DE GRUYTER OPEN

http://www.jrdiabet.ro
Rom J Diabetes Nutr Metab Dis. **22**(3):289-296
doi: 10.1515/rjdnmd-2015-0035

HAEMOGLOBIN A1C AND IRON DEFICIENCY ANAEMIA OUR UNDERSTANDING THROUGH THE DECADES

Nadia Hussain ✉

Department of Physiology, College of Medicine and Health Sciences, UAE University

received: June 30, 2015 *accepted*: August 23, 2015
available online: September 15, 2015

Abstract

HbA1c concentrations are affected by several factors including red blood cell turnover. The International Expert Committee has highlighted this observation for the benefit of physicians who evaluate HbA1c in diabetics. There are many types of anaemia that affect glycated haemoglobin (HbA1c) values but iron deficiency anaemia, one of the most common, has been proved to show higher than true values of HbA1c. The mechanism of how iron deficiency anaemia affects HbA1c has yet to be understood. Several studies have been conducted in order to unravel the mechanisms but there still remains a dearth of information. Future research needs to focus on the mechanistic reasons why HbA1c is higher in patients with iron deficiency anaemia in particular. This can pave the way for possible large scale studies to address the HbA1c enhancing effect and the mechanism of increased HbA glycation in iron deficiency properly.

key words: *diabetes; haemoglobin; iron deficiency anaemia; HbA1c*

Introduction

Haemogloblin A1c (HbA1c) is a glycated form of haemoglobin (Hb) that is formed when the NH2-terminal valine residue of the β chain of globin is glycated [1]. It is most often used to assess glycaemic control over the previous three months.

HbA1c and other glycated haemoglobins, constitute the HbA1 fraction of adult hemoglobin (HbA). HbA1c is the predominant hemoglobin found in HbA1 fractions [1].

Since 2010, there has been a change in using HbA1c for more than just glycaemic control and it is now accepted for the diagnosis of diabetes [2].

The Diabetes Control and Complications Trial (DCCT) and the United Kingdom Prospective Diabetes Study (UKPDS) were two major clinical trials that showed a clear link between metabolic control (as measured by HbA1c) and the risk of chronic diabetes complications [3,4]. Worldwide diabetic centers recommend and use specific HbA1c targets in terms of DCCT/UKPDS HbA1c.

The American Diabetes Association (ADA) guidelines recommend HbA1c levels below 7% in all diabetic patients [5].

The value of HbA1c depends on three main factors; the Hb present in reticulocytes when these are released from the bone marrow, the glycation rate of Hb as the erythrocytes age (since Hb glycation rate is a function of glucose

✉ P.O.Box; 17666, Al Ain, UAE; Phone: 0097142580066; Fax: 00971422580099
corresponding author e-mail: hussain1214@uaeu.ac.ae

concentration to which Hb is exposed), and the mean age of erythrocytes in the circulation [6-9].

HbA1c is currently used to evaluate the level of metabolic control, assess the risk of developing diabetic complications, and measure the quality of diabetes care. However, there are several medical conditions (not related to glycaemia) that can lead to falsely higher or lower values. The presence of Hb variants, structural haemoglobinopathies and thalassemia syndromes affect HbA1c. Any state that decreases the age span of the red blood cells will lead to lower results than the actual value of HbA1c, no matter what assay is used to measure HbA1c [10].

Hb variants such as haemoglobin S, haemoglobin C and haemoglobin E influence certain assay methods used to measure HbA1c. Elevated haemoglobin F, which is also associated with thalassemia syndromes also affects some assay methods [11]. Other factors that affect HbA1c assays include haemolytic anaemias, haemoglobinopathies, chronic blood loss, pregnancy, opiate consumption, vitamin C ingestion, vitamin E ingestion, chronic ingestion of salicyclates, alcoholism, uremia, hyperbilirubinemia, aberrant lipid profiles, hemolytic anaemia and also in recovery from acute blood loss [11-19].

There are also ethnic and racial differences in HbA1c. Studies that compare HbA1c in groups with type 2 diabetes (T2DM) patients have shown higher HbA1c levels in African Americans, Hispanics, and Asian/Pacific Islanders when compared to Caucasians. Factors that might affect glycaemia such as age, gender, adiposity, fasting and post-load glucose were all taken into account. In fully adjusted models, up to 8% of the variance in HbA1c was observed [20].

Anaemia and its effect on HbA1c has not been studied extensively. The presence of anaemia can lead to increased red blood cell turnover and lower values of HbA1c than actual values. The red blood cell turnover can be decreased too and this will increase glycation rate of the Hb N-terminal valine and give higher than expected values of HbA1c [21].

Iron deficiency anaemia (IDA) is one of the most common types of anaemia found worldwide [22]. The WHO (World Health Organization) estimates that 2.1 billion people globally have iron deficiency anaemia which is approximately 30% of the world population at the time [22-24]. The prevalence of iron deficiency is higher in low and high income countries. Adolescents, children and women are the most susceptible. In these countries, diabetes is also a rapidly increasing issue [25].

Studies conducted in the 1980 – 1990s

One of the earliest studies investigating the effects of IDA on HbA1c measurements was performed by Horton and Husiman and showed that the mean concentration of HbA1c was 4.9% in IDA patients compared to 5.3% in healthy individuals [12]. Also, Brooks et al. discovered that non-diabetic individuals with IDA had increased mean concentrations of Hb after 6 weeks of iron therapy. The mechanism that led to increased glycosylated HbA1 levels in IDA was not clear, but it was postulated that the quarternary structure of the haemoglobin molecule was altered in IDA. This allowed the β globin to be more readily glycosylated in the face of low iron levels. It was also speculated that if haemoglobin glycosylation is linked to microvascular complications, then diabetics with IDA could be at greater risk [26].

However, Sluiter et al [27] postulated that formation of glycated haemoglobin is irreversible and the HbA1c concentration in a red blood cell increases with the cell age. In individuals with normal blood glucose and

normal red blood cell life span, the HbA1c levels should be normal. In those individuals where the red blood cells have a shorter life span, a phenomenon that often occurs after iron treatment, HbA1c levels will decrease. If the IDA is chronic, red blood cell production will decrease leading to anaemia and a longer life span of red blood cells in the circulation. This would lead to an increased HbA1c [27].

Mitchell et al. [28] used the mean cell haemoglobin to calculate the absolute amount of HbA1 in each red cell before iron therapy and 6 weeks later. The amounts of HbA1c were 1.9 pg and, respectively 1.95 pg, which pointed to no significant difference of iron therapy on HbA1c. Thus it was considered unlikely that red blood cell age was a significant factor in IDA treatment [28].

In a patient who had IDA and T2DM, Davis et al reported that the glycated Hb concentration decreased from 15.4% to 11%, independent of glycaemic control which was assessed by blood glucose concentration [29].

Using the affinity chromatography to measure glycosylated haemoglobin in non-diabetic IDA patients, it was found that the mean glycosylated Hb was not significantly different from normal. The values did not alter significantly after iron treatment. It was assumed that the HbA1c value differences noted in previous studies could have appeared because of the post-translational modifications of haemoglobin rather than glycosylation in IDA [28]. The modified haemoglobin would co-elute with HbA1 and affect readings in cation exchange chromatography assay methods. However, this modification however, would not affect affinity gel assays because this technique depends on the binding between the gel and glucose residues on the globin chains alone [30].

Using colorimetric assays, ion exchange chromatography, and affinity chromatography to measure HbA1c in non-diabetic IDA patients and controls showed no significant differences between any of the methods [31].

Other studies showed similar results but treatment with iron and vitamin B12 decreased HbA1c significantly and altered red blood cell indices. It was postulated that in IDA, the red blood cell survival rate is normal, but survival rate decreased in vitamin B12 deficiency. This affects mature and immature red blood cells. The decrease in HbA1c levels after treatment could be due to an increase in bone marrow production of red blood cells and production of new immature red blood cells. It was concluded that in patients with IDA and vitamin B12 deficiency, glycosylated haemoglobin can illustrate the changes in the red blood cell population observed when mostly immature ones are being made [32].

However, another study showed an inverse relationship between hemoglobin levels and HbA1c in paediatric patients with and without T1DM, with one third of them having IDA. Higher HbA1c concentrations were found in diabetic patients with IDA, independent of glycaemic control. Iron supplementation decreased HbA1 in both non diabetic and diabetic groups. This could be because of young red blood cells in the blood appear after iron therapy and lead to a “dilution effect” and lowering of HbA1c. The group stated there was no correlation between HbA1c and any other parameter than Hb. Structural or affinity changes in Hb, rather than iron concentration, reflect in changes of HbA1c [33].

Studies conducted in the 2000’s and beyond

The effect of IDA on Hb subtype levels has also been studied using complete blood counts. This was done in a study using IDA individuals before and after iron therapy. Iron treatment improved RBC, Hb, Hct (haematocrit), MCV

(mean corpuscular volume) and MCH (mean corpuscular volume) values but HbA1c levels decreased as much as 17%. Iron replacement used in IDA diabetics could increase the reliability of HbA1c measurements. It was postulated that in normal individuals, there is a balance between HbA and serum glucose and if the glucose remained constant, a decrease in Hb concentration could cause an increase in the glycated fraction [34].

A study that compared non diabetics with IDA to control groups found a higher mean HbA1c in IDA individuals. HbA1c significantly decreased after a 3 month course of iron therapy [35].

Glycosylated haemoglobin has also been studied for its potential use as an index to distinguish between IDA and thalassemia minor. In a study on non-diabetic individuals, HbA1c levels were measured in β thalassemia, IDA and healthy controls. Median glycosylated hemoglobin was lower in β-thalassemia minor than IDA patients but there was no difference between IDA and control groups. In addition, in the IDA groups there was no significant correlation between HbA1c and other haemotological paramenters. The normal HbA1c concentrations in IDA could be due to normal red blood cell survival rate and normal levels of glycosylated haemoglobin in mature red blood cells [36].

Menopause has also been studied in respect with its link to IDA and HbA1c. Koga et al. [37] evaluated red blood cell indices and glycated haemoglobin in pre-menopausal women and showed that RBC count is positively associated with HbA1c, but the case is the opposite for Hb, MCH and MCV. In the post-menopausal group, none of the indices could be linked to HbA1c. The MCH index had the highest correlation coefficient for association with HbA1c in premenopausal women because a decrease of 1 pg in MCH corresponded to an increase of approximately 0.03% in HbA1c [37]. In IDA, MCV and MCH modifications are observed before total Hb and RBC count are affected [38]. In premenopausal women, menstrual blood loss can cause IDA. It was concluded that the relatively iron deficient state of premenopausal women with lower MCH would be responsible for the higher observed HbA1c levels. In IDA, a decrease in Hb concentration could lead to an increase in the glycation fraction [34,35]. The HbA1c catabolism could be reduced in premenopausal women with lower MCH because if the red blood cell life span is increased then the HbA1c levels also increase. However, the study was not able to conclude this because information on red blood cell life span was contradictory [39-41].

Later Koga et al. [42] conducted a study to relate iron metabolism indices with HbA1c in premenopausal women. The women had normal glucose tolerance and were grouped into those with IDA or iron deficient state (IDS) and the normal iron state (NIS). HbA1c levels were shown to be inversely associated with serum iron, serum transferrin saturation and serum ferritin. HbA1c levels were significantly higher in IDA and IDS than in the NIS group. It was concluded that iron deficiency increases HbA1c in premenopausal women regardless if the anaemia is yet present or not [42].

Pregnant women have also been studied to find a correlation between HbA1c levels and glycaemia in late pregnancy. In this stage, most women already have IDA. Using erythrocyte indices and iron metabolism indices, two studies by Hashimoto et al. [43] included non-diabetic pregnant women and later diabetic pregnant women not supplemented with iron. In both studies, the HbA1c levels were significantly increased in late pregnancy. HbA1c levels showed a negative correlation with MCH, serum

transferrin saturation and serum transferrin. It was concluded that the late pregnancy increase in HbA1c levels was because of the IDA presence at this stage [43].

A study involving non-diabetic pregnant women with and without IDA and an age matched control group, analysed the influence of iron metabolism indices on HbA1c and found a significant correlation between HbA1c and red blood cell and iron metabolic indices. Amongst the women who had confirmed IDA, HbA1c, OGTT (oral glucose tolerance test), red blood cell and iron metabolic indices were measured before and after iron treatment. The results indicated that HbA1c levels were higher in women with IDA, and after iron supplementation, the HbA1c levels decreased. A significant correlation between HbA1c and red blood cell and iron metabolic indices was observed [44].

In the National Health and Nutrition Examination Survey (NHANES), observations of iron deficiency and HbA1c levels amongst non-diabetic adults showed that IDA increased the HbA1c level slightly and this occurred at the lower end of HbA1c levels i.e. between <5.5% and 5.5–6.0%. It could not be shown whether this was also the case in the higher end of the HbA1c spectrum [45].

Using cross-sectional data on HbA1c levels from the NHANES study, observations on IDA and non-IDA states showed there was a significant positive correlation between Hb concentrations and HbA1c among participants with and without iron deficiency [46]. There was a negative correlation between erythrocyte size and Hb with HbA1c which supported the theory that iron deficiency increases Hb glycation [47].

Another study that included T2DM patients with chronic kidney disease receiving treatment with intravenous iron and/or erythrocyte stimulating agents (ESA) found that mean HbA1c values fell in both groups. This showed that there was a statistically important decrease in HbA1c after treatment, regardless of glycaemic control [48].

A study by Ford et al. [46] analyzing the influence of iron-deficiency anemia and non-iron-deficiency anemia on HbA1c levels among adults in the US revealed a significant positive correlation between Hb concentrations and HbA1c. After adjusting for age, gender, and race, the mean HbA1c was 5.28% in participants with Hb < 100 g/L and 5.72% in participants with Hb > 170 g/L. The adjusted mean concentrations of HbA1c were 5.56% and 5.46% among participants with and without iron deficiency, respectively (P = 0.095). They suggested, in contrast to previous studies, that IDA had little population effect on HbA1c. The difference in concentrations of HbA1c between extremes of concentrations of Hb was 0.2%. It was concluded that people with anemia who are close to the diagnostic threshold should be retested or undergo another diagnostic method [46].

Another more recent study showed that when comparing HbA1c levels in patients with IDA, it was found that the mean baseline level of HbA1c in anaemic patients is lower and increases after treatment. The subsequent increase in HbA1c after iron supplementing was in contrast to the studies conducted previously. This was explained by the fact that their study group belonged to a lower socio-economic level where the cause of IDA was nutritional deficiency, rather than bleeding and malabsorption, and this could have affected the results [49].

Finally an Indian study that analyzed the effect of glycaemic and non-glycaemic parameters on HbA1c concentrations showed that if HbA1c is used to diagnose prediabetes and diabetes in iron deficient populations, this

leads to a false high prevalence [50]. Additionally other haemotological parameters that predict higher HbA1c are anaemia and red blood cell indices of IDA such as microcytosis, low MCH, low MCHC (mean corpuscular haemoglobin concentration) or high RDW (red blood cell distribution width) and low ferritin concentrations [50].

Conclusion

Worldwide, iron deficiency is the most common nutritional deficiency and this is especially true for the low and middle income countries. In these regions, diabetes is also a rapidly increasing phenomenon. HbA1c is a popular tool for the screening and diagnosing of diabetes. Other tests such as the OGTT are time consuming and repeated laboratory tests can become expensive for poorer populations. Non-glycaemic factors that affect assays such as conditions that affect red blood cell turnover affect HbA1c readings. Most studies indicate that, although other forms of anaemia lower HbA1c, iron deficiency anaemia appears to have an enhancing effect on HbA1c.

The mechanism of how IDA affects HbA1c remains elusive which indicates the need for further research to validate existing theories. Until large scale studies are conducted and the mechanism of increased HbA glycation in IDA states is not understood, we conclude that the reliability of HbA1c in IDA is questionable for diagnosing diabetes. People with anaemia who are close to the diagnostic threshold may need to be retested or to be diagnosed with an alternative method. HbA1c values that do not correlate with the clinical scenario or in diabetics with IDA should be approached with caution.

REFERENCES

1. **Telen MJ, Kaufman RE.** The mature erythrocyte function. In: Wintrobe`s Clinical Hematology, Greer JP, Foerster J (Eds), Lippincot Williams & Wilkins, Philadelphia, USA, pp 193-227, 1998.

2. **International Expert Committee.** International Expert Committee report on the role of the A1C assay in the diagnosis of diabetes. *Diabetes Care* 32: 1327-1334, 2009.

3. **UK Prospective Diabetes Study (UKPDS) Group.** Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet* 352(9131): 837-853, 1998.

4. **The Diabetes Control and Complications Trial Research Group.** The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Eng J Med* 329: 977-986, 1993.

5. **American Assocation of Diabetes.** Standards of medical care in diabetes. *Diabetes Care*, 30: S4-S41, 2007.

6. **Jeffcoate SL.** Diabetes control and complications: the role of glycated haemoglobin, 25 years on. *Diabet Med* 21: 657-665, 2004.

7. **Gerlo E, Gorus F.** Calibration of ion-exchange HPLC measurements of glycohemoglobin: effect on interassay precision. *Clin Chem* 43: 2353-2357, 1997.

8. **Landgraf R.** The relationship of postprandial glucose to HbA1c. *Diabetes Metab Res Rev* 20: S9-S12, 2004.

9. **Gorus F, Mathieu C, Gerlo E.** How should HbA1c measurements be reported? *Diabetologia* 49: 7-10, 2006.

10. **American Diabetes Association**. Tests of glycemia in diabetes. *Diabetes Care* 27: S91-3, 2004.

11. **National Glycohemoglobin Standardization Program.** Factors that interfere with HbA1c results 2014. Accessed at: http://www.ngsp.org/factors.asp.

12. **Horton BF, Huisman TH.** Studies on the heterogeneity of haemoglobin. VII. Minor haemoglobin components in haematological diseases. *Br J Haematol* 11: 296-304, 1965.

13. **Eberentz-Lhomme C, Ducrocq R, Intrator S, Elion J, Nunez E, Assan R.** Haemoglobinopathies: a pitfall in the assessment of glycosylated haemoglobin by

ion-exchange chromatography. *Diabetologia* 27: 596-598, 1984.

14. Bernstein RE. Glycosylated hemoglobins: hematologic considerations determine which assay for glycohemoglobin is advisable. *Clin Chem* 26: 174-175, 1980.

15. Starkman HS, Wacks M, Soeldner JS, Kim A. Effect of acute blood loss on glycosylated hemoglobin determinations in normal subjects. *Diabetes Care* 6: 291-294, 1983.

16. Lind T, Cheyne GA. Effect of normal pregnancy upon the glycosylated haemoglobins. *Br J Obstet Gynaecol* 86: 210-213, 1979.

17. Hanson U, Hagenfeldt L, Hagenfeldt K. Glycosylated hemoglobins in normal pregnancy: sequential changes and relation to birth weight. *Obstet Gynecol* 62: 741-744, 1983.

18. Phelps RL, Honig GR, Green D, Metzger BE, Frederiksen MC, Freinkel N. Biphasic changes in hemoglobin A1c concentrations during normal human pregnancy. *Am J Obstet Gynecol* 147: 651-653, 1983.

19. Lum G. Artefactually low hemoglobin A1c in a patient with hemolytic anemia. *Lab Medicine* 41: 267-270, 2010.

20. Kirk JK, Bell RA, Bertoni AG et al. Ethnic disparities: control of glycemia, blood pressure, and LDL cholesterol among US adults with type 2 diabetes. *Ann Pharmacother* 39: 1489-1501, 2005.

21. Gallagher EJ, Le Roith D, Bloomgarden Z. Review of hemoglobin A(1c) in the management of diabetes. *J Diabetes* 1: 9-17, 2009.

22. DeMaeyer E, Adiels-Tegman M. The prevalence of anaemia in the world. *World Health Stat Q* 38: 302-316, 1985.

23. McLean E, Cogswell M, Egli I, Wojdyla D, de Benoist B. Worldwide prevalence of anaemia, WHO Vitamin and Mineral Nutrition Information System, 1993-2005. P*ublic Health Nutr* 12: 444-454, 2009.

24. Stevens GA, Finucane MM, De-Regil LM et al. Global, regional, and national trends in haemoglobin concentration and prevalence of total and severe anaemia in children and pregnant and non-pregnant women for 1995-2011: a systematic analysis of population-representative data. *Lancet Glob Health* 1: e16-25, 2013.

25. International Diabetes Federation. International Diabetes Federation Atlas 6th edition**,** 2010. Accessed at: http://www.idf.org/diabetesatlas.

26. Brooks AP, Metcalfe J, Day JL, Edwards MS. Iron deficiency and glycosylated haemoglobin A. *Lancet* 8186(2):141, 1980.

27. Sluiter WJ, van Essen LH, Reitsma WD, Doorenbos H. Glycosylated haemoglobin and iron deficiency. *Lancet* 2(8193): 531-532, 1980.

28. Mitchell TR, Anderson D, Shepperd J. Iron deficiency, haemochromatosis, and glycosylated haemoglobin. *Lancet* 2 (8197): 747, 1980.

29. Davis RE, McCann VJ, Nicol DJ. Influence of iron-deficiency anaemia on the glycosylated haemoglobin level in a patient with diabetes mellitus. *Medical J Aust* 1: 40-41, 1983.

30. van Heyningen C, Dalton RG. Glycosylated haemoglobin in iron-deficiency anaemia. *Lancet* 1(8433): 874, 1985.

31. Rai KB, Pattabiraman TN. Glycosylated haemoglobin levels in iron deficiency anaemia. *Indian J Med Res* 83: 234-236, 1986.

32. Gram-Hansen P, Eriksen J, Mourits-Andersen T, Olesen L. Glycosylated haemoglobin (HbA1c) in iron- and vitamin B12 deficiency. *J Intern Med* 227: 133-136, 1990.

33. Tarim O, Küçükerdoğan A, Günay U, Eralp O, Ercan I. Effects of iron deficiency anemia on hemoglobin A1c in type 1 diabetes mellitus. *Pediatr Int* 41: 357-362, 1999.

34. 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.

35. Coban E, Ozdogan M, Timuragaoglu A. Effect of iron deficiency anemia on the levels of hemoglobin A1c in nondiabetic patients. *Acta Haematol* 112: 126-128, 2004.

36. Aslan D, Gursel T. The usefulness of glycosylated hemoglobin (HbA1C) in discriminating between iron deficiency and thalassemia. *Pediatr Hematol Oncol* 23: 307-315, 2006.

37. Koga M, Morita S, Saito H, Mukai M, Kasayama S. Association of erythrocyte indices with glycated haemoglobin in pre-menopausal women. *Diabet Med* 24: 843-847, 2007.

38. Harvey LJ, Armah CN, Dainty JR et al. Impact of menstrual blood loss and diet on iron deficiency among women in the UK. *Br J Nutr* 94: 57-564, 2005.

39. Temperley IJ, Sharp AA. The life span of erythrocytes in iron-deficiency anaemia. *J Clin Pathol* 15:346-349, 1962.

40. Verloop MC, Van der Wolk, Heier AJ. Radioactive iron studies in patients with iron deficiency anemia with concurrent abnormal hemolysis. *Blood* 15: 791-806, 1960.

41. Rasch CA, Cotton EK, Griggs RC, Harris JW. Shortened survival of autotransfused 51Cr-labeled erythrocytes in infants with severe iron-deficiency anemia. *Semin Hematol* 13: 181-186, 1976.

42. Koga M, Saito H, Mukai M, Matsumoto S, Kasayama S. Influence of iron metabolism indices on glycated haemoglobin but not glycated albumin levels in premenopausal women. *Acta Diabetol* 47(Suppl1): 65-69, 2010.

43. Hashimoto K, Osugi T, Noguchi S et al. A1C but not serum glycated albumin is elevated because of iron deficiency in late pregnancy in diabetic women. *Diabetes Care* 33: 509-511, 2010.

44. Rafat D, Rabbani TK, Ahmad J, Ansari MA. Influence of iron metabolism indices on HbA1c in non-diabetic pregnant women with and without iron-deficiency anemia: effect of iron supplementation. *Diabetes Metab Syndr* 6: 102-105, 2012.

45. 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* 33: 780-785, 2010.

46. 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* 3: 67-73, 2011.

47. Sharifi F, Nasab NM, Zadeh HJ. Elevated serum ferritin concentrations in prediabetic subjects. *Diab Vasc Dis Res* 5: 15-18, 2008.

48. Ng JM, Cooke M, Bhandari S, Atkin SL, Kilpatrick ES. The effect of iron and erythropoietin treatment on the A1C of patients with diabetes and chronic kidney disease. *Diabetes Care* 33: 2310-2313, 2010.

49. Sinha N, Mishra TK, Singh T, Gupta N. Effect of iron deficiency anemia on hemoglobin A1c levels. *Ann Lab Med* 32: 17-22, 2012.

50. Hardikar PS, Joshi SM, Bhat DS et al. Spuriously high prevalence of prediabetes diagnosed by HbA1c in young Indians partly explained by hematological factors and iron deficiency anemia. *Diabetes Care* 35: 797-802, 2012.