Original Communication

Efficiency of the sublingual route in treating B12 deficiency in infants

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Abstract: *Objective:* To evaluate the efficiency of the sublingual route for the treatment of vitamin B12 deficiency in infants. *Background:* Vitamin B12 deficiency is common in children. In breastfed infants, the main reason is maternal B12 deficiency. Parenteral administration is commonly prescribed. However, patient compliance is not satisfactory due to repeated painful parenteral applications. It is also known that the oral route is efficient in high doses. In recent years, the sublingual route has been tried. This route stands out due to its easy applicability and low cost. However, there are few efficacy studies in infants for the sublingual route. *Materials and methods:* The study included 49 infants aged 6–12 months. All infants with marginal or deficient B12 levels (<300 pg/mL) were incidentally detected and treated with sublingual methylcobalamin. Each dose was 1000 μg and administered once a day in the first week, every other day in the second week, twice a week in the third week, and once a week in the last week. Serum vitamin B12 levels were measured before and after the treatment. Paired Sample T-Test was used to compare variables. *Results:* All infants had normal physical development and had no hematological or neurological issues. It was learned from the parents that the infants tolerated treatment well, and no side effects related to the treatment, such as vomiting or rash, were observed. Before and after the treatment, the mean vitamin B12 levels were 199±57 pg/mL and 684±336 pg/ml, respectively. The difference between the means was statistically significant ($p<0.001$). *Conclusion:* According to the study, it seems possible to treat vitamin B12 deficiency via a sublingual route in infants. In addition, methylcobalamin can be an alternative to the commonly used cyanocobalamin.

Keywords: Infant, sublingual route, treatment, vitamin B12 deficiency

Introduction

Vitamin B12, also known as cobalamin, is an essential water-soluble nutrient necessary for many body functions. It has a substantial role in DNA synthesis, methylation and mitochondrial metabolism, erythropoiesis, maintaining the myelination of nerves, and in cognitive processes in the nervous system [1]. Vitamin B12 has different forms that are cofactors for complex reactions in the cell. Methylcobalamin and adenosylcobalamin are active forms of cobalamin. Methylcobalamin is the cofactor for methionine synthase, and its deficiency causes megaloblastic anemia. Adenosylcobalamin is also a cofactor for methylmalonyl-CoA mutase, and this pathway is responsible for the neurological effects of vitamin B12 deficiency [2–5].

Humans cannot synthesize vitamin B12 through endogenous processes [6]. Therefore, it needs to be ingested with animal-origin foods. It is found in large amounts in liver, beef, lamb, and seafood, and in small quantities in chicken, eggs, and dairy products. By contrast, vegetable sources do not contain any forms of cobalamin [1]. Adults need about 1 μg a day of vitamin B12 [7]. Children need a smaller amount of vitamin B12 because of their lower body mass. The Recommended Dietary Intake (RDI) is the amount required to maintain adequate serum vitamin B12 concentrations. The RDI for adults is 2.4 μg/day [6]. However, the RDI based on the estimated intake of vitamin B12 from breast milk for healthy infants is 0.4 μg/day in the first six months and 0.5 μg/day in the second six months of life [8].

Vitamin B12 available in foods is absorbed sufficiently in the ileum via the intrinsic factor (IF) [9]. Another route of cobalamin absorption is passive diffusion. Only 1% of vitamin B12 is absorbed by passive diffusion [10]. Hence, much more oral B12 is required in cases of malabsorption or lacking the IF.

Vitamin B12 binds to some proteins in the serum. About 20% of circulating cobalamin is bound to Transcobalamin (TC) [1]. The cobalamin-TC complex, known as holotranscobalamin (HoloTC), delivers vitamin B12 to the cells. HoloTC represents metabolically active vitamin B12. Most circulating cobalamin is bound to the Haptocorrin [11]. However, the physiological role of haptocorrin is still unknown [12].

Vitamin B12 deficiency is common; fundamental reasons are malabsorption and dietary inadequacy. Especially in developing countries, it has also become a big problem in children and young adults today due to the limited consumption of animal products [13]. In breastfed infants, the main reason is maternal vitamin B12 deficiency [12, 14]. Breastfed infants get vitamin B12 from breast milk during the first months of life, and B12 levels decrease around sixth months in exclusively breastfed infants [15]. Especially in mothers following a vegan diet or suffering from gastritis or other malabsorption disorders, breast milk may not contain adequate amounts of vitamin B12. In addition, congenital disabilities such as Imerslund-Gräsbeck syndrome rarely cause cobalamin deficiency in infants [16].

Vitamin B12 deficiency causes a wide range of disorders, from asymptomatic to life-threatening. The leading concrete consequences of vitamin B12 deficiency are megaloblastic anemia and neurological disorders [6]. In children, commonly seen findings are developmental delay or regression, hypotonia, lethargy, hyperirritability, tremors, and microcephaly [12].

The intramuscular (IM) route is the most widely used treatment method in infants today. In patients with severe vitamin B12 deficiency, this route is primarily chosen using different application schemes. Likewise, the IM route is generally selected in infants. Depending on the severity of vitamin B12 deficiency, the cobalamin treatment may be administered in doses ranging from 250 to 1000 μg three to seven times per week for 1–2 weeks, then once a week for one month [17]. Vitamin B12 is absorbed in a rapid and substantial rate by this route. Although this seems advantageous, amounts exceeding the transcobalamin binding capacity are excreted in the urine [1]. Besides, this route also involves some troubles such as repeated painful parenteral applications, bleeding, inconvenience, needing a healthcare provider, and high costs [18, 19]. Therefore, patient compliance is unsatisfactory. For this reason, sometimes, the oral route is preferred. Many studies stated that effective treatment is possible with high doses of oral vitamin B12 [10, 20–22].

Various forms of cobalamin are selected for treating vitamin B12 deficiency. Hydroxocobalamin and cyanocobalamin are inactive forms of cobalamin and transform into active forms in the body [1, 7, 12]. Cyanocobalamin is a synthetic form of cobalamin containing cyanide. It is the most common form, especially in parenteral treatments. Although toxicity has not been reported with low amounts of cyanide, this form is still under discussion due to the toxic effects cyanide may cause [23]. Cobalamins need to undergo specific reactions to transform into active forms. However, additional steps are needed for cyanocobalamin. Moreover, some processes are also required to remove the resulting cyanide from the body. However, methylcobalamin and adenosylcobalamin are active forms, and side effects or toxicity have not been reported [24].

On the other hand, intranasal or sublingual (SL) routes have been tried recently. However, only a few studies on these routes are available [25–28].

As far as we are aware, this is the first study in infants younger than age one year. This study aimed to investigate the efficacy of the SL route of vitamin B12 supplementation in infants treated with SL vitamin B12 by comparing vitamin B12 levels before and after the treatment.

Materials and methods

Data collection

The study was designed as a retrospective and descriptive study and conducted at a pediatric follow-up outpatient clinic. All infants aged 6–12 months underwent a routine laboratory screening test consisting of complete blood count (CBC), serum vitamin B12, and ferritin in their regular follow-up. These routine tests performed in 2018 and 2019 were scanned, and the results of 826 subjects were analyzed. Vitamin B12 levels of 174 infants were below 300 pg/mL, and 49 were treated with a SL B12. Vitamin B12 levels and hemogram parameters before and after treatment were retrieved from the laboratory database. However, vitamin B12 levels after treatment could only be obtained for 42 infants. During follow-up after the second B12 result, no further vitamin B12 tests were carried out for these patients.

Inclusion and exclusion criteria

The study included healthy infants aged 6–12 months. Infants who received any vitamin supplementation other than vitamin D and had any chronic disease or malabsorption syndrome that would affect cobalamin metabolism were excluded from the study. Breastfed infants whose mothers used vitamin B12 supplements were not included in the study.

Laboratory assessment

Total serum vitamin B12 was analyzed by the chemiluminescence method using Cobas immunoassay analyzers (Roche Diagnostics). Commonly, a concentration of <200 pg/mL (148 pmol/L) is defined as a deficiency and a concentration of 200–300 pg/mL (148–221 pmol/L) is defined as marginal status [29].

Treatment method

All infants with marginal or deficient B12 levels (<300 pg/mL) were treated with a methylcobalamin SL form (Ocean Methyl B12, 1000 μg spray). Similar to the recommendations of the local Diagnosis and Treatment Guideline published by the Turkish Society of Hematology [30], the treatment was administered to infants by their parents once a day in the first week, every other day in the second week, twice a week in the third week, and once a week in the last week. Every infant received 14 doses of SL vitamin B12, each dose being 1000 μg. One week after completion of the treatment, the second serum vitamin B12 level was measured. Subsequently, only clinical findings were followed up without measuring any B12 level.

Data analysis

Data were evaluated using IBM SPSS 21 for Windows. Equality of variances was checked with Levene's Test for Equality of Variances, and normality of distribution was examined with the Kolmogorov-Smirnov test. Descriptive statistical analyses were used to obtain the means and standard deviations of continuous variables. Paired Sample T-Test was used to compare variables observed before and after treatment. In addition, the Chi-square test was used to compare frequencies. P values <0.05 were accepted as significant.

Ethics approval

The Ethics Committee of Sisli Memorial Hospital, Istanbul approved the present study (File no: 2019/004).

Results

The present study examined infants between 6–12 months of age, and 826 vitamin B12 test results were obtained in the course of two years, 174 of which (21%) were below 300 pg/mL. The vitamin B12 level was in the marginal range for 117 patients (14.2%), and vitamin B12 deficiency was seen in 57 (6.9%) of these patients. Forty-nine infants with marginal or deficient vitamin B12 were treated with SL methylcobalamin. Post-treatment vitamin B12 levels were obtained for 42 patients (Figure 1).

The mean age of the infants included in the study was 6.9 ±1.2 months, and 17 of them (41%) were female. During the first 6 months, all infants were breastfed; some were supported with formula (breast milk only: 62%; breast milk plus formula: 38%). Afterward, they received complementary foods in addition to breast milk or formula. All of them showed normal physical development and had no symptoms or signs indicating vitamin B12 deficiency.

All infants were treated with SL B12. It was learned from the parents that the infants did not refuse the treatment and tolerated it well, and no side effects related to the treatment, such as vomiting or rash, were observed.

The mean B12 level for all infants (n=42) was 199±57 pg/mL before treatment and 684±336 pg/mL after treatment. The difference between the mean values was statistically significant ($p<0.001$). After treatment, the mean B12 level increase was 485±330 pg/mL. This value was 389±226 for females and 549±376 for males. Although the change was greater in male infants, the difference between the sexes was not statistically significant. The mean vitamin B12 level before B12 treatment was significantly lower in exclusively breastfed babies. Vitamin B12 levels according to feeding status are shown in Table 1.

Hematological laboratory findings were normal before and after treatment. No significant change was observed (Table 2).

Discussion

The present study demonstrated that the SL route successfully raised the serum vitamin B12 level in infants at the end of a 1-month period. Besides, the results obtained showed that SL methylcobalamin corrected vitamin B12 deficiency in the target population as much as the commonly chosen cyanocobalamin. This method has certain advantages compared to oral or IM treatment [26]. Patient compliance is good because it is safe, comfortable, painless, easy, and low cost. Drugs are absorbed rapidly and directly into the systemic circulation due to the thinner epithelium and mucus layer in the sublingual area. At the same time, the drug's bioavailability is high as it directly enters the systemic circulation without first being exposed to hepatic metabolic processes or degradation [31–33].

Many studies compare oral and IM treatment methods [34–37]. Kuzminski et al. have found the oral route to be as effective as parenteral administration. Moreover, the study stated that daily oral treatment might be superior to monthly IM treatment. However, the study had a small number of participants [10]. Similarly, some researchers also found the oral route to be effective in children [20–22].

On the other hand, some studies tried the SL route for treating vitamin B12 deficiency [25–28]. To our knowledge, the first study on SL treatment was published in 1998 by Delpre et al. [25]. The study demonstrated that SL treatment is effective in adults. After a while, in a study including 30 adults, Sharabi et al. [38] compared SL and oral routes for vitamin B12 treatment in doses of 500 μg. In this study, both SL and oral treatment significantly increased serum vitamin

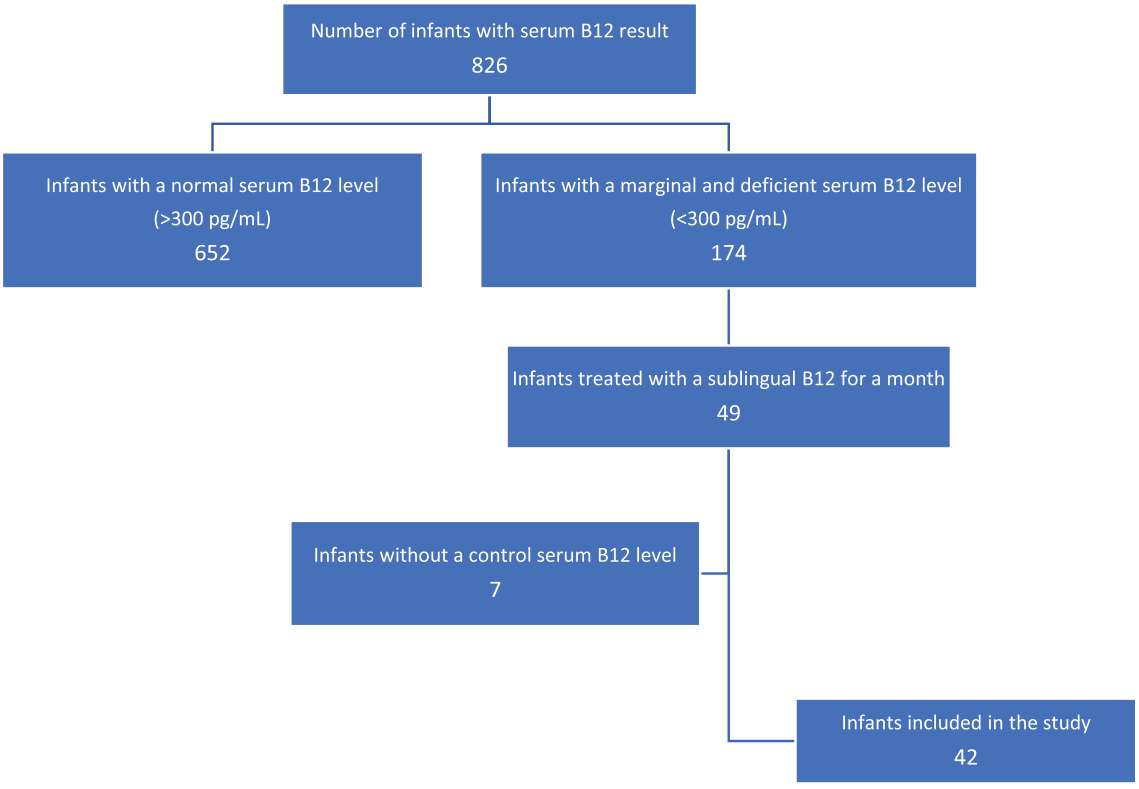

Figure 1. Flow diagram of the sampling procedure.

Table 1. Vitamin B12 levels according to feeding status

	Breast milk only	Breast milk and formula	
B12 levels	n=26	n=16	p
Before treatment	181±59	229±39	<0.01
After treatment	630±291	771±393	0.19
Change	450±284	541±398	0.39

The results are presented in pg/mL as mean±standard deviation.

Table 2. Before and after treatment, hematological laboratory findings

	SI reference intervals [44]	Values before treatment	Values after treatment	p
RBC count (10^6/μL)	3.6–5.5	4.6±0.4	4.7±0.5	0.68
Hemoglobin (g/dL)	10.1–13.7	11.3±0.8	11.4±0.9	0.32
Hematocrit (%)	26–41	33.8±2.8	34.1±3.1	0.36
MCV (fL)	68–88	73.5±4.5	73.9±9.0	0.45
Leukocyte count (10^3/μL)	3.7–14.6	9.0±2.6	9.8±3.9	0.77
Platelet count (10^3/μL)	169–562	385±122	350±123	0.13

The results are presented as mean±standard deviation. RBC: red blood cell; MCV: mean corpuscular volume.

B12 levels. There was no significant difference between treatment methods. In another study performed by Parry-Strong et al. [39], SL methylcobalamin treatment was effective in adult diabetic patients using metformin. Also, in a multi-participant adult study conducted by Bensky et al. [26], SL treatment was found to be effective, and its superiority over IM therapy was emphasized. At the same time, this was the most extensive study about SL B12 treatment. All of these were adult studies. SL B12 studies in adults are summarized in Table 3.

In a case reported in 2014, a child with short bowel syndrome was successfully treated via the SL route [28]. Then,

Table 3. Summaries of sublingual B12 studies in adults

	n	SL B12 dose and form	Duration	Before	After	Change	p
Delpre G. [25]	18	2000 μg/day Cyanocobalamin SL tablet	7–14 days	127.9±42.6	515.7±235	387.7	0.0001
Sharabi A et al.* [38]	30	500 μg/day Cyanocobalamin SL tablet	4 weeks	127.4±40.6	390.3±100.3	262.9	0.0001
Parry-Strong A et al.* [39]	19	1000 μg/day Methylcobalamin SL tablet	3 months	230±52.8	504.3±140.0	273.6	0.002
Bensky MJ et al. [26]	3451	1000 μg/day Cyanocobalamin SL tablet	9 months	298±155	551±203	252	<0.001

The results are presented in pg/mL as mean±standard deviation and in pg/mL unit. SL: sublingual. *The values were converted to metric units (pmol/L to pg/mL).

Table 4. Summaries of sublingual B12 studies in children

	n	SL B12 dose and form	Duration	Before	After	Change	p
Kartal et al. [27]	43	1000 μg Cyanocobalamin SL tablet	1 month	137.2±36.5	483.4±144.8	346.2	<0.001
	Aged 5–18 years	Total 17 doses					
Kartal et al. [27]	39	1000 μg Methylcobalamin SL spray	1 month	146.7±40.5	565.5±108.1	418.8	<0.001
	Aged 5–18 years	Total 17 doses					
Varkal et al.	42	1000 μg Methylcobalamin SL spray	1 month	199±57	684±336	485	<0.001
	Aged 6–12 months	Total 14 doses					

The results are presented in pg/mL as mean±standard deviation. SL: sublingual.

for the first time, a study compared two substances via SL route (cyanocobalamin and methylcobalamin) and one by IM route (cyanocobalamin) in children aged 5–18 [27]. In the study, 1 mg cyanocobalamin was administered to the IM treatment group every other day for the first week and once a week for the next three weeks, and 1 mg cyanocobalamin or methylcobalamin, respectively, was given to the SL groups every day for the first week and every other day for the next three weeks. Serum vitamin B12 levels increased significantly at the end of one month in all groups. Moreover, SL methylcobalamin was significantly more effective than SL cyanocobalamin. Also, all treatment routes resulted in considerably better hematologic findings. Besides, the study emphasized that IM treatment provided faster hematologic improvement, although it was not statistically significant. SL B12 studies in children are summarized in Table 4.

While the adequate serum level of B12 is still under debate, there is no consensus regarding the treatment dose and not enough information in the literature about an efficient SL dose in children. Therefore, in the present study, the SL B12 treatments were administered with a dose of 1 mg, as recommended by the Turkish Society of Hematology. However, in an adult study performed by Cristian Del Bo et al. [40], it is stated that lower doses of cobalamin are also sufficient for treatment by the SL route. In their study, SL cyanocobalamin was administered to two randomly divided groups at doses of 350 μg/week (mean age 43 years) and 2000 μg/week (mean age 42 years) for three months. At the end of the second week, no significant difference was observed between the two groups in the increase of serum B12. However, they differed significantly at the end of the first, second, and third months. The increases in the low-dose group remained lower in these periods. Nonetheless, according to the inference based on data at the end of the third month, both treatment methods normalized B12 levels and metabolic markers such as holotranscobalamin, methylmalonic acid, succinic acid, and homocysteine. The study provides substantial evidence about the treatment dose of B12. It is also known that the quantity of vitamin B12 absorbed increases with the amount of intake, whereas the absorption rate decreases with increasing doses [18, 41].

The strength of this research is that it assessed B12 therapy by SL route for the first time in infants. This route is low-cost, easy, painless, and does not require the intervention of a healthcare professional. Also, methylcobalamin, which is not commonly chosen, was used in this study. However, it is unclear whether this form is more effective than the alternatives.

There are several limitations to the study. First, this research included a small number of participants and had no control group, limiting the possibility of generalizing the results. Secondly, the study did not use a prospective design to investigate treatments with different doses and routes and did not include any long-term follow-up. Therefore, the study does not allow for definitive conclusions about the optimum dose and duration. Thirdly, the patients included in the study had no serum homocysteine (Hcy) and methylmalonic acid (MMA) levels measured. Elevated MMA and Hcy indicate impaired cobalamin metabolism and low B12 status. Although serum vitamin B12 status is assessed through serum concentration, some authors claim that MMA and Hcy measurements are more appropriate for evaluating vitamin B12 status [42, 43].

Conclusion

According to this study, it seems possible to treat vitamin B12 deficiency by SL route in infants. Several published studies support this argument. The treatment option is low-cost, painless, easily applicable, and does not require a healthcare professional to be present. While these are important advantages of the treatment method, comprehensive studies are needed to get clear answers regarding the most appropriate form, dosage, and timing for B12 deficiency treatment.

References

1. O'Leary F, Samman S. Vitamin B12 in health and disease. Nutrients. 2010;2:299–316.
2. Gherasim C, Lofgren M, Banerjee R. Navigating the B12 road: Assimilation, delivery, and disorders of cobalamin. J Biol Chem. 2013;288:13186–93.
3. Brito A, Hertrampf E, Olivares M, Gaitán D, Sánchez H, Allen LH, et al. Folate, vitamin B12 and human health. Rev Med Chil. 2012;140:1464–75.
4. Stover PJ. Physiology of folate and vitamin B12 in health and disease. Nutr Rev. 2004;62(6 Pt 2):S3–12; discussion S13.
5. Marsh ENG, Meléndez GDR. Adenosylcobalamin enzymes: Theory and experiment begin to converge. Biochim Biophys Acta – Proteins Proteomics. 2012;1824:1154–64.
6. Green R. Vitamin B12 deficiency from the perspective of a practicing hematologist. Blood. 2017;129:2603–11.
7. Stabler SP, Allen RH. Vitamin B12 deficiency as a worldwide problem. Annu Rev Nutr. 2004;24:299–326.
8. Allen LH. Impact of vitamin B-12 deficiency during lactation on maternal and infant health. Adv Exp Med Biol. 2002;503:57–67.
9. Carmel R. Mild transcobalamin I (haptocorrin) deficiency and low serum cobalamin concentrations. Clin Chem. 2003;49: 1367–74.
10. Kuzminski AM, Del Giacco EJ, Allen RH, Stabler SP, Lindenbaum J. Effective treatment of cobalamin deficiency with oral cobalamin. Blood. 1998;92:1191–8.
11. Ozhan B, Evrengul H, Yilmaz Agladioglu S, Umit Yasar S, Demir S. Vitamin D status of children in a university hospital in West Turkey. Hong Kong Med J. 2016;21:251–6.
12. Stabler SP. Clinical practice. Vitamin B12 deficiency. N Engl J Med. 2013;368:149–60.
13. Green R, Allen LH, Bjørke-Monsen AL, Brito A, Guéant JL, Miller JW, et al. Vitamin B12 deficiency. Nat Rev Dis Prim. 2017;3:1–20.
14. Honzik T, Adamovicova M, Smolka V, Magner M, Hruba E, Zeman J. Clinical presentation and metabolic consequences in 40 breastfed infants with nutritional vitamin B12 deficiency – What have we learned? Eur J Paediatr Neurol 2010;14:488–95.
15. Monsen ALB, Refsum H, Markestad T, Ueland PM. Cobalamin status and its biochemical markers methylmalonic acid and homocysteine in different age groups from 4 days to 19 years. Clin Chem. 2003;49:2067–75.
16. Nielsen MJ, Rasmussen MR, Andersen CBF, Nexø E, Moestrup SK. Vitamin B 12 transport from food to the body's cells – A sophisticated, multistep pathway. Nat Rev Gastroenterol Hepatol. 2012;9:345–54.
17. Bjørke-Monsen AL, Ueland M. Cobalamin status in children. J Inherit Metab Dis. 2011;34(1):111–9.
18. Carmel R. How I treat cobalamin (vitamin B12) deficiency. Blood. 2008;112:2214–21.
19. Masucci L, Goeree R. Vitamin B12 intramuscular injections versus oral supplements: A budget impact analysis. Ont Health Technol Assess Ser. 2013;13:1–24.
20. Bahadir A, Reis PG, Erduran E. Oral vitamin B12 treatment is effective for children with nutritional vitamin B12 deficiency. J Paediatr Child Health. 2014;50:721–5.
21. Altay Ç, Çetin M. Oral treatment in selective vitamin B12 malabsorption. J Pediatr Hematol Oncol. 1997;19:245–6.
22. Sezer RG, Bozaykut A, Akoğlu HA, Özdemir GN. The efficacy of oral vitamin B12 replacement for nutritional vitamin B12 deficiency. J Pediatr Hematol Oncol. 2018;40:69–72.
23. Geller RJ, Barthold C, Saiers JA, Hall AH. Pediatric cyanide poisoning: Causes, manifestations, management, and unmet needs. Pediatrics. 2006;118(5):2146–58.
24. Methylcobalamin. Altern Med Rev. 1998;3:461–3.
25. Delpre G, Stark P, Niv Y. Sublingual therapy for cobalamin deficiency as an alternative to oral and parenteral cobalamin supplementation. Lancet. 1999;354:740–1.
26. Bensky MJ, Ayalon-Dangur I, Ayalon-Dangur R, Naamany E, Gafter-Gvili A, Koren G, et al. Comparison of sublingual vs. intramuscular administration of vitamin B12 for the treatment of patients with vitamin B12 deficiency. Drug Deliv. Transl Res. 2019;9:625–30.
27. Tuğba-Kartal A, Çağla-Mutlu Z. Comparison of sublingual and intramuscular administration of vitamin B12 for the treatment of vitamin B12 deficiency in children. Rev Invest Clin. 2020;72:380–5.
28. Kotilea K, Quennery S, Decroës V, Hermans DA. Successful sublingual cobalamin treatment in a child with short-bowel syndrome. J Pediatr Pharmacol Ther. 2014;19:60–3.
29. Allen LH. How common is vitamin B-12 deficiency? Am J Clin Nutr 2009;89:693–6.
30. Turkish Society of Hematology. Vitamin B12 deficiency diagnosis and treatment guide – B12 Vitamini Eksikliği Tanı ve Tedavi Kılavuzu. Version 1. Istanbul: Galenos Yayınevi; 2019.
31. Narang N, Sharma J. Sublingual mucosa as a route for systemic drug delivery. Int J Pharm Pharm Sci. 2011;3:18–22.
32. Vitetta L, Zhou J, Manuel R, Dal Forno S, Hall S, Rutolo D. Route and type of formulation administered influences the absorption and disposition of vitamin B12 levels in serum. J Funct Biomater. 2018;9:1–9.
33. Hua S. Advances in nanoparticulate drug delivery approaches for sublingual and buccal administration. Front Pharmacol. 2019;10:1328.
34. Sanz-Cuesta T, González-Escobar P, Riesgo-Fuertes R, Garrido-Elustondo S, del Cura-González I, Martín-Fernández J, et al. Oral versus intramuscular administration of vitamin B12 for the treatment of patients with vitamin B12 deficiency: a pragmatic, randomised, multicentre, non-inferiority clinical trial undertaken in the primary healthcare setting (Project OB12). BMC Public Health. 2012;12:1–11.
35. Castelli MC, Friedman K, Sherry J, Brazzillo K, Genoble L, Bhargava P, et al. Comparing the efficacy and tolerability of a new daily oral vitamin B12 formulation and intermittent intramuscular vitamin B12 in normalizing low cobalamin levels: a randomized, open-label, parallel-group study. Clin Ther. 2011;33:1–16.
36. Verma D, Chandra J, Kumar P, Shukla S, Sengupta S. Efficacy of oral methylcobalamin in treatment of vitamin B12 deficiency anemia in children. Pediatr Blood Cancer. 2017; 64:1–4.

37. Wang H, Li L, Qin LL, Song Y, Vidal-Alaball J, Liu TH. Oral vitamin B12 versus intramuscular vitamin B12 for vitamin B12 deficiency. Cochrane Database Syst Rev. 2018;3(3):CD004655.
38. Sharabi A, Cohen E, Sulkes J, Garty M. Replacement therapy for vitamin B12 deficiency: Comparison between the sublingual and oral route. Br J Clin Pharmacol. 2003;56:635–8.
39. Parry Strong A, Haeusler S, Weatherall M, Krebs J. Sublingual vitamin B12 compared to intramuscular injection in patients with type 2 diabetes treated with metformin: A randomised trial. N Z Med J. 2016;129:67–75.
40. Del Bo C, Riso P, Gardana C, Brusamolino A, Battezzati A, Ciappellano S. Effect of two different sublingual dosages of vitamin B 12 on cobalamin nutritional status in vegans and vegetarians with a marginal deficiency: A randomized controlled trial. Clin Nutr. 2019;38:575–83.
41. Adams JF, Ross SK, Mervyn L, Boddy K, King P. Absorption of cyanocobalamin, coenzyme b12, methylcobalamin, and hydroxocobalamin at different dose levels. Scand J Gastroenterol. 1971;6:249–52.
42. Pfeiffer CM, Caudill SP, Gunter EW, Osterloh J, Sampson EJ. Biochemical indicators of B vitamin status in the US population after folic acid fortification: Results from the National Health and Nutrition Examination Survey 1999–2000. Am J Clin Nutr. 2005;82:442–50.
43. Klee GG. Cobalamin and folate evaluation: Measurement of methylmalonic acid and homocysteine vs vitamin B12 and folate. Clin Chem. 2000;46:1277–83.
44. Wong ECC, Brugnara C, Straseski JA, Kellogg MD, Khosrow A, Pediatric reference intervals (8th ed.). Elsevier; 2021.

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All authors contributed to the concept and design of the study, acquisition and analysis of the data, drafting of the manuscript, and critical revision of the manuscript for important intellectual content. All authors had full access to the data, contributed to the study, approved the final version for publication, and take responsibility for its accuracy and integrity.

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