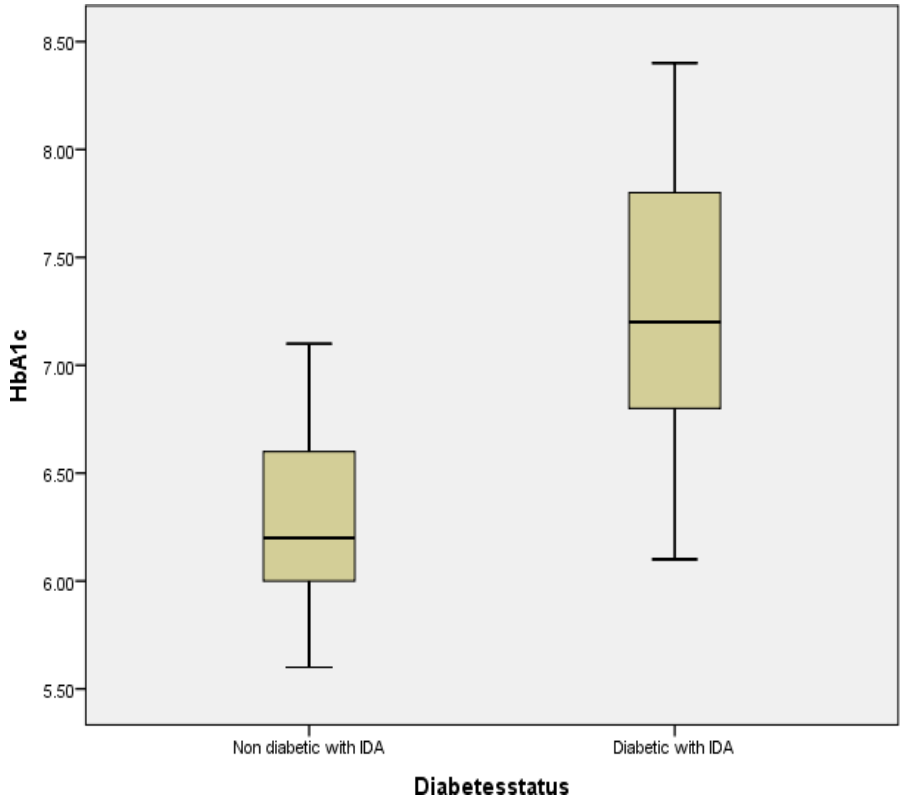

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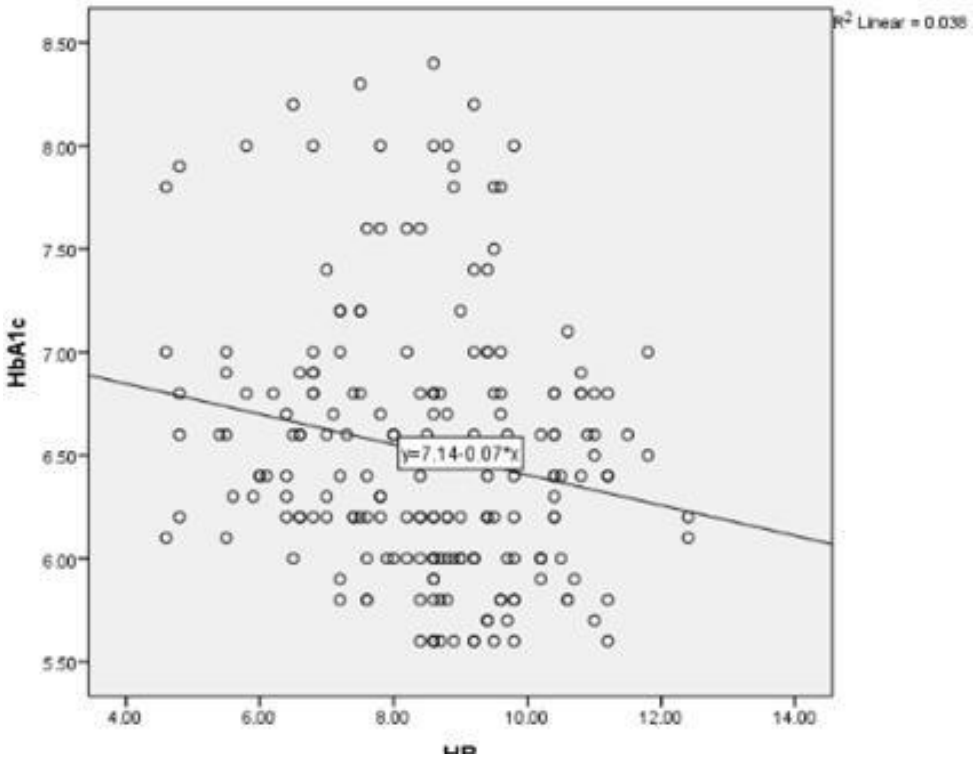
Figure 2: Regression plot for Hb andHbA1c



The regression plot shows that as haemoglobin levels fall, HbA1c levels significantly increase

4. DISCUSSION:

We conducted a cross sectional study on 200 Iron deficiency anaemia patients 50 with Diabetes mellitus in group A and 150 with non-diabetic in group B our study is to compare the effects of IDA on HBA1c levels. In this study HbA1c in Non diabetics with IDA is negatively correlated significantly with Body mass index, hemoglobin, PCV, MCHC and not correlated with age, MCV, MCH, Serum ferritin, FBS, PPBS.

Similar to our study in 2014 a study was done by Vishal Kalasker et al[22] on the effect of iron deficiency anemia on glycosylated hemoglobin levels in non-diabetic Indian adults. They postulated that Hb concentrations are positively corrected with HbA1c concentration and that HbA1c concentration tended to be lower in the presence of iron deficiency anemia. But they concluded that iron deficiency anemia is unlikely to be a major concern in diagnosing diabetes using concentration of HbA1c according to the ADA guideline.

Rajagopal, Ganapathy, Arunachalam, Raja, & Ramraj[23] and Shanthi, Revathy, Devi, & Subhashree[24] both examined a non-diabetic population that included males and females. In both of the studies, their iron deficient group had higher levels of A1C than the non-iron deficient group.

N.S Neki et al[25] did a study to analyse change in HbA1c level with treatment of iron deficiency anaemia. 50 confirmed 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%).

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The baseline HbA1c was found to be greater in individuals with associated iron deficiency anemia (6.2%) in a study by Vydehi Veera malla et al[26] on diabetic patients. After receiving iron supplements for two months, the HbA1c dramatically decreased (5.1%).
HbA1c in diabetics with IDA is negatively correlated significantly with age, hemoglobin, PCV, MCH, MCV, Serum ferritin and MCHC and not correlated with BMI, FBS, PPBS.
According to a study by Alap L. Christy et al,[27] in diabetics with regulated plasma glucose levels, iron deficiency anemia raises HbA1c levels. They hypothesised that elevated HbA1cvaluesarepositivelycorrelatedwith iron deficient anemia.
Study done by Van Heyningen et al[28] showed there was no significant influence of iron deficiency anemia over HbA1c concentrations. They suggested that differences observed in previous studies could be due to the various laboratory methods used in estimating the HbA1c.
El-Agouza et al [29] reported that with iron deficiency anemia HbA1c levels also increase and it decreased after treatment. That is if the plasma glucose was maintained, the lower hemoglobin concentration would lead to rise in HbA1c levels.
Coban and others [30] study comprised of 100 participants - 50 had IDA while the rest 50 were healthy controls chosen after matching for age and sex. Patients with IDA were given oral supplementation of ferrous sulphate 100 mg a day for 3 months. HbA1c values dropped significantly after iron treatment ($p<0.001$). This study suggests that IDA must be corrected before using HBA1c for making a diagnostic or therapeutic decision.

5. CONCLUSIONS:

Our study concludes that iron deficiency was associated with higher proportions of HbA1c Hence, the iron status must be considered during the interpretation of the HbA1c in diabetics and also non-diabetic subjects. Our data also imply that clinicians should be careful when identifying prediabetes and diabetes in anemic patients. IDA characteristics are strongly correlated with HbA1c in non-diabetics. Therefore, IDA related variables should be assessed before diagnosing diabetes. We recommend correcting the anemia in patients with IDA before using HbA1c for diagnostic purposes.

6. REFERENCES:

1. Andrews, N.C. The iron transporter DMT1. Int. J. Biochem. Cell Biol. 1999, 31, 991–994. [CrossRef] [PubMed]
2. Adams, P.C.; Reboussin, D.M.; Barton, J.C.; McLaren, C.E.; Eckfeldt, J.H.; McLaren, G.D.; Dawkins, F.W.; Acton, R.T.; Harris, E.L.; Gordeuk, V.R.; et al. Hemochromatosis and iron-overload screening in a racially diverse population. N. Engl. J. Med. 2005, 352, 1769–1778. [CrossRef]
3. Salonen, J.T.; Tuomainen, T.-P.; Nyyssönen, K.; Lakka, H.-M.; Punnonen, K. Relation between iron stores and non-insulin dependent diabetes in men: Case-control study. BMJ 1998, 317, 727–730. [CrossRef] [PubMed]
4. U.S. National Library of Medicine. Available online: https://pubmed.ncbi.nlm.nih.gov/31082013/ (accessed on 5 August 2023).
5. Abbaspour, N.; Hurrell, R.; Kelishadi, R. Review on iron and its importance for human health. J. Res. Med. Sci. 2014, 19, 164–174. [PubMed]
6. Liu, J.; Li, Q.; Yang, Y.; Ma, L. Iron metabolism and type 2 diabetes mellitus: A meta-analysis and systematic review. J. Diabetes Investig. 2020, 11, 946–955. [CrossRef]

ISSN: 0975-3583, 0976-2833 VOL15, ISSUE9, 2024

7. World Health Organization. Use of Glycated Haemoglobin (HbA1c) in Diagnosis of Diabetes Mellitus. Available online: https:// www.who.int/publications-detail-redirect/use-of-glycated-haemoglobin-(- hba1c)-in-diagnosis-of-diabetes-mellitus (accessed on 5 August 2023).
8. Massi-Benedetti, M. Changing targets in the treatment of type 2 diabetes. Curr. Med. Res. Opin. 2006, 22, S5–S13. [CrossRef]
9. The International Expert Committee. International Expert Committee Report on the role of the A1C assay in the diagnosis of diabetes. Diabetes Care 2009, 32, 1327–1334. [CrossRef]
10. Christy, A.L.; Manjrekar, P.A.; Babu, R.P.; Hegde, A.; Rumkini, M.S. Influence of Iron Deficiency Anemia on Hemoglobin A1C Levels in Diabetic Individuals with Controlled Plasma Glucose Levels. Iran Biomed. J. 2014, 18, 88–93. [CrossRef]
11. Prasad, K.; Gupte, S. Correlation of Iron Deficiency Anemia on HbA1C Levels: Comparison among Patients living with Diabetic and Non-diabetics. Int. J. Health Clin. Res. 2020, 3, 160–162.
12. Chaudhari, A.S.; Sontakke, A.N.; Trimbake, S.B. HbA1c status in Type II Diabetes Mellitus with and without Iron Deficiency Anemia. Int. J. Biochem. Res. Rev. 2020, 29, 114–120. [CrossRef]
13. Madhu, S.V.; Raj, A.; Gupta, S.; Giri, S.; Rusia, U. Effect of iron deficiency anemia and iron supplementation on HbA1c levels—Implications for diagnosis of Prediabetes and diabetes mellitus in Asian Indians. Clin. Chim. Acta 2017, 468, 225–229. [CrossRef]
14. Urrechaga, E. Influence of iron deficiency on Hb A1C levels in type 2 diabetic patients. Diabetes Metab. Syndr. 2018, 12, 1051–1055. [CrossRef]
15. Hong, J.W.; Ku, C.R.; Noh, J.H.; Ko, K.S.; Rhee, B.D.; Kim, D.-J. Association between the presence of iron deficiency anemia and hemoglobin A1C in Korean adults. Medicine 2015, 94, e825. [CrossRef]
16. Jimenez, K.; Kulnigg-Dabsch, S.; Gasche, C. Management of Iron Deficiency Anemia. Gastroenterol. Hepatol. 2015, 11, 241–250
17. Brooks, A.P.; Metcalfe, J.; Day, J.L.; Edwards, M.S. Iron deficiency and glycosylated haemoglobin A. Lancet 1980, 2, 141. [CrossRef]
18. Gram-Hansen, P.; Eriksen, J.; Mourits-Andersen, T.; Olesen, L. Glycosylated haemoglobin (HbA1c) in iron- and vitamin B12 deficiency. J. Intern. Med. 1990, 227, 133–136. [CrossRef]
19. Muldowney, F.P., Crooks, J., Wayne, E.J., The total red cell mass in thyrotoxicosis and myxedema. 16: 309-314 (1957).
20. Eid, W.E., Potalla, J.V., Value of Hemoglobin A1c in Diagnosing Diabetes Mellitus within a Chronic Disease Management System Illustrated by theReceiver Operating Characteristic Curve. Endocrine Practice. 16(1): 14-20 (2010).
21. Lavanya Rajagopal, Sundaram Arunachalam 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.Annals of Pathology and Laboratory Medicine, Vol. 04, No. 02, March - April, 2017.
22. Vishal Kalasker , Sudhamadhuri, Kodliwadmath M V, Harish Bhat. 'Effect of Iron Deficiency Anemia on Glycosylated Hemoglobin Levels in Non Diabetic Indian Adults', Int J Med Health Sci, (jan 2014) Vol-3;(Issue-1),pp 41-43]
23. Rajagopal, L.; Ganapathy, S.; Arunachalam, S.; Raja, V.; Ramraj, B. Does Iron Deficiency Anaemia and its Severity Influence HbA1C Level in Non Diabetics? An Analysis of 150 Cases. J. Clin. Diagn. Res. 2017, 11, EC13–EC15. [CrossRef]

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24. Shanthi, B., Revathy, C., Manjula Devi, A. J., & Subhashree. (2013). Effect of Iron Deficiency on Glycation of Haemoglobin in Nondiabetics. Journal of Clinical and Diagnostic Research : JCDR, 7(1), 15–17.
25. 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.
26. 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.
27. Christy AL, Manjrekar PA, Babu RP, Hegde A, Rukmini MS. Influence of iron deficiency anemia on hemoglobin A1c levels in diabetic individuals with controlled plasma glucose levels. Iran Biomed J. 2014;18(2):88-92
28. van Heyningen C, Dalton RG. Glycosylated haemoglobin in irondeficiency anaemia. Lancet 1(8433):874, 1985
29. El-Agouza I, Abu Shahla A, Sirdah M. The effect of iron deficiency anaemia on the levels of haemoglobin subtypes: possible consequences for clinical diagnosis. Clin Lab Haematol 24: 285-289, 2002.
30. Coban E, Ozdogan M, Timuragaoglu A. Effect of iron deficiency anemia on the levels of hemoglobin A1c in nondiabetic patients. Acta Hematol 2004;112:126 128 [PubMed]