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Evaluation of the salivary level of glucosyltransferase-B in relation to sera levels of iron, ferritin, hepcidin, and vitamin D in patients with beta-thalassemia major

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Abstract

Introduction: Beta-thalassemia major is a severe genetic blood disorder characterized by defective hemoglobin production, leading to chronic anemia and frequent blood transfusions, which in turn cause iron overload and related complications.

Objectives: The study explores the association between salivary glucosyltransferase-B (GTF-B) levels and iron metabolism markers, including ferritin, hepcidin, and vitamin D, in patients with beta-thalassemia major.

Patients and Methods: This cross-sectional study included 45 adolescents aged 12-17 years with beta-thalassemia major and 45 healthy controls. Saliva and blood samples were collected from participants. Salivary GTF-B levels were measured using enzyme-linked immunosorbent assay (ELISA), while serum levels of hepcidin, ferritin, and vitamin D were assessed using the Cobas E411 and C111 systems. Statistical analysis was performed using SPSS version 26.

Results: This study found significantly lower levels of salivary GTF-B in beta-thalassemia patients (13.221±3.307 pg/mL) compared to controls (17.714±3.923 pg/mL; $P<0.001$). Serum iron and ferritin were significantly higher in beta-thalassemia patients (221.151±55.472 µg/dL versus 4158.367±542.637 ng/mL) compared to control subjects (73.540±39.257 µg/dL), (25.110±10.933 ng/mL), respectively ($P<0.001$). Serum hepcidin levels were higher in the study group (127.133±40.482 ng/mL) than in controls (114.071±34.698 ng/mL) but without statistical significance ($P=0.420$). Vitamin D levels were significantly lower in beta-thalassemia patients (9.831±4.693 ng/mL) compared to controls (16.404±12.934 ng/mL; $P=0.002$).

Conclusion: Beta-thalassemia major patients exhibit altered levels of salivary GTF-B and serum markers of iron metabolism and vitamin D. The findings suggest potential disruptions in oral health biomarkers and iron regulatory mechanisms in these patients.

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Introduction

Beta-thalassemia is a genetic disorder caused by mutations in the beta-globin gene, leading to impaired production of beta chains in hemoglobin (1). These mutations result in three main classifications: beta-thalassemia minor, intermedia, and major. Beta-thalassemia minor, which is asymptomatic or presents with mild symptoms, arises from a heterozygous mutation (beta-plus thalassemia) (2). On the other hand, beta-thalassemia major, resulting from a homozygous mutation (beta-zero thalassemia), is characterized by the complete absence of beta chains (3). This severe form manifests clinically as jaundice, growth retardation, hepatosplenomegaly, and various endocrine disorders (1). Between these two extremes is beta-thalassemia intermedia, which often presents with mild to moderate clinical symptoms (4).

Typically, symptoms of beta-thalassemia manifest within the first two years of life (5). However, alpha thalassemia major can be detected during pregnancy due to the absence of fetal hemoglobin production (6). Early detection is critical as it allows for the implementation of preventive measures to reduce maternal complications such as toxemia, preterm birth, and the need for cesarean sections. Effective management of thalassemia hinges on prevention, which can be achieved through genetic screenings of parents to identify mutations before conception (5).

Key point

This study investigates the relationship between salivary glucosyltransferase-B (GTF-B) levels and serum markers (iron, ferritin, hepcidin, and vitamin D) in patients with beta-thalassemia major, highlighting significant alterations in oral and systemic biomarkers.

Patients with thalassemia frequently experience symptoms like reduced appetite, jaundice, and enlarged spleen or liver, alongside bone complications (5,7). To manage severe cases, regular blood transfusions are essential to maintain hemoglobin levels around 9 to 10 mg/dL, thereby ensuring overall well-being and controlling erythropoiesis while preventing extramedullary hematopoiesis (8). However, these transfusions lead to iron overload, as the body lacks a mechanism to eliminate excess iron (6). This condition results in a cascade of complications including cardiovascular issues, pulmonary hypertension, bone disease, endocrine disorders, and liver problems. For patients with enlarged spleens, splenectomy might be necessary, although it increases susceptibility to infections (9).

Bone health is a significant concern in thalassemia patients, as chronic anemia can lead to osteoporosis, resulting in fragile bones prone to fractures and significant pain (10). Moreover, individuals may develop facial bone deformities such as maxillary hypertrophy, known as the "chipmunk face," and dental issues like misalignment and spacing of teeth (11). Chronic iron overload is a central issue in thalassemia management due to frequent blood transfusions. Hepcidin, a peptide hormone produced by hepatocytes, plays a crucial role in regulating iron levels in the body (12). It controls the action of ferroportin, the cellular iron exporter, thereby regulating iron absorption from the intestine and iron release from storage. Elevated hepcidin levels, triggered by high plasma iron or stored iron, inhibit dietary iron absorption, exacerbating iron accumulation. Conversely, hepcidin suppression in iron deficiency enhances dietary iron absorption to restore iron reserves. Over time, excessive hepcidin can lead to anemia with restricted iron availability, whereas insufficient hepcidin results in excessive iron buildup in the liver (5).

Vitamin D deficiency is prevalent among beta-thalassemia major patients, exacerbated by iron overload and its effects on intestinal absorption and hepatic hydroxylation. Studies have shown significant rates of vitamin D deficiency in children and adolescents with β-thalassemia, contributing to various bone-related problems (13). This deficiency becomes more pronounced with age, as individuals with β-thalassemia have substantially lower vitamin D levels compared to healthy peers. Adequate vitamin D levels are critical for reducing fracture risk and maintaining bone health in thalassemia patients. A vitamin D level below 20 ng/mL indicates a deficiency of this hormone (14).

In patients with thalassemia, a lack of vitamin D can lead to severe complications such as ventricular hypertrophy, atherosclerosis, heart failure, and rhythm abnormalities (15). Interestingly, a recent study by Pistis et al highlights that, vitamin D has a suppressive effect on hepcidin expression in healthy individuals, suggesting potential therapeutic implications for managing iron overload in thalassemia patients (16).

Saliva plays a crucial role in maintaining oral health and physiology. It acts as a reliable biomarker detection tool for host protective components. Salivary proteins perform various functions including cleaning teeth, preventing abrasion and attrition, delaying demineralization, promoting remineralization, neutralizing acids, and protecting the oral cavity from infection (17).

One critical aspect of oral health is the role of glucosyltransferases (GTFs) in the virulence of *Streptococcus mutans*, a significant contributor to dental caries. These enzymes synthesize glucans on tooth and bacterial surfaces, facilitating the adhesion of streptococci and other organisms (18). *Streptococcus mutans* produces three GTF gene products (19). There are three types of GTF enzymes involved in the synthesis of glucans in saliva; GTF-B, which polymerizes an insoluble glucan rich in 1,3-linked glucose and GTF-D, which synthesizes a soluble glucan primarily consisting of 1,6-linked glucosyl units and also GTF-C, which produces a polymer with both 1,3- and 1,6-linked glucose moieties (20). These glucans are prevalent in saliva and are essential for the development of caries, linking them to caries activity. Furthermore, many diseases originate at mucosal surfaces, which are typically protected by antimicrobial proteins. Secretory IgA, the predominant antibody in upper airway secretions, plays a vital role in host defense against microbial infection. Its presence in saliva indicates the immune state of the oral mucosa and is crucial for blocking viral infections from entering the body through mucosal surfaces (21).

Objectives

To investigate the relationship between salivary GTF-B levels and the serum levels of iron, ferritin, hepcidin, and vitamin D in patients with beta-thalassemia major.

Patients and Methods

Study population

In this cross-sectional study, clinical examinations and laboratory biochemical tests were conducted from November 2023 to June 2024. The study received the necessary approvals from the Ministry of Health and the Ministry of Education. The control group consisted of 45 healthy school students, while the study group comprised 45 adolescents aged 12-17 with beta-thalassemia major recruited from Al-Sadr city, Ibn Al-Baladi hospital.

The inclusion criteria required participants to have a medical diagnosis is of beta-thalassemia major and be between the ages of 12 and 17 years. The study was open to both males and females. To maintain consistency and avoid confounding factors, the exclusion criteria eliminated individuals on medications for other chronic diseases, those unwilling to participate, individuals with adverse habits like tobacco chewing or smoking, and those who had received periodontal therapy within the past six months.

Saliva collection

One to 3 mL of entire, unstimulated saliva were taken from the patients between 8 and 10 AM. The subjects were instructed not to eat or drink for three hours prior to the saliva collection procedure, to wash their mouth with distilled water for one minute, and to relax for five minutes before beginning saliva collection; subjects were then instructed to spit saliva into sterilized cups. Then, saliva was centrifuged at 3500 rpm for 10 minutes, and the supernatant was frozen at -20 °C until the human GTF-B was analyzed using the enzyme immunoassay (ELISA) method.

Blood collection

Each patient provided five milliliters of venous blood, which was collected aseptically. The blood was then transferred to a sterile gel tube, and serum was separated by centrifugation at 3000 rpm for 10 minutes. The separated serum was then split into small aliquots and stored at -20 °C until analyses were conducted. GTF-B and hs-Hepc25 (high sensitivity hepcidin 25) were measured using ELISA, while serum iron was assessed using the Cobas C111 machine. The Cobas E411 system was conducted to measure serum ferritin and vitamin D concentrations.

Statistical analysis

The statistical analysis was conducted using SPSS program version 26 and Microsoft Excel 2019. The normality test revealed parametric data, which were presented as mean ± SD. Statistical tests included the two-sample independent T-test to determine differences between two groups and ANOVA to assess variations among multiple groups. The chi-square test was employed to assess the association between two qualitative variables. Pearson's correlation, a parametric test, was conducted to determine the linear correlation between variables. Statistical significance was set at a level of $P < 0.05$.

Results

The gender distribution in the study and control groups is presented in Figure 1, showing the number and percentage of males and females in each group. In the study group, there are 17 males (37.78%) and 28 females (62.22%), while in the control group, there are 13 males (28.89%) and 32 females (71.11%). The chi-square value is 0.800 with a P value of 0.371, indicating no significant difference in gender distribution between the groups.

The comparison of age (in years) between participants in the study group (adolescents with beta-thalassemia major) and the control group (healthy adolescents) is shown in Table 1. The minimum age in the study group is 12 years, while in the control group, it is 15 years. The maximum age in both groups is 17 years. The mean ± SD value is 14.889 ± 1.511 years for the study group and 16.378 ± 0.716 years for the control group. Although the control group is slightly older than the study group, the difference is not statistically significant ($P = 0.065$).

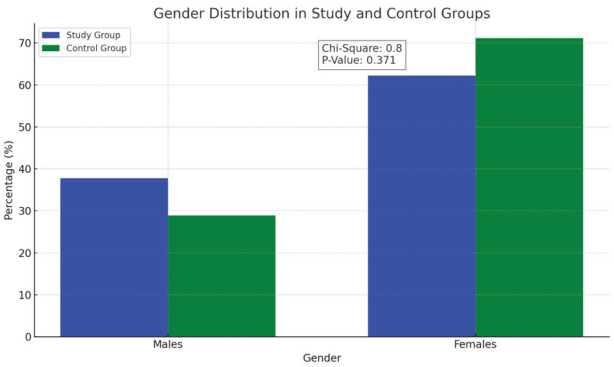

Figure 1. Distribution of gender among groups.

The comparison of salivary GTF-B levels between the study and control groups is illustrated in Table 2 and Figure 2. The mean ± SD of GTF-B levels is significantly lower ($P = 0.001$) in the study group (13.221 ± 3.307 pg/mL) compared to the control group (17.714 ± 3.923 pg/mL).

The comparison of levels of hepcidin-25 in sera between the study and control groups are shown in Table 3 and Figure 2. The mean ± SD value was slightly higher in the study group (127.133 ± 40.482 ng/mL) than in the control group (114.071 ± 34.698 ng/mL), but the statistical analysis showed a non-significant difference between the groups ($P = 0.420$). The mean iron level is significantly higher ($P < 0.001$) in the study group (221.151 ± 55.472 µg/dL) compared to the control group (73.540 ± 39.257 µg/dL). Additionally, the mean value of ferritin level is also significantly higher in the study group (4158.367 ± 542.637 ng/mL) versus the control group (25.110 ± 10.933ng/mL; $P < 0.001$). However, the mean vitamin D level is lower in the study group (9.831 ± 4.693 ng/mL) compared to the control group (16.404 ± 12.934 ng/mL), with a statistically

Table 1. Distribution of age among groups

Groups		Statistics
Study	Control	
14.889	16.378	Mean
1.511	0.716	±SD
12.00-07.00	15.00-17.00	Range
1.915		T-test
0.065		*P* value

Table 2. Descriptive and statistical analysis of salivary GTF-B (pg/mL) among study and control groups

Groups		Statistics
Study	Control	
13.221	17.714	Mean
3.307	3.923	±SD
5.550-20.750	10.440-26.090	Range
5.875		T-test
0.001		*P* value

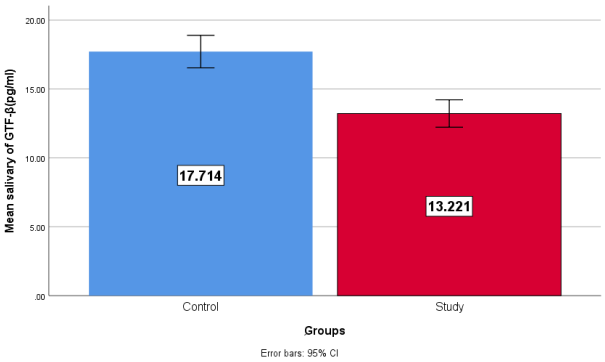

Figure 2. The mean value of GTF-B level among groups.

significant difference ($P=0.002$) as demonstrated in Table 3 and Figure 3.

The findings in Table 4 showed that only serum iron and ferritin in the study group have significant negative correlations with GTF-B ($P=0.044$, $P=0.018$) respectively.

In regards to the other tested markers shown in Table 5, positive significant correlations were found between serum iron and both ferritin and hepcidin ($P<0.001$, $P=0.022$) in the sera of the study group.

Discussion

The main goal of this study was to assess immunological markers, specifically salivary GTF-B, hepcidin-25, and vitamin D levels in relation to iron and ferritin in individuals with beta-thalassemia major compared to healthy controls. The average age of the study group (adolescents with beta-thalassemia major) was 14.889 ± 1.511 years, while the control group (healthy adolescents) had a mean age of 16.378 ± 0.716 years. Although the control group was slightly older, this age difference was not statistically significant ($P=0.065$). Similarly, Abdelmotaleb et al found no significant difference in age between the thalassemia group and the control groups (p = 0.46). In their study, 85 Egyptian children were divided into two groups: 55 children with β-thalassemia major and 30 healthy controls matched for age and gender (22). Both groups had a wide age range, and there was no statistically significant age difference between the study and control groups. The study also examined gender distribution across thalassemia and iron deficiency groups, finding no statistically significant difference between the study population and the control group. The thalassemia intermedia group had an equal distribution of males and females, while other groups had roughly equal distributions. These studies reveal that weight, height, and body mass index may vary considerably between thalassemic patients and healthy controls, while age and gender may not (22). The current study's observation aligns with these findings, as the age difference between the study and control groups was not statistically significant ($P=0.065$).

Our study revealed that the level of GTF-B was significantly lower in the beta-thalassemia group compared to the control group. Beta thalassemia is a hereditary blood disorder characterized by reduced hemoglobin production (23). The decreased levels of GTF-B observed in our study may be linked to the altered physiological conditions present in individuals with beta thalassemia. One possible explanation for this finding is that the chronic anemia and frequent blood transfusions associated with beta

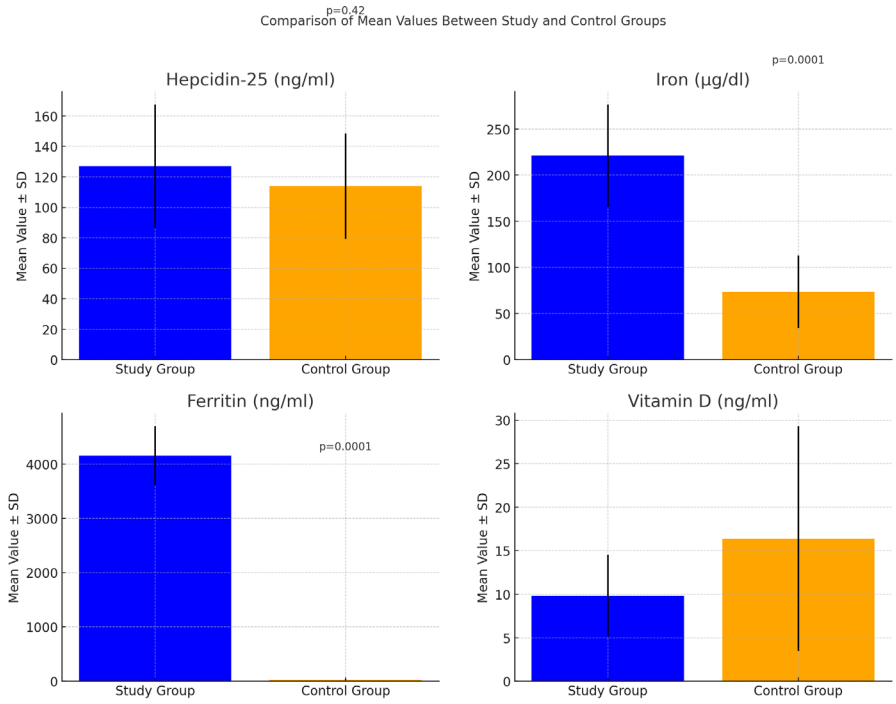

Figure 3. The mean values of tested parameters of groups under study

Table 3. Descriptive and statistical test of tested parameters of all groups

Parameter	Group		T-test	*P* value
	Study	Control		
	Mean ±SD	Mean ±SD		
Hepcidin-25 (ng/mL)	127.133±40.482	114.071±34.698	0.811	0.420
Iron (µg/dL)	221.151±55.472	73.540±39.257	14.571	0.0001
Ferritin (ng/mL)	4158.367±542.637	25.110±10.933	10.904	0.0001
Vitamin D (ng/mL)	9.831±4.693	16.404±12.934	3.205	0.002

thalassemia could impact the overall metabolic and enzymatic activities in the body, including those related to oral health. GTF-B is a pathogenic biomarker that plays a crucial role in the etiology and pathogenesis of caries. It synthesizes insoluble glucans primarily composed of α1,3-linked glucose moieties, which act as binding sites for streptococci and aid in the colonization of tooth surfaces (24). The data from the current study are consistent with the findings of Dwivedi et al, who reported that the buildup of serum non-transferrin-bound iron (NTBI) generates singlet oxygen that reacts rapidly with microbial cell components such as DNA , causing the damage that ultimately leads to reduced GTF-B gene expression (25). Additionally, Chua et al, found that repeated exposure of bacterial cells to oxidative stress is associated with the induction of cyclic guanosine monophosphate signaling, resulting in increased biofilm production (26).

In the current investigation, it was found that hepcidin-25 levels in sera were higher in the study group compared to the control group, although not significantly different. Regular transfusions increase iron loading and block erythropoietic drive, leading to increased hepcidin in β-thalassemia patients (27). Ismail et al in 2019 found that β-thalassemia patients with both thalassemia major and β-Thalassemia intermedia had considerably higher serum hepcidin levels than healthy youngsters. Hepcidin has a complex regulation in β-thalassemia patients, with its production being controlled by opposing effects from erythropoiesis, anemias, and iron overload. Hepcidin is up-regulated by increased body iron levels, infection and inflammation, and down-regulated by factors such as anemia, hypoxia, iron deficiency, ineffective erythropoiesis and increased levels of erythropoietin (28). The study revealed that the study group (beta thalassemia major) had significantly higher serum iron levels ($P<0.001$) (221.151 ± 55.472 µg/dL) compared to the control group (73.540 ± 39.257 µg/dL). This result is consistent with the study by Basu et al, who concluded that improper dietary iron absorption raises body iron burden by 2–5 g per year in non-transfused severe thalassemia patients, while frequent transfusions quadruple iron buildup (29). Beta-thalassemia increases intestinal iron absorption due to inefficient erythropoiesis and hepcidin downregulation, leading to gradual iron overload over time (29). Patients with beta-thalassemia major require ongoing blood transfusions, which also contribute to iron accumulation (29). Excessive iron absorption and transfusion-related iron overload exceed the body's capacity to utilize and store iron, resulting in serum photodynamic antibacterial therapy (30).

The beta-thalassemia major group exhibited significantly higher ferritin levels in their sera compared to the control group, this may be attributed to a genetic

Table 4. Pearson's correlation between biomarkers and GTF-B

Groups		GTF-B (pg/mL)	
		r	*P*
Control	Vitamin D	0.036	0.816
	Iron	-0.062	0.685
	Ferritin	0.006	0.967
	Hepcidin -25	-0.041	0.789
Study	Vitamin D	-0.148	0.331
	Iron	-0.302	0.044
	Ferritin	-0.271	0.072
	Hepcidin-25	-0.353	0.018

Table 5. Pearson's correlation between biomarkers and GTF-B

Groups		Iron		Ferritin		Hepcidin -25	
		r	*P*	*r*	*P*	*r*	*P*
Control	Vitamin D	0.229	0.130	0.158	0.299	-0.127	0.404
	Iron			-0.054	0.725	0.264	0.079
	Ferritin					0.101	0.511
	Hepcidin -25						
Study	Vitamin D	-0.054	0.722	0.044	0.776	-0.113	0.460
	Iron			0.516	<0.001	0.341	0.022
	Ferritin					0.187	0.219
	Hepcidin -25						

r, Pearson's correlation.

Study Highlights

- Patients with beta-thalassemia major exhibit altered levels of salivary GTF-B compared to healthy controls.
- Elevated serum levels of iron, ferritin, and hepcidin were observed in the beta-thalassemia group.
- Serum vitamin D levels were significantly lower in patients with beta-thalassemia major.
- The study emphasizes the impact of systemic conditions like beta-thalassemia on oral health biomarkers.
- Findings suggest potential diagnostic and therapeutic targets for managing oral health in beta-thalassemia patients.

factor such as a mutation or deficiency in the hemoglobin gene, which impairs hemoglobin production and leads to severe anemia and iron buildup in tissues, glands, and organs. This increased iron toxicity can be managed with iron chelation therapy, which is excreted by the kidneys in cases of severe anemia. Regular blood transfusions are necessary to compensate for the lack of red blood cells essential for cellular function, Elevated ferritin levels indicate illness (31). Due to rising iron levels, these finding are consistent with research by Hussein et al, who observed higher ferritin levels in beta-thalassemia patients compared to healthy individuals, with excess iron being eliminated through the kidneys during severe anemia (32).

Iron binds to transferrin, a carrier protein that transports iron to tissues during normal function. In situations of excess iron, transferrin becomes saturated, leading to an excess of free iron. Free iron is highly reactive and generates reactive oxygen species, which can cause cell damage (33). Organ damage is the primary cause of illness and mortality in individuals with β-thalassemia (32). The study group exhibited significantly lower mean vitamin D levels (9.831 ± 4.693 ng/mL) compared to the control group (16.404 ± 12.934 ng/mL) with a statistically significant difference ($P = 0.002$). Vitamin D, acting as a prohormone, plays a crucial role in regulating calcium homeostasis and enhancing intestinal calcium absorption (34).

Findings show that there is no significant correlation between biomarkers, except for iron which has a positive correlation with serum ferritin. Serum iron also shows a weak positive correlation with hepcidin-25. Additionally, serum iron is negatively correlated with GTF-B with statistical significance, and hepcidin-25 is weakly negatively correlated with GTF-B. There are no significant correlations between the biomarkers in the control group. Ferritin is a protein that stores iron in the body. The correlation between serum iron and ferritin suggests a potential link between iron levels and ferritin levels in the body (35). Similarly, the correlation between serum iron and hepcidin-25 indicates a possible connection between iron levels and hepcidin-25 levels in the body (36). Previous studies have not explored the relationship between these biomarkers in the current study. Highlighting the need for further research to fully understand the mechanisms underlying these correlations.

Conclusion

This study emphasizes significant changes in salivary and serum biomarkers in adolescents with beta-thalassemia major, highlighting the intricate relationship between chronic disease and overall health. Specifically, we noticed a decrease levels of salivary GTF-B levels, which could increase the risk of dental problems, and an increased serum ferritin level, indicating iron overload due to regular transfusions. While hepcidin levels were elevated, they did not differ significantly from those of healthy individuals, indicating a complex reaction to iron regulation in thalassemia. Additionally, the significant lack of vitamin D emphasizes the necessity of nutritional support and supplementation.

Limitations of the study

The limitations of this study include a relatively small sample size due to the short time period and difficulties encountered in collecting samples. Additionally, the age range of participants was limited to 12-17 years. The study did not account for nutritional status, which could influence biomarker levels.

Authors' contribution

Conceptualization: Shahad Fayiz Abd.
Data curation: Shahad Fayiz Abd.
Formal analysis: Shahad Fayiz Abd.
Investigation: Shahad Fayiz Abd.
Methodology: Shahad Fayiz Abd.
Project administration: Shahad Fayiz Abd.
Resources: Shahad Fayiz Abd.
Software: Shahad Fayiz Abd.
Supervision: Shahad Fayiz Abd and Maha Adel Mahmood.
Validation: Shahad Fayiz Abd.
Visualization: Shahad Fayiz Abd.
Writing–original draft: Shahad Fayiz Abd.
Writing–review & editing: Shahad Fayiz Abd and Maha Adel Mahmood.

Conflicts of interest

The authors declare that they have no competing interests.

Ethical issues

The research protocol was approved by the basic sciences department's scientific committee at the College of Dentistry, University of Baghdad, on January 1, 2024 (Project No. 890824). This study was conducted based on the ethical standards stipulated in the Declaration of Helsinki. Also, a written consent form and patient information sheet were provided to each participant for gaining the acceptance of child's parents or his/her caregiver. Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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