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Iron deficiency anemia and glycated hemoglobin among adolescents: A case-control study

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Abstract. Glycated hemoglobin (HbA1c) is a hemoglobin fraction used to diagnose diabetes mellitus and its related complications. Previous studies have shown inconsistent results regarding the association between HbA1c and iron deficiency anemia (IDA). Thus, the present study aimed to provide insight into this association. For this purpose, a school-based case-control study was conducted from September to December, 2022. The present study included both adolescents with IDA (the study population) and healthy adolescents without anemia (the control group). Hemoglobin, HbA1c and serum ferritin levels were measured using standard methods. A total of 47 cases (adolescents with IDA) and 94 controls (adolescents without IDA) were enrolled in the present study. The two groups exhibited no significant differences in age, body mass index (BMI), or blood glucose levels. In total, 27 of the cases (57.4%) and 48 of the controls (51.1%) were female (P=0.591). The median (interquartile range) of the HbA1c was 5.4% (5.3=5.7%) in the cases and 5.5% (5.1=5.8%) in the controls (P=0.890). In multivariate linear analysis, age, sex, BMI and iron deficiency (coefficient = 0.008; 95% CI, -0.16-0.17; P=0.929) were not associated with HbA1c. On the whole, the present study found no difference in HbA1c in adolescents with and without IDA.

Introduction

Anemia is a global health issue; in 2019, the World Health Organization (WHO) estimated that 29.9% of all women of reproductive age, 39.9% of all children and 60.2% of all African children are anemic (1). Anemia can lead to several adverse health effects that have a negative impact on cognitive functioning in children and on work capacity in adults (1,2). Iron deficiency anemia (IDA) comprises 66.2% of all types of anemia, rendering it a crucial health issue, particularly for women of reproductive age and children (3,4). Several factors for anemia have been reported, including dietary causes, hemoglobinopathies, malaria and other infectious diseases (3).

Glycated hemoglobin (HbA1c) is a hemoglobin fraction that is used as a diagnostic tool for diabetes mellitus and its related complications (5). Previous studies have shown inconsistent results regarding the association between HbA1c, iron depletion and IDA (6-11). While some studies have reported a low level of HbA1c in IDA (12,13), others have found that anemia/IDA is associated with high HbA1c levels (6-11).

In Sudan, there is a high prevalence of anemia among various age groups, including children (49.4%) (14), adults (25.2%) (15) and pregnant women (37.0%) (16). There is also a high prevalence of diabetes mellitus in Sudan; 20% of adults have diabetes mellitus and 80.0% of these adults have uncontrolled diabetes mellitus (17). The high prevalence of anemia may underestimate the diagnosis of diabetes mellitus. It is crucial to assess whether HbA1c levels are influenced by IDA and whether this can lead to the revaluation of the cut-off for HbA1c in diagnosing diabetes mellitus, as well as the level of control of diabetes mellitus. Such results would prove helpful for clinicians who provide patient care, health planners and academicians. The present study was conducted in an aim to compare the HbA1c levels in adolescents with IDA to those in their peers without IDA.

Patients and methods

Study population. A school-based case-control study was conducted in Northern Sudan from September to December, 2022. The number of adolescents from each school was determined by the total number of adolescents in that school, following a probability proportional to size. Thus, schools with more students greatly contributed to the study sample. The assigned sample size was selected from the list of students in each school through the use of a simple random technique (lottery method). The present study was carried out in accordance with the Declaration of Helsinki and good clinical practices. The study obtained ethics approval from the Ethical Board of the Faculty of Medicine, University of Khartoum, Khartoum, Sudan, under reference no. 9, 2021. All included students and their guardians agreed to participate and

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signed a written informed consent form. The author followed all measures to ensure the participants' privacy, safety and confidentiality, such as excluding personal identifiers during data collection.

Inclusion and exclusion criteria. The study population comprised of adolescents (aged 10-19 years) with IDA and no other diseases; the controls were adolescents with no such anemia (and otherwise healthy, as described below). On the other hand, students whose guardians or parents did not provide their consent to participate in the study and those who were ill, pregnant, or lactating were excluded from the study.

Study variables and measures. The questionnaire included data on various sociodemographic characteristics, such as age in years, sex (male or female), parental educational levels (less than secondary or greater than or equal to secondary), the occupational status of the mother (housewife or employed), the occupational status of the father (laborer or skilled worker), anthropometric measurements, including weight and height [expressed as body mass index (BMI) z-score] and hematological parameters, including serum ferritin. The investigator trained five medical research assistants to collect the data; all trainings were complete prior to the commencement of the study. The training emphasized proper measurement techniques for weight and height, effective utilization of the study questionnaire, and adherence to the study protocol.

The medical research assistants (three males and two females) approached the selected adolescents after the participants and their guardians had agreed to participate and signed an informed consent form. The selected adolescents were informed about the aims of the study and provided with all necessary information, including their voluntary participation in the study and their right to withdraw at any time without needing to give a reason. The study also took preventive measures to ensure the privacy and confidentiality of the participants, such as excluding personal identifiers during data collection, further reinforcing the study's ethical integrity.

Weight, height, anemia and serum ferritin levels were measured using standard procedures, as detailed below (sociodemographic data, BMI and hematological parameters).

Weight and height measurements. The weights of the adolescents were measured in kg using standard procedures (i.e., well-calibrated scales adjusted to zero prior to each measurement). Weight was measured to the nearest 100 g. Shoes and excess clothing were removed before the adolescents stood with minimal movement, with their hands by their sides. Height was measured to the nearest 0.1 cm, with the adolescents standing straight with their backs against the wall and their feet together. The BMI for the age z-score was determined based on the WHO standards (18).

Blood sample processing. To analyze hemoglobin and serum ferritin levels, 5 ml blood were obtained from each student and transferred into an EDTA tube under aseptic conditions. These samples were used as part of a complete blood count. An automated hematology analyzer (Sysmex KX-21, Sysmex Corporation) was used to measure the hemoglobin levels (19). Based on the recommendation of the WHO for adolescents, a hemoglobin concentration <12 g/dl in females and 13 g/dl in males was considered a sign of anemia (20). Furthermore, the hemoglobin values were categorized as mild (>10 g/dl), moderate (7-9.9 g/dl) and severe (<7 g/dl) (21) anemia.

The remaining blood samples (3 ml) were centrifuged (at 3,000 x g, for 10 min at room temperature) and the serum was stored at -20˚C in the laboratory. Serum ferritin levels were subsequently measured using a radioimmunoassay gamma counter (Riostad, Berthold Technologies GmbH & Co. KG) and kits provided by Beijing Isotope Nuclear Electronic Co. (22). HbA1c levels were measured in all participants, including those who were later diagnosed with mild, moderate, or severe anemia, using enzymatic methods on the ARCHITECT clinical chemistry analyzer (Abbott Pharmaceutical Co. Ltd.).

In accordance with previous studies (23,24), a serum ferritin level ≥15 μg/ was considered normal, while a level <15 μg/l was considered low (iron deficiency). In the present study, IDA was defined as the presence of anemia as per the definition of the WHO (a hemoglobin level <12 g/dl and serum ferritin level <15 μg/l). To ensure accurate and consistent results, both the automated hematology analyzer and the radioimmunoassay gamma counter were calibrated on a monthly basis. Additionally, quality control measures were taken prior to daily operation of these instruments.

Sample size calculation. OpenEpi Menu software was used to compute the desired sample size (25). The present study required a sample of 47 cases and 94 controls (a ratio of 1:2). The mean (standard deviation) of HbA1c was set at 5.5% (0.3) for the cases and 5.7% (0.4) for the controls. This sample size would have a confidence interval (CI) of 95% (1.96) and a margin of error of 5% (0.05).

Statistical analysis. The data were entered into IBM Statistical Product and Service Solutions (SPSS) for Windows (Version 22.0; IBM Corp.) for analysis. Continuous data, such as age, BMI, and hemoglobin and serum ferritin levels, were evaluated for normality using the Kolmogorov-Smirnov test. They were found to be non-normally distributed; therefore, they were expressed as a median [interquartile range (IQR)]. Additionally, the Mann-Whitney U test was used to compare the continuous variables between adolescents with IDA and adolescents without IDA. The Chi-squared test was sued to compare proportions between two groups). Univariate analysis was conducted initially, and all variables were then shifted for multivariate logistic regression to adjust for covariates. Adjusted multivariate analysis was performed on anemia and iron deficiency as categorical variables, as dependent variables, and independently, as well as on sociodemographic characteristics (age, sex, BMI z-score as independent variables). Adjusted odds ratios (AORs) and 95% CIs were calculated as they were applied. A two-sided P-value <0.05 was considered to indicate a statistically significant difference.

Results

General characteristics of the study subjects. A total of 141 adolescents were enrolled in the present study. Of these, 75 (53.2%) were females, and 66 (46.8%) were males. The median (IQR) of the adolescents' age was 14.1 (12.3-16.1) years (Table I).

Table I. Comparison of the age, body mass index, HbA1c levels, parents' education and occupation of adolescents with and without iron deficiency anemia in Sudan in 2022.

Variables	Total adolescents (n=141)	Adolescents with IDA (n=47)	Adolescents without IDA (n=94)	P-value
Age, years; median (interquartile range)[a]	14.1 (12.3-16.1)	14.1 (12.4-16.2)	14.3 (12.2-16.1)	0.832
Body mass index, kg/m^2; median (interquartile range)[a]	17.0 (15.3-19.8)	16.9 (15.0-19.8)	17.1 (15.4-19.9)	0.875
Blood glucose, mg/dl^2; median (interquartile range)[a]	80.1 (71.5-90.0)	83.0 (75.0-95.0	80.0 (70.0-88.0)	0.174
HbA1c, %; median (interquartile range)[a]	5.5 (5.2-5.7)	5.4 (5.3-5.7)	5.5 (5.1-5.8)	0.890
Serum ferritin, μg/l; median (interquartile range)[a]	18.3 (10.2-37.4))	10.0 (10.0-11.6)	29.1 (18.1-53.7))	<0.001
Sex, n (%)[b]				
Male	66 (46.8)	20 (42.6)	46 (48.9)	0.591
Female	75 (53.1)	27 (57.4)	48 (51.1)	
Mother's education, n (%)[b]				
> Secondary level	97 (68.8)	32 (68.1)	65 (69.1)	0.522
≤ Secondary level	44 (31.2)	15 (31.9)	29 (30.9)	
Mother's occupation, n (%)[b]				
Housewife	119 (84.8)	39 (83.0	80 (85.1)	0.460
Employed	22 (15.6)	8 (17.0)	14 (14.9)	
Father's education, n (%)[b]				
> Secondary level	102 (72.3)	34 (72.3)	68 (72.3)	0.999
≤ Secondary level	39 (27.7)	13 (27.7)	26 (27.7	
Father's occupation, n (%)[b]				
Skilled	49 (34.8)	16 (34.0)	33 (35.1)	0.999
Labourer	92 (65.2)	31 (66.0)	61 (64.9)	

[a]Data were analyzed using the Mann-Whitney U test; [b]data were analyzed using the Chi-squared test. HbA1c, glycated hemolgobin.

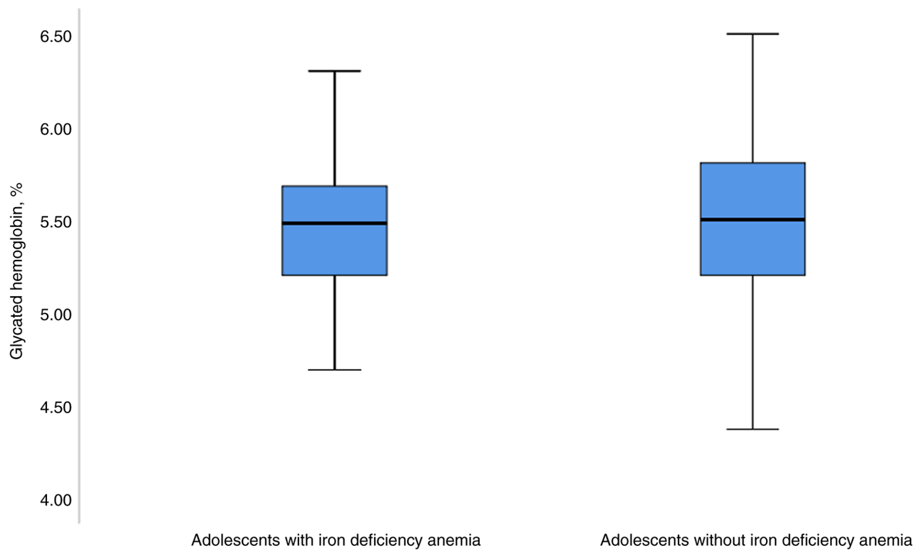

Figure 1. Glycated hemoglobin levels in adolescents with iron deficiency anemia and those without this condition.

In total, 47 cases (adolescents with IDA) and 94 controls (adolescents without IDA) were enrolled in the present study. The two groups exhibited no significant differences in age, BMI, or blood glucose levels, using Mann-Whitney U test (Table I). Of note, 27 of the cases (57.4%) and 48 of the controls (51.1%) were females (P=0.591).

Table II. Univariate and multivariate linear regression analysis of the factors associated with HbA1c in adolescents in Sudan, 2022.

	Univariate linear analysis			Multivariate linear analysis		
Variables	Coefficient	95% confidence interval	P-value	Coefficient	95% confidence interval	P-value
Age, years	-0.007	-0.038-0.024	0.658	-0.007	-0.04-0.03	0.717
Body mass index, kg/m^2	-0.002	-0.022-0.017	0.826	1.11	-0.023-0.023	0.999
Sex						
Male	Reference					
Female	-0.014	-0.170-0.143	0.865	-0.004	-0.17-0.16	0.958
Iron deficiency anemia						
No	Reference					
Yes	0.008	-0.1570-0.174	0.921	0.008	-0.16-0.17	0.929

Factors associated with HbA1c levels. The median (IQR) of HbA1c was 5.4% (5.3-5.7%) in the cases and 5.5% (5.1-5.8%) in the controls; the Mann-Whitney U test did not reveal any significant differences between the two groups (P=0.890; Fig. 1 and Table I). In the univariate linear analysis, age, sex, BMI and iron deficiency (IDA: coefficient, 0.008; 95% CI, -0.1570-0.174; P=0.921) were not found to be associated with HbA1c (Table II). Moreover, in the multivariate linear analysis, age, sex, BMI and iron deficiency (IDA: coefficient, 0.008; 95% CI, -0.16-0.17; P=0.929) were also not found to be associated with HbA1c (Table II).

Discussion

The main findings of the present study were that no difference was found in HbA1c between adolescents with and without IDA, and HbA1c was not associated with IDA. This is consistent with the findings of a recent meta-analysis that included six studies and demonstrated that IDA had no effect on HbA1c in patients with diabetes mellitus (26). However, the results of the meta-analysis revealed that IDA had an effect on HbA1c in non-diabetic individuals (26). By contrast, it has been reported that in 263 patients without diabetes mellitus, HbA1c levels were significantly lower in patients with IDA than in their peers who had no such anemia (6). Moreover, a moderate correlation has been reported between HbA1c and serum iron levels in children with type 1 diabetes (13).

As aforementioned, the results of previous studies on HbA1c levels in iron deficiency have been inconsistent. Building upon earlier results that demonstrated that IDA is associated with high HbA1c (in diabetic patients), a previous study found that a high level of glycated hemoglobin was markedly corrected (decreased) following treatment with iron supplements (6). By contrast, other research has suggested that a low level of HbA1c is associated with IDA (12). Furthermore, a recent systematic review concluded that iron replacement therapy reduced HbA1c in patients with uncontrolled diabetic mellitus who had an iron deficiency (27). However, in another study, the mean HbA1c level was higher in anemic than non-anemic Indian euglycemic patients (7). In addition, HbA1c was significantly increased in iron-deficient patients (non-diabetic) compared to the control group (8).

In a large-scale retrospective study, patients with IDA had higher HbA1c levels than those without anemia (10). A later study confirmed these results and found that patients with nutritional anemia (such as IDA) exhibited a higher mean level of HbA1c, which was then reduced in response to the treatment of anemia (9). Intra *et al* (10) demonstrated that the mean HbA1c was higher in anemic patients than in non-anemic patients.

In Dutch children with diabetes, the mean HbA1c did not differ in patients with an iron deficiency (28). However, an inverse finding was reported among Egyptian children with type 1 diabetes, who had significantly higher levels of HbA1c than non-anemic diabetic children (11). In addition, serum iron has been negatively correlated with glycated hemoglobin in children and adolescents with type 1 diabetes mellitus (13). The diverse sociodemographic characteristics of the enrolled participants and the varied prevalence of anemia and severe anemia in different areas may explain these variable results. Moreover, some of these studies assessed HbA1c and iron deficiency in diabetic patients, while others investigated these variables among healthy cohorts.

Although the exact mechanism by which HbA1c increases in association with IDA remains unclear; it is considered that the reduction in iron and ferritin levels affects the rate of red blood cell turnover. This leads to a prolonged half-life of the red blood cells eligible for destruction, thereby increasing the rate of hemoglobin glycation (29). Additionally, hemoglobin with a lower iron content is more vulnerable to oxidative stress, resulting in elevated malondialdehyde levels, which further accelerate hemoglobin glycation (30).

From a clinical perspective, in cases of IDA, it is essential to correct hemoglobin and iron levels before interpreting HbA1c, as falsely elevated results are likely. Furthermore, it is advisable to measure hemoglobin levels whenever elevated HbA1c is detected to rule out potential false-positive results.

The present study has certain limitations that warrant consideration. Potential confounding factors that could influence HbA1c levels, such as recent dietary habits, physical activity and a family history of diabetes, were not examined. As a result, further research is needed to address these variables and provide a more comprehensive understanding of the relationship between IDA and HbA1c levels. In conclusion, the present study did not find any notable differences in HbA1c levels between adolescents with IDA and those without IDA.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Author's contributions

The author AM was involved in the conceptualization of the study, in the design of the study methodology, in data curation and formal analysis, as well as in the investigation and procedural aspects of the study. The author AM was also drafted and reviewed the manuscript. AM confirms the authenticity of all the raw data. The author has read and approved the final manuscript.

Ethics approval and consent to participate

The present study was carried out in accordance with the Declaration of Helsinki and good clinical practices. The study obtained ethics approval from the ethical board of the Faculty of Medicine, University of Khartoum, Khartoum, Sudan, under reference no. 9, 2021. All included students and their guardians agreed to participate and signed a written informed consent form. The author followed all measures to ensure the participants' privacy, safety, and confidentiality, such as excluding personal identifiers during data collection.

Patient consent for publication

Not applicable.

Competing interests

The author declares that he has no competing interests.

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