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Original Research Article

Association of Haemoglobin and Glycated Haemoglobin Level among Iron Deficient versus Non-Iron Deficient Non Diabetic Patients in a Tertiary Care Centre of Gujarat

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Abstract

Background: Iron deficiency Anaemia (IDA) is characterised by reduction in the number of Red Blood Cells (RBCs) or Hb. concentration due to incomplete Hb synthesis. Glycated Haemoglobin (HbA1c) is person's average blood glucose (sugar) levels for the last two to three months. Patients with haemolytic disease or other conditions with shortened RBC survival exhibit a substantial reduction in HbA1c. Similarly, individuals with recent significant blood loss have falsely low values owing to a higher fraction of young erythrocytes. Some studies have reported iron depletion is related to increased Glycation of Hb. resulting in false high values of HbA1c.

Objectives: This study was conducted with objective of to compare the effect of Hb% and HbA1c level among iron deficient and non-iron deficient nondiabetic patients.

Material and Methods: This cross-sectional and comparative study was conducted in the Department of Biochemistry, Pathology and Medicine of tertiary care hospital of Gujarat. Total 184 non-diabetic patients with confirmed diagnosis of IDA were included as cases. Another 184 healthy individuals without IDA and diabetes were included as control. Statistical analysis was done by student's t-test (unpaired t-test) after testing for homogeneity of variance and p-value was calculated (p-value <0.05 significant).

Result: Mean HbA1c in group A was more as compared to group B and this difference was highly significant (p<0.0001). It shows that iron deficient patients had higher value of HbA1c as compared to non-iron deficient subjects

Conclusion: Our study concluded that iron deficiency leads to increase in HbA1c. Using HbA1c as a common diagnostic tool for diabetes we must keep in mind the IDA status for better endocrinological profile and medication of patients. HbA1c should always be interpreted cautiously in anaemic patients.

Keywords: Diabetes Mellitus, Glycated Haemoglobin, Haemoglobin, Iron Deficiency Anaemia.

Introduction

Iron deficiency Anaemia (IDA) occurs when your body does not have enough iron. Iron helps make red blood cells. Iron deficiency anaemia is the commonest form of anaemia in all types of anaemia. Anaemia is a serious global public health problem that mainly affects young children and pregnant women. Anaemia is characterised by reduction in the number of Red Blood Cells (RBCs) or Hb. concentration due to incomplete Hb. synthesis that results in microcytic and hypochromic RBCs [1]. Criteria for anaemia is Hb value <12 g/dl in women and <13 g/dl in men as per World Health Organisation (WHO) guideline [2]. In developing countries, causes for anaemia are multi-factorial like nutritional deficiencies (iron, folate, and vitamin B12 deficiencies), infections and chronic illness. The term "Diabetes Mellitus" describes a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The effects of diabetes mellitus include long–term damage, dysfunction and failure of various organs [3]. Glycated Haemoglobin (HbA1c) is person's average blood glucose (sugar) levels for the last two to three months. Formation of HbA1c is essentially irreversible. Its concentration in the blood depends on both the life span of the RBC (average life span is 120 days) and the blood glucose concentration. Values of HbA1c are unaffected by day-to-day glucose fluctuations, recent exercise or food ingestion [4]. Interpretation of HbA1c depends on RBCs having a normal lifespan. Patients with haemolytic disease or other conditions with shortened RBC survival exhibit a substantial reduction in HbA1c [5]. Similarly, individuals with recent significant blood loss have falsely low values owing to a higher fraction of young erythrocytes. Some studies have reported iron depletion is related to increased Glycation of Hb. resulting in false high values of HbA1c [6]. The effect of IDA on HbA1c estimation is yet to be established as the studies conducted so far have no specific results [7,8]. This study was conducted with objective of to compare the effect of Hb% and HbA1c level among iron deficient and non-iron deficient non-diabetic patients.

Materials and Methods

This was cross-sectional and comparative study, conducted in the Department of Biochemistry and Pathology of tertiary care hospital of Gujarat, India for 6 months from October 2021 to March 2022. Study was approved from the Institutional Ethics Committee Study was started after getting informed consent from the study participants. In 6 months approximately 100000 samples were processed in the Department of Biochemistry & Pathology. Out of these only 40% of samples were for CBC (including Haemogram), Blood Sugar, HbA1c and Serum Iron. Hence taking 95% as confidence level, 5% as margin of error, 40% population proportion and 100000 population size, the size of consecutive samples taken was 368 [9]. Out of which 184 cases of IDA with non diabetic (Group A) and 184 healthy subjects as control Non IDA and Non diabetic (Group B) were consecutively selected from OPD and IPD of Department of Medicine. Both male and females of age 18 to 65 years were included as case as well as control. Non-diabetic patients with confirmed diagnosis of IDA were included as cases. Healthy individuals without IDA, diabetes was included as control. Hb% (gm/dl) for inclusion as cases was <12 for female and <13 for male. Those who were less than 18 years and more than 65 years, having diabetes, renal disease, Pregnant women, history of recent blood transfusion and having anaemia due to blood loss or

heamoglobinopathies were excluded from study. After obtaining informed consent from study participants, blood sample was collected from each of the study subject for Haemogram (CBC)- White Blood Cell (WBC) count, Total 1. leucocyte count, Differential leucocyte count, RBCs, Platelet count, Packed Cell Volume (PCV), Red Cell Distribution Width (RDW), Hb%, MCH, MCV, MCHC. Estimation of Blood sugar- Fasting and Postprandial (PP), HbA1c, blood urea, serum creatinine, Estimation of Iron profile- Serum iron, Serum ferritin, TIBC. A 10 ml of blood was collected from both groups in overnight fasting condition in the morning hours. The antecubital vein of the arm was preferred for sample collection. Blood was collected in plain vial, EDTA and Fluoride vial. The tubes were appropriately labelled and transferred to the laboratory. Blood was collected in plain vial and Fluoride vial was allowed to clot by placing the vacutainer tube with red and lavender cap, respectively in a rack at the room temperature for at least 30 minutes. Then vials were centrifuged at 2000 rpm for 10 minutes and serum was separated. The clear serum was analysed within eight hours. Grossly haemolysed samples were avoided for analysis. After proper mixing of EDTA blood haemogram (CBC) was done on Advia 2120 Hematology System by Siemens Healthineers India [10]. Serum iron [11], Serum TIBC [12], glycated haemoglobin [13], fasting and PP blood glucose [14] and kidney function test [15,16] was done on AU 5800 chemistry analyser by Beckman Coulter. Serum ferritin17 was estimated by Chemiluminescence Immunoassay (CLIA) method in Beckman Coulter Access2 instrument by Beckman Coulter. Internal and external quality control of all parameters was regularly maintained by controls provided by Bio-Rad and CMC Vellore. Collected data was entered in Microsoft Excel 2010. Mean and the Standard Deviation (SD) for all the variables of both the groups were calculated using MedCal C v.12.5.0. Statistical analysis was done by student's t-test (unpaired t-test) after testing for homogeneity of variance and p-value was calculated (p-value <0.05 significant).

Results

As per table 1 in group A, 82 (44.57%) participants were male and 102 (55.43%) were female. In group B, 95 (51.63%) participants were male and 89 were female (48.37%). Both groups are comparable (p=0.21). Mean age in group A was 42.38 years and mean age for group B was 39.86 years, that was statistically not significant (p=0.09).

As shown in table 2, mean of Hb%, MCV, MCH and MCHC were significantly higher in group B compared to group A (p-value <0.0001) because group B was taken as non-iron deficient patients. Mean serum iron and mean serum ferritin was higher in group B as compared to group A and the difference was highly significant(p<0.0001). Mean serum TIBC was more in group A compared to group B and the difference was highly significant. Mean HbA1c in group A was more as compared to group B and this difference was highly significant (p<0.0001). It shows that iron deficient patients had higher value of HbA1c as compared to non-iron deficient subjects.

Table 1: Sociodemographic Data

Gender	Group A(n=184)	Group B(n=184)	p- Value
Male	82 (44.57%)	95 (51.63%)	0.21
Female	102 (55.43%)	89 (48.37%)	
Age in Years (Mean ± SD)	42.38±13.72	39.86±15.32	0.09

Table 2: Comparison of Haemogram and Iron profile

Haemogram	Group A(n=184) Mean ± SD	Group B(n=184) Mean ± SD	p- Value
Hb%(g/dl)	8.02 ± 2.46	12.86 ± 1.12	<0.0001
MCV (fl)	69.28 ± 11.02	86.22 ± 7.22	<0.0001
MCH (pgm	26.10 ± 3.78	29.86 ± 2.16	<0.0001
MCHC (gmHb/dl)	28.72 ± 2.98	34.26 ± 2.12	<0.0001
Iron profile			
Serum Iron (µgm/dl)	33.42 ± 19.22	74.18 ± 23.46	<0.0001
Serum Ferritin (µgm/dl)	19.81 ± 30.12	62.06 ± 46.38	<0.0001
Serum TIBC (µgm/dl)	392.80 ± 128.20	269.24 ± 86.68	<0.0001

As shown in table 3, Glycated Haemoglobin is significantly higher in Group A compared to Group B (P <0.0001).

Table 3: Comparison of HbA1c

Parameters	Group A(n=184) Mean ± SD	Group B(n=184) Mean ± SD	p-Value
Glycated Haemoglobin (HbA1c) (gm Hb%)	5.98 ± 0.72	5.34 ± 0.30	<0.0001

Discussion

A study by Shanthi B et al., found that the mean HbA1c value in the patients with IDA was more than the control group (p <0.001) [6] that is comparable with our study. Kim C et al., reported an increase in HbA1c in iron-deficient people and lead to an upward shift of HbA1c distribution. In the present study similar results were obtained but the HbA1c level was in normal range [18]. Parlapally RP et al., found that the mean HbA1c level in the patients with IDA (6.13% ± 0.57%) was higher than that in the control group (5.12 ±0.30%) (p<0.001) [19] Adeoye S et al., found that non-diabetic anaemic patients had lower mean of HbA1c (5.3 vs 5.7). Result of Adeoye S et al., was different from present study [20]. Sinha N et al., found that mean baseline HbA1c level in anaemic patients was significantly lower than that of non-anaemic group, result of our study was different compared to our study [21].

Conclusions

Our study concluded that iron deficiency (decreased Hb%) leads to increase in HbA1c. In Indian population Iron Deficiency Anaemia and Diabetes Mellitus are extremely common. Using HbA1c as a common diagnostic tool for diabetes we must keep in mind the IDA status for better endocrinological profile and medication of patients. HbA1c should always be interpreted cautiously in anaemic patients. Mechanism of high HbA1c level in IDA is not established and various theories exist to explain this, however more large-scale studies are required to find out the proper mechanism underlying this relationship.

References

1. De LM, Pena-Rosas RJP, Cusick S. (Eds.) (2011). Haemoglobin Concentrations for the Diagnosis of Anemia and Assessment of Severity; Vitamin and Mineral Nutrition Information System. (p 1). Volume; WHO/NMH/NHD/MNM/11.1. Geneva, Switzerland: World Health Organization. Available from https://apps.who.int/iris/bitstream/hand

le/10665/85839/WHO_NMH_NHD_MNM_11.1_eng.pdf?ua=1
2. Anaemia [Internet]. Who.int. 2022 [cited 12 May 2022]. Available from: https://www.who.int/health-topics/anaemia
3. Diabetes [Internet]. Who.int. 2022 [cited 10 May 2022]. Available from: http://www.who.int/diabetes/action_online/basics/en/
4. Sacks DB. Diabetes mellitus. Glycated Hemoglobin. In: Burtis CA, Ashwood ER, Bruns DE, (Eds.).Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 5th ed. St. Louis, Elsevier Saunders, 2012. p.1441-7.
5. Bry L, Chen PC, Sacks DB. Effects of hemoglobin variants and chemically modified derivatives on assays for glycohemoglobin [Review]. Clin Chem. 2001; 47:153-63.
6. Shanthi B, Revathy C, Manjula Devi AJ, Subhashree, Shanthi B, Revathy C, et al. Effect of iron deficiency on glycation of haemoglobin in nondiabetics. 2013;7(1):15-17.
7. Lavanya Rajagopal L, Shivashekar Ganapathy S, Sundaram Arunachalam, S, Veena Raja V, Balaji Ramraj B. Does iron deficiency anaemia and its severity influence HbA1C level in nondiabetics An analysis of 150 cases. J Clin Diagn Res. 2017;11(2):EC13-EC15.
8. Van Heyningen C, Dalton RG, Glycosylated haemoglobin in iron-deficiency anaemia, Lancet. 1985;1(8433):874.
9. Sample Size Calculator [Internet]. Calculator.net. 2022 [cited 10 Feb 2022]. Available from: https://www.calculator.net/sample-sizehttps://www.calculator.net/sample-size
10. Harris N, Kunicka J, Kratz A. The ADVIA 2120 hematology system: flow cytometry-based analysis of blood and body fluids in the routine hematology laboratory. Lab Hematol. 2005;11(1):47-61.
11. Beckmancoulter.com. 2022 [cited 12 Feb 2022]. Available from: https://www.beckmancoulter.com/wsrportal/techdocs?docname=/cis/A18491/%%/EN_FE.pdf
12. Beckmancoulter.com. 2022 [cited 07 March 2022]. Available from: https://www.beckmancoulter.com/wsrportal/techdocs?docname=/cis/A18504/%25%25/%25_A18504-%25%25_English.pdf
13. Beckmancoulter.com. 2022 [cited 09 March 2022]. Available from: https://www.beckmancoulter.com/wsrportal/techdocs?docname=/cis/A18497/AG/EN_HbA1c.pdf
14. Beckmancoulter.com. 2022 [cited 10 Feb 2022]. Available from: https://www.beckmancoulter.com/wsrportal/techdocs?docname=/cis/A18496/%25%25/%25_A18496-%25%25_English.pdf
15. Beckmancoulter.com. 2022 [cited 07 April 2022]. Available from: https://www.beckmancoulter.com/wsrportal/techdocs?docname=/cis/A18483/AG/EN_CREm.pdf
16. Beckmancoulter.com. 2022 [cited 05 Feb 2022]. Available from: https://www.beckmancoulter.com/wsrportal/techdocs?docname=/cis/A18564/%25%25/EN_UREA.pdf
17. Beckmancoulter.com. 2022 [cited 04 March 2022]. Available from:https://www.beckmancoulter.com/wsrportal/techdocs?docname=/cis/988631/%%/FER_988631-%25%25_English.pdf
18. 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.
19. Parlapally RP, Kumari KR, T Srujana T. A study of effect of iron deficiency anaemia on glycation of haemoglobin in nondiabetics. International Journal of Scientific Study. 2016;4(5):194-96.

20. Adeoye S, Abraham S, Erlikh I, Sarfraz S, Borda T, Yeung L. Anemia and hemoglobin A1c level: Is there a case for redefining reference ranges and therapeutic goals? British Journal of Medical Practioners. 2014;7(1):a706.
21. Sinha N, Mishra TK, Singh T, Gupta N. Effect of iron deficiency anaemia on haemoglobin A1c levels. Ann Lab Med. 2012;32(1):17-22.