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.

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 glycaemia (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 glycaemia (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 glycaemia 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 glycaemic level (5,6).

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 measurement of HbA_1c (10).

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Iron deficiency and HbA1c

measurement of hematological, biochemical, and nutritional parameters. We started measuring HbA1c 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 HbA1c was collected at a subsequent home visit.

Laboratory analysis

Hemoglobin and hematological parameters were measured on a Beckman Coulter analyzer (AcT Diff, Miami, FL). HbA1c was measured using high-performance liquid chromatography (Bio-Rad D-10; Bio-Rad Laboratories, Hercules, CA) calibrated against the National Glycosylated Hemoglobin and hematological parameters were measured on a Beckman Coulter Hemoglobin and hematological parameters were measured on a Beckman Coulter analyzer (AcT Diff, Miami, FL). HbA1c was measured against the National Glycosylated Hemoglobin and hematological parameters were measured on a Beckman Coulter analyzer (AcT Diff, Miami, FL).

HbA1c (%) 5.4 ± 0.4

Prediabetes 27 (23.3) 50 (20.6)

Diabetes 3 (2.6) 3 (1.2)

Hematological

Hemoglobin (g/dL) 13.0 ± 2.0

Anemic 39 (33.6) 82 (33.7)

MCV (fL) 87.8 ± 9.0

RDW (%) 15.0 ± 2.0

MCH (pg) 27.9 ± 3.4

MCHC (pg) 31.8 ± 1.5

Platelets (10^3/μL) 311.1 ± 79.4

Erythrocytes (10^6/μL) 4.7 ± 0.5

WBCs (10^3/μL) 7.3 ± 1.7

Circulating nutrients

Plasma B12 (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)

Table 1—Characteristics of the study participants

Participants in whom OGTT and HbA1c were measured on the same day

Participants for whom HbA1c 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 ± 9.8

Weight (kg) 61.7 ± 13.2 59 ± 13.3

BMI (kg/m²) 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)

HbA1c (%) 5.4 ± 0.4 5.4 ± 0.3

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Definitions

For the OGTT, glycemic status was classified according to WHO criteria (9). The classification of glycemia by HbA1c 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 B12, 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 HbA1c measurements to define prediabetes and diabetes, compared with the OGTT data. Associations between HbA1c 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. HbA1c measurements were available for 243 participants.
(136 males); these were no different from the full sample of 351 participants with respect to BMI, glyceremia (OGTT), hematological, and biochemical measurements (P > 0.05, data not shown). In 116 subjects, HbA1c 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 11–23). There were no differences between the 116 and 243 participants with respect to sex, BMI, 2-h glucose, HbA1c, hemoglobin, ferritin, vitamin B12, 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) HbA1c for the group was 5.4% (4.4–6.7). By ADA HbA1c 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 HbA1c criteria, and 6 prediabetic or diabetic participants were misclassified as normal by HbA1c criteria (Table 2). In the ROC analysis, the area under the curve was 0.74 (Supplementary Fig. 1). HbA1c 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 2—Glycemic classification by WHO OGTT and ADA HbA1c criteria in the study group (n = 116) and in the anemic group (n = 39)

| WHO OGTT                        | ADA HbA1c |        |        |        |
|--------------------------------|-----------|--------|--------|--------|
|                                | 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.

CONCLUSIONS—We started measuring HbA1c 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 HbA1c (25.9%) compared with the results of an OGTT (10.4%).
Iron deficiency and HbA$_{1c}$

This discrepancy was even greater among anemic participants (33 vs. 12%). Only 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 $\beta$-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 $\beta$-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 highest 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 3—Characteristics of participants with normal glucose tolerance and hyperglycemia (prediabetes and diabetes) according to ADA HbA$_{1c}$ criteria

|                      | Normoglycemic (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)***                               |
| **Hematological**    |                                 |                                            |
| **Hemoglobin (g/dL)**| 13.2 ± 1.8                      | 12.1 ± 2.3*                                |
| **Anemic**           | 25 (29.1)                       | 14 (46.7)                                  |
| **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$^3$/μL)** | 303.5 ± 75.2                   | 332.8 ± 88.1                               |
| **Erythrocytes (10$^6$/μL)** | 4.6 ± 0.5                     | 4.5 ± 0.4                                  |
| **WBCs (10$^3$/μL)** | 7.3 ± 1.6                       | 7.5 ± 2.0                                  |
| **Circulating nutrients** |                                 |                                            |
| **Plasma B$_12$ (pmol/L)** | 175.0 (134.7–238.0)            | 151.0 (117.2–198.2)                        |
| <150                 | 32 (37.2)                       | 14 (46.7)                                  |
| **Plasma folate (nmol/L)** | 9.4 (7.2–13.57)                | 11.4 (6.8–17.8)                            |
| <7                   | 18 (20.9)                       | 8 (26.7)                                   |
| **Plasma ferritin (ng/mL)** | 27.1 (8.0–52.8)                | 21.0 (4.6–65.3)                            |
| <15                  | 30 (34.9)                       | 13 (34.3)                                  |
| **Creatinine (mg%)** | 0.7 ± 0.1                       | 0.7 ± 0.1                                  |
| **eGFR (ml/min)**    | 129.2 (112.0–151.0)             | 132.4 (112.1–156.7)                        |

Data are mean ± SD or n (%) unless otherwise indicated. *Data are 50th (25th–75th) percentiles. Values refer to significance of the difference between groups calculated by ANOVA or Mann-Whitney U test. *P < 0.05, **P < 0.01, ***P < 0.001.
Circulating nutrients

Parameter |
|-----------|
| Glycemic |
| Demographic |

it would reduce the HbA1c concentrations. (25,26), cannot explain our results because trait (prevalence any subject. The commonest hemoglobin-
abnormalities of OGTT.

Hematological parameters, and prevalence of factors and performed a standard OGTT. HbA1c was measured by an internationally accepted method with attention to quality control. The method allows detection of hemoglobinopathies (HbS and HbC), which interfere with HbA1c 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 HbA1c concentrations. There were some limitations to the study. HbA1c 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 non-
glycemic nutritional influence on HbA1c concentrations in young nondiabetic Indians. This complicates the use of HbA1c 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 B12, and folic acid) on HbA1c 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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References

1. International Expert Committee. International Expert Committee report on the role of the A1C assay in the diagnosis of diabetes. Diabetes Care 2009;32:1327–1334.

2. World Health Organization. Use of Glycated Haemoglobin (HbA1c) in the Diagnosis of Diabetes Mellitus: Abbreviated Report of WHO Consultation. Geneva, World Health Org., 2011.

3. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2011;34(Suppl. 1):S62–S69.

4. World Health Organization Prevention and Control of Iron Deficiency Anaemia in Women and Children: Report of the UNICEF/WHO Regional Consultation February 1999. Geneva, World Health Org., 2001.

5. Coban E, Ozdogan M, Timuragaoğlu A. Effect of iron deficiency anemia on the levels of hemoglobin A1c in non diabetic patients. Acta Haematol 2004;112:126–128.

6. Koga M, Morita S, Saito H, Mukai M, Kasayama S. Association of erythrocyte indices with glycated haemoglobin in premenopausal women. Diabet Med 2007;24:843–847.

7. Tarim O, Küçükderoğlu A, Günay U, Eralp O, Erkan I. Effects of iron deficiency anemia on hemoglobin A1c in type 1 diabetes mellitus. Pediatr Int 1999;41:357–362.

8. Bawdekar A, Yajnik CS, Fall CH, et al. Insulin resistance syndrome in 8-year-old Indian children: small at birth, big at 8 years, or both? Diabetes 1999;48:2422–2429.

9. World Health Organization. Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycemia: Report of WHO/ IDF Consultation. Geneva, World Health Org., 2006.

10. eGFR Calculator [Internet]. Available from http://www.renal.org/eGFRcalc/. Accessed 7 October 2011.

11. Kelleher BP, Walshe KG, Scott JM, O’Broin SD. Microbiological assay for vitamin B12 with use of a colistin-sulfate-resistant organism. Clin Chem 1987;33:52–54.

12. Kelleher BP, Broin SD. Microbiological assay for vitamin B12 performed in 96-well microtitre plates. J Clin Pathol 1991;44:592–595.

13. Horne DW, Patterson D. Lactobacillus casei microbial assay of lactic acid derivatives in 96-well microtitrte plates. Clin Chem 1988;34:2357–2359.
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14. Tamura T, Freeberg LE, Cornwell PE. Inhibition of EDTA of growth of Lactobacillus casei in the folate microbiological assay and its reversal by added manganese or iron. Clin Chem 1990;36:1993

15. World Health Organization. Iron Deficiency Anaemia: Assessment, Prevention and Control. A Guide for Programme Managers. Geneva, World Health Org., 2001 (WHO/NHD/01.3)

16. Refsum H, Smith AD, Ueland PM, et al. Facts and recommendations about total homocysteine determinations: an expert opinion. Clin Chem 2004;50:3–32

17. Clarke R, Grimley Evans J, Schneede J, et al. Vitamin B12 and folate deficiency in later life. Age Ageing 2004;33:34–41

18. Kim C, Bullard KM, Herman WH, Beckles GL. Association between iron deficiency and A1C Levels among adults without diabetes in the National Health and Nutrition Examination Survey, 1999-2006. Diabetes Care 2010;33:780–785

19. Gallagher EJ, Le Roith D, Bloomgarden Z. Review of hemoglobin A1c in the management of diabetes. J Diabetes 2009;1:9–17

20. Gould BJ, Davie SJ, Yudkin JS. Investigation of the mechanism underlying the variability of glycated haemoglobin in non-diabetic subjects not related to glycaemia. Clin Chim Acta 1997;260:49–64

21. Khera PK, Joiner CH, Carruthers A, et al. Evidence for interindividual heterogeneity in the glucose gradient across the human red blood cell membrane and its relationship to hemoglobin glycation. Diabetes 2008;57:2445–2452

22. Brooks AP, Metcalfe J, Day JL, Edwards MS. Iron deficiency and glycosylated haemoglobin A. Lancet 1980;2:141

23. International Diabetes Federation. Diabetes Atlas [Internet], 2009. Available from http://www.diabetesatlas.com/map

24. Lee JM, Wu EL, Tarini B, Herman WH, Yoon E. Diagnosis of diabetes using hemoglobin A1c: should recommendations in adults be extrapolated to adolescents? J Pediatr 2011;158:947–952

25. Colah R, Gorakshakar A, Phanasgaonkar S, et al. Epidemiology of beta-thalassaemia in Western India: mapping the frequencies and mutations in sub-regions of Maharashtra and Gujarat. Br J Haematol 2010;149:739–747

26. Madan N, Sharma S, Sood SK, Colah R, Bhatia LH. Frequency of β-thalassaemia trait and other hemoglobinopathies in northern and western India. Indian J Hum Genet 2010;16:16–25