MAK HILL Publications Research Journal of Medical Sciences

Research Article
doi: 10.36478/makrjms.2024.9.628.632

Assessment of Vitamin D and Glycated Hemoglobin in Non-Diabetic Anemic Patients: A Clinical Study

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OPEN ACCESS

Key Words
Anemia, non-diabetic, vitamin D, HbA1c

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Received: 10 August 2024
Accepted: 11 September 2024
Published: 16 September 2024

Citation: Karan Chaudhari, Hemali Jha, Milav Bhavsar and Shinky Mehta, 2024. Assessment of Vitamin D and Glycated Hemoglobin in Non-Diabetic Anemic Patients: A Clinical Study. Res. J. Med. Sci., 18: 628-632, doi: 10.36478/makrjms. 2024.9.628.632

ABSTRACT
Research has shown elevated HbA1c levels in anemic individuals, even though the lifespan of their erythrocytes is reduced. Lower Vitamin D levels have been suggested as an independent risk factor for type 2 diabetes mellitus (T2DM), often associated with elevated HbA1c. This study aims to evaluate and compare the levels of HbA1c, hemoglobin (Hb) and Vitamin D in non-diabetic anemic individuals with those in healthy, age-and sex-matched control subjects. A case-control study was designed, including 78 patients with anemia as cases and 80 healthy individuals as controls. Patients with diabetes, hemolytic anemia, parasitic infections, chronic alcohol consumption, or kidney disease were excluded. Routine blood tests, including fasting and postprandial glucose levels, along with HbA1c, Hb and Vitamin D concentrations, were measured in all participants. Vitamin D was assessed via ELISA, while fasting and postprandial glucose were measured using the GOD-POD method. Hemoglobin concentration was determined by Drabkin's method and HbA1c was measured using immunoturbidimetry. In patients with iron deficiency anemia (IDA), the average HbA1c level was significantly higher than the control group. There were no significant differences in fasting and postprandial blood sugar levels between the groups. A negative correlation was found between Vitamin D and HbA1c in both male and female subjects and between HbA1c and hemoglobin levels. A positive correlation was also observed between Vitamin D and hemoglobin concentrations. HbA1c is influenced by factors beyond blood glucose levels, particularly iron deficiency. It is important to rule out iron deficiency anemia before making clinical decisions based on HbA1c values.

INTRODUCTION

Hemoglobin A1c (HbA1c) is a glycated form of hemoglobin that results from a non-enzymatic reaction between glucose and the N-terminal valine of both β-chains of the hemoglobin (Hb) molecule. It represents the predominant type of glycated hemoglobin[1]. Glycation of Hb intensifies when blood glucose levels remain elevated over time. Given that red blood cells have a lifespan of approximately 120 days, HbA1c reflects average blood glucose levels over the preceding 2-3 months[2].

In patients with diabetes mellitus (DM), HbA1c levels are typically elevated and are positively associated with metabolic control[3]. According to the American Diabetes Association, the recommended target for HbA1c is below 7% in diabetic individuals[4]. HbA1c values exceeding 7% are indicative of an increased risk of developing microvascular complications. Nevertheless, various conditions beyond diabetes, such as hemolytic anemia[5], hemoglobinopathies[6], acute and chronic hemorrhage[7], pregnancy[8], elevated uric acid[9] and Vitamin D[10], can influence HbA1c levels.

Vitamin D has been noted for its biological actions, including muscle strengthening[11], cellular differentiation[12], immune system modulation[13], inhibition of renin production[14] and insulin synthesis[15]. Moreover, it has been found to influence bone marrow function[16], with Vitamin D concentrations being higher in bone marrow than in plasma[17]. Despite these intriguing findings, there is a scarcity of clinical research investigating whether Vitamin D sufficiency impacts blood hemoglobin levels. Mitchell[17] and Sluiter[18] and identified a connection between iron deficiency anemia (IDA) and HbA1c levels, proposing that changes in HbA1c in IDA may result from alterations in both the structure of hemoglobin and the proportion of HbA1c in young versus older red blood cells. However, studies exploring Vitamin D status in anemic adults, particularly in the Indian population, are limited. Data from the National Health and Nutrition Examination Survey (USA) revealed that children with Vitamin D levels below 30 ng/mL had nearly double the risk of anemia compared to those with normal Vitamin D levels[19].

Globally, iron deficiency anemia is recognized by the World Health Organization as the most prevalent deficiency disorder[19]. One of the adverse effects of IDA is the abnormal glycation of proteins[20], a process that affects protein structure and function. These changes lead to pathological consequences that depend on the protein's function and concentration in various tissues[21]. The glycation of proteins is influenced by both glucose levels and protein half-life[22]. Research has demonstrated that iron supplementation significantly reduces HbA1c levels[23]. Therefore, this study was undertaken to investigate whether Vitamin D plays a role in elevating HbA1c levels in non-diabetic anemic patients and to assess whether HbA1c levels are indeed elevated in this population.

MATERIALS AND METHODS

A case-control study was conducted involving 78 patients diagnosed with anemia as cases and 80 healthy individuals as controls. Blood samples were collected from anemic patients aged between 45 and 50 years, consisting of 19 males and 31 females, as well as from age-matched healthy participants. The cases were selected from patients attending the outpatient department. Anemic individuals were identified based on hemoglobin levels (Hb <11g/dL), serum ferritin concentrations of <9 ng/mL for females and 15 ng/mL for males and a peripheral blood smear showing a microcytic hypochromic pattern. Patients with diabetes, hemolytic anemia, parasitic infections, chronic alcohol consumption, or kidney disease were excluded from the study.

Blood samples were drawn after an overnight fast. An automated hematology analyzer was employed to measure complete blood counts, including hemoglobin, hematocrit, mean corpuscular volume, and mean corpuscular hemoglobin. Serum ferritin levels were analyzed using a kit with a semi-automated analyzer and peripheral blood smears were reviewed for all participants. HbA1c levels were assessed using immunoturbidimetry.

Data were analyzed using MS Excel 2016 and EpiInfo software for windows. Results were presented as mean±standard deviation (SD). To assess the statistical significance of the differences between groups, the Student's t-test was employed. Pearson's correlation coefficient was used to evaluate correlations. A p-value of 0.05 was set as the threshold for minimal statistical significance.

RESULTS AND DISCUSSIONS

Both fasting and postprandial blood glucose levels indicated non-diabetic status. Serum ferritin levels, reflecting iron deficiency, were reduced in individuals with iron deficiency (3.5±1.7 ng/ml), which corresponded with a hypochromic microcytic appearance in peripheral blood smears. HbA1c levels were notably elevated in comparison to controls, with the mean HbA1c in the IDA group being 7.6±0.5%, significantly higher than the control group's 5.1±0.7% ($P<0.001$). No significant differences were observed in fasting or postprandial glucose levels between the IDA and control groups. In the control group, Hb, serum ferritin, fasting and postprandial glucose and HbA1c levels were all within normal ranges (Table 1). A positive correlation was observed between Hb and

Vitamin D, whereas negative correlations were found between Vitamin D and HbA1c and between HbA1c and Hb (Table 2).

Table 1:Comparison of Lab Parameters in Study Groups

Parameters	IDA Group (n=78)	Control Group (n=80)	p-value
Hemoglobin, g/dl	9.1 ± 1.3	14.7 ± 0.82	<0.01
MCV, fl	68.7 ± 3.8	79.2 ± 3.4	<0.01
MCH, pg	19.6 ± 2.0	31.2 ± 1.5	<0.01
Hematocrit, %	30.6 ± 1.9	39.2 ± 2.5	<0.01
Ferritin, ng/ml	3.5 ± 1.7	24.4 ± 5.7	<0.01
HbA1c, %	7.6 ± 0.5	5.1 ± 0.7	<0.01
FBS, mg/dl	94.5 ± 8.9	90.5 ± 9.1	<0.01
PP2BS, mg/dl	106.0 ± 6.0	102.0 ± 5.4	<0.01

Table 2: Gender Wise Correlation Between Various Parameters

Parameters	Males (n=88)	Females (n=70)
Hb and Vitamin D	r = 0.6, p = <0.01	r = 0.4, p = <0.01
Vitamin D and HbA1c	r = -0.3, p = <0.01	r = -0.4, p = <0.01
HbA1c and Hb	r = -0.5, p = <0.01	r = -0.5, p = <0.01

The current study demonstrated that IDA was linked with elevated HbA1c levels. Brooks *et al.* found that HbA1c concentrations were higher in non-diabetic adults with iron deficiency, but these levels normalized following iron supplementation[24]. Conversely, Hansen *et al.* reported that HbA1c levels were normal in iron deficiency but decreased below normal after iron supplementation[25]. Rai *et al.* explored the changes in the hemoglobin (Hb) molecule, suggesting that the glycation of the β-globin chains occurs more readily in IDA[26]. The elevated HbA1c levels observed in non-diabetic anemic patients were primarily attributed to the reduction in hemoglobin levels[27]. Given the common occurrence of IDA, it can influence HbA1c levels when measured by immunoturbidimetry. Therefore, it is crucial to correct IDA before making any diagnostic or therapeutic decisions based on HbA1c values. HbA1c is a widely accepted marker for assessing long-term blood glucose control in diabetic patients and has been shown to predict the risk of developing various chronic complications associated with diabetes[28,29].

This study found a significant inverse relationship between HbA1c and serum Vitamin D levels in both males and females. Vitamin D levels were lower in cases of IDA and there was a positive correlation between Vitamin D and hemoglobin (Hb) that was statistically significant. These findings underscore the potential benefit of daily Vitamin D supplementation in reducing diabetes risk, as previously suggested by Knekt[30]. Additionally, this study confirms an elevated risk of anemia in individuals with low Vitamin D levels compared to those with normal levels. A cross-sectional study conducted in the USA showed that children with Vitamin D levels below 30 ng/mL were nearly twice as likely to have anemia as those with normal levels[31]. Vitamin D plays a role in modulating cytokine production, which has anti-inflammatory effects, particularly relevant in anemia of chronic disease. In vivo and in vitro studies have shown that calcitriol (1,25-dihydroxyvitamin D [25(OH)]) reduces cytokine production[18].

Another potential mechanism is that Vitamin D may directly stimulate erythroid precursors. Vitamin D receptors have been identified in various non-renal tissues, including bone marrow[32]. Ensuring adequate tissue levels of Vitamin D may provide a sufficient substrate for local production of 25(OH)D in hematopoietic tissues through the extrarenal activity of the 1alpha-hydroxylase enzyme. Hematons have been shown to contain higher concentrations of Vitamin D compared to bone marrow[33].

Alemzadeh *et al.* discovered a significant correlation between 25(OH)D and HbA1c levels in Caucasians but not in African Americans[33,34]. This study concluded that the association between Vitamin D status and HbA1c may be age-dependent. Vitamin D has been recognized to play a role in insulin production, secretion and resistance. Recent studies have indicated that adequate Vitamin D levels are essential for the proper functioning of various organs and tissues. Vitamin D receptors have been identified in cardiac and smooth muscle, pancreatic beta cells and immune cells. It has been demonstrated that pancreatic beta cells and immune-regulating cells possess the enzyme required for Vitamin D synthesis, the vitamin receptor, and calcium-binding proteins dependent on Vitamin D, further highlighting its role in insulin secretion.

The study had a small sample size and potential selection bias, as patients were not randomly recruited in this hospital-based study. HbA1c was measured using immunoturbidimetry and the concentration of HbA1c has been found to increase in anemic patients, despite the reduced lifespan of red blood cells. It has been hypothesized that the quaternary structure of hemoglobin may also be altered, affecting the accuracy of these measurements. Other methods, such as colorimetric methods, ion-exchange chromatography, and affinity chromatography, could provide alternative ways to measure HbA1c levels[26]. Further studies involving larger sample sizes are needed to elucidate the relationship between Vitamin D and the mechanisms of anemia. Randomized clinical intervention trials are essential to confirm the role of Vitamin D in reducing diabetes risk.

CONCLUSION

Iron deficiency anemia (IDA) is associated with elevated HbA1c levels, which may lead to a misdiagnosis of uncontrolled diabetes mellitus (DM) in anemic patients. It is essential to consider Vitamin D status when interpreting HbA1c levels in diabetic individuals. Iron supplementation is particularly crucial in diabetic patients with iron deficiency, as it can improve the accuracy and reliability of HbA1c measurements.

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