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Effect of iron deficiency anaemia on HbA1c levels is dependent on the degree of anaemia

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ABSTRACT

Objectives: Studies suggest that iron deficiency anaemia (IDA) is associated with higher HbA1c levels. We conducted a control–case study to investigate the effect of IDA on HbA1c levels, measured by two commonly used methods, in non-diabetic individuals.

Design and methods: A total of 122 patients were included, 61 patients with IDA and 61 patients without anaemia. HbA1c was measured by both ion exchange HPLC Variant II Turbo BioRad and immunoturbidimetry (IT) Tina Quant II Roche Diagnostics in each sample. HbA1c results were compared between groups. For correlation analysis, patients were considered altogether.

Results: There was a significant difference between the results of HbA1c in patients with IDA [HPLC 5.6 ± 0.4% (38 ± 4.4 mmol/mol) and IT 5.7 ± 0.4% (39 ± 4.4 mmol/mol)] and those measured in patients without anaemia [HPLC 5.3 ± 0.4% (34 ± 4.4 mmol/mol) and IT 5.3 ± 0.3% (34 ± 3.3 mmol/mol)], ($p < 0.001$). Significant negative correlations were observed between total haemoglobin (Hb), haematocrit, mean corpuscular volume (MCV) and ferritin with HbA1c values measured by IT ($r = -0.557$; $r = -0.539$; $r = -0.488$; $r = -0.499$; $p < 0.01$; respectively). These negative correlations were weaker with HbA1c measured by HPLC ($r = -0.272$; $r = -0.250$; $r = -0.273$; $r = -0.229$ for Hb, haematocrit, MCV and ferritin; $p < 0.05$; respectively). HbA1c results were higher in patients with moderate and severe anaemia. However mild anaemia did not show significant effects on HbA1c results measured by both methods.

Conclusions: IDA affects HbA1c results and this effect is dependent on anaemia degree. These upward changes are statistically significant but they may be not clinically relevant when the overall variability of the HbA1c test is considered. The presence of slight anaemia is likely to have a minor effect on HbA1c levels favouring its use to diagnose diabetes in patients with mild anaemia.

1. Introduction

Glycated haemoglobin (HbA1c) is the parameter of choice to evaluate the long term degree of glycaemic control in patients with diabetes mellitus (DM) [1]. Glycaemic levels are a determining factor in the development and progression of diabetic complications and HbA1c is able to predict the risk of developing many of these chronic complications [2,3]. In addition, HbA1c ≥ 6.5% (48 mmol/mol) is recommended as the cutoff point for diagnosing DM [4–6].

HbA1c is a form of haemoglobin with a glucose residue attached to the terminal NH_2 group (valine residue) of one or both HbA beta chains.Red blood cells are freely permeable to the plasma glucose molecules, and haemoglobin is practically exposed to the same glucose concentrations as plasma. Therefore, the levels of HbA1c reflect more specifically the glycaemic control from the past 2 to 3 months, the red blood cells half life time, preceding the measurement [7–9]. Clinically, HbA1c is used to determine improvement or worsening in glycaemic control by comparing HbA1c serial results to determine if the patients achieve their HbA1c targets and, recently, it has been also recommended to diagnose DM [1,5,6].

Depending on the methodology used to measure HbA1c, several factors can affect or interfere in the HbA1c results [1,8–10]. Traditionally, some diseases and pathological states, such as anaemia and haemoglobinopathies, are considered potential factors that can significantly alter HbA1c results [1,11].

Anaemia is a public health problem that affects worldwide populations. Its primary cause is iron deficiency (ID). Approximately one third of the patients with anaemia have iron deficiency (IDA) [12].

A recent review in this topic presented the controversies about this issue and highlighted the need for further studies in this field to confirm and elucidate the role of anaemia on HbA1c results [13]. Recently, we carried out a meta-analysis and showed that IDA and/or ID had a positive effect on HbA1c levels in patients without DM, but with a large confidence interval, and no statistical significance. As a result of the high

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heterogeneity among the available studies, the effects of IDA and/or ID remained inconclusive [14].

Some studies suggest that IDA is associated with higher HbA1c concentrations in diabetic and non-diabetic patients and that the therapy to re-establish iron stores leads to diminished HbA1c [15–19].On the other hand, it was not reported significant changes in HbA1c concentrations in non-diabetic patients with IDA [20,21] and others failed to show this HbA1c rising effect [22].

Also, HbA1c levels are associated with erythrocyte indices such as total haemoglobin (Hb), mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH), independently of glucose levels, in pre-menopausal women even when they are not anaemic [23]. In addition, pre-menopausal women with iron deficiency with or without anaemia showed higher HbA1c levels than women without iron deficiency and/or anaemia [24]. Moreover, HbA1c levels are elevated in late pregnancy due to iron deficiency in diabetic women [25].

There are several studies on the role of anaemia on the HbA1c levels but their results are conflicting [13,14].

Because of the important role of HbA1c on DM diagnosis and the high prevalence of anaemia worldwide, the aim of this study was to investigate the effect of IDA on HbA1c levels in patients without DM, measured by two commonly methods used in the routine of clinical laboratories worldwide.

2. Material and methods

2.1. Patients

Patients were selected based on their laboratory data from September 2008 to March 2012 in the Clinical Pathology Service at Hospital de Clinicas de Porto Alegre and were divided into two groups: Group 1 included patients with IDA — ferritin < 15 μg/mL, haemoglobin (Hb) < 13 g/dL (if male) or <12 g/dL (if female) and mean corpuscular volume (MCV) <80 fL [12] and Group 2 included patients without anaemia [serum ferritin >15 μg/mL and <150 μg/mL (if female) or <200 μg/mL (if male), plus a complete blood count within the reference values]. The groups were matched by sex and age. In order to exclude DM, the patients had to have no history of DM and two fasting glycaemia ≤ 126 mg/dL, performed close to the date of the complete blood count, and HbA1c < 6.5% (48 mmol/mol) [5,6].

All other conditions known to interfere or lead to misinterpretation of HbA1c results were excluded [1,11]. The protocol was approved by the Ethics Committee of Hospital de Clinicas de Porto Alegre (GPPG 06511).

2.2. Laboratory investigations

Whole blood samples were collected by vacuum into tubes containing EDTA for HbA1c, complete blood count and reticulocyte analyses. HbA1c was measured by two methods: ion exchange HPLC Variant II Turbo (Bio-Rad Laboratories, Hercules, CA) and immunoturbidimetry, with the Tina Quant II kit, in the Modular P analyser (Roche Diagnostics, Germany). Both methods were calibrated and standardized by the National Glycohemoglobin Standardization Programme (NGSP), International Federation of Clinical Chemistry (IFFC) [26], aligned to DCCT [2] and they were interchangeable.

The results of the complete blood counts and reticulocyte counts were obtained in the ABX Pentra DX 120, ABX Pentra DF 120 and Sysmex XE 2100 equipment (Roche Diagnostics, Germany).

Serum samples, collected by vacuum in tubes without anticoagulant and with separator gel, were used to analyse triglycerides, glucose and urea by colorimetric enzymatic methods in the Modular P analyser (Roche Diagnostics, Germany). Serum ferritin was determined by electrochemiluminescence immunoassay (ECLIA) in the Modular E-170 equipment (Roche Diagnostics, Germany).

2.3. Statistical analysis

Data are expressed as mean and SD when normally distributed and as median (interquartile range) for non-Gaussian variables. The Student t test, ANOVA, Pearson and Kendall correlations were used as appropriate. For comparison, initially, HbA1c levels by different methods in patients with and without anaemia were compared. Afterward, patients with anaemia were separated into three groups, according to their total Hb [27]: 1) patients classified as having “mild anaemia” (females with Hb ≥ 11 g/dL and <12 g/dL and males with Hb ≥ 11 g/dL and <13 g/dL); 2) patients with “moderate anaemia” (Hb ≥ 8 g/dL and <11 g/dL, for both sexes) and 3) patients with “severe anaemia” (Hb < 8 g/dL, for both sexes). HbA1c results in each group, measured by the two methods, were compared with HbA1c results in the group without anaemia. For correlation analysis, patients were considered altogether. The SPSS 19.0 programme was used for data analysis. Significance of 5% was adopted.

3. Results

A total of 122 patients with age varying from 18 to 77 years were enrolled in this study. Sixty one individuals were classified with IDA (Cases) and 61 without anaemia (Controls).Each group were formed by 43 (78%) females. The laboratory characteristics of these patients are shown in Table 1.

There were no differences in the levels of fasting glycaemia, triglycerides, and urea between the two groups. Also, no significant difference was found in the HbA1c results by the two different methods within the same group (P = 0.192). As expected, there were significant differences in the levels of haematological parameters related to IDA. Levels of total haemoglobin, haematocrit, MCV and ferritin were lower in patients with anaemia (P < 0.001). There was no difference in the reticulocytes count between the groups.

However, HbA1c values in the group without anaemia were statistically different from those in the group with anaemia, when analysed by HPLC [5.3 ± 0.4% (34 ± 4.4 mmol/mol) and 5.6 ± 0.4% (38 ± 4.4 mmol/mol), P < 0.001, respectively] and by IT [5.3 ± 0.3% (34 ± 3.3 mmol/mol) and 5.7 ± 0.4% (39 ± 4.4 mmol/mol), P < 0.001, respectively]. The mean differences (absolute value of HbA1c) were 0.3% (3.3 mmol/mol) and 0.4% (4.4 mmol/mol), for HbA1c measured by HPLC and IT, respectively (Table 1).

Additionally, when patients were classified according to degree of anaemia, 19 presented mild anaemia, 26 showed moderate anaemia and 16 presented severe anaemia. HbA1c results were higher with the

Table 1
Laboratory characteristics of patients with iron deficiency anaemia and patients without anaemia.

	No anaemia	Anaemia
N	61	61
Age (years)	48 ± 14	49 ± 14
Sex (male/female)	18/43	18/43
Ferritin (μg/mL)*	58.7 (21.3–305.2)	5.8 (1.1–14.0)
Haematocrit (%)*	40.2 ± 3.2	31.6 ± 4.7
Haemoglobin (g/dL)*	13.2 ± 1.1	9.4 ± 1.9
MCV (fL)*	87.8 ± 13.2	71.1 ± 6.9
Reticulocytes (millions/μL)	0.045 (0.021–0.051)	0.041 (0.015–0.086)
Glycaemia 1 (mg/dL)	94 ± 10	92 ± 9
Glycaemia 2 (mg/dL)	90 ± 9	88 ± 14
Triglycerides (mg/dL)	91 (41–302)	99 (33–316)
Urea (mg/dL)	31 ± 13	32 ± 11
HbA1c HPLC (%)*	5.3 ± 0.4	5.6 ± 0.4
(mmol/mol)	34 ± 4.4	38 ± 4.4
HbA1c Immunoturbidimetry (%)*	5.3 ± 0.3	5.7 ± 0.4
(mmol/mol)	34 ± 3.3	39 ± 4.4

Data are expressed as mean ± SD or median (interquartile range). MCV = mean corpuscular volume.
* P < 0.001.