Review

HbA1c and iron deficiency: A review

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A R T I C L E   I N F O

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A B S T R A C T

Conditions that affect erythrocyte turnover influence HbA1c concentrations and the International Expert Committee has warned clinicians to be aware of any conditions that could affect the turnover of red blood cells. Although many forms of anemia are associated with lowering of HbA1c, iron deficiency has been shown to shift HbA1c slightly upward. The exact mechanism through which iron deficiency anemia affects HbA1c levels, however, still remains unclear. The explanations provided above are merely speculations, warranting further studies to confirm and elucidate the role of these factors. As little work has been done in this field so far, future and large scale studies are required which may address HbA1c enhancing effect and the mechanism of increased HbA1c glycation in iron deficiency properly.

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1. Introduction

Hemoglobin A1c (HbA1c) is a glycated hemoglobin, formed by glycation of NH2-terminal valine residue of the β-chain of globin and can be used as an indicator of a patient’s glycemic status over the previous 3 months [1]. Before 2010 virtually all diabetes societies recommended blood glucose analysis as the exclusive method to diagnose diabetes. Notwithstanding these guidelines, the last few years physicians have been using HbA1c to screen for and diagnose diabetes [2]. HbA1c determination used clinically to evaluate glycemic control depends on 3 factors: (1) the HbA1c in reticulocytes when they are released from the bone marrow; (2) the synthetic rate of HbA1c (or Hb glycation rate) as red blood cells (RBCs) become older, a function of glucose concentration to which Hb is exposed; and (3) the mean age of RBCs in the circulation.

Measurement of HbA1c for diagnosis is appealing as with one number, a total, integrated view of glycemia over time is derived but it has some inherent limitations. These issues have become the focus of considerable attention with the recent publication of the Report of the International expert committee that recommended the use of A1c for diagnosis of diabetes [3], a position that has been endorsed by American Dental Association, the Endocrine Society and in a more limited fashion by American Association of Clinical Endocrinologists/American College of Endocrinology [4].

Currently, HbA1c is widely accepted as an index of mean glycemia, a measure of risk for the development of diabetes complications, and a measure of the quality of diabetes care but there are number of factors, that can either falsely lower HbA1c or raise HbA1c test results independent of glycemia. These include structural hemoglobinopathies, thalassemia syndromes, and chemical alterations of hemoglobin. Moreover, any condition that decreases mean erythrocyte age will falsely lower HbA1c test results regardless of the assay method used [5]. Examples of such conditions include hemolytic anemia and recovery from acute
Influence of Iron Deficiency Anemia on Hemoglobin A1C Levels in Diabetic Individuals with Controlled Plasma Glucose Levels

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ABSTRACT

Introduction: Hemoglobin A1C (HbA1c) reflects patient’s glycemic status over the previous 3 months. Previous studies have reported that iron deficiency may elevate A1C concentrations, independent of glycemia. This study is aimed to analyze the effect of iron deficiency anemia on HbA1c levels in diabetic population having plasma glucose levels in control. Methods: Totally, 120 diabetic, iron-deficient anemic individuals (70 females and 50 males) having controlled plasma glucose levels with same number of iron-sufficient non-anemic individuals were streamlined for the study. Their data of HbA1c (Bio-Rad D-10 HPLC analyzer), ferritin (cobas e411 ECLI A hormone analyzer), fasting plasma glucose (FPG, Roche Hitachi P800/917 chemistry analyzer) were recorded. Statistical analysis was carried out by student’s t-test, chi-square test, and Pearson’s coefficient of regression. Results: We found elevated HbA1c (6.8 ± 1.4%) in iron-deficient individuals as compared to controls, and elevation was more in women (7.02 ± 1.58%). On further classification on the basis of FPG levels, A1C was elevated more in group having fasting glucose levels between 100-126 mg/dl (7.33 ± 1.55%) compared to those with normal plasma glucose levels (<100 mg/dl). No significant correlation was found between HbA1c and ferritin and hemoglobin. Conclusion: This study found a positive correlation between iron deficiency anemia and increased A1C levels, especially in the controlled diabetic women and individuals having FPG between 100-126 mg/dl. Hence, before altering the treatment regimen for diabetic patient, presence of iron deficiency anemia should be considered. Iran. Biomed. J. 18 (2): 88-93, 2014

Keywords: Iron deficiency anemia, Hemoglobin A1C (HbA1c), Diabetes

INTRODUCTION

Iron deficiency is one of the most prevalent forms of malnutrition. Globally, 50% of anemia is attributed to iron deficiency. Ferritin is the storage form of iron, and it reflects the iron status accurately [1]. An earlier study showed that reduced iron stores have a link with increased glycation of hemoglobin A1C (HbA1c), leading to false-high values of HbA1c in non-diabetic individuals [2]. HbA1c is the most predominant fraction of HbA1, and it is formed by the glycation of terminal valine at the β-chain of hemoglobin [3]. It reflects the patient’s glycemic status over previous 3 months. HbA1c is widely used as a screening test for diabetes mellitus, and American Diabetes Association has recently endorsed HbA1c ≥ 6.5% as a diagnostic criterion for diabetes mellitus [4]. Its alteration in other conditions, such as hemolytic anemia, hemoglobinopathies, pregnancy, and vitamin B12 deficiency has been explained in a study conducted by Sinha et al. [5]. Although iron deficiency is the most common nutritional deficiency, reports of the clinical relevance of iron deficiency on HbA1c levels have been inconsistent [2, 5].

In a study carried out by Brooks et al. [2], the HbA1c values were estimated in 35 non-diabetic patients with iron deficiency anemia before and after treatment with iron. They significantly observed elevated HbA1c values in iron deficiency anemia patients and decreased levels after treatment with iron [2]. Similar results were also found in studies carried out by Gram-Hansen et al. [6] and Coban et al. [7]. Investigations performed on diabetic chronic kidney disease patients, and diabetic pregnant women showed increased A1C levels in iron deficiency anemia, which was reduced following iron therapy [8, 9]. In a study by Tarim et al. [10], it was...
Effect of Iron Deficiency Anemia on the Levels of Hemoglobin A1c in Nondiabetic Patients

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Key Words
Anemia · Iron deficiency · Glycated hemoglobin · HbA1c

Abstract
The major form of glycohemoglobin is hemoglobin A1c (HbA1c). The HbA1c fraction is abnormally elevated in chronic hyperglycemic diabetic patients and correlates positively with glycemic control. Previous studies suggest that iron deficiency anemia (IDA) affects the levels of HbA1c. The aim of this study was to determine the effect of IDA on HbA1c levels in nondiabetic patients. The population studied consisted of 50 patients (30 women, 20 men, mean age 35.7 ± 11.9 years) with IDA and 50 healthy subjects that were matched for age and sex. Patients who had glucose tolerance abnormalities (impaired glucose tolerance or diabetes mellitus), hemoglobinopathies, hemolytic anemia, chronic alcohol ingestion and chronic renal failure were excluded from the study. Hematologic investigations, fasting and postprandial glucose and HbA1c levels were measured in all subjects before iron therapy. All patients with IDA were treated with iron 100 mg/day for 3 months. We repeated the laboratory investigation after iron therapy. Before iron treatment, the mean HbA1c (7.4 ± 0.8%) level in patients with IDA was higher than in a healthy group (5.9% ± 0.5) (p < 0.001). In patients with IDA, HbA1c decreased significantly after iron treatment from a mean of 7.4% ± 0.8 to 6.2% ± 0.6 (p < 0.001). Iron deficiency must be corrected before any diagnostic or therapeutic decision is made based on HbA1c.

Introduction
Glycohemoglobin is produced by a ketoamine reaction between glucose and the N-terminal amino acid of both β-chains of the hemoglobin molecule. The major form of glycohemoglobin is hemoglobin A1c (HbA1c) [1, 2]. Measurement of glycated hemoglobin is the standard method of assessing long-term glycemic control. When plasma glucose is consistently elevated, nonenzymatic glycation of hemoglobin increases; this alteration reflects the glycemic history over the previous 2–3 months, since erythrocytes have an average lifespan of 120 days [3, 4]. The HbA1c fraction is abnormally elevated in patients with chronic hyperglycemic diabetes mellitus and correlates positively with metabolic control [5].

Anemia is a common problem in the world. Approximately one third of patients with anemia have iron deficiency [6, 7]. Previous studies suggest that iron deficiency anemia (IDA) affects HbA1c levels. The aim of this study was to determine the effect of IDA on the levels of HbA1c in nondiabetic patients.
Iron-deficiency anemia, non-iron-deficiency anemia and HbA1c among adults in the US

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Abstract

Background: Conditions that affect erythrocyte turnover affect HbA1c concentrations. Although many forms of anemia are associated with lowering of HbA1c, iron deficiency tends to increase HbA1c. We examined the effect of iron and hemoglobin (Hb) status on HbA1c and on the relationship between concentrations of fasting glucose and HbA1c in a national sample of adults in the US.

Methods: Cross-sectional data from 8296 adults aged ≥20 years who participated in NHANES 1999–2002 were used.

Results: The prevalence of low Hb (defined as <120 and <118 g/L in women aged 20–69 and ≥70 years, respectively, and <137, <133, and <124 g/L in men aged 20–49, 50–69, and ≥70 years, respectively) was 5.5%. There was a significant positive correlation between Hb concentrations and HbA1c concentrations after adjusting for age, gender, and race or ethnicity, with HbA1c rising from a mean of 5.28% among participants with Hb <100 g/L to 5.72% among 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). However, there was no evidence of differences in the relationship between fasting glucose and HbA1c when groups of anemic and non-anemic individuals with and without iron deficiency were examined individually.

Conclusions: Caution should be used when diagnosing diabetes and prediabetes among people with high or low Hb when the HbA1c level is near 6.5% or 5.7%, respectively, as changes in erythrocyte turnover may alter the test result. However, the trend for HbA1c to increase with iron deficiency does not appear to require screening for iron deficiency in ascertaining the reliability of HbA1c in the diagnosis of diabetes and prediabetes in a given individual.

Keywords: hemoglobin A1c protein, hemoglobins, iron-deficiency anemia.

Introduction

In 2009, an expert panel recommended using HbA1c rather than blood glucose to diagnosis diabetes mellitus with the caveat that conditions that affect the turnover of red blood cells could lead to erroneous values of HbA1c.1 The American Diabetes Association has recently concurred with the recommendation,2 as has the American Association of Clinical Endocrinologists, although calling attention to the potential for the test to be misleading in the presence of conditions such as anemia and renal insufficiency.3 Anemia may be
Spuriously High Prevalence of Prediabetes Diagnosed by HbA$_1c$ in Young Indians Partly Explained by Hematological Factors and Iron Deficiency Anemia

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OBJECTIVE—To examine the influence of glycemic and nonglycemic parameters on HbA$_1c$ concentrations in young adults, the majority of whom had normal glucose tolerance.

RESEARCH DESIGN AND METHODS—We compared the diagnosis of normal glucose tolerance, prediabetes, and diabetes between a standard oral glucose tolerance test (OGTT; World Health Organization 2006 criteria) and HbA$_1c$ concentrations (American Diabetes Association [ADA] 2009 criteria) in 116 young adults (average age 21.6 years) from the Pune Children’s Study. We also studied the contribution of glycemic and nonglycemic determinants to HbA$_1c$ concentrations.

RESULTS—The OGTT showed that 7.8% of participants were prediabetic and 2.6% were diabetic. By ADA HbA$_1c$ criteria, 23.3% were prediabetic and 2.6% were diabetic. The negative predictive value of HbA$_1c$ was 93% and the positive predictive value was 20% (only 20% had prediabetes or diabetes according to the OGTT; this figure was 7% in anemic participants). Of participants, 34% were anemic, 37% were iron deficient (ferritin <15 ng/mL), 40% were vitamin B$_12$ deficient (vitamin B$_12$ <150 pmol/L), and 22% were folate deficient (<5 mg/L). On multiple linear regression analysis, HbA$_1c$ was predicted by higher 2-h glucose ($R^2 = 23.6\%$) and lower hematocrit ($R^2 = 7.7\%$). When hematological parameters were replaced by ferritin, vitamin B$_12$, and folate, HbA$_1c$ was predicted by higher glicemia ($R^2 = 23.6\%$) and lower ferritin ($R^2 = 4.3\%$).

CONCLUSIONS—The use of HbA$_1c$ to diagnose prediabetes and diabetes in iron-deficient populations may lead to a spuriously exaggerated prevalence. Further investigation is required before using HbA$_1c$ as a screening tool in nutritionally compromised populations.

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The use of HbA$_1c$ to diagnose prediabetes and diabetes is an attractive option in prospective epidemiological studies because it may avoid the need for repeated oral glucose tolerance tests (OGTTs). The American Diabetes Association (ADA) and World Health Organization (WHO) have recently approved the use of HbA$_1c$ for screening and diagnosis of diabetes (1-3). Both organizations have suggested that concentrations $\geq 6.5\%$ be considered diabetes, and the ADA has suggested $5.7-6.4\%$ as diagnostic of prediabetes (3).

The concentration of HbA$_1c$ depends on not only prevailing glycemia but also the life span of erythrocytes. Nutritional deficiencies are a major factor affecting erythrocyte survival. Among these, iron deficiency is the most common and affects $>50\%$ of the world’s population (4). Previous studies show that iron deficiency increases erythrocyte survival and therefore disproportionately elevates HbA$_1c$ concentrations at a given glycemic level (5,6). These were small studies in nondiabetic subjects. There is one similar report in type 1 diabetic patients (7). WHO and ADA have acknowledged this limitation of using HbA$_1c$ in the diagnosis of prediabetes and diabetes in nutritionally compromised populations, but not the magnitude of the effect.

In the current study, we aimed to investigate the diagnostic performance of HbA$_1c$ against a standard OGTT in young adults in a prospective birth cohort (Pune Children’s Study [PCS]) and study the influence of hematological, nutritional, and other factors on HbA$_1c$ concentrations.

RESEARCH DESIGN AND METHODS—The study participants were from the PCS (8), which follows children born between 1987 and 1989 in the King Edward Memorial Hospital (KEMH). The study has investigated their growth, glucose tolerance, and cardiovascular risk factors since 1991. In the present round, started in January 2009, we studied these children as 21-year-old young adults. KEMH Research Centre’s ethics committee approved the study, and all participants gave informed consent.

The participants reported to the KEMH Diabetes Unit the evening before the study. Height and weight were measured according to a standard protocol. The next morning, a 75-g OGTT (9) was performed. Blood samples were drawn for the measurement of fasting, 30-min, and 2-h plasma glucose. The fasting sample was also used for the
A1C but Not Serum Glycated Albumin Is Elevated in Late Pregnancy Owing to Iron Deficiency

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OBJECTIVE — A1C levels have been shown to be elevated in relation to glycemia in late pregnancy, although the precise mechanisms remain undetermined. We hypothesized that iron deficiency is involved in the A1C increase in late pregnancy.

RESEARCH DESIGN AND METHODS — In study 1, A1C, serum glycated albumin, erythrocyte indexes, and iron metabolism indexes were determined in 47 nondiabetic pregnant women not receiving iron supplementation who were divided into four groups according to gestational period (group I, 21–24 weeks; group II, 25–28 weeks; group III, 29–32 weeks; and group IV, 33–36 weeks). In study 2, these determinants were obtained at two gestational periods (20–23 weeks and 32–33 weeks) in 17 nondiabetic pregnant women.

RESULTS — In study 1, A1C levels were higher in groups III and IV than those in groups I and II, whereas serum glycated albumin levels were not different among these four groups. Hemoglobin, mean corpuscular hemoglobin (MCH), serum transferrin saturation, and serum ferritin were lower in groups III and IV. A1C levels were negatively correlated with MCH, serum transferrin saturation, and serum ferritin. In study 2, A1C levels were significantly increased at gestational weeks 32–33 from those at weeks 20–23, whereas serum glycated albumin levels did not differ between the two gestational periods. MCH, serum transferrin saturation, and serum ferritin were decreased at gestational weeks 32–33. A1C levels showed a negative correlation with MCH, serum transferrin saturation, and serum ferritin.

CONCLUSIONS — A1C levels were elevated in late pregnancy owing to iron deficiency. Serum glycated albumin may offer a better index for monitoring glycemic control in pregnancy.


In pregnant women displaying diabetes and women with gestational diabetes mellitus, intensive glycemic control during pregnancy is needed to lower the risk of intrauterine fetal death, fetal growth disorders, and maternal complications (1,2). The extent of nonenzymatic glycation of proteins increases in diabetic patients. Of these glycated proteins, A1C is widely used as the current standard marker for monitoring chronic glycemic control (3,4) and represents an important target for treatment of diabetic patients (5). Phelps et al. (6) showed biphasic changes in A1C levels during pregnancy, with A1C levels being lowest at gestational week 24. A longitudinal study also demonstrated similar biphasic changes in A1C levels (7).

A1C measurements are known to be profoundly affected by erythrocyte turnover, as are plasma glucose levels (8,9). Blood dilution–related anemia is known to be frequently observed in pregnancy. In late pregnancy, iron deficiency anemia is also often observed, caused by the increased demands for iron (10). A1C levels have been shown to be higher in relation to glycemia in patients with iron deficiency anemia (11–13). We have recently shown that A1C levels are higher in premenopausal women with an iron-deficient state, even in the absence of anemia (14). We therefore hypothesized that A1C levels are set higher in relation to glycemia in late pregnancy, during which most women are iron deficient. To confirm this possibility, we studied the relationship between A1C and iron metabolism in nondiabetic pregnant women.

For clinical issues, a study performed in pregnant diabetic women is important. However, in diabetic women fluctuations of plasma glucose may directly influence A1C levels beyond the effect of iron metabolism, making it difficult to analyze the direct effects of gestational course on A1C levels. Thus, in this study, we aimed to examine the relationship between A1C and iron metabolism in nondiabetic pregnant women, in whom the influence of plasma glucose levels is minimal. Serum glycated albumin, a different indicator for chronic glycemia, was also studied in these subjects.

RESEARCH DESIGN AND METHODS — In a cross-sectional study (study 1), we studied 47 pregnant Japanese women at gestational weeks 21–36. All subjects had been seen at Aizenbashi Hospital from February to July 2007, and ambulatory plasma glucose levels were <100 mg/dl. Mean ± SD age was 29.5 ± 5.7 years. All subjects had not been and were not receiving iron and vitamin supplementations during pregnancy. Subjects were divided into four groups according to gestational period: group I (n = 20), gestational weeks 21–24; group II (n = 9), gestational weeks 25–28; group III (n = 11), gestational weeks 29–32; and group IV (n = 7), gestational weeks 33–36. A1C, erythrocyte (red blood cell [RBC]) count, hematocrit, hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), serum iron, serum transferrin saturation, serum ferritin, and glycated albumin were determined.
Association Between the Presence of Iron Deficiency Anemia and Hemoglobin A1c in Korean Adults

The 2011–2012 Korea National Health and Nutrition Examination Survey

Jae W. Hong, Cheol R. Ku, Jung H. Noh, Kyung S. Ko, Byoung D. Rhee, and Dong-Jun Kim

Abstract: Few studies have investigated the clinical effect of iron deficiency anemia (IDA) on the use of the Hemoglobin A1c (HbA1c) as a screening parameter for diabetes or prediabetes. We investigated the association between IDA and HbA1c levels in Korean adults.

Among the 11,472 adults (≥19 years of age) who participated in the 2011–2012 Korea National Health and Nutrition Examination Survey (a cross-sectional and nationally representative survey conducted by the Korean Center for Disease Control for Health Statistics), 807 patients with diabetes currently taking anti-diabetes medications were excluded from this study. We compared the weighted HbA1c levels and weighted proportion (%) of HbA1c levels of ≥5.7%, ≥6.1%, and ≥6.5% according to the range of fasting plasma glucose (FPG) levels and the presence of IDA.

Among 10,665 participants (weighted n = 35,229,108), the prevalence of anemia and IDA was 7.3% and 4.3%, respectively. The HbA1c levels were higher in participants with IDA (5.70% ± 0.02%) than in normal participants (5.59% ± 0.01%, P < 0.001), whereas there was no significant difference in FPG levels. In participants with an FPG level of <100 mg/dL and 100 to 125 mg/dL, the weighted HbA1c level was higher in those with IDA (5.59% ± 0.02% and 6.00% ± 0.05%) than in normal participants (5.44% ± 0.01% and 5.82% ± 0.01%) after adjusting for confounders such as age, sex, FPG level, heavy alcohol drinking, waist circumference, and smoking status as well as after exclusion of an estimated glomerular filtration rate of <60 mL/min/1.73 m² (P < 0.001, <0.05). The weighted proportions (%) of an HbA1c level of ≥5.7% and ≥6.1% were also higher in participants with IDA than in normal participants (P < 0.001, <0.05). However, the weighted HbA1c levels in individuals with an FPG level ≥126 mg/dL and a weighted proportion (%) of an HbA1c level of ≥6.5% showed no significant differences according to the presence of IDA.

In conclusion, the presence of IDA shifted the HbA1c level upward only in the normoglycemic and prediabetic ranges, not in the diabetic range. Therefore, IDA should be considered before using HbA1c as a screening test for prediabetes.

INTRODUCTION

Hemoglobin A1c (HbA1c) is formed by glycation of the NH2-terminal valine residue of the β-chain of hemoglobin (Hb). It is commonly used as a marker of glucose control when monitored over 3 months, and provides information on the risk of long-term complications in patients with diabetes. HbA1c is also used to diagnose diabetes and to identify individuals that are at high risk for developing diabetes. The American Diabetes Association (ADA) has suggested that an HbA1c level of ≥6.5% is the diagnostic cutoff point for diabetes and that a level of 5.7% to 6.4% is the threshold for prediabetes. Measurement of HbA1c levels to screen for diabetes or prediabetes has many advantages over measurement of the fasting plasma glucose (FPG) level or performance with the 75-g oral glucose tolerance test (OGTT). However, HbA1c levels can be influenced by a variety of factors, including age, ethnicity, smoking, and conditions that alter red cell turnover and glucose homeostasis.

One condition that affects erythrocyte turnover is anemia. Anemia may be associated with more rapid erythrocyte turnover, which decreases the HbA1c level, or with slower turnover or changes in the 3-dimensional configuration of Hb, which elevates the HbA1c level. Iron and vitamin B12 deficiency, renal failure, and bone marrow suppression in alcoholism inhibit erythropoiesis and increase the mean survival duration of erythrocytes, leading to an increase HbA1c levels. Furthermore, numerous hemoglobinopathies, including HbAS, HbAC, HbE, and HbD, can also influence HbA1c levels.

The prevalence of anemia is estimated about 10% to 30% in patients with diabetes. Approximately one-third of patients with anemia exhibit iron deficiency. Previous studies have shown that iron deficiency elevates HbA1c levels independent of glycemia. Koga et al. showed that the HbA1c levels in subjects with iron deficiency anemia (IDA) were higher than those of subjects with normal iron levels. Shanti et al. also showed that iron deficiency was associated with higher proportions of HbA1c in subjects without diabetes. However, because these studies were performed mostly in subjects without diabetes, they could not conclude whether the presence of IDA affected the HbA1c level in diabetic or prediabetic patients.
Association Between Iron Deficiency and A1C Levels Among Adults Without Diabetes in the National Health and Nutrition Examination Survey, 1999-2006

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OBJECTIVE — Iron deficiency has been reported to elevate A1C levels apart from glycemia. We examined the influence of iron deficiency on A1C distribution among adults without diabetes.

RESEARCH DESIGN AND METHODS — Participants included adults without self-reported diabetes or chronic kidney disease in the National Health and Nutrition Examination Survey 1999–2006 who were aged ≥18 years and had complete blood counts, iron studies, and A1C levels. Iron deficiency was defined as at least two abnormalities including free erythrocyte protoporphyrin >70 µg/dl, erythrocytes, transferrin saturation <16%, or serum ferritin <15 µg/l. Anemia was defined as hemoglobin <13.5 g/dl in men and <12.0 g/dl in women.

RESULTS — Among women (n = 6,666), 13.7% had iron deficiency and 4.0% had iron deficiency anemia. Whereas 316 women with iron deficiency had A1C ≥5.5%, only 32 women with iron deficiency had A1C ≥6.5%. Among men (n = 3,869), only 13 had iron deficiency and A1C ≥5.5%, and only 1 had iron deficiency and A1C ≥6.5%. Among women, iron deficiency was associated with a greater odds of A1C ≥5.5% (odds ratio 1.39 [95% CI 1.11–1.73]) after adjustment for age, race/ethnicity, and waist circumference but not with a greater odds of A1C ≥6.5% (0.79 [0.33–1.93]).

CONCLUSIONS — Iron deficiency is common among women and is associated with shifts in A1C distribution from <5.5 to ≥5.5%. Further research is needed to examine whether iron deficiency is associated with shifts at higher A1C levels.

A1C is formed by the glycation of the terminal valine of the β-chain of hemoglobin. It is used commonly as a screening test for diabetes in clinical practice (1). A1C may be less susceptible than other measures of glycemia to temporary fluctuations caused by diet, physical activity, or illness as well as differences in local testing standards; as a result, an expert committee has recently endorsed A1C ≥6.5% as diagnostic for diabetes (1).

Previous studies have reported that depletion of iron stores may alter the glycation rate of hemoglobin and elevate A1C concentrations, independent of glycemia (2). Iron deficiency may be present without associated anemia (3). Although iron deficiency is the most common nutritional deficiency (3), the clinical relevance of iron deficiency on the use of A1C as a screening test for diabetes has not been studied. Reproductive-age women are particularly vulnerable to iron deficiency, reflecting iron loss through menstruation and pregnancy. In the Third National Health and Nutrition Examination Survey (NHANES) 1988–1994 and later NHANES waves, >11% of women had iron deficiency (3,4).

Using a recent population-based sample of U.S. adults, we examined the distribution of A1C by iron deficiency status among adults without known diabetes. We hypothesized that adults with iron deficiency would be more likely to have elevated A1C levels, even after consideration of fasting plasma glucose. We also hypothesized that any differences would persist after adjustment for other factors associated with A1C and iron deficiency, including age, race/ethnicity, and waist circumference.

RESEARCH DESIGN AND METHODS — We used data from the NHANES 1999–2006 conducted by the National Center for Health Statistics of the Centers for Disease Control and Prevention to assess the health and nutritional status of the U.S. population. The NHANES 1999–2006 included a nationally representative probability sample of the U.S. civilian noninstitutionalized population, identified through a complex multistage cluster sampling design (5).

During a household interview, participants provided information on sociodemographics and health status, and physicians and health care technicians conducted a standard examination on sampled subjects within 4 weeks of the interview. For the purposes of this analysis, we included NHANES 1999–2006 participants aged ≥18 years who had a complete blood count, iron studies, and A1C levels. We excluded individuals with known self-reported diabetes (n = 1,029) and pregnant women (n = 1,144). Con-
Association Between the Presence of Iron Deficiency Anemia and Hemoglobin A1c in Korean Adults
The 2011–2012 Korea National Health and Nutrition Examination Survey
Jae W. Hong, Cheol R. Ku, Jung H. Noh, Kyung S. Ko, Byoung D. Rhee, and Dong-Jun Kim

Abstract: Few studies have investigated the clinical effect of iron deficiency anemia (IDA) on the use of the Hemoglobin A1c (HbA1c) as a screening parameter for diabetes or prediabetes. We investigated the association between IDA and HbA1c levels in Korean adults.

Among the 11,472 adults (≥19 years of age) who participated in the 2011–2012 Korea National Health and Nutrition Examination Survey (a cross-sectional and nationally representative survey conducted by the Korean Center for Disease Control and Health Statistics), 807 patients with diabetes currently taking anti-diabetes medications were excluded from this study. We compared the weighted HbA1c levels and weighted proportion (%) of HbA1c levels of ≥5.7%, ≥6.1%, and ≥6.5% according to the range of fasting plasma glucose (FPG) levels and the presence of IDA.

Among 10,665 participants (weighted n = 35,229,108), the prevalence of anemia and IDA was 7.3% and 4.3%, respectively. The HbA1c levels were higher in participants with IDA (5.70+0.02%) than in normal participants (5.59+0.01%; <0.001), whereas there was no significant difference in FPG levels. In participants with an FPG level of <100 mg/dL and 100 to 125 mg/dL, the weighted HbA1c level was higher in those with IDA (5.59+0.02% and 6.00+0.05%) than in normal participants (5.44+0.01% and 5.82+0.01%) after adjusting for confounders such as age, sex, FPG level, heavy alcohol drinking, waist circumference, and smoking status as well as after exclusion of an estimated glomerular filtration rate of <60 mL/min/1.73 m² (<0.001, <0.01). The weighted proportions (%) of an HbA1c level of ≥5.7% and ≥6.1% were also higher in participants with IDA than in normal participants (P<0.001, <0.03). However, the weighted HbA1c levels in individuals with an FPG level ≥126 mg/dL and a weighted proportion (%) of an HbA1c level of ≥6.5% showed no significant differences according to the presence of IDA.

In conclusion, the presence of IDA shifted the HbA1c level upward only in the normoglycemic and prediabetic ranges, not in the diabetic range. Therefore, IDA should be considered before using HbA1c as a screening test for prediabetes.

INTRODUCTION

Hemoglobin A1c (HbA1c) is formed by glycation of the N-terminal valine residue of the β-chain of hemoglobin (Hb). It is commonly used as a marker of glucose control when monitored over 3 months, and provides information on the risk of long-term complications in patients with diabetes. HbA1c is also used to diagnose diabetes and to identify individuals that are at high risk for developing diabetes. The American Diabetes Association (ADA) has suggested that an HbA1c level of ≥6.5% is the diagnostic cutoff point for diabetes and that a level of 5.7% to 6.4% is the threshold for prediabetes. Measurement of HbA1c levels to screen for diabetes or prediabetes has many advantages over measurement of the fasting plasma glucose (FPG) level or performance with the 75-g oral glucose tolerance test (OGTT). However, HbA1c levels can be influenced by a variety of factors, including age, ethnicity, smoking, and conditions that alter red cell turnover and glucose homeostasis.

One condition that affects erythrocyte turnover is anemia. Anemia may be associated with more rapid erythrocyte turnover, which decreases the HbA1c level, or with slower turnover of or changes in the 3-dimensional configuration of Hb, which elevates the HbA1c level. Iron and vitamin B12 deficiency, renal failure, and bone marrow suppression in alcoholism inhibit erythropoiesis and increase the mean survival duration of erythrocytes, leading to increase HbA1c. However, hemolytic anemia, chronic liver disease, and increased hemolysis from splenomegaly increase reticulocyte and decrease the mean age of erythrocyte, which can decrease HbA1c level. Furthermore, numerous hemoglobinopathies, including HbAS, HbAC, HbE, and HbD, can also influence HbA1c level.

The prevalence of anemia is estimated about 10% to 30% in patients with diabetes. Approximately one-third of patients with anemia exhibit iron deficiency. Previous studies have shown that iron deficiency elevates HbA1c levels independent of glycemia. Koga et al. showed that the HbA1c levels in subjects with iron deficiency anemia (IDA) were higher than those of subjects with normal iron levels. Shanti et al. also showed that iron deficiency was associated with higher proportions of HbA1c in subjects without diabetes. However, because these studies were performed mostly in subjects without diabetes, they could not conclude whether the presence of IDA affected the HbA1c level in diabetic or pre-diabetic patients.
ABSTRACT
Background: Protein glycation is a spontaneous reaction that is believed to play a key role in the pathogenesis of many clinical disorders. The glycation of proteins is enhanced by elevated glucose concentrations. The major form of protein glycation with a clinical consideration is glycated haemoglobin (HbA1c). The HbA1c fraction is abnormally elevated in chronic hyperglycaemic diabetic patients and it correlates positively with the glycaemic control. However, increased glycated haemoglobin levels have been documented in iron deficiency anaemic patients without any history of diabetes.
Aims and Objective: The aim of this study was to determine the effect of IDA on the HbA1c levels in nondiabetic patients, so as to consider IDA as an important factor which influenced the HbA1c levels, while monitoring the glycaemic status of diabetics.
Methodology: Fifty non-diabetic, anaemic patients and 50 age-matched healthy subjects were enrolled in this study. The patients who had glucose tolerance abnormalities (impaired glucose tolerance or diabetes mellitus), haemoglobinopathies, haemolytic anaemia, infestation, chronic alcohol ingestion and chronic renal failure were excluded from the study. Haematologic investigations were done and the fasting and postprandial glucose and HbA1c levels were measured in all the subjects.
Results: The mean HbA1c (7.6 ± 0.5%) level in the patients with IDA was higher than that in the control group (5.9% ± 0.8) (p < 0.001). There were no differences in the levels of fasting and postprandial glucose between the IDA and the control groups (p > 0.05). The haemoglobin, serum ferritin, fasting and postprandial glucose, and the HbA1c levels were normal in the control group (p > 0.05).
Conclusion: HbA1c is not affected by the blood sugar levels alone, and there are various confounding factors when HbA1c is measured, especially that of iron deficiency, which is the commonest of the deficiency diseases worldwide. It is hence prudent to rule out IDA before making a therapeutic decision, based on the HbA1c levels.

Key Words: Iron deficiency, HbA1c, Protein glycation

INTRODUCTION
Glycated haemoglobin is produced by a ketoamine reaction between glucose and the N-terminal valine of both B-chains of the haemoglobin molecule. The major form of glycated haemoglobin is haemoglobin A1c [HbA1c] [1,2]. The measurement of glycated haemoglobin is the standard method for assessing the long-term glycaemic control. When plasma glucose is consistently elevated, the nonenzymatic glycation of haemoglobin increases; the alteration reflects the glycaemic history over the previous 2–3 months, since erythrocytes have an average lifespan of 120 days [3,4]. The HbA1c fraction is abnormally elevated in patients with chronic hyperglycaemic diabetes mellitus and it correlates positively with the metabolic control [5]. According to the American Diabetes Association (ADA) guidelines, the value of HbA1c should be kept below 7% in all the diabetics [6]. The values which are greater than 7% indicate an increased chance of progression to the diabetic complications, especially the microvascular ones.
HbA1c is majorly affected by the blood glucose levels alone. However, certain studies have proven that the HbA1c levels are altered by various other coexisting factors, along with diabetes, especially that of iron deficiency anaemia, which is a major public health problem in developing countries like India.
According to the World Health Organization (WHO), iron deficiency is the commonest of the deficiency diseases worldwide [7]. One of the well-studied pathological ill-effects of IDA in the biological system is the glycation of proteins [8]. The nonenzymatic glycation of proteins has pronounced effects on the structure and the function of proteins. The pathological consequences of these alterations depend on the nature of the proteins which are involved, as well as on their functions and concentrations in specific tissue localizations [9].
The two known factors which can modulate the glycation of proteins are the prevailing concentration of glucose and the half life of the protein [10]. But evidences in the literature have documented increased glycated protein levels in some non-diabetic pathological states, like iron deficiency anaemia. Some authors have also found that on supplementation with iron therapy, there was a significant decrease in the levels of glycated haemoglobin [11]. Evidence has accumulated, which supports the hypothesis that the glycation reaction, apart from the traditional chronic hyperglycaemia, can be modulated by the iron status of the patient. If the degree of glycation of other proteins in anaemic patients was similar to that of the glycated haemoglobin, it would have important clinical implications. Thus, the objective of the present study was to determine whether the HbA1c levels were increased among the anaemic patients without diabetes. If so, the iron deficiency had to be corrected before any diagnostic or therapeutic decision was made based on the HbA1c level.
Effect of iron deficiency anaemia on HbA1c levels is dependent on the degree of anaemia

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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 Quan 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 ± 44 mmol/mol) and IT 5.7 ± 0.4% (39 ± 44 mmol/mol)] and those measured in patients without anaemia [HPLC 5.3 ± 0.4% (34 ± 44 mmol/mol) and IT 5.3 ± 0.3% (34 ± 33 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.

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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 NH2 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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