# 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 (<150 pmol/L), and 22% were folate deficient (<7 nmol/L). On multiple linear regression analysis, $HbA_{1c}$ was predicted by higher 2-h glucose ($R^2$ = 25.6%) and lower hemoglobin ($R^2$ = 7.7%). When hematological parameters were replaced by ferritin, vitamin $B_{12}$, and folate, $HbA_{1c}$ was predicted by higher glycemia ($R^2$ = 25.6%) 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 ≥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

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measurement of hematological, biochemical, and nutritional parameters. We started measuring $HbA_{1c}$ concentrations from February 2010, after the ADA recommendations were published (1). In 116 participants, the measurements were performed on the same day as the OGTT; in 127 participants who had already attended the study, a blood sample for only $HbA_{1c}$ was collected at a subsequent home visit.

### Laboratory analysis

Hemoglobin and hematological parameters were measured on a Beckman Coulter analyzer (AcT Diff, Miami, FL). $HbA_{1c}$ was measured using high-performance liquid chromatography (Bio-Rad D-10; Bio-Rad Laboratories, Hercules, CA) calibrated against the National Glycosylated Standardization Program. Coefficients of variations (CVs) were 1.3% at an $HbA_{1c}$ concentration of 5.8% and 1.2% at a concentration of 10.0%. Bio-Rad External Quality Assurances Services results were within ±0.1% of the group mean.

Blood samples were centrifuged (4°C, 2,500*g* × 15 min) within 1 h of collection, and plasma was stored at −80°C. Plasma ferritin concentrations were measured using an ELISA (Novatec Immundiagnostica GmbH, Dietzenbach, Germany) on the Victor-2 system (PerkinElmer, Turku, Finland) with a CV of 2%. Plasma glucose was measured by glucose oxidase peroxidase, and creatinine and alanine aminotransferase (ALT) concentrations were measured using standard kits on an analyzer (Hitachi 902, Tokyo, Japan) with a CV <5% for both. Estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease formula (10). Plasma cobalamin (vitamin $B_{12}$) and folate were measured by microbiological assay using a colistin sulfate–resistant strain of *Lactobacillus leichmannii* (11,12) and a chloramphenicol-resistant strain of *Lactobacillus casei* (13,14), respectively. CVs for vitamin $B_{12}$ and folate measurement were <8%.

### Definitions

For the OGTT, glycemic status was classified according to WHO criteria (9). The classification of glycemia by $HbA_{1c}$ was performed according to ADA criteria (prediabetes: 5.7–6.4%; diabetes: ≥6.5%) (3). Anemia was defined as a hemoglobin concentration <12 g/dL in females and <13 g/dL in males (15). Iron, vitamin $B_{12}$, and folate deficiencies were defined as plasma ferritin, cobalamin, and folate concentrations <15 ng/mL (15), <150 pmol/L (16), and <7 nmol/L, respectively (17). Microcytosis refers to a mean corpuscular volume (MCV) <80 fL and macrocytosis as MCV >100 fL.

### Statistical methods

Data are presented as mean ± SD for normally distributed variables and as 50th (25th–75th) centiles for skewed variables. Skewed variables were log normalized for further analysis. Parametric and nonparametric comparisons were performed using ANOVA and Mann-Whitney *U* tests as appropriate. We performed a receiver operating characteristic (ROC) function analysis and calculated sensitivity, specificity, and positive and negative predictive values of $HbA_{1c}$ measurements to define prediabetes and diabetes, compared with the OGTT data. Associations between $HbA_{1c}$ and glycemic and nonglycemic factors were assessed using Pearson correlation coefficients, followed by multiple linear regression analysis. The level of significance was set at $P < 0.05$. Statistical analyses were performed using SPSS 16 (SPSS Inc., Chicago, IL).

**RESULTS**—A total of 351 participants attended the 21-year follow-up (72% of the original cohort). The average age at the time of the testing was 21.6 years (range 21.0–23.0). Of the participants, 3 were known to have diabetes and were excluded from the analysis. $HbA_{1c}$ measurements were available for 243 participants

**Table 1—*Characteristics of the study participants***

| | Participants in whom OGTT and $HbA_{1c}$ were measured on the same day | Participants for whom $HbA_{1c}$ was available |
|---|---|---|
| *n* | 116 | 243 |
| Demographic | | |
| Age (years) | 21.6 ± 0.5 | 21.4 ± 0.4 |
| Boys | 65 (56.0) | 136 (56.0) |
| Height (cm) | 166.6 ± 9.4 | 165.4 ± 9.8 |
| Weight (kg) | 61.7 ± 13.2 | 59.4 ± 13.3 |
| BMI (kg/m$^2$) | 22.1 ± 4.1 | 21.6 ± 4.1 |
| OGTT | | |
| Fasting glucose (mg/dL) | 93.3 ± 8.4 | 91.9 ± 7.9 |
| 2-h glucose (mg/dL) | 107.5 ± 32.4 | 104.8 ± 29.4 |
| Impaired fasting glucose | 0 (0) | 1 (0.4) |
| Impaired glucose tolerance | 9 (7.8) | 13 (5.3) |
| Diabetes | 3 (2.6) | 5 (2.1) |
| $HbA_{1c}$ (%) | 5.4 ± 0.4 | 5.4 ± 0.3 |
| Prediabetes | 27 (23.3) | 50 (20.6) |
| Diabetes | 3 (2.6) | 3 (1.2) |
| Hematological | | |
| Hemoglobin (g/dL) | 13.0 ± 2.0 | 13.1 ± 2.1 |
| Anemic | 39 (33.6) | 82 (33.7) |
| MCV (fL) | 87.8 ± 9.0 | 85.0 ± 9.5 |
| RDW (%) | 15.0 ± 2.0 | 14.9 ± 2.0 |
| MCH (pg) | 27.9 ± 3.4 | 28.3 ± 3.7 |
| MCHC (pg) | 31.8 ± 1.5 | 33.2 ± 2.1* |
| Platelets (10$^3$/μL) | 311.1 ± 79.4 | 335.5 ± 85.2 |
| Erythrocytes (10$^6$/μL) | 4.7 ± 0.5 | 4.6 ± 0.6 |
| WBCs (10$^3$/μL) | 7.3 ± 1.7 | 7.3 ± 1.7 |
| Circulating nutrients | | |
| Plasma $B_{12}$ (pmol/L)† | 173.0 (134.0–227.8) | 167.0 (133.0–216.0) |
| <150 | 46 (39.7) | 92 (37.9) |
| Plasma folate (nmol/L)† | 10.1 (7.2–15.3) | 11.1 (7.8–17.0) |
| <7 | 26 (22.4) | 42 (17.3) |
| Plasma ferritin (ng/mL)† | 25.8 (7.9–53.8) | 23.2 (6.6–46.7) |
| <15 | 43 (37.1) | 98 (40.3) |
| Creatinine (mg/%) | 0.7 ± 0.2 | 0.8 ± 0.1* |
| eGFR† | 129.2 (112.0–151.0) | 112.4 (99.4–133.5) |

Data are mean ± SD or *n* (%) unless otherwise indicated. *$P < 0.05$ for the difference between groups. †Data are 50th (25th−75th) centiles.

(136 males); these were no different from the full sample of 351 participants with respect to BMI, glycemia (OGTT), hematological, and biochemical measurements ($P > 0.05$, data not shown). In 116 subjects, $HbA_{1c}$ was measured on the same day as the OGTT; in the remainder, it was measured during a subsequent home visit, a mean of 18 months later (range 11–25). There were no differences between the 116 and 243 participants with respect to sex, BMI, 2-h glucose, $HbA_{1c}$, hemoglobin, ferritin, vitamin $B_{12}$, and folate concentrations (Table 1). Our primary analysis relates to the 116 who had measurements made on the same day; analyses for the full 243 are shown in Supplementary Data.

Among the 116 participants, the OGTT showed that 7.8% had prediabetes (all impaired glucose tolerance) and 2.6% had diabetes. The mean (range) $HbA_{1c}$ for the group was 5.4% (4.4–6.7). By ADA $HbA_{1c}$ criteria, 23.3% had prediabetes and 2.6% had diabetes. A total of 24 participants who were normoglycemic by OGTT criteria were misclassified as having prediabetes or diabetes by $HbA_{1c}$ criteria, and 6 prediabetic or diabetic participants were misclassified as normal by $HbA_{1c}$ criteria (Table 2). In the ROC analysis, the area under the curve was 0.74 (Supplementary Fig. 1). $HbA_{1c}$ had 50% sensitivity, 77% specificity, 20% positive predictive value, and 93% negative predictive value for diagnosis of prediabetes and diabetes compared with the OGTT. In other words, $HbA_{1c}$ correctly diagnosed prediabetes or diabetes in 50% of case subjects and correctly diagnosed normoglycemia in 77% of case subjects. In total, 93% of case subjects diagnosed as normoglycemic by $HbA_{1c}$ also had a normal OGTT; however, only 20% of case subjects diagnosed as prediabetes or diabetes by $HbA_{1c}$ criteria had prediabetes or diabetes according to the OGTT. There were similar findings among the full sample of 243 participants with $HbA_{1c}$ measurements (Supplementary Table 1).

Approximately one-third of the participants were anemic, iron deficient, and vitamin $B_{12}$ deficient, while one-quarter were folate deficient. None of the subjects showed abnormal hemoglobin peaks (HbS and HbC) during high-performance liquid chromatography measurement of $HbA_{1c}$. Of the anemic participants, 43.6% had microcytosis and 2.5% had macrocytosis, 66.7% were iron deficient, 30.8% were vitamin $B_{12}$ deficient, and 15.4% were folate deficient; 30% had multiple micronutrient deficiencies (at least two of the above). The predictive qualities of $HbA_{1c}$ were even lower among the anemic participants (Table 2). In the ROC analysis, the area under the curve was 0.54 (Supplementary Fig. 1). $HbA_{1c}$ had 25% sensitivity, 62% specificity, 7% positive predictive value, and 88% negative predictive value for the diagnosis of hyperglycemia compared with the OGTT. There were similar findings among the full sample of 243 participants (Supplementary Table 1).

Table 3 compares the characteristics of participants who were normoglycemic ($HbA_{1c} < 5.7\%$) and those with prediabetes or diabetes ($HbA_{1c} \geq 5.7\%$). Anthropometric measurements (height and weight) and plasma creatinine concentrations were similar in the two groups. However, prediabetic and diabetic participants had higher BMI; mean corpuscular hemoglobin (MCH), and MCH concentration (MCHC); and higher erythrocyte distribution width (RDW) compared with those who were normoglycemic ($P < 0.05$ for all). These hematological differences are suggestive of a higher prevalence of iron deficiency in the participants classified by $HbA_{1c}$ as prediabetic or diabetic. Ferritin and vitamin $B_{12}$ concentrations tended to be lower, and folate higher, in the prediabetic and diabetic group compared with the normal group. The findings were similar in the larger sample ($n$ = 243), among whom serum ferritin concentrations were significantly lower in the prediabetic and diabetic compared with the normal group (Supplementary Table 2).

**Table 2—*Glycemic classification by WHO OGTT and ADA $HbA_{1c}$ criteria in the study group* (n = *116) and in the anemic group* (n = *39)***

| | ADA $HbA_{1c}$ | | |
|---|---|---|---|
| WHO OGTT | Normal | Prediabetes and diabetes | Total |
| Study group | | | |
| Normal | 80 | 24 | 104 |
| Prediabetes and diabetes | 6 | 6 | 12 |
| Total | 86 | 30 | 116 |
| Anemic group | | | |
| Normal | 22 | 13 | 35 |
| Prediabetes and diabetes | 3 | 1 | 4 |
| Total | 25 | 14 | 39 |

Data are *n*.

### Predictors of $HbA_{1c}$

On univariate analysis, $HbA_{1c}$ concentrations were directly related to BMI ($P < 0.05$). In addition to the expected association with higher glycemia, higher $HbA_{1c}$ concentrations were associated with lower hemoglobin ($r = -0.24$), MCV ($r = -0.22$), MCH ($r = -0.30$), and MCHC ($r = -0.32$) ($P < 0.05$ for all). There were no associations with age, sex, plasma creatinine concentrations, eGFR, ALT, and white blood cells (WBCs). We used multiple linear regression analysis to study independent determinants of $HbA_{1c}$ (Table 4). All models were adjusted for age, sex, and BMI. In model 1, we studied the contribution of glycemia (2-h plasma glucose) during the OGTT. In model 2A, we added hemoglobin concentrations to model 1. In model 2B, we added erythrocyte indices to model 2A. In model 2C, we replaced hematological parameters by circulating nutrients (ferritin, vitamin $B_{12}$, and folate).

$HbA_{1c}$ was positively predicted by 2-h plasma glucose, which explained 25.6% of the variance (Table 4, model 1). Hematological parameters made the following contributions to the variability of $HbA_{1c}$: hemoglobin 7.7% (model 2A), MCHC and RDW together 13.1% (model 2B), and lower plasma ferritin concentrations 4.3% (model 2C); vitamin $B_{12}$ and folate were not significantly related to $HbA_{1c}$. Inclusion of birth weight, ALT, and WBC did not make any contribution. The interaction term 2-h glucose × ferritin was significant ($P < 0.05$). The total $R^2$ in this analysis was <40%, suggesting a major contribution by additional factors that were not included in our analysis. The equivalent figure for the full sample of 243 participants was 24.8% (Supplementary Table 3).

**CONCLUSIONS**—We started measuring $HbA_{1c}$ in our birth cohort after the ADA recommended it as a diagnostic test for prediabetes and diabetes (1). In 21-year-old Indian men and women, we observed an unexpectedly high prevalence of prediabetes and diabetes by $HbA_{1c}$ (25.9%) compared with the results of an OGTT (10.4%). This discrepancy was even greater among anemic participants (33 vs. 12%). Only

**Table 3—*Characteristics of participants with normal glucose tolerance and hyperglycemia (prediabetes and diabetes) according to ADA $HbA_{1c}$ criteria***

| | Normal glucose tolerance ($HbA_{1c}$ <5.7%) | Prediabetes and diabetes ($HbA_{1c}$ ≥5.7%) |
|---|---|---|
| *n* | 86 | 30 |
| Demographic | | |
| Age (years) | 21.6 ± 0.4 | 21.6 ± 0.4 |
| Boys | 52 (60.5) | 13 (43.3) |
| Height (cm) | 167.3 ± 9.4 | 164.5 ± 9.3 |
| Weight (kg) | 60.5 ± 12.5 | 64.9 ± 14.5 |
| BMI ($kg/m^2$) | 21.5 ± 3.6 | 23.9 ± 4.8** |
| OGTT | | |
| Fasting glucose (mg/dL) | 91.7 ± 6.8 | 97.0 ± 10.7** |
| 2-h glucose (mg/dL) | 100.0 ± 22.2 | 128.8 ± 45.4*** |
| Impaired fasting glucose | 0 (0) | 0 (0) |
| Impaired glucose tolerance | 6 (7) | 3 (10) |
| Diabetes | 0 (0) | 3 (10) |
| $HbA_{1c}$ (%) | 5.2 (0.2) | 5.9 (0.2)*** |
| Prediabetes | 27 (23.3) | 50 (20.6) |
| Diabetes | 3 (2.6) | 3 (1.2) |
| Hematological | | |
| Hemoglobin (g/dL) | 13.0 ± 2.0 | 13.1 ± 2.1 |
| Anemic | 39 (33.6) | 82 (33.7) |
| MCV (fL) | 88.6 ± 8.3 | 85.1 ± 10.3 |
| RDW (%) | 14.7 ± 1.9 | 15.7 ± 2.1* |
| MCH (pg) | 28.3 ± 3.1 | 26.6 ± 3.9* |
| MCHC (pg) | 32.0 ± 1.4 | 31.1 ± 1.5* |
| Platelets ($10^3/\mu L$) | 303.5 ± 75.2 | 332.8 ± 88.1 |
| Erythrocytes ($10^6/\mu L$) | 4.6 ± 0.5 | 4.5 ± 0.4 |
| WBCs ($10^3/\mu L$) | 7.3 ± 1.6 | 7.5 ± 2.0 |
| Circulating nutrients | | |
| Plasma $B_{12}$ (pmol/L)† | 173.0 (134.0–227.8) | 167.0 (133.0–216.0) |
| <150 | 46 (39.7) | 92 (37.9) |
| Plasma folate (nmol/L)† | 10.1 (7.2–15.3) | 11.1 (7.8–17.0) |
| <7 | 26 (22.4) | 42 (17.3) |
| Plasma ferritin (ng/mL)† | 25.8 (7.9–53.8) | 23.2 (6.6–46.7) |
| <15 | 43 (37.1) | 98 (40.3) |
| Creatinine (mg/%) | 0.7 ± 0.2 | 0.8 ± 0.1* |
| eGFR (mL/min)† | 129.2 (112.0–151.0) | 112.4 (99.4–133.5) |

Data are mean ± SD or *n* (%) unless otherwise indicated. *P* values refer to significance of the difference between groups calculated by ANOVA or Mann-Whitney *U* test. †Data are 50th (25th−75th) centiles. $*P < 0.05$. $**P < 0.01$. $***P < 0.001$.

20% of those diagnosed as prediabetic and diabetic by $HbA_{1c}$ had prediabetes and diabetes according to the OGTT, and among the anemic, this figure was only 7%. In this young, apparently healthy, and nondiabetic group, 2-h glucose concentrations explained only 25.6% of the variance in $HbA_{1c}$ concentrations, and hematological parameters contributed up to 13.1%, leaving over half of the variance unexplained. Hematological parameters that predicted higher $HbA_{1c}$ included anemia and erythrocyte indices indicative of iron deficiency (microcytosis, low MCH, low MCHC, and high RDW) and low ferritin concentrations.

In clinical practice, $HbA_{1c}$ is used in diabetic patients as an index of long-term glycemic control. It is formed by glycation of the $NH_2$-terminal valine residue of the β-chain of globin (18). In addition to ambient glycemia, factors affecting erythrocyte life span affect $HbA_{1c}$ concentrations. For a comparable glycemic exposure, conditions that shorten erythrocyte life span reduce $HbA_{1c}$ concentrations (hemolytic anemias, infections, blood loss, hypersplenism, malaria, and pregnancy). On the other hand, prolongation of erythrocyte survival (iron deficiency, splenectomy, aplastic anemia, and certain hemoglobinopathies) elevates $HbA_{1c}$ concentrations (19). Kidney and liver disease have complex effects on $HbA_{1c}$ concentrations. Such clinical abnormalities do not explain our findings because our subjects were healthy, and inclusion of age, sex, degree of obesity, renal and hepatic function (in the normal range), WBC count, and birth weight in the multiple linear regression analysis of $HbA_{1c}$ did not improve the variance. In addition, interindividual differences in erythrocyte permeability to glucose and intracellular concentrations of its metabolites have been shown to influence rate of glycation and could explain some of the variance (20,21). In addition to increasing erythrocyte survival, iron deficiency could alter these parameters. It is also suggested that iron deficiency may alter the quaternary structure of the hemoglobin molecule and facilitate glycation of the β-globin chain (22). Finally, as yet uninvestigated genetic and environmental factors, which may influence erythrocyte dynamics, including inflammation, could also contribute to the remaining variance.

Iron deficiency is the commonest nutritional deficiency worldwide, affecting ~50% of the world population (4). The prevalence is higher in low- and middle-income countries compared with high-income countries, and women, children, and adolescents are the most susceptible. Diabetes is rapidly increasing in low- and middle-income countries, and the young and the poor are increasingly affected (23). The use of $HbA_{1c}$ for diagnosis of hyperglycemia in such populations is an attractive alternative to the cumbersome OGTT (1). Limitations imposed by nonglycemic nutritional influences should invite further research into the application of $HbA_{1c}$ in the diagnosis of prediabetes and diabetes in undernourished populations. Similar associations between iron deficiency and elevated $HbA_{1c}$ concentrations have been shown in other studies in nondiabetic as well as type 1 diabetic patients (5,7,18,22). A causal role for iron deficiency in elevating $HbA_{1c}$ concentration is supported by a fall in levels after iron supplementation (5). There is some recognition of these issues in both the ADA position statement (1,3) and the WHO report (2), but little appreciation of the magnitude of the misclassification and the implications of this to prevalence statistics, as well as to the individual who is incorrectly diagnosed with prediabetes or diabetes. A large study in adolescent obese American children also shows low sensitivity and low positive predictive value for $HbA_{1c}$ in the diagnosis of prediabetes and diabetes (24).

**Table 4—*Multiple linear regression models to define demographic, glycemic, hematological, and nutritional determinants of $HbA_{1c}$* (n = 116)**

| | Model 1 | | Model 2A | | Model 2B | | Model 2C | |
|---|---|---|---|---|---|---|---|---|
| Parameter | % $R^2$ | β | % $R^2$ | β | % $R^2$ | β | % $R^2$ | β |
| Demographic | | | | | | | | |
| Sex | — | — | — | — | — | — | — | — |
| Age | — | — | — | — | — | — | — | — |
| BMI | — | — | — | — | — | — | — | — |
| Glycemic | | | | | | | | |
| 2-h glucose | 25.6 | 0.50** | 25.6 | 0.51** | 25.6 | 0.51** | 25.6 | 0.52** |
| Hematological | | | | | | | | |
| Hemoglobin | — | — | 7.7 | −0.27** | — | — | — | — |
| MCV | — | — | — | — | — | — | — | — |
| RDW | — | — | — | — | 10.6 | 0.22* | — | — |
| MCHC | — | — | — | — | 2.5 | −0.18* | — | — |
| MCH | — | — | — | — | — | — | — | — |
| Platelet | — | — | — | — | — | — | — | — |
| Circulating nutrients | | | | | | | | |
| Plasma ferritin | — | — | — | — | — | — | 4.3 | −0.20* |
| Vitamin $B_{12}$ | — | — | — | — | — | — | — | — |
| Plasma folate | — | — | — | — | — | — | — | — |
| Total $R^2$ (%) | 25.6 | — | 33.3 | — | 38.7 | — | 29.9 | — |

*$P < 0.05$. **$P < 0.01$.

Our study has several strengths. The majority of our cohort were nondiabetic and had no confounding comorbidities (e.g., renal, thyroid, and hepatic disease or pregnancy), offering a unique opportunity to study $HbA_{1c}$ in the young and healthy group, among whom its use as a screening tool would be most desirable. We measured a wide range of parameters, including demographic, anthropometric, hematological, biochemical, and nutritional (ferritin, vitamin $B_{12}$, and folate) factors and performed a standard OGTT. $HbA_{1c}$ was measured by an internationally accepted method with attention to quality control. The method allows detection of hemoglobinopathies (HbS and HbC), which interfere with $HbA_{1c}$ measurements, and we did not find such interference in any subject. The commonest hemoglobinopathy in our population, β-thalassemia trait (prevalence <4% in our population) (25,26), cannot explain our results because it would reduce the $HbA_{1c}$ concentrations. There were some limitations to the study. $HbA_{1c}$ measurements were available on only a proportion of the participants. However, this is unlikely to affect our results because the study group did not differ from the total group with respect to age, sex, hematological parameters, and prevalence of abnormalities of OGTT.

Our results support a substantial nonglycemic nutritional influence on $HbA_{1c}$ concentrations in young nondiabetic Indians. This complicates the use of $HbA_{1c}$ in the diagnosis of prediabetes in nutritionally compromised populations (i.e., more than half of the world's population). We plan to study the effects of a nutritional intervention (iron, vitamin $B_{12}$, and folic acid) on $HbA_{1c}$ in our population. It would also be informative to extend the study to diabetic patients to determine the potential effect on clinical practice and the interpretation of clinical trials.

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