Glycated haemoglobin and iron deficiency anaemia: a case-control study

Abstract
Several studies have suggested an association between iron deficiency anaemia and higher HbA1c levels, but the results are conflicting and the matter is under debate. We conducted a retrospective case-control study to investigate both the effects of iron deficiency and the reduction of haemoglobin level on HbA1c measurement in subjects with iron deficiency anaemia.

Laboratory data were collected from a sample of subjects consecutively assessed from 1990–2016 in the Italian Hospital of Desio, Lombardy. All non-pregnant subjects aged over 12 years with one HbA1c measurement, a complete blood count, fasting blood glucose, and ferritin values during the same blood collection were enrolled.

A total of 2625 subjects met the study criteria. In all, 109 individuals were diagnosed as affected by iron deficiency anaemia, while 2516 had normal iron and haemoglobin values. The adjusted means of HbA1c were significantly higher in subjects with iron deficiency anaemia (36.87mmol/mol [5.53%]), compared to those measured in individuals without anaemia (34.75mmol/mol [5.34%]); (p<0.0001). Multiple linear regression analysis showed that haemoglobin values are inversely associated with HbA1c levels.

To the best of our knowledge, this paper is the first attempt to propose, for subjects affected by iron deficiency anaemia, a correction of HbA1c values based on haemoglobin level after a complete blood count, which may be required when diagnosing pre-diabetes and monitoring diabetes. Copyright © 2018 John Wiley & Sons.

Key words
anaemia; diabetes; glycated haemoglobin; iron deficiency; erythrocyte indices

Introduction
Glycated proteins are important markers for monitoring glycaemia in patients with diabetes.1,2 Glycated haemoglobin, whose major form is haemoglobin A1c (HbA1c),3 is the most known and used clinical indicator for the follow up of long-term glycaemic control, and it has been recently identified as a criterion for the diagnosis of diabetes by the World Health Organization (WHO).4

Glycated haemoglobin is formed by a spontaneous reaction between glucose and the N-terminal valine residue of both β-chains of the haemoglobin molecule.5 The glycated haemoglobin produced is proportional to the plasma glucose levels, and once glycated it remains in this stable product reflecting the average level of glucose over the previous two to three months, since the red-cell lifespan is about 120 days.4 However, HbA1c levels are influenced by various pathological conditions, such as haemolytic anaemia,5 haemoglobinopathies,6 and pregnancy,7 and they are also altered by other factors, such as vitamin B12, folate and iron deficiency,8–12 causing misleading interpretations of the results. Moreover, it has been demonstrated that red-cell life is also a factor that influences HbA1c results.13–15

Anaemia is a severe global public health problem, and it affects 1.6 billion individuals worldwide. In particular, iron deficiency is the greatest contributing factor to the global widespread of this disease.16 Recently, a review described the controversies concerning the role of anaemia on HbA1c.17 In fact, some studies suggested that in patients with or without diabetes, iron deficiency anaemia (IDA) is associated with higher HbA1c levels.17–19 Other works reported that, in patients not affected by diabetes but with iron deficiency, HbA1c concentrations had no significant changes.8,16 On the other hand, Sinha et al. observed that HbA1c level in subjects with IDA was significantly lower than in the control group.10 Few works evaluated the relationship between erythrocyte indices and HbA1c levels; in particular, statistical analyses were unclear or not assessed.10,20,21 Koga et al. showed that in pre-menopausal women without IDA, HbA1c levels had a negative correlation with three
Iron deficiency anaemia and HbA1c

Laboratory database

Consecutive subjects (n=5479)
Age >12 years. Non-pregnant. Available glycated haemoglobin measurement, complete blood cell count, fasting blood glucose (FBG), and ferritin in the same blood collection

Subjects selected (n=2625)

Cases group (n=109)
Ferritin ≤10ng/ml (female)
Ferritin ≤17ng/ml (male)
Haemoglobin ≤11.5g/dL (female)
Haemoglobin ≤13.0g/dL (male)
Mean corpuscular haemoglobin ≤28pg/cell. This group was then randomly split in Fit dataset (n=54) and Validation dataset (n=55) in order to test the reproducibility of the relationship between HbA1c and haemoglobin values

Subjects excluded (n=2854)
Individuals with diabetes, infectious diseases, malignancies, haematological diseases, non-iron deficiency anaemia, liver diseases, chronic renal failure, cardiovascular diseases, autoimmune disorders, and haemochromatosis.* FBG >100mg/dL; white blood cells >11 and <4x10³/µl; ferritin >200ng/ml

Controls group (n=2516)
Ferritin >10ng/ml (female)
Ferritin >17ng/ml (male)
Haemoglobin >11.5g/dL (female)
Haemoglobin >13.0g/dL (male)
Mean corpuscular haemoglobin >28pg/cell. This group was then randomly split in Fit dataset (n=1258) and Validation dataset (n=1258) in order to test the reproducibility of the relationship between HbA1c and haemoglobin values

Figure 1. Flow diagram of the study for case-control selection. *Subjects were excluded from the analysis based on diagnoses, which were coded according to the International Classification of Disease, 9th revision, Clinical Modification (ICD-9-CM)

Materials and methods

Study design and selection of participants. This retrospective study started from a large, computerised database.

Laboratory data were collected from a sample of subjects consecutively assessed from January 1990 to November 2016 in the Italian Hospital of Desio, Lombardy. Firstly, we enrolled subjects aged >12 years, non-pregnant, with one serum glycated haemoglobin (HbA1c) measurement, a complete blood count, and fasting blood glucose (FBG) and ferritin values during the same blood collection. (Figure 1.) Secondly, all individuals affected by diabetes, infectious diseases, malignancies, haematological diseases, non-iron deficiency anaemia, liver diseases, chronic renal failure, cardiovascular diseases, autoimmune disorders, and haemochromatosis were excluded on the basis of diagnoses coded according to the International Classification of Disease (9th revision, Clinical Modification). Moreover, subjects with white blood cells <4x10³/µl and >11x 10³/µl, ferritin <200ng/ml, FBG level >5.5mmol/L (100mg/dL)² were excluded. Finally, subjects with ferritin ≤10ng/ml (if female) or ≤17ng/ml (if male), Hb ≤11.5g/dL (if female) or ≤13.0g/dL (if male), and MCH ≤28pg/cell were defined as subjects with IDA (named ‘Cases’). Individuals with ferritin levels 10–200ng/ml (if female) or 17–200ng/ml (if male), Hb >11.5g/dL (if female) or >13.0g/dL (if male), and MCH >28pg/cell were defined as non-anaemic subjects with normal iron state (named ‘Controls’). (Figure 1.)

Laboratory analysis. From 1990–2016, different analytical instruments were used to perform analysis. HbA1c levels were obtained using ion-exchange high performance liquid chromatography on different Menarini/ARKRAY ADAMS A1c series HA (Menarini Diagnostics, Firenze, Italy) haemoglobin analysers. All HbA1c results were reported in the Diabetes Control and Complications Trial/National Glycohemoglobin Standardization Program (%) units and derived IFCC units (mmol/mol). The levels of haemoglobin (Hb), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), mean corpuscular haematocrit (MCH), and mean corpuscular haemoglobin concentration (MCHC),
**Table 1.** Participants’ haematological and biochemical characteristics. All values are expressed as mean (and mean ± SD) except where stated.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Subjects affected by IDA (Cases*): n=109</th>
<th>Non-anaemic subjects (Controls**: n=2516</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at the time of tests: years (median [5th/95th percentile])</td>
<td>43 (24/84)</td>
<td>43 (20/72)</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>9.9 (8.6–11.3)</td>
<td>14.4 (13.2–15.6)</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>32.3 (28.5–36.1)</td>
<td>42.0 (39.0–45.0)</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>75.2 (68.0–82.3)</td>
<td>87.6 (84.0–91.1)</td>
</tr>
<tr>
<td>MCH (pg/cell)</td>
<td>23.5 (20.5–26.4)</td>
<td>30.1 (28.9–31.3)</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>31.1 (29.6–32.7)</td>
<td>34.4 (33.3–35.5)</td>
</tr>
<tr>
<td>RBC (x 10^6/µl)</td>
<td>4.25 (3.73–4.78)</td>
<td>4.80 (4.40–5.20)</td>
</tr>
<tr>
<td>WBC (x10^3/µl)</td>
<td>6.7 (5.0–8.5)</td>
<td>6.4 (4.7–7.5)</td>
</tr>
<tr>
<td>FBG (mg/dL)</td>
<td>87 (79–96)</td>
<td>85 (76–94)</td>
</tr>
<tr>
<td>Ferritin (ng/ml)</td>
<td>7.0 (3.6–10.5)</td>
<td>65.1 (25.6–107.0)</td>
</tr>
</tbody>
</table>

*Cases. Individuals affected by iron deficiency anaemia (Hb ≤13.0g/dL for men and ≤11.5g/dL for women, ferritin level ≤17ng/ml for men and ≤19ng/ml for women, and MCH ≤28 pg/cell).
**Controls. Non-anaemic individuals (Hb >13.0g/dL for men and >11.5g/dL for women, MCH >28pg/ cell, ferritin level between 17–200ng/ml).

Iron deficiency anaemia and HbA1c

Haematocrit (Ht), red blood cell count, white blood cell count, and differential leucocytes count were measured by automated counters, such as Technicon H2 and ADVIA 120 (Bayer, Germany), and Sysmex XE 2100 (Sysmex, Germany). All measurements were performed on whole blood samples collected by vacuum into tubes containing EDTA.

Ferritin levels were measured by electrochemiluminescence immunoassay method (ECLIA), and FBG level was measured by enzymatic reaction. Analysis was performed using automated chemistry analysers, such as Hitachi, Modular and Cobas (Roche Diagnostics, Germany). All measurements were performed on plasma samples collected by vacuum in tubes with lithium heparin as anticoagulant.

Statistical analysis. A database using SAS software (version 9.4; SAS Institute Inc, Cary, NC, USA) was maintained. HbA1c values were fitted with a general linear model including groups, using ‘Cases’ and ‘Controls’ as terms, and age at the time of tests, gender, FBG, and year and month of blood collection (to correct imbalances of the different methodologies and instruments used during the time considered in this study) as covariates. HbA1c values were log-transformed to approximate normal distribution and homoscedasticity. Results were expressed as back-transformation of least-square means (i.e. the adjusted means of HbA1c [%] and HbA1c [mmol/mol]).

According to the Bonferroni principle, a 0.05 comparison-wise risk of type I error was used. Then, using SAS procedure to generate random numbers, the study population was randomly split into two different sub-datasets, which were named Fit dataset and Validation dataset (Figure 1). Fit dataset was used to define the relationship between Hb and HbA1c values, while Validation dataset was used to test the validity of the observed relationship.

**Results**

A total of 5479 consecutive subjects were enrolled. Of these, 2854 were excluded for diseases and/or abnormal test results (Figure 1). The final group consisted of 2625 individuals, 1433 (55%) males and 1192 (45%) females. In all, 109 individuals were diagnosed with IDA (Cases), while 2516 had normal Hb and iron levels (Controls). Participants’ haematological and biochemical characteristics are shown in Table 1. The adjusted mean values of HbA1c were statistically higher (36.87mmol/mol [5.53%]) in Cases compared to those measured in Controls (34.75mmol/mol [5.34%]); (p<0.0001). (Figure 2.) To perform the following analysis, the dataset was randomly split into two different sub-datasets. The characteristics of haematological and biochemical variables of participants are shown in Table 2.

Multiple regression analysis was used to assess the relationships between HbA1c, Hb and the covariates: age at the time of tests, gender, year and month of blood collection, and FBG among all 1312 individuals belonging to the Fit dataset. The linear regression was the best model to correlate HbA1c and Hb. A negative correlation between HbA1c and Hb was observed (p<0.0001). (Figure 3.) Ferritin was negatively correlated with HbA1c mmol/mol (p<0.0001), and HbA1c % (p<0.0001), while gender (male), age at the time of tests,
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FBG, and white cell count were positively correlated (p<0.0001); (Figure 3.) In addition, the correction applied in the model by the covariate year and month of blood collection was justified since it was positively correlated (p<0.01), suggesting that during the time of the study the improvements of instrumentation slightly increased HbA1c values. Estimated parameters (slopes), which were adjusted for age at the time of tests, gender, year and month of blood collection, FBG, ferritin, and white blood cell count, were -0.003±0.0009 (standard error, SE) per g/dL of Hb for log-HbA1c (mmol/mol) (p=0.0004), and -0.002±0.0006 (SE) per g/dL of Hb for log-HbA1c (%); (p=0.0002). (Figure 3.) We then carried out the same linear regression analysis on 1312 individuals belonging to the Fit dataset. Student’s t-test revealed no significant differences between results obtained using the Fit and Validation datasets (comparison of slopes, p=0.89; comparison of intercepts, p=0.90).

Using the regression coefficients of estimated parameters of percentage and absolute HbA1c obtained using the Fit dataset (Figure 3), we calculated the reduction of HbA1c values estimated at an Hb concentration of 13g/dL. This value was assumed as corresponding to non-anaemic status, and we compared these results to those measured in 203 individuals different from those used in the study population, and affected by both IDA and other pathologies (Figure 4). Bland-Altman plots were used to show the percentage reduction of estimated HbA1c values (Figure 4). Enhanced Bland-Altman plots were used to illustrate the differences between observed and estimated HbA1c values plotted against the different measured Hb values (Figure 4). For example, in a subject with haemoglobin concentration of 8g/dL, an HbA1c measurement of 48.00mmol/mol (6.5%) corresponds to an estimated HbA1c of 46.41mmol/mol (6.36%). Finally, in order to interpret HbA1c results in a more accurate and clinically meaningful way in subjects with IDA, in Table 3 we showed the values with which an HbA1c measurement (mmol/mol) must be diminished, supposing a normal HB level of 13g/dL, starting from different range measurements of Hb. Collectively, using the 48.00mmol/mol as cut-off and our correction factors, nine subjects out of 203 (4.4%) would be diagnosed as non-affected by diabetes.

Discussion

WHO recommends using HbA1c to diagnose type 2 diabetes and to monitor blood glucose, for its convenience and high reproducibility and correlation with diabetes-related complications. A large number of factors, such as age, ethnicity, genetic, and many diseases influence HbA1c.15,20,21,25 Anaemia is considered one of the most contributing factors that affect HbA1c measurements and many efforts have been made to investigate their relationship, which depends on the cause of anaemia.11 In this study, we wanted to consider the effect of iron deficiency on HbA1c values in individuals with IDA, in particular around the diagnostic cut-off of 48mmol/mol (6.5%),2 in order to propose a correction of HbA1c measurements. Our results showed that these subjects presented significantly higher HbA1c levels, when compared to the individuals without anaemia. These data are

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**Figure 2.** Box plots comparing Cases and Controls data of HbA1c—(A): HbA1c (%); and (B): HbA1c (mmol/mol). Values shown are median (line within box), 25th and 75th percentiles (bottom and top of box, respectively), and mean (open diamond). (C): HbA1c (%) and HbA1c (mmol/mol) comparison data between Cases and Controls.

**Table 3.** Comparison of estimation of HbA1c in subjects with IDA, in Table 3 we showed the values with which an HbA1c measurement (mmol/mol) must be diminished, supposing a normal Hb level of 13g/dL, starting from different range measurements of Hb. Collectively, using the 48.00mmol/mol as cut-off and our correction factors, nine subjects out of 203 (4.4%) would be diagnosed as non-affected by diabetes.

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in agreement with those obtained in other studies, showing that IDA causes falsely elevated HbA1c measurements.11,26

The exact mechanism underlying the effect of iron deficiency on HbA1c is not yet known. However, two hypotheses have been proposed. Firstly, iron deficiency state is a condition that affects red-cell lifespan and, in the case of anaemia, the production of erythrocytes is decreased, leading to an older population of red blood cells that are in contact with glucose for longer, resulting in falsely higher HbA1c measurements. Second, an association between non-enzymatic glycation processes and oxidation stress has been reported.57,28 In the iron deficiency state, enzymes involved in the antioxidant defence system are functionally limited and, concomitantly, an increase in lipid peroxidation was observed.28 A biomarker of oxidative stress is the malondialdehyde (MDA), which results from lipid peroxidation of polyunsaturated fatty acids.29 In patients with IDA, levels of MDA were significantly higher compared to those measured in the control group.28 In vitro, glycated haemoglobin levels increased when erythrocytes were incubated with MDA, due to a not completely understood non-enzymatic mechanism between MDA and Hb.27 The increase was significantly blocked when erythrocytes were pre-treated with antioxidant, suggesting a mutual correlation between lipid peroxidation and non-enzymatic glycation of Hb.27 Although in vivo studies have not yet been performed to confirm this pathophysiological process, it is important to consider all possible influences on HbA1c measurements before any diagnostic decision.

Moreover, three studies reported a significant reduction in HbA1c values after iron therapy in anaemic patients, suggesting that iron deficiency must be corrected before diagnosis of pre-diabetes and diabetes.12,17,30 On the other hand, Sinha et al. showed that HbA1c values were significantly lower in subjects affected by IDA than those measured in a non-anaemic group. After iron treatment, in anaemic individuals HbA1c levels were significantly higher compared to the initial values, suggesting that nutritional deficit might be an important cause of altered HbA1c measurements in addition to iron deficiency.10 The effects of oral iron treatment were also observed on markers of oxidative stress, resulting in a decrease of MDA level.28 If the concentration of MDA decreases, the non-enzymatic glycation of haemoglobin tends to reduce, limiting the interference effects of MDA on HbA1c measurements.27

What relationship exists between erythrocyte abnormalities and HbA1c values? Two previous studies demonstrated that a correlation between HbA1c and Hb, MCV, and MCH levels exists.22,31 Starting from data of the National Health and Nutrition Examination Surveys, Ford et al. observed that among all participants HbA1c increased progressively from a mean of 5.28% (patients with Hb <10g/dL) to 5.72% (patients with Hb >17g/dL).8 Using a linear regression analysis, the difference between adjusted mean concentrations of HbA1c among individuals with IDA (5.56%) and those without (5.46%) was only 0.1% (p=0.095).8 However, because of the small number of

Table 2. Haematological and biochemical characteristics of the individuals belonging to the two datasets. All values are expressed as mean (and ± SD) except where stated.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Validation dataset</th>
<th>Non-anaemic subjects</th>
<th>Controls**</th>
<th>Cases. Individuals affected by IDA (Hb ≤13.0g/dL for men and ≤11.5 g/dL for women, ferritin level ≤17ng/ml for men and ≤10ng/ml for women, and MCH ≤28pg/cell).</th>
<th>Non-anaemic subjects: n=1258</th>
<th>Controls**: Non-anaemic individuals (Hb &gt;13.0g/dL for men and &gt;11.5g/dL for women, MCH &gt;28pg/cell, ferritin level between 17 and 200ng/ml).</th>
<th>Non-anaemic subjects: n=55</th>
</tr>
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<tbody>
<tr>
<td>Age at the time of tests: years (median [5th/95th percentile])</td>
<td>43 (20/71)</td>
<td>32.5 (27.9–37.1)</td>
<td>32.5 (29.1–35.1)</td>
<td>Age at the time of tests: years (median [5th/95th percentile])</td>
<td>43 (20/71)</td>
<td>32.5 (27.9–37.1)</td>
<td>32.5 (29.1–35.1)</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>9.9 (8.8–11.0)</td>
<td>14.4 (13.2–15.6)</td>
<td>10.0 (8.4–11.6)</td>
<td>Hb (g/dL)</td>
<td>9.9 (8.8–11.0)</td>
<td>14.4 (13.2–15.6)</td>
<td>10.0 (8.4–11.6)</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>32.1 (29.1–35.1)</td>
<td>42.0 (39.0–45.0)</td>
<td>32.5 (29.1–35.1)</td>
<td>Haematocrit (%)</td>
<td>32.1 (29.1–35.1)</td>
<td>42.0 (39.0–45.0)</td>
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<td>MCV (fl)</td>
<td>75.0 (67.4–82.6)</td>
<td>87.6 (84.0–91.2)</td>
<td>75.4 (68.7–82.1)</td>
<td>MCV (fl)</td>
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<td>75.4 (68.7–82.1)</td>
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<td>MCH (pg/cell)</td>
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<td>30.1 (28.9–31.3)</td>
<td>23.6 (20.7–26.5)</td>
<td>MCH (pg/cell)</td>
<td>23.4 (20.4–26.4)</td>
<td>30.1 (28.9–31.3)</td>
<td>23.6 (20.7–26.5)</td>
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<tr>
<td>MCHC (g/dL)</td>
<td>31.1 (29.6–32.6)</td>
<td>34.4 (33.3–35.5)</td>
<td>31.2 (29.6–32.8)</td>
<td>MCHC (g/dL)</td>
<td>31.1 (29.6–32.6)</td>
<td>34.4 (33.3–35.5)</td>
<td>31.2 (29.6–32.8)</td>
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<tr>
<td>RBC (x 10^12/µl)</td>
<td>4.25 (3.78–4.72)</td>
<td>4.80 (4.40–5.20)</td>
<td>4.26 (3.68–4.84)</td>
<td>RBC (x 10^12/µl)</td>
<td>4.25 (3.78–4.72)</td>
<td>4.80 (4.40–5.20)</td>
<td>4.26 (3.68–4.84)</td>
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<td>WBC (x10^3/µl)</td>
<td>6.7 (5.0–8.4)</td>
<td>6.1 (4.7–7.5)</td>
<td>6.8 (4.9–8.7)</td>
<td>WBC (x10^3/µl)</td>
<td>6.7 (5.0–8.4)</td>
<td>6.1 (4.7–7.5)</td>
<td>6.8 (4.9–8.7)</td>
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<td>FBG (mg/dL)</td>
<td>87 (78–96)</td>
<td>85 (76–94)</td>
<td>88 (80–96)</td>
<td>FBG (mg/dL)</td>
<td>87 (78–96)</td>
<td>85 (76–94)</td>
<td>88 (80–96)</td>
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<tr>
<td>Ferritin (ng/ml)</td>
<td>7.0 (4.1–9.9)</td>
<td>66.9 (24.6–109.2)</td>
<td>7.1 (3.0–11.2)</td>
<td>Ferritin (ng/ml)</td>
<td>7.0 (4.1–9.9)</td>
<td>66.9 (24.6–109.2)</td>
<td>7.1 (3.0–11.2)</td>
</tr>
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</table>

*Cases. Individuals affected by IDA (Hb ≤13.0g/dL for men and ≤11.5 g/dL for women, ferritin level ≤17ng/ml for men and ≤10ng/ml for women, and MCH ≤28pg/cell).
**Controls. Non-anaemic individuals (Hb >13.0g/dL for men and >11.5g/dL for women, MCH >28pg/cell, ferritin level between 17 and 200ng/ml).
Iron deficiency anaemia and HbA1c

Patients with Hb <10g/dL, it is important to note that they studied the correlation between Hb and HbA1c only in patients with mild anaemia (men: 10 < Hb < 13g/dL; women: 10 < Hb < 12g/dL). Recently, Simmons and Hlaing studied the relationships between HbA1c concentrations, MCV and MCHC, as haematological parameters. Using a generalised linear model, a negative correlation between HbA1c and MCHC was observed, with a gradient from an adjusted mean HbA1c of 36mmol/mol (5.4%) with an MCHC of 32g/dL to an HbA1c of 30mmol/mol (4.9%) with an MCHC of 37g/dL. The difference between the two measurements was 6mmol/mol (0.5%). In addition, the correlation with MCV was also negative, but the gradient presented a difference of only 1mmol/mol (0.1%).

More recently, in a retrospective study, Grossman et al. found that the correlation between HbA1c and Hb, haematocrit, and nutritional factor causing anaemia in elderly subjects, was inconsistent. In general, we can argue that abnormalities of erythrocyte indices, and not only iron deficiency, are remarkable confounders in the HbA1c measurements, as described above. Our data contribute to the literature on influences of non-glycaemic factors on HbA1c measurements, confirming that altered haematological parameters affect HbA1c levels in patients with Hb <10g/dL.

**Table 1.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimated parameter</th>
<th>Standard error</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
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<td>Intercept</td>
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<td>0.0076</td>
<td>0.3925</td>
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<td>Sex</td>
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<td>0.0016</td>
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<td>0.0001</td>
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<td>0.0001</td>
<td>0.0093</td>
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<tr>
<td>Hb (g/dL)</td>
<td>-0.0021</td>
<td>0.0006</td>
<td>0.0002</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>-0.0002</td>
<td>0.0001</td>
<td>0.0002</td>
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<td>0.0004</td>
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<td>Fasting blood glucose (mg/dL)</td>
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<td>&lt;0.0001</td>
</tr>
<tr>
<td>Ferritin (ng/ml)</td>
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<td>0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*Bold typeface indicates p-values <0.05.

**Figure 3.** Correlation between HbA1c (A): HbA1c (%); and (B): HbA1c (mmol/mol) — and haemoglobin (Hb) values among all 1312 subjects belonging to the Fit dataset. Analyses were corrected using as covariates: age at the time of tests, gender, year and month of blood collection, ferritin, fasting blood glucose, and white blood cells. (C): Estimated parameters of linear regression between HbA1c (% and mmol/mol) and Hb values among all 1312 individuals.

**Figure 4.** (A): The Bland-Altman plot between observed and estimated HbA1c (mmol/mol) expressed as percentage difference plotted against the mean of the two values. (B): Enhanced Bland-Altman plot where the differences between observed and estimated HbA1c (mmol/mol) were plotted against measured haemoglobin concentrations. Similar results were obtained between observed and estimated HbA1c (%).
patients with IDA. In particular, we observed that Hb values had a significant negative correlation with glycated haemoglobin levels. To our knowledge, this study is the first attempt in which estimated correction factors were proposed to clinicians to correct measured HbA1c in patients affected by IDA (Table 3).

In our work, the increase in adjusted values of HbA1c among individuals with IDA compared to those without IDA was 2.12mmol/mol (0.19%), well under the value of 5.5mmol/mol (0.5%), which is considered clinically relevant, and therefore a re-assessment is recommended.32 However, it is important to note that in patients with IDA and a measured Hb of 7.0g/dL, an HbA1c value of 48.00mmol/mol (6.5%) must be reduced to 47.42mmol/mol (6.45%). Moreover, within-subject, biological variation of measured HbA1c is 1.9%,33,34 therefore in the examples described above the two estimated HbA1c levels could vary between the range 45.11–46.85mmol/mol (6.21–6.45%) and 46.52–48.32mmol/mol (6.33–6.57%), respectively, compared to the range of measured HbA1c 47.09–48.91mmol/mol (6.46–6.62%).

A limitation of our study is that it was a retrospective study performed in a single hospital. A greater number of individuals with IDA is needed in order to improve the accuracy of our estimates of the relationships between HbA1c and Hb values.

We conclude that the effects of IDA must be considered while monitoring people with pre-diabetes and diabetes.

### Table 3. Estimated correction of HbA1c values

<table>
<thead>
<tr>
<th>Measured haemoglobin concentration range (g/dL)</th>
<th>Percentage decrease of HbA1c (mmol/mol) supposing a normal haemoglobin concentration of 13g/dL (median [5th/95th percentile])*</th>
<th>Lower glycated haemoglobin values in the case of HbA1c 48mmol/mol (median [5th/95th percentile])**</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.0–8.0</td>
<td>-4.2 (-4.6/-3.9)</td>
<td>45.98 (45.79/46.13)</td>
</tr>
<tr>
<td>8.1–9.0</td>
<td>-3.3 (-3.7/-3.2)</td>
<td>46.41 (46.22/46.46)</td>
</tr>
<tr>
<td>9.1–10.0</td>
<td>-2.6 (-2.9/-2.3)</td>
<td>46.75 (46.61/46.90)</td>
</tr>
<tr>
<td>10.1–11.0</td>
<td>-1.9 (2.2/-1.6)</td>
<td>47.09 (46.94/47.23)</td>
</tr>
<tr>
<td>11.1–12.0</td>
<td>-1.2 (-1.5/-0.8)</td>
<td>47.42 (47.28/47.62)</td>
</tr>
<tr>
<td>12.1–13.0</td>
<td>-0.3 (-0.7/0.0)</td>
<td>47.86 (47.66/48.00)</td>
</tr>
</tbody>
</table>

*These corrections should be applied to HbA1c levels obtained using ion-exchange HPLC ARKRAY ADAMS A1c series HA (Menarini Diagnostics, Firenze, Italy) haemoglobin analysers.

**Reduction of the cut-off HbA1c 48mmol/mol at different haemoglobin concentrations using estimated percentage differences between measured and estimated HbA1c values.

### In this study, we observed that the magnitude of the change is quite small unless anaemia is severe, but we offered the opportunity to correct HbA1c values in individuals affected by IDA. However, alternatively, an oral glucose tolerance test or an iron therapy could be considered important before any diagnostic or therapeutic decision based on HbA1c measurements in these subjects. Currently, local clinicians use the results of such analysis to avoid misdiagnosing diabetes in subjects affected by IDA.

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### Declaration of interests

There are no conflicts of interest declared. Funding: none declared.

### References

References are available in Practical Diabetes online at www.practicaldiabetes.com.
Iron deficiency anaemia and HbA1c

References


