Al-Rafidain Dental Journal

Volume 26, Issue 1, 2026, pp.132-148. ISSN (Print): 1812-1217 ISSN (Online): 1998-0345

DOI: https://doi.org/10.33899/rdentj.v26i1.60504

Research Article

Correlation between Dental Caries, Dental Plaque, Gingival Diseases and Vitamin. B12 level in serum in 10-13-years old Children: A Cross-sectional Study

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Article History
Received: 25 April 2025
Revised: 15 August 2025
Accepted: 2 October 2025
Published online: 1 March 2026

Abstract

Aims: The present study aimed to assess the value of vitamin B_{12} blood levels in children and their effect on the general health of teeth and gums.

Materials and Methods: Across sectional study was carried out by collecting data from 60 children. The data were collected by using the DMFT index, the gingival index (GI), the plaque index (PI) by Loe and Silness, and measuring serum vitamin B12 level in pg/ml. The data about serum B12 levels were obtained through referring the children into Hivi Pediatric Teaching Hospital for investigating the vitamin B12 level. The reference range value of vitamin B12 level for children aged 10-13 was 250-900 pg/ml. The group investigating below the level of 250 pg/ml was recorded as the vitamin B12-deficient group. All data were recorded and analyzed using Statistical Package for Social Science (SPSS, IBM, Chicago, USA, version 28). The Pearson correlation coefficient (r) method was used to find out the strength of correlation of vitamin B12 levels with DMFT, GI, and PI scores.

Results: Among the randomly recruited 60 children, 84% of the participants observed deficient vitamin B12 levels 69.7% of them were boys, and 78.4% of them were girls. After group adjustment, in the deficient group, the vitamin B12 levels were observed to have a significant negative correlation with DMFT or dmf, while with GI, vitamin B12 levels were observed to have a highly significant negative correlation and no significant correlation with PI. In the normal group, the vitamin B12 levels showed no significant correlation with DMFT or dmf, while with GI, vitamin B12 levels showed a significant negative correlation and no significant correlation with PI.

Conclusion: This study demonstrates a clear correlation between Vitamin B12 deficiency and poor oral health outcomes in children aged 10 to 13 years.

Keywords: Dental caries, Gingival diseases, Healthy children, Oral health, Vitamin B_{12}

How to cite: Tahir DSH, Mousa HD, Qasim HJ, Karam BM. Correlation between Dental Caries, Dental Plaque, Gingival Diseases and Vitamin. B12 level in serum in 10-13-year-old children: A Cross-sectional Study. Al-Rafidain Dent J. 2026;26(1):132-148.

INTRODUCTION

Numerous oral illnesses, such as tooth decay, gum inflammation, and burning mouth syndrome, may contribute to vitamin B12 deficiency (1). These oral diseases can be prevented or minimized by timely examination of vitamin B_{12} levels in children around 10 to 13 years old. However, few epidemiologic studies focus on the relationships among the status of vitamin B_{12} and caries and gingival disease in children aged 10 to 13 years (2). Vitamin B_{12} is a macronutrient that is not synthesized by plants and is not present in an animal-free or vegan diet. However, extensive sanitary preparation of food can also lead to vitamin B_{12} deficiency (3). B_{12} content has been reported in dental plaque, and as a result, it could be considered a potential factor influencing oral health(4).

The absorption of B_{12} depends on normal gastric functions, and the stomach-intrinsic system is responsible for the release of the vitamin from food. Prolonged deficiency of this vitamin may lead to dental germination, hypoplasia in enamel, and gingivitis(4). Vitamin B_{12} is an important aqueous soluble vitamin that plays a key function in DNA synthesis, red blood cell formation, and supports the normal function of the nervous system. It has an important role in numerous complex physiological functions throughout the body (5).

The synthesis of DNA is represented in the integration of both thymine and folate; both structures allow the formation of red blood cells with the help of vitamin B_{12}(6). The production of the cytosolic enzyme methionine depends on the vitamin B_{12} as a cofactor, synthesis of methionine from homocysteine is by the aid of this enzyme(7), besides, vitamin B_{12} plays role as a cofactor for the enzyme that is responsible for the conversion of the methylmalonyl-CoA to succinyl CoA which intermediates numerous biochemical routs, citric acid cycle represents one of them(8). Vitamin B_{12} has many biological roles, thereby preserving the organ to function normally, and is also important for the CNS. The importance of B_{12} is often realized when the deficiency symptoms manifest(9). Vitamin B_{12} plays an indispensable role in various metabolic pathways and has a significant impact on systemic homeostasis, including the regulation of immune responses, red cell maturation, and myelination (10). Vitamin B_{12} deficiency mainly influences growth and development and can result in hematological alterations, affecting 2-3% of the pediatric population(11). The B_{12} level is correlated with growth, intellectual development, and overall well-being. Raised levels of B_{12} in the blood serum can be caused by excessive vitamin B_{12} intake. (12).Vitamin B_{12} deficiency is known to be associated with hematological abnormalities and neurological complications. Inadequate dietary intake, an inadequate stomach acid level, or low intrinsic factor secretion could cause vitamin B_{12} deficiency. Treatment includes oral or intramuscular supplementation measures(13).

Dental caries is an infectious microbial disease of the teeth resulting from the interaction of dietary sugars, dental plaque, and the host, whose enamel and dentin are progressively demineralized and destroyed as a consequence of acid produced from the fermentation of dietary sugars by oral microbiota (14).

On the other hand, periodontal diseases are chronic inflammatory diseases mediated by the host's immune response to pathogenic microorganisms. Most cross-sectional studies find a prevalence of mild to severe gingivitis between 46% and 61% at 12 years of age (15).

Vitamin B_{12} and folic acid, when they are at low levels, can lead to increased expression of matrix metalloproteinases and increased expression of cytokines, which are responsible for the destruction of the periodontal ligament, leading to gingival inflammation (16-17).

On the other hand, diet plays a significant role in the development and progression of periodontitis. Consequently, correlation analysis among serum B_{12} levels with dental caries and gingival maturity investigates which of the assumptions about the potential link of vitamin B_{12} with dental development in children had not been previously validated (18-19).

MATERIALS AND METHODS

Ethical Approval

This study protocol was approved by the Research Ethics Committee, Duhok, Iraq (Ref. No. **30102024-9-7**). Written informed consents from the parents and voluntary assent from the children were obtained. All participants' parents were given detailed information about the purpose of the study and their rights as research subjects.

Study design

This cross-sectional study was conducted by collecting data from randomly selected 60 children aged 10 to 13 years, who were attending the Pediatric Department at the Dentistry College, Duhok University. Clarify serum vitamin B_{12} levels were distributed among 60 children for both genders (boys and girls), then more participants were recruited to adjust the sample size to 30 children (n=30) for each group, deficient and normal.

In the oral exam, which was done by the same calibrated dentist, relevant oral hygiene-related data, including (dental caries status, gingival inflammation, and dental plaque), were recorded for all permanent teeth and primary teeth for each child, by assessing DMFT, gingival index, and plaque index by (Loe and Silness). Besides, the participants were referred to the Hivi Pediatric Teaching Hospital for investigating serum vitamin B_{12} levels. For this study, the reference range value of vitamin B_{12} level for children aged 10-13 was 250-900 pg/ml. The children, who were investigated below the level of 250 pg/ml, were recorded as the vitamin B_{12}-deficient group (20).

The **Gingival Index (GI) by Lüe and Silness (1963)** is a clinical measure used to assess the severity of gingivitis. It evaluates the condition of the gingiva based on color, consistency, and bleeding on probing (21).

The Gingival Index is scored on a scale from 0 to 3 for each tooth or site examined, with the following values:

0 – No inflammation: The gingiva is healthy, with no redness, swelling, or bleeding.

1 – Mild inflammation: There is slight redness, swelling, and possibly a slight bleeding on probing, but the condition is reversible.

2 – Moderate inflammation: There is moderate redness, swelling, and bleeding on probing, and the condition is more pronounced.

3 – Severe inflammation: The gingiva appears very red, swollen, and may bleed spontaneously without probing, indicating advanced gingivitis.

Each tooth is typically assessed at four sites: mesial, distal, buccal, and lingual. **The Gingival Index score** for an individual is usually the average of the scores from these 4 sites.

The Plaque Index (PI) by Silness and Lüe (1964) is a clinical tool used to assess the thickness of dental plaque present on teeth. It evaluates the accumulation of plaque on the tooth surfaces. [21]

The Plaque Index is scored on a scale from 0 to 3 for each tooth or site examined, based on the following criteria:

0 – No plaque: No plaque is visible or detectable on the tooth surface.

1 – Thin film of plaque: A thin film of plaque cannot be seen by the naked eye, but can be seen by using a probe on the tooth surface; it does not cover the entire surface of the tooth.

2 – Moderate plaque: A moderate amount of plaque is visible or palpable, covering a significant portion of the tooth surface.

3 – Abundant plaque: A large amount of plaque is visible or palpable, covering most or all of the tooth surface.

Plaque is assessed at four sites of each tooth: the mesial, distal, buccal, and lingual surfaces. The individual plaque score is the average of these 4 sites.

The DMFT was calculated by adding up the number of decayed (D), missing (M), and filled (F) teeth in an individual's mouth.

The **WHO protocol** was reserved as a basis for the dental examinations. The caries index used in this study was DMFT for permanent teeth and (dmft) for primary teeth. The collection of data was done by using a mixed dentition chart (Figure 1), and the components of the caries index (22). were as follows:

DMFT:

D,d component: (Decayed teeth that measured)

1-Carious tooth.

2-Only the roots are left.

3-Defect filling.

4-Temporary filling.

5-Filled tooth surface with another surface decayed.

M,m component: (Missing teeth due to caries)

Missing teeth that are not measured with this index DMF:

1- Tooth that was extracted for reasons other than caries:

a- Orthodontic treatment.

b- Impaction.

c- Periodontal disease.

2- Unerupted teeth.

3- Congenitally missing.

4- Avulsed teeth due to trauma or accident.

F,f component (Filled teeth due to caries)

Teeth that were restored for reasons other than dental caries were not measured with this index:

a- Trauma (fracture).

b- Hypoplasia (for esthetic purposes).

c- Bridge abutment (retention).

d- Seal a root canal due to trauma.

e- Fissure sealant.

f- Preventive fillings

		UPPER				
		M	O	D	B	L
	02					
	03					
E	04					
D	05					
C	06					
B	07					
A	08					
A	09					
B	10					
C	11					
D	12					
E	13					
	14					
	15					

		LOWER				
		M	O	D	B	L
	18					
	19					
E	20					
D	21					
C	22					
B	23					
A	24					
A	25					
B	26					
C	27					
D	28					
E	29					
	30					
	31					

Figure (1): Mixed dentition chart

The examinations of the teeth were carried out in the clinics of the pediatric department by using a dental mirror and a sharp probe, under good LED light and in an upright position. All teeth of all participants were examined in a systematic approach by the same examiner, starting from the upper right teeth and proceeding through the lower right teeth. The periodontal probe used for the examination of both gingival inflammation and dental plaque was the Williams periodontal probe. It was held very comfortably to produce the lightest pressure and at a 60-degree angle to the long axis of the tooth.

Inclusion and exclusion standards

Participants, who were medically fit and submissive, were recruited, while those with any systemic diseases and those not receptive to accept proposed treatment plan were excluded from the study.

Statistical analysis

The data were analyzed using Statistical Package for Social Science (SPSS, IBM, Chicago, USA, version 28). The Pearson correlation coefficient (r) method was used to find out the correlation of vitamin B_{12} levels with DMFT, GI, and PI scores. A *P*-value less than 0.05 was considered significant, while a *P*-value less than 0.01 was considered highly significant. A two-tailed test was used, considering both extremes (positive and negative) of the probability distribution.

RESULTS

Figure (2) revealed vitamin B_{12} levels of distribution among randomly selected 60 children. 84% of them observed less than 250pg/ml vitamin B_{12} level, among them deficiency was found in about 69.7% in boys, whereas in girls it was 78.4%. Figure (2)

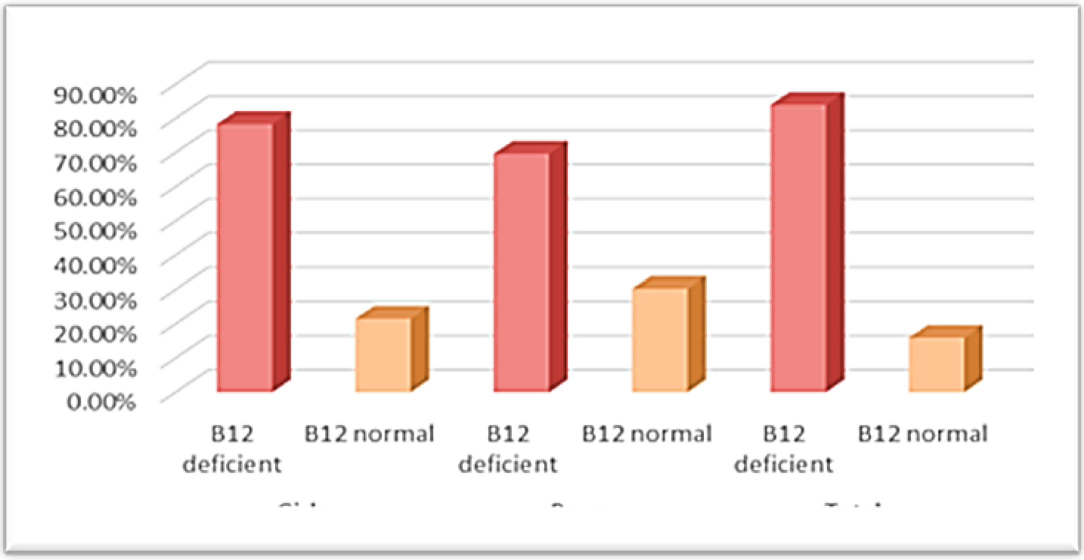

Figure (2): Gender distribution and Percentage of Vitamin B_{12}

The mean and standard deviation (SD) of the deficient vitamin B_{12} levels group and the parameters (DMFT, GI, and PI scores) are illustrated in (Table 1). vit B_{12} level = 187.5±47.6, DMFT= 4.19±2.93, GI=1.9±0.51 and PI= 1.36±0.52.

Table (1): Mean and standard deviation of vit B_{12} levels, DMFT, GI, and PI of the deficient group

Variable	Mean± SD (pg/mL)	Parameters	Mean ±SD
Vit B_{12}		DMFT	4.19(2.93)
n=30	187.5(47.6)	GI	1.9(0.51)
		PI	1.36(0.52)

Pearson correlation coefficient (r) for deficient vit B_{12} group comparing the strength of correlation between vit B_{12} level with DMFT, GI, and PI scores is illustrated in (Table 2). The correlation between vitamin B_{12} levels and DMFT was statistically significant, the correlation between vitamin B_{12} levels and GI was highly significant, while the correlation between vitamin B_{12} levels and PI was not significant.

Table (2): Correlation between the parameters (DMFT, GI, and PI scores) and deficient vitamin B_{12} levels.

Variable	Parameters	r-value	P-value
Vit B_{12}	DMFT	−0.377	0.04* (p<0.05)
n=30	GI	−0.534	0.0097** (p<0.01)
	PI	−0.325	0.074 (p> 0.05)

*: Significant difference; **: Highly significant difference using Pearson correlation coefficient (r)

The mean and standard deviation (SD) of the normal vitamin B_{12} levels group and the parameters (DMFT, GI, and PI scores) are illustrated in (Table 3). vit B_{12} level = 274±32.1, DMFT= 2.04±3.87, GI= 1.1±0.32 and PI= 1.35±0.2.

Table (3): Mean and standard deviation of vit B_{12} levels, DMFT, GI, and PI of the normal group.

Variable	Mean±SD(pg/mL)	Parameters	Mean ±SD
		DMFT	2.04(3.87)
Vit B_{12}		GI	1.1(0.32)
n=30	274(32.1)	PI	1.35(0.2)

Pearson correlation coefficient (r) for the normal vit B_{12} group comparing the strength of correlation between vit B_{12} level with DMFT, GI, and PI scores is illustrated in (Table 4). The correlation between vitamin B_{12} levels and DMFT was not significant, the correlation between vitamin B_{12} levels and GI was observed to be significant, while the correlation between vitamin B_{12} levels and PI was not significant.

Table (4): Correlation between the parameters (DMFT, GI, and PI scores) and normal vitamin B_{12} levels.

Variable	Mean±SD	Parameters	r-value	P-value
		DMFT	−0.359	0.051 (p> 0.05)
Vit B_{12}		GI	-0.371	0.044* (p<0.05)
n=30	274(32.1)	PI	-0.330	0.075 (p> 0.05)

*: Significant difference; **: Highly significant difference using Pearson correlation coefficient (r)

DISCUSSION

The present study revealed that low vitamin B_{12} levels were significantly higher dental caries and gingival diseases in 10- to 13-year-old children.

Vitamin B_{12} is involved in various metabolic pathways, including L-methylmalonyl-CoA to succinyl-CoA and homocysteine to methionine metabolism, the synthesis of nucleic acids, the formation of blood, and maintaining overall cellular health and function (23). Vitamin B_{12} deficiency has also been linked to neurological disorders and developmental problems. Ensuring adequate levels of vitamin B_{12} through diet and supplementation can help prevent dental issues and promote optimal oral health in children (18). Additionally, further research is needed to explore the underlying mechanisms and potential interventions to mitigate the impact of low salivary vitamin B_{12} levels on dental health outcomes (24,25). Additionally, Vitamin B_{12} is essential for modulating the immune response, including the production of cytokines and the functioning of macrophages. A weakened immune response in Vitamin B_{12}-deficient children may exacerbate microbial infections in the oral cavity, contributing to the observed correlation between deficiency and poor oral health outcomes.

Many epidemiological studies on serum and plasma vitamin B_{12} levels in children have been conducted to investigate the correlation between vitamin B_{12} status and dental caries and gingival diseases, but there have been many contradictory results (26-33). Many epidemiological studies in the literature have presented the association between salivary vitamin B_{12} levels and dental caries and gingival diseases (26,34,35).

A study found that dental caries was observed in 96.2% of children categorized in the 1–25th percentile, which stood for low salivary vitamin B_{12} levels, and it decreased with an increase in salivary vitamin B_{12} levels. Out of these, 143 children suffered from dental caries, and 96 children belonged to those with the lowest vitamin B_{12} levels. Therefore, vitamin B_{12} levels play a crucial role in dental caries. Salivary vitamin B_{12} levels are lower in children, which might be associated with the size of the salivary gland. Therefore, these results reveal that low salivary vitamin B_{12} levels in saliva are a risk factor for dental caries and gingival diseases in children (27).

The average salivary and serum vitamin B_{12} levels were found to be 2.2 and 309.7 pg/mL, respectively. Furthermore, the children were distributed into three salivary vitamin B_{12} tertiles, namely 200–1463, 466–701, and 1471–1918 pg/mL, to categorize their levels more precisely. These findings suggest that salivary vitamin B_{12} can be utilized as an excellent method to measure a precise level of vitamin B_{12} (28).

Vitamin B_{12} deficiency progresses to cardiovascular disease and variable neurological and dental manifestations. Vitamin B_{12} is essential for DNA synthesis, as well as fat and carbohydrate metabolism, and its deficiency reportedly alters the formation and repair of oral structures; hypoplasia of enamel, cytokine-mediated osteoclast stimulation, and maintenance of Streptococcus salivarius are all linked to its deficiency, which can lead to an increased risk of caries development, abnormal teeth development, and increased periodontitis. Vitamin D plays an essential role in oral health, and its deficiency is associated with significant changes in oral health diseases (29).

CONCLUSIONS

Within the limitations of the current study, it is possible to conclude that:

This study provides relatively lower levels of plasma vitamin B_{12} in a selected sample of the 10–13-year-old children population, and it showed a significant association between plasma vitamin B_{12} levels and dental caries, and provides preliminary evidence that lower levels of plasma vitamin B_{12} may be related to the severity of gingival inflammation in 10-13-year-old children.

Acknowledgment: This study was supported by the College of Dentistry, University of Duhok, Kurdistan Region, Iraq.

Authors' Contribution

Tahir DSH., Mousa HD., Qasim HJ., Karam BM. completed the conceptualization, data curation, formal analysis, finding acquisition, investigation, resources, software, validation, visualization, writing- original draft, writing- review, and editing. Tahir DSH., Mousa HD., Qasim HJ., Karam BM completed the Conceptualization, Investigation, Methodology, Project administration, Supervision, Visualization, Writing, Writing- review & editing. Manton finished the Conceptualization, investigation, methodology, project administration, supervision, visualization, and writing- review & editing.

Funding: This study is self-funded

Ethical statement: This study protocol was approved by the Research Ethics Committee, Duhok, Iraq (Ref. No. **30102024-9-7**). Written informed consents from the parents and voluntary assent from the children were obtained. All participants' parents were given detailed information about the purpose of the study and their rights as research subjects.

Conflict of interest

The author declares that there are no conflicts of interest regarding the publication of this manuscript

Availability of data and materials: All data generated or analyzed during this study are included in this published article and its supplementary information files.

Declaration of Generative AI and AI-assisted technologies

During the preparation of this work, the authors used a grammar tool to improve the readability of the manuscript. The authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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الارتباط بين تسوس الأسنان، واللويحة السنية، وأمراض اللثة، ومستوى فيتامين B12 في مصل الدم لدى الأطفال بعمر 10–13 سنة: دراسة مقطعية

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الملخص

الأهداف: هدفت الدراسة الحالية إلى تقييم قيمة مستويات فيتامين B12 في الدم لدى الأطفال وتأثيرها في الصحة العامة للأسنان واللثة. **المواد وطرائق العمل**: أُجريت دراسة مقطعية من خلال جمع البيانات من 60 طفلًا. تم جمع البيانات باستخدام مؤشر تسوس الأسنان المفقودة والمحشوة (DMFT/dmf)، ومؤشر اللثة (GI)، ومؤشر اللويحة السنية (PI) وفقًا لطريقة لو وسيلنس، إضافة إلى قياس مستوى فيتامين B12 في مصل الدم بوحدة بيكوغرام/مل. تم الحصول على بيانات مستويات فيتامين B12 في المصل عن طريق إحالة الأطفال إلى مستشفى هيفي التعليمي للأطفال لإجراء فحص مستوى فيتامين B12.

تراوح المجال المرجعي الطبيعي لمستوى فيتامين B12 لدى الأطفال بعمر 10–13 سنة بين 250 و900 بيكوغرام/مل، في حين سُجِّلت القيم التي تقل عن 250 بيكوغرام/مل ضمن مجموعة نقص فيتامين B12. جرى تسجيل جميع البيانات وتحليلها باستخدام الحزمة الإحصائية للعلوم الاجتماعية (SPSS، شركة IBM، شيكاغو، الولايات المتحدة الأمريكية، الإصدار 28). واستُخدم معامل ارتباط بيرسون (r) لتحديد قوة الارتباط بين مستويات فيتامين B12 وكل من مؤشرات DMFT وGI وPI. **النتائج**: أظهرت النتائج ان من بين 60 طفلًا جرى اختيارهم عشوائيًا، لوحظ أن 84% من المشاركين يعانون من نقص في مستويات فيتامين B12، وكان 69.7% منهم من الذكور و78.4% من الإناث. بعد ضبط المجموعات، أظهرت مجموعة نقص فيتامين B12 وجود ارتباط سلبي معنوي بين مستويات فيتامين B12 ومؤشر DMFT/dmf، في حين لوحظ ارتباط سلبي عالي المعنوية بين مستويات فيتامين B12 ومؤشر اللثة (GI)، ولم يُسجَّل ارتباط معنوي مع مؤشر اللويحة السنية (PI). أما في المجموعة ذات المستويات الطبيعية من فيتامين B12، فلم يُظهر مستوى الفيتامين ارتباطًا معنويًا مع مؤشر DMFT/dmf، بينما سُجِّل ارتباط سلبي معنوي مع مؤشر اللثة (GI)، ولم يُلاحظ أي ارتباط معنوي مع مؤشر اللويحة السنية (PI). **الاستنتاجات**: تُظهر هذه الدراسة وجود علاقة واضحة بين نقص فيتامين B12 وتدهور صحة الفم لدى الأطفال الذين تتراوح أعمارهم بين 10 و13 عامًا.

الكلمات المفتاحية: تسوس الأسنان، أمراض اللثة، أطفال أصحاء، صحة الفم، فيتامين ب12