Combination of bolus dose vitamin D with routine vaccination in infants: a randomised trial

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ABSTRACT

Introduction: The present study was designed to compare two methods of vitamin D supplementation in infants: every two months as a routine vaccination versus a daily dose.

Methods: A randomised clinical trial was performed on 120 healthy breastfed infants between January and September 2007 in Yazd, Iran. The infants were randomly divided into three groups with different doses of vitamin D_3 supplementation: 200 IU daily, 400 IU daily and a bolus of 50,000 IU every two months. A blood sample was taken and evaluated for 25-hydroxy vitamin D and calcium levels when the infants were six months old. The data was reported as the mean and standard deviation.

Results: No significant differences were observed between the serum level of 25-hydroxy vitamin D in the groups administered with 200 IU and 400 IU vitamin D daily. However, the serum level of 25-hydroxy vitamin D reached significance in the third group (p is less than 0.001). All the blood calcium measured was below 11 mg/dl in the bolus group. A few complications such as diarrhoea and agitation, all of which were self-limited, were seen in the bolus group. No other significant side effects were reported in the other groups.

Conclusion: This study demonstrates that a bolus of 50,000 IU of vitamin D every two months with a routine child vaccination program provides the ideal serum level of vitamin D. This method produces no serious side effects and offers a highly convenient way to supply vitamin D, especially among non-compliant parents.

Keywords: 25-hydroxy vitamin D, bolus dose, dietary supplements, infant

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INTRODUCTION

In recent years, the many benefits of vitamin D have begun to be increasingly recognised. The direct and indirect role that this vitamin plays on at least 200 genes and on the immune system, as well as its preventive role against diabetes, hypertension, autoimmune disease and dental disease, are only a few examples of its importance.

Vitamin D supplementation seems to be necessary for breastfed infants since breast milk, in contrast to formula milk, does not contain enough vitamin D to satisfy the requirements of infants. The recommended daily dosage of vitamin D for neonates is about 400 units, which is equivalent to 5 ml of fish liver oil.[1,2] However, various daily doses of vitamin D, ranging from 100 IU to 1,000 IU, have also been recommended for infants.[3-11] The American Academy of Paediatrics in 2003 as well as Canadian experts have recommended that just 200 IU (800 units for the northern areas) of vitamin D is sufficient to maintain the blood level above 11 ng/ml in order to prevent rickets.[8] It has been observed, however, that despite a daily intake of 400 IU of vitamin D, some infants have a blood level of 25-hydroxy (OH) vitamin D lower than 32 ng/ml in the winter, even in countries in which mothers have a vitamin D-fortified diet.[12] This resulted in the development of a new recommendation that supports a 400 IU daily dose of vitamin D, and an even higher dose in the northern areas.[13]

Although the ideal blood level of 25-OH vitamin D remains unknown especially among infants, it is known that a serum level of 11 ng/ml is sufficient to prevent rickets, but a higher level of 25-OH vitamin D (more than 30 ng/ml) is required to control secondary hyperparathyroidism and to increase calcium absorption.[14] Owing to the unique pharmacokinetics model of vitamin D, a serum level higher than 40 ng/ml is essential to saturate the 25-hydroxylase enzyme and to optimise the kinetics of 25-OH vitamin D production.[15]

According to studies conducted by Holick, a blood level of 25-OH vitamin D higher than 30 ng/ml is required to maximise health benefits, a blood level of 25-OH vitamin D of 20–29 ng/ml is classified as "relative insufficiency" and less than 20 ng/ml, as "insufficiency".

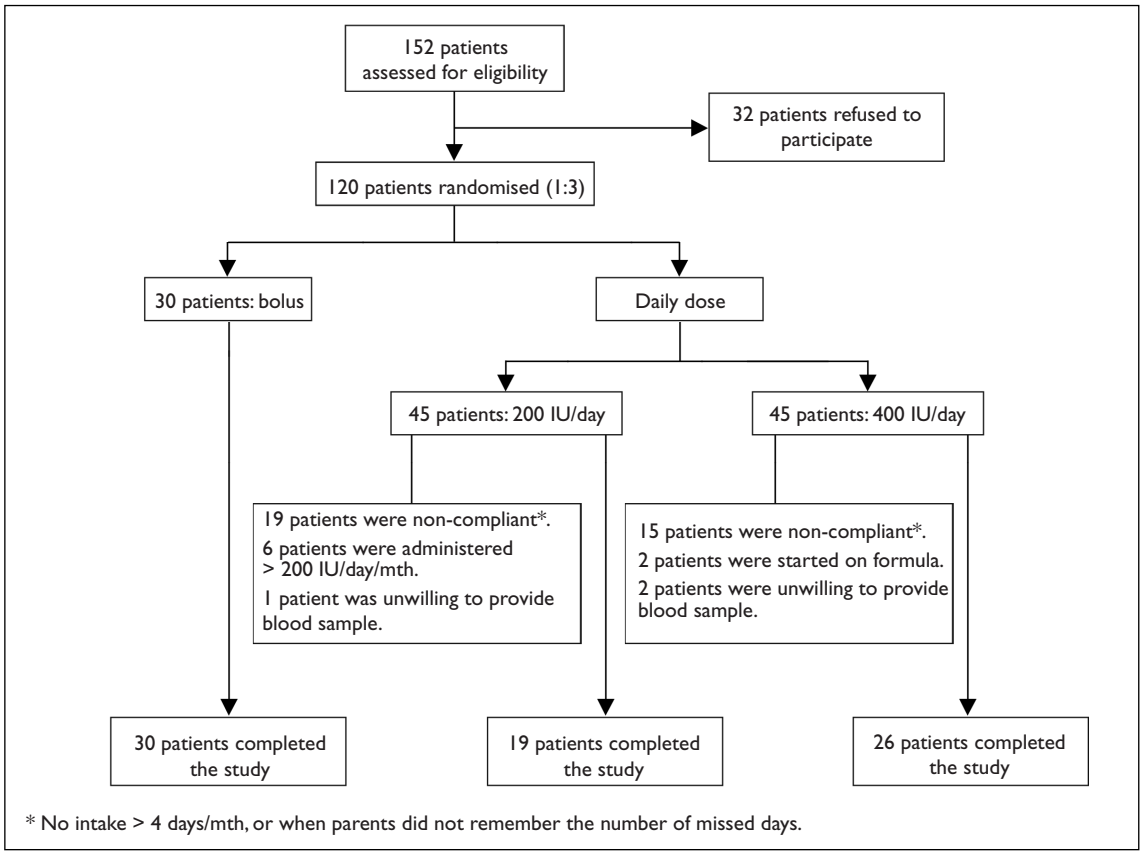

Fig. 1 Flow chart of the vitamin D clinical trial.

Some other studies have also suggested that 30–60 ng/ml is an ideal serum level for vitamin D and that levels above 100–150 ng/ml could be toxic.[16-19]

The breast milk of mothers does not contain a sufficient amount of vitamin D, and supplementation of this nutrient is therefore vital to ensure the health of breastfed infants. A daily oral dosage is an uncomplicated and effective way to ensure the adequate intake of vitamin D; nevertheless, it is inconvenient and could be missed, especially by non-compliant parents. Since there is a large gap between the effective and toxic serum levels of vitamin D, it may be worth considering the bolus doses, especially in areas such as the Middle East, where the majority of mothers are vitamin D deficient and a large number of newborns are born with vitamin D deficiencies. This study was therefore designed to compare the efficacy of combining vitamin D supplementation through a routine infant vaccination program (every two months) with daily oral doses. This study was carried out in primary care clinics in Yazd, Iran.

METHODS

This study was a randomised clinical trial that was conducted between January and September 2007. Approval to conduct the study was obtained from the ethics committee of the Shahid Sadoughi University of Medical Sciences. All parents were informed of the nature of the study. A total of 120 healthy breastfed infants weighing 2,500–4,000 g from three primary care clinics in the urban areas of Yazd city participated in this study. The infants' mothers were all healthy and were not under medication at the time of the study.

The infants were randomised based on a computer-generated randomisation list (restricted randomisation), with a randomisation ratio 3:1, so that for each infant in the bolus group, three infants in the daily group were selected, which distributed them equally within the 200 IU and 400 IU daily dosage groups. Group I (n = 45) was administered Vitamin D 200 IU daily, Group II (n = 45) was administered 400 IU daily and Group III (n = 30) was administered 50,000 IU bolus vitamin D once every two months. The infants were maintained on the same allocated dosage throughout the follow-up period if the parent continued with the protocol of the study. The paediatrician responsible for the infant allocated the next available number on entry into the trial, and each parent collected the vitamin drop and complete instructions directly from the pharmacy. The code was revealed to the researchers at the end of the analysis of the results.

Table I. Distribution of 25-OH vitamin D levels in the three groups of infants who received different doses of vitamin D.

	Amount of Vitamin D supplement		
	200 IU daily (I)	400 IU daily (II)	50,000 IU bolus/2 mths (III)
No. of infants	19	26	30
Mean 25-OH serum level ± SD (range) (ng/ml)	31.3 ± 8.5 (20–51)	38.4 ± 11.4 (23–64)	53.7 ± 19.5 (28–102)

One-way ANOVA: p = 0.001; Tukey's test: (I) vs. (II): p = 0.5; (I) vs. (III): p = 0.001; (II) vs. (III): p = 0.005
25-OH: 25 hydroxy; SD: standard deviation

The parents were supplied with free vitamin D_3 drops and complete instructions every month. They were eliminated from the study if they failed to give their infants more than 15% of the daily doses (> 4 days in a month [36 infants]), or if they did not remember the number of missed days, or in the case of Group I, if more than 200 IU of vitamin D was consumed per day (6 infants). No other form of supplementation was utilised. Infants who switched to formula milk or consumed fortified solid foods were also eliminated from the study (2 infants). The majority of withdrawals occurred in the first and last months of follow-up, when the parent either discontinued the administration of the vitamin due to common illnesses such as diarrhoea or because they had forgotten to administer the dose (Fig. 1).

The first drop of vitamin D was administered to the infants on their first visit (during their first month from 15–30 days of life) to the local primary care unit. In the case of Group III (50,000 IU bolus), the first dose was administered to the infants on their first visit, between 15–30 days after birth, followed by two more doses at two and four months of age (with their routine vaccination). At each visit, the infants were provided with five drops of 50,000 IU vitamin D_3 (Pearl vitamin D_3, Alhavy Iran Company, Tehran, Iran). All the infants were followed up monthly by the paediatrician, who had been blinded to the patient assignment, for a routine check-up as well as to monitor their growth and check for any potential complications.

When the infants were six months old, a blood sample was taken at the time of their vaccination and evaluated by the chemiluminescent immunoassay, Diasorin (Diasorin spA, Via Crescentino, Vercelli, Italy) to measure the 25-OH vitamin D levels. The blood calcium levels of Group III patients were also determined. The parents of three infants expressed an unwillingness to provide a blood sample from their infants, and these patients were subsequently eliminated from the study. At the end of the study, 19 blood samples were obtained from Group I patients, 26 from Group II patients and 30 from Group III patients. Nine blood samples were also obtained from infants (from Groups I and II) who did not follow the protocol and did not receive regular vitamin D supplementation (36 infants).

Data from an earlier study was used to estimate the sample size required, considering $\sigma_1 = 9.2$ and $\sigma_2 = 15$ (standard deviation [SD] of the serum level of 25-OH vitamin D in the daily and bolus groups, respectively). It was important to determine whether the bolus administration mode could significantly retain at least a mean difference of 10.5 (d = 10.5) in the serum level of 25-OH vitamin D, using a study power of 0.90 (β = 0.20) and (α = 0.05). 22 infants were required in each group. Taking into account a follow-up loss of 20% for the bolus group and 50% for the daily groups, a total of 120 subjects were randomly assigned to the three different trial groups described above.

The vitamin D data was distributed normally. The data was reported as mean ± SD and analysed using the Statistical Package for the Social Sciences version 9 (SPSS Inc, Chicago, IL, USA), Student's *t*-test, ANOVA and Tukey's test. A value of $p < 0.05$ was considered to be statistically significant.

RESULTS

A total of 40 female and 35 male infants participated in the study. No differences were found between their birth weight, head circumference and height at the beginning and end of the study (p = 0.6). The gender distribution was uniform across the groups (p = 0.8).

Table I shows the serum level of 25-OH vitamin D in all three groups. No significant differences were found between Groups I and II; however, both these groups had a significantly lower level of 25-OH vitamin D than Group III ($p < 0.05$). The blood calcium levels of all 30 infants in the bolus group were reported to be below 11 mg/dl.

There were three reports of side effects in Group I (diarrhoea and irritability) and four in Group II after vaccination. In comparison, six infants in Group III had

Table II. Distribution of 75 infants who received different doses of vitamin D according to their 25-OH vitamin D levels.

Vitamin D dosage	25-OH vitamin D level (ng/ml)	No. (%)
200 IU/day (n =19)	20–29	12 (63)
	30–60	7(37)
	> 60	0
400 IU/day (n = 26)	20–29	6 (23)
	30–60	18 (69)
	> 60	2 (8)
50,000 IU/2 mths (n = 30)	20–29	1 (3)
	30–60	23 (77)
	> 60	6 (20)

25-OH: 25-hydroxy

diarrhoea for 2–3 days, and this occurred after each bolus for four of the six infants. Three infants became irritable for 3–4 days after administration of one bolus. In one infant, irritability occurred after each bolus of vitamin D. However, it was self-limited and none of the infants required further medical attention.

Table II shows the number of infants in each treatment group according to Holick's definition of vitamin D deficiency. The serum level of 25-OH vitamin D was < 20 ng/ml in none of the groups. Two infants in the bolus group had a serum level > 100 ng/ml (101 ng/ml and 102 ng/ml). Among the nine infants who did not take a regular drop of vitamin D, all had a serum level < 30 ng/ml, with five < 20 ng/ml and two < 11 ng/ml. The coefficient of variation in repeated measurements was < 11%.

DISCUSSION

Although adding extra doses of vitamin D supplementation to the mothers' daily diet makes it possible to achieve an acceptable level of vitamin D in mothers' breast milk, many studies have indicated that the best way to satisfy an infant's need is to directly supply the child with vitamin D supplementation.[20] This study has demonstrated that the daily administration of 200 IU and 400 IU of vitamin D is sufficient to provide a blood level of 20 ng/ml, which some studies have considered to be the ideal level.[2] However, considering that there is a large safe gap for vitamin D toxicity, as well as the recent recommendations of some studies for a level higher than 30 ng/ml as the ideal level for adults, it may be more desirable to administer doses higher than 200 IU or 400 IU. In fact, this study found that even in the summer, some of the infants had a serum level of less than 30 ng/ml, despite having been supplied with the daily recommended doses of vitamin D.

There are limited studies on bolus supplementation in infants. A 1985 study found that a single dose of 600,000 IU led to toxicity and doses of 100,000 IU every three months provided a satisfactory level.[21] The results of our study also indicate that a bolus of 50,000 IU of vitamin D every two months along with routine vaccination in a child not only results in a serum vitamin D level of more than 20 ng/ml, but is also devoid of any major side effects and complications. In addition, it is highly convenient, especially among non-compliant parents. In this study, all 30 infants in the bolus group completed the course of the study, but at least one third of the participants in the daily group did not receive daily doses of vitamin D due to illness or inattentive parents, and thus had to be eliminated from the study. Some of these infants also had low levels of vitamin D.

The side effects of the bolus doses, such as crying and diarrhoea observed in infants, were minimal and non-specific. It was difficult to distinguish these from the side effects of the vaccination, although in one of the infants, the agitation occurred even without the infant having undergone the simultaneous vaccination (first visit). Four cases of diarrhoea were reported, all of which were self-limited and did not require additional medical support, although this complaint may well be related to the simultaneous administration of the oral polio vaccine. None of the infants in the bolus group had a high calcium level. This result is similar to that reported by another study, where doses ≤ 200,000 IU were administered in neonates.[22]

There are several limitations to this study. First, the elimination rate of infants in one of the daily groups was higher than expected. This could have been a result of our strict definition of non-compliance (more than four days per month). Parents may have easily missed the doses due to common viral illnesses in the infant or other personal problems. Second, an intention to treat analysis was not performed. Our study conclusion, which was based on the best case scenario in the daily group, showed that ideally, the daily dose should be similar to the bolus dose (level > 20 ng/ml is considered optimal). Hence, the analysis would not have affected the conclusion. Third, the vitamin D levels of both the infants and the mothers were not checked at the beginning of the study. According to another local study and another study conducted in Iran, more than half of the mothers were vitamin D deficient at the time of delivery.[22,23] Although this study was not designed to show the changing pattern of the vitamin D levels, and it was unclear when the vitamin levels normalised in this study group, it appears that the bolus dose may achieve an adequate level sooner than the daily doses, especially

in areas with a high prevalence of vitamin D deficiency in mothers. Routine vitamin D supplementation cannot normalise the blood parameter of vitamin D deficiency even in formula-fed infants with low vitamin D levels.[24] Finally, this study was unable to adequately address the side effects of the bolus dose, except in one case where irritability occurred without the vaccine in the first dose. A placebo randomised trial is required in order to clarify this issue.

Thus, the bolus method of administering 50,000 IU vitamin D_3 every two months is an easy and convenient method of supplementing infants with vitamin D. It is reliable and provides an adequate level of vitamin D without incurring any serious side effects. The bolus method is economically more desirable than the daily method, as it may reduce costs by up to 100 times. With a population of 100,000 infants and based on the current costs of drugs in Iran, this could save up to US$500,000 in six months.

In conclusion, it is possible to provide close to the ideal level of vitamin D for infants through the use of daily supplementation. However, it was observed that even with compliance, some of the infants had a vitamin D serum level below 30 ng/ml, even in summer in a city like Yazd. Administering 50,000 IU bolus of vitamin D_3 every two months (equivalent to 800 IU daily), especially in combination with routine vaccination in children, is a convenient way to provide a sufficient level of vitamin D that may also cost less. Moreover, its side effects are limited, with no extra need for medical intervention. However, further investigations are required to evaluate the potential complications of bolus therapy in infants and to compare this method with a program of monthly administration of 20,000 bolus, in order to determine the most desirable method of vitamin D supplementation that can be integrated with routine vaccination or day clinical care to improve patient compliance to this important supplement.

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