

Relationship between glycosylated hemoglobin and vitamin B12 deficiency anemia

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ABSTRACT

OBJECTIVE: Previous studies showed that vitamin B12 deficiency anemia causes a false increase in glycosylated hemoglobin (HbA1c) and that HbA1c decreases with B12 treatment. However, no study has been conducted on how much an increase in hemoglobin (Hgb) level causes a decrease in HbA1c level after treatment.

METHODS: The study included 37 patients who were not diagnosed with diabetes, did not use anti-diabetic drugs, were pre-diabetic according to HbA1c level, and were diagnosed with vitamin B12 deficiency anemia in the patient group and 40 healthy volunteers of similar age and gender characteristics in the control group. The patient group was given 1 mg/day of cyanocobalamin (vitamin B12) orally for 3 months. Patients' Hgb, mean corpuscular volume, fasting plasma glucose, HbA1c, and vitamin B12 values were compared at the beginning and at the end of the 3rd month.

RESULTS: In the patient group, it was determined that 0.94 mg/dL increase in Hgb after vitamin B12 treatment caused a 0.24 decrease in HbA1c (%). The initial HbA1c of the patient group was 6.01±0.20 and the 3rd-month HbA1c was 5.77±0.33; the initial and 3rd-month Hgb values were 11.31±0.28 and 12.26±0.33, respectively; the initial and 3rd-month vitamin B12 (ng/L) levels were 112.43±7.18 and 408.48±119.61, respectively; and there was a significant difference between the initial and 3rd-month values ($p<0.001$, $p<0.001$, $p<0.001$, respectively). Moreover, 35% of the patients in the patient group had no diagnosis of prediabetes according to the HbA1c level at the end of the 3rd month.

CONCLUSION: Elimination of vitamin B12 deficiency anemia before making a diagnosis or treatment decision according to HbA1c level will prevent patients from misdiagnosis of diabetes and unnecessary treatment changes in diabetic patients.

Keywords: Diabetes mellitus; glycosylated hemoglobin; vitamin B12 deficiency anemia.

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Prediabetes is defined as a condition, where the glucose level is higher than normal but not high enough to be diagnosed as diabetes (DM). Guidelines defines prediabetes as a plasma fasting blood glucose of 100–125 mg/dL, a blood glucose level of 140–200 mg/dL 2 h after a 75-g oral glucose tolerance test, or glycosylated hemoglobin (HbA1c) in the range of 5.7–6.4% [1]. Prediabetes is a high-risk condition for the development of

DM, as it turns into diabetes at a rate of 5–10% annually [2]. The Statistics Report of the Centers for Disease Control and Prevention National Diabetes suggested that between 2009 and 2012, 37% of adults aged over 20 and 51% of adults aged over 65 in the United States had prediabetes as defined by fasting glucose or HbA1c levels [3]. Prediabetes is a public health problem and its prevalence is increasing worldwide [4].

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Vitamin B12 deficiency arises most commonly from food-based malabsorption of cobalamin. Studies have shown that the prevalence of vitamin B12 deficiency anemia varies with age and increases with advancing age. Deficiency is much more common in developing countries [5].

HbA1c, which is used as a clinical indicator of the average blood glucose level of the past 3 months, is one of the laboratory tests used in the diagnosis and follow-up of DM [6, 7]. The American Diabetes Association (ADA) guidelines recommend keeping the HbA1c level below 6.5% to prevent diabetes complications. Despite national standards to eliminate technical errors in the HbA1c test, studies showed that factors such as race, age, diet, medication, and comorbidities can also change HbA1c levels [8]. While there may be a false increase in HbA1c levels in nutritional anemias and abnormal hemoglobinopathies (such as HbF and HbS) due to iron, vitamin B12, and Folate deficiency, a false decrease can be observed in HbA1c levels in hemolytic anemia [9]. HbA1c value increases as erythrocyte (RBC) lifespan increases [10]. Vitamin B12 deficiency anemia can cause false high HbA1c levels as it increases the survival time of RBC [10, 11].

Anemia and DM are diseases that are common in the society and constitute a public health problem, and they are highly likely to be seen together in the same patient [12]. Studies showed that the HbA1c level decreases with the treatment of vitamin B12 deficiency anemia [13–15]. However, there is no study examining how much of the increase in Hgb level after treatment leads to a decrease in HbA1c. While there is only one study on the patient population with iron deficiency anemia (IDA) [16], there is no study on the patient population with vitamin B12 deficiency anemia. This study aimed to demonstrate whether there is a decrease in HbA1c after the treatment of vitamin B12 deficiency anemia and if there is how much Hgb increase causes a decrease in HbA1c.

MATERIALS AND METHODS

Study Population

In the study, 37 patients with vitamin B12 deficiency anemia who were considered to be prediabetic in their examinations and 40 prediabetic patients without vitamin B12 deficiency were included in the study. A fasting blood glucose of <100 mg/dL and HbA1c of 5.7–6.5

Highlight key points

- HbA1c is unreliable in patients with vitamin B12 deficiency anemia.
- Treatment of vitamin B12 anemia causes an increase of 0.94 mg/dL in Hgb and a decrease of 0.24 in HbA1c (%).
- Deep anemia makes HbA1c even more insecure.
- HbA1c is more reliable after a thorough hematological evaluation.

were considered prediabetes. Anemia was defined as a hemoglobin concentration of 12 g/dL in women and 13 g/dL in men [17]. Iron and vitamin B12 deficiencies were defined as plasma ferritin and cobalamin concentrations of 15 ng/mL [17] and 150 pmole/L [18], respectively. Microcytosis was expressed as a mean corpuscular volume (MCV) of <80 fL and macrocytosis was expressed as MCV >100 fL. Inclusion and exclusion criteria of the study were as follows.

Patients with prediabetes according to HbA1c level (5.7–6.4%) were included in our study [1]. For the patient group, Hgb <female: 12 g/dl, male: <13 g/dl, MCV >100 (fL), vitamin B12 <150 pmol/L, for the control group, patients with Hgb female >12 g/dl, male >13 g/dl, MCV <100 (fL), and vitamin B12 >150 pmol/L were included in the study. Patients who were previously diagnosed with diabetes or were using a drug known to affect blood sugar levels (oral anti-diabetic, orlistat, GLP-1 analog, etc.) were excluded from the study. Patients in the patient group were given 1 mg/day of vitamin B12 (cyanocobalamin) orally for 3 months in accordance with the guidelines [19]. Patients in the control group of the study did not receive any treatment for 3 months. Hgb, MCV, fasting plasma glucose (FPG), HbA1c, vitamin B12, and body mass index values of the patients were measured at the beginning and at the end of the 3rd month and compared.

Biochemical Analysis

Blood samples were taken at the beginning of the study and at the end of the 3rd month, following overnight fasting of at least 10 h, in the morning. A Sysmex automated hematology analyzer was used for the whole blood counts. HbA1c levels were determined by high-performance liquid chromatography with a TOSOH. Serum vitamin B12 level was studied with electrochemiluminescence immunoassay method (Beckman Coulter UniCel D×1 600).

TABLE 1. Demographic parameters

Characteristic	Study group (n=37)	Control group (n=40)	p
Mean age, years	44.84±4.34	43.03±4.58	0.08
Sex (%)			0.963
Male	27	27.5	
Female	73	72.5	

Statistical Analysis

Statistical analyses were performed with SPSS version 23.0 (IBM Corporation, Armonk, NY, USA). Continuous variables were expressed as mean±standard deviation and categorical variables were presented as frequency and percentage. The normal distribution was evaluated with Shapiro–Wilk’s test. The Mann–Whitney U or independent samples t-test was used for the analysis of continuous variables, depending on whether the parameters showed normal distribution or not. Paired t-test was used for comparisons of pre- and post-treatment values. Pearson and Spearman rho tests were used for correlation analysis. $P < 0.05$ within a confidence interval of 95% was considered statistically significant.

Ethics Committee Approval

Our study was approved by the university ethics committee (date: 09.02.2021, number: 2021/02). This study was conducted at the Kutahya Health Sciences University Hospital and written consent was obtained from each of the patients.

RESULTS

Demographic Data

The patient group consisted of 37 patients; 27 of them were female (73%) and ten were male (27.02%). The mean age was 44.84±4.34 years. The control group consisted of 40 patients: 29 female (72.5%) and 11 male (27.5%). Their mean age was 43.03±4.58 years. There was no difference in terms of demographic characteristics ($p=0.08$ $p=0.963$, respectively) (Table 1).

Laboratory Results of the Patient and Control Groups

There was a statistically significant difference in HbA1c, MCV, Vitamin B12, and Hgb values in the patient group between the beginning and end of the 3rd month

TABLE 2. Comparison of the initial and 3rd month data of the patient group

	Initial	3 rd month	p
HbA 1c (%)	6.01±0.20	5.77±0.33	<0.001
FPG (mg/dL)	93.97±3.65	93.92±3.34	0.940
Vitamin B12 (ng/L)	112.43±7.18	408.49±119.61	<0.001
Hgb (g/dL)	11.31±0.28	12.26±0.33	<0.001
MCV (fL)	104.05±4.03	93.24±3.98	<0.001
BMI (kg/m ²)	25.51±2.79	25.53±2.79	0.758

HbA1c: Glycosylated hemoglobin; FPG: Fasting plasma glucose; Hgb: Hemoglobin; MCV: Mean corpuscular volume; BMI: Body mass index.

TABLE 3. Comparison of the initial and 3rd month data of the control group

	Initial	3 rd month	p
HbA 1c (%)	5.62±0.27	5.61±0.25	0.830
FPG (mg/dL)	93.80±4.20	92.80±4.55	0.188
Vitamin B12 (ng/L)	279.10±96.44	276.48±99.01	0.729
Hgb (g/dL)	13.27±0.77	13.17±0.66	0.176
MCV (fL)	84.87±2.87	85.34±2.77	0.375
BMI (kg/m ²)	26.06±1.87	26.07±1.87	0.854

HbA1c: Glycosylated hemoglobin; FPG: Fasting plasma glucose; Hgb: Hemoglobin; MCV: Mean corpuscular volume; BMI: Body mass index.

($p < 0.001$, $p < 0.001$, $p < 0.001$, and $p < 0.001$, respectively). There was no difference in the control group ($p > 0.05$). Details are shown in Tables 2 and 3.

In addition, it was determined that a 0.94 mg/dL increase in Hgb value and a 10.81 fL decrease in MCV value were found to correspond to a 0.24% decrease in HbA1c value at the end of the 3rd month after Vitamin B12 treatment in the patient group (Table 4). Moreover, 13 of 37 patients who were prediabetic after B12 replacement had HbA1c values below 5.7, indicating prediabetes. This shows that approximately 35% of patients are mistakenly diagnosed as prediabetic if HbA1c is used as a diagnostic criterion.

DISCUSSION

Our study demonstrated that the HbA1c level decreased statistically significantly with the treatment of vitamin B12 deficiency anemia in prediabetic patients. It was determined that each increase of 0.94 g/dL in Hgb achieved with vitamin B12 replacement corresponds to a 0.24% HbA1c decrease.

TABLE 4. Comparison of changes in HbA1c and hematological parameters between the patient and control groups

	Patient group, mean [(min–max)]	Control group, mean [(min–max)]	p
ΔHbA1c	-0.24 [(-1.0)–(0.2)]	-0.005 [(-0.3)–(0.3)]	<0.001
ΔHgb (g/dL)	0.94 (0.3–2.0)	-0.1 [(-1.7)–(0.7)]	<0.001
ΔMCV (fL)	-10.81 [(-20.0)–(-2.0)]	0.46 [(-8)–(9)]	<0.001
ΔSerum B12 (ug/dL)	296 (94–520)	-2.62 [(-122)–(116)]	<0.001

HbA1c: Glycosylated hemoglobin; Hgb: Hemoglobin; MCV: Mean corpuscular volume; Δ: Third month value-initial value.

The first study in the literature to evaluate the relationship between B12 deficiency and HbA1c was conducted by Gram-Hansen et al. [15], it was reported that patients with anemia of both iron (n=10) and vitamin B12 (n=10) deficiency had a significant decrease in HbA1c concentrations after treatment. Capoor et al. [20], on the other hand, compared the basal HbA1c levels of 30 patients with IDA, 30 patients with vitamin B12 deficiency anemia, and 30 control group patients and found that patients with IDA and vitamin B12 deficiency had higher HbA1c levels compared to the control group. In a similar study, basal HbA1c concentrations of 60 patients with IDA, 60 patients with vitamin B12 deficiency anemia, and 60 control group patients were compared. Higher HbA1c was reported in the vitamin B12 deficiency anemia and IDA groups [14].

In a recent study, 100 IDA patients and 100 patients with vitamin B12 deficiency anemia were compared with 100 healthy controls before and after 3-month treatment, and similarly, it was found that HbA1c decreased significantly after vitamin B12 deficiency anemia and IDA treatment [13].

Although the methods of other studies in the literature and, in our study, are different, their share similar objectives. The aim is the evaluate suspicion and the possibility of error against the use of HbA1c level in the diagnosis and follow-up of diabetes in patients with nutritional anemia. The guidelines state that diabetic patients should consider conditions that may affect RBC survival before making any treatment decision based on HbA1c level [21]. Evaluation of HbA1c after a detailed hematological examination will contribute to the accurate diagnosis and follow-up of diabetes [22]. Gestational diabetes mellitus (GDM) is one of the most widely encountered metabolic disturbances [23]. HbA1c levels obtained following treatment of vitamin

B12 deficiency anemia in a pregnant woman would allow for the correct diagnosis of GDM-pre-GDM.

Although it is known that the treatment of vitamin B12 deficiency anemia causes an increase in Hgb level and a decrease in HbA1c level, it is not clear how much an increase in Hgb level causes a decrease in HbA1c. No study has examined this subject in patients with vitamin B12 deficiency anemia. In a single study conducted with patients with IDA, one of the nutritional anemias, it was reported that an increase of 2.2 mg/dL in Hgb leads to a 0.4% decrease in the HbA1c level [16]. As shown in this study, HbA1c level significantly decreases in vitamin B12 deficiency anemia, just like in IDA, after vitamin B12 replacement and a 0.94 mg/dL increase in Hgb level corresponds to a 0.24% decrease in HbA1c level, possibly indicating that the more severe the anemia, the more HbA1c loses its reliability in the diagnosis and follow-up of DM.

In our study, HbA1c levels of 13 patients out of 37 patients who were considered prediabetic according to the HbA1c criteria were measured to be lower than the prediabetic limits after the recovery of B12 deficiency and anemia. This indicates that approximately 35% of prediabetic patients with vitamin B12 deficiency anemia are misdiagnosed as prediabetes. Although these results we obtained are very valuable, the number of patients included in our study was low; thus, multicenter studies with larger samples should be conducted. Furthermore, we think that our study is valuable in determining how much an increase in Hgb causes how much a decrease in HbA1c, as it can suggest a different ratio that can be included in the guidelines for DM diagnosis and follow-up.

It was suggested that vitamin B12 deficiency anemia causes false high HbA1c levels secondary to increased survival time of RBC [9, 11]. Although there was no parameter to show this in our study, we think

that HbA1c decreased due to the normalization of the survival time of RBC secondary to the treatment of vitamin B12 anemia. Nonetheless, in a study, it was determined that high HbA1c was associated with higher MCV [24]. In line with this work, it can be suggested that the increase in RBC volume may contribute to false increases in HbA1c in vitamin B12 deficiency anemia since increased MCV in vitamin B12 deficiency anemia expands the erythrocyte surface area and interacts with plasma glucose compared to normal erythrocytes. Our study is original also in this aspect.

Conclusion

In this study, which was conducted with patients with vitamin B12 deficiency anemia, it was shown that HbA1c levels significantly decrease with B12 replacement therapy. Elimination of vitamin B12 deficiency anemia before making a diagnosis or treatment decision based on HbA1c level may prevent patients from being misdiagnosed with DM and from treatment changes in diabetic patients whose blood glucose is not regulated. Administering additional treatment to diabetic patients with vitamin B12 deficiency according to the elevation of HbA1c will not only increase the risk of developing hypoglycemia, but will also bring additional drug costs to the countries.

Ethics Committee Approval: The Kutahya Health Sciences University Non-Interventional Clinical Research Ethics Committee granted approval for this study (date: 09.02.2021, number: 2021/02-16).

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