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EFFECT OF IRON AND VITAMIN B12 DEFICIENCY ANEMIA ON HEMOGLOBIN A1C LEVELS

General Medicine

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ABSTRACT

Hemoglobin A1c (HbA1c) is central to the diagnosis of diabetes, but its validity assumes normal erythrocyte turnover.1 Nutritional anemias are prevalent in India and may distort HbA1c independently of glycemia.2,3 This study assessed the effect of iron deficiency anemia (IDA) and of vitamin B12 deficiency anemia on HbA1c in non-diabetic adults, and compared both with non-anemic controls. A hospital-based cross-sectional study at L.N. Medical College and J.K. Hospital, Bhopal, enrolled 390 non-diabetic adults with fasting blood sugar below 100 mg/dL by consecutive sampling: 184 with IDA, 76 with vitamin B12 deficiency anemia and 130 non-anemic controls. Complete blood count, iron profile, serum B12 and HbA1c by HPLC were estimated, and data analysed by ANOVA with post-hoc Tukey's test, chi-square test and Pearson's correlation. Mean HbA1c was highest in IDA (5.83 ± 0.6%), intermediate in vitamin B12 deficiency (5.41 ± 0.5%) and lowest in controls (5.00 ± 0.4%); both anemic groups differed from controls ($p<0.001$) despite normal fasting glucose. Hemoglobin correlated inversely with HbA1c in IDA ($r = –0.781$) and B12 deficiency ($r = –0.643$), as did ferritin, transferrin saturation and serum B12 (all $p<0.001$), whereas serum iron and MCV did not. HbA1c reached the 5.7% prediabetes threshold in 62.5% of IDA patients versus 26.3% of B12-deficient patients and 3.8% of controls ($p<0.001$). Nutritional anemia spuriously elevates HbA1c, iron deficiency more than B12 deficiency, and should be identified and corrected before HbA1c is interpreted for the diagnosis of diabetes.

KEYWORDS

HbA1c, Iron Deficiency Anemia, Vitamin B12 Deficiency, Anemia, Glycemic Misclassification, Prediabetes

INTRODUCTION

HbA1c reflects mean glycemia over the preceding two to three months and is endorsed at a threshold of 6.5% for diagnosing diabetes mellitus.[1] Its reliability rests on an assumption of normal red cell survival, and that assumption fails in nutritional anemia. In iron deficiency, restricted haem synthesis prolongs erythrocyte lifespan and extends the exposure of hemoglobin to circulating glucose, favouring glycation.[2] In vitamin B12 deficiency, ineffective erythropoiesis yields macrocytes of variable survival, and the net effect is less predictable.[3]

This matters in India, where nutritional anemia and dysglycemia coexist at high prevalence and HbA1c is increasingly a stand-alone screening test. Published work remains discordant, and few studies have compared both deficiency states against a common non-anemic reference group. This study therefore quantifies and compares the influence of iron and vitamin B12 deficiency anemia on HbA1c in non-diabetic adults.

OBJECTIVES

To study the effect of iron deficiency anemia on HbA1c, to study the effect of vitamin B12 deficiency anemia on HbA1c, and to compare both with non-anemic controls.

MATERIALS AND METHODS

Study Design and Setting: An observational, hospital-based, cross-sectional study was carried out in the Department of General Medicine, L.N. Medical College & Research Centre and J.K. Hospital, Bhopal, over 18 months. Ethics Committee approval and written informed consent were obtained.

Participants: Adults aged 18 years and above with laboratory-confirmed iron or vitamin B12 deficiency anemia, plus non-anemic controls, were recruited by consecutive non-probability sampling. Only non-diabetic individuals with fasting blood sugar below 100 mg/dL and no history of diabetes were included. Patients with diagnosed diabetes, impaired fasting glucose, anti-diabetic therapy, or hemolytic, aplastic or chronic-disease anemia were excluded. Calculated sample size was 385; 390 were enrolled.

Diagnostic Criteria: IDA was defined by hemoglobin below 12 g/dL in females and 13 g/dL in males with microcytic hypochromic indices (MCV <80 fL, MCH <27 pg), ferritin below 30 µg/L in males and 15 µg/L in females, serum iron below 65 µg/dL, TIBC above 400 µg/dL and transferrin saturation below 20%. Vitamin B12 deficiency anemia required anemia with MCV above 100 fL, megaloblastic changes on smear and serum B12 below 200 pg/mL.

Investigations and Analysis: After 8–10 hours of fasting, complete blood count, peripheral smear, iron profile, serum vitamin B12 (chemiluminescence immunoassay), fasting plasma glucose and HbA1c by high-performance liquid chromatography were performed. Analysis used R 4.5.1: one-way ANOVA with post-hoc Tukey's test, chi-square test and Pearson's correlation, $p<0.05$ significant.

RESULTS

Of 390 participants, 184 (47.2%) had IDA, 76 (19.5%) had vitamin B12 deficiency anemia and 130 (33.3%) were controls. Females predominated (64.6%) and 40.0% were aged 31–40 years. Laboratory profiles confirmed the expected microcytic and macrocytic patterns, with normal fasting glucose throughout (Table 1).

Table 1: Laboratory Profile of the Study Groups (Mean ± SD)

Parameter	IDA (n=184)	Vitamin B12 deficiency (n=76)	Control (n=130)
Hemoglobin (g/dL)	6.9 ± 1.7	8.3 ± 1.9	14.6 ± 1.4
MCV (fL)	69.3 ± 9.4	112.7 ± 12.1	89.1 ± 5.3
MCH (pg)	20.7 ± 4.1	38.4 ± 5.1	30.1 ± 2.4
Serum ferritin (µg/L)	11.2 ± 6.8	—	87.2 ± 42.6
Serum iron (µg/dL)	35.4 ± 12.7	—	—
TIBC (µg/dL)	507.8 ± 78.3	—	—
Transferrin saturation (%)	9.8 ± 3.9	—	—
Serum vitamin B12 (pg/mL)	—	109.3 ± 32.6	498.7 ± 132.4
Fasting blood sugar (mg/dL)	90.7 ± 9.4	85.4 ± 9.0	83.9 ± 8.2
HbA1c (%)	5.83 ± 0.6	5.41 ± 0.5	5.00 ± 0.4

HbA1c differed significantly across the three groups (ANOVA, $p<0.001$). The mean value was 5.83 ± 0.6% in IDA and 5.41 ± 0.5% in vitamin B12 deficiency, against 5.00 ± 0.4% in controls, giving mean differences from control of 0.83% and 0.41% respectively; post-hoc Tukey testing confirmed both comparisons at $p<0.001$. Across three HbA1c strata (4.4–5.0%, 5.1–5.6%, 5.7–6.2%) the distribution shifted rightward in parallel: 62.5% of IDA and 26.3% of B12-deficient patients reached the 5.7% prediabetes threshold, against 3.8% of controls ($\chi^2 = 127.12$, df = 4, $p<0.001$) (Table 2). All participants had fasting glucose below 100 mg/dL, so this elevation occurred without any accompanying rise in measured glycemia.

Table 2: HbA1c Levels and Distribution Across Study Groups

Group	n	Mean HbA1c ± SD (%)	95% CI	HbA1c 5.7–6.2% n (%)	p-value
Iron deficiency anemia	184	5.83 ± 0.6	5.74–5.92	115 (62.5)	<0.001
Vitamin B12 deficiency	76	5.41 ± 0.5	5.30–5.52	20 (26.3)	<0.001
Control (non-anemic)	130	5.00 ± 0.4	4.93–5.07	5 (3.8)	—

p-values are versus the control group (ANOVA with post-hoc Tukey's test).

Hemoglobin correlated strongly and inversely with HbA1c in IDA (r = –0.781) and moderately in B12 deficiency (r = –0.643); ferritin, transferrin saturation and serum B12 were likewise inversely correlated, whereas serum iron and MCV were not (Table 3). By ICMR grade, the 260 anemic participants comprised 47.7% severe, 39.6% moderate and 12.7% mild anemia, with no very severe cases. HbA1c peaked in moderate anemia (5.87 ± 0.54%) rather than severe (5.64 ± 0.61%) or mild (5.45 ± 0.57%). This pattern was pronounced in IDA (moderate 5.99 ± 0.48%, severe 5.73 ± 0.63%, mild 5.49 ± 0.69%) but absent in B12 deficiency, where values were near-uniform across grades (5.36–5.46%).

Table 3: Correlation of Anemia Parameters with HbA1c

Parameter	IDA r	p	B12 r	p
Hemoglobin	–0.781	<0.001	–0.643	<0.001
Serum ferritin	–0.624	<0.001	—	—
Transferrin saturation	–0.658	<0.001	—	—
Serum iron	–0.067	0.369	—	—
Serum vitamin B12	—	—	–0.571	<0.001
MCV	0.021	0.776	–0.028	0.808

DISCUSSION

Both nutritional anemias raised HbA1c in the absence of hyperglycemia, and iron deficiency did so roughly twice as strongly as vitamin B12 deficiency. The IDA excess of 0.83% above control matches Srinath et al., who recorded 5.87 ± 0.76% against 5.03 ± 0.34% in non-diabetic IDA patients,[4] and Alzahrani et al., who reported 5.75% (95% CI 5.68–5.82) versus 5.32% among 324 non-diabetic subjects.[5] The accepted mechanism is prolonged erythrocyte survival under iron-restricted erythropoiesis, lengthening the interval over which hemoglobin meets glucose.[2]

The vitamin B12 finding is less uniformly supported. Pilla et al., studying 100 iron-deficient, 100 B12-deficient and 100 controls, found HbA1c raised in both anemias and falling after replacement.[6] Alzahrani et al., however, found megaloblastic anemia (5.38%) no different from control.[5] Depth of anemia plausibly reconciles this: their B12 cohort averaged 11.3 g/dL hemoglobin against 6.1 g/dL in Pilla's series. Our B12 group, at 8.3 g/dL, lies between the two and showed an intermediate elevation of 0.41%, consistent with a hemoglobin-dependent effect. Aydin et al. recorded a fall from 6.01 ± 0.20% to 5.77 ± 0.33% after three months of supplementation,[3] and Garg and Thakre reclassified 90.1% of prediabetic B12-deficient adults as normoglycemic after treatment,[7] indicating a reversible and therefore artefactual elevation.

Neither MCV nor serum iron correlated with HbA1c, whereas hemoglobin, ferritin and transferrin saturation did; comparable inverse hemoglobin-HbA1c correlations are reported in non-diabetic women.[8] This is expected: serum iron fluctuates diurnally whereas ferritin and transferrin saturation index stored iron, and cell size is irrelevant if erythrocyte lifespan rather than morphology is the driver. The non-linear severity relationship fits the same mechanism, compensatory reticulocytosis in severe anemia introducing younger, less glycated cells that partly offset the effect at moderate grades.

The clinical consequence is quantifiable. Had HbA1c been used alone, 62.5% of these non-diabetic IDA patients would have been labelled prediabetic. Neki et al. showed such elevations reverse with iron therapy, confirming the label would be false,[9] and Solomon et al. found the same distortion in 174 diabetic patients, where it exaggerates poor control.[10] Klonoff argues generally that hemoglobin and red cell turnover abnormalities must be weighed before HbA1c is trusted.[11] Where nutritional anemia is common, hemoglobin and iron status should precede diagnostic use of HbA1c, with fructosamine or glycated albumin as alternatives when anemia cannot be corrected.

Limitations include the single-centre cross-sectional design, no post-treatment HbA1c, a single fasting glucose rather than an oral glucose tolerance test, and the small B12 group.

CONCLUSION

Iron deficiency anemia produces a clinically meaningful false elevation of HbA1c in non-diabetic adults, and vitamin B12 deficiency anemia does so to a lesser degree. Hemoglobin is the strongest correlate in both conditions, while MCV is not. Because 62.5% of non-diabetic iron-deficient patients here would have been misclassified as prediabetic on HbA1c alone, anemia should be identified and corrected before HbA1c is interpreted for diabetes.

Declarations
Conflict of Interest: None.
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Ethical Approval: Obtained.

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