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Original Research

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A Randomized Controlled Trial of Oral Vitamin D Supplementation in Pregnancy to Improve Maternal Periodontal Health and Birth Weight

Farhan Raza Khan[1], Tashfeen Ahmad[2], Rabia Hussain[3], Zulfiqar Ahmed Bhutta[4]

Contributors:
[1]Assistant Professor, Department of Dentistry, Aga Khan University, Karachi, Pakistan; [2]Assistant Professor, Department of Surgery, Aga Khan University, Karachi, Pakistan; [3]Professor, Department of Pathology, Aga Khan University, Karachi, Pakistan; [4]Professor, Division of Women & Child Health, Aga Khan University, Karachi, Pakistan.

Correspondence:
Dr. Khan FR, Department of Dentistry, Aga Khan University, Karachi 74800, Pakistan. Fax: 0092-21-4934294, Phone: 0092-3052225117. Email: farhan.raza@aku.edu

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***Abstract*:**

Background: Studies show that the presence of maternal periodontal disease and hypovitaminosis D are associated with multiple health outcomes in neonates including low birth weight (LBW). It's speculated that vitamin D supplementation in pregnancy may improve birth weight and maternal periodontal health. An interventional study was planned to determine if oral vitamin D supplementation to pregnant women reduces the incidence of LBW among neonates and to determine if oral vitamin D supplementation improves periodontal probing depth (PD), bleeding on probing (BoP) and clinical attachment loss (AL) among pregnant women.

Materials and Methods: A community-based randomized controlled trial was conducted at Jhelum, Pakistan. Pregnant females (n=85) at ~12 weeks of gestation were recruited. Periodontal parameters such as PD, BoP and the AL were assessed. Blood samples were drawn to measure vitamin D levels and blood indices. The birth weight of the babies born to study participants was also recorded. The study was registered at clinical trials. gov (# NCT01422122).

Results: There were 85 pregnant women in the final analysis. Of which 36 were supplemented with vitamin D and 49 were given placebo. Participants were comparable at baseline. Periodontal disease was detected in 5/85 (6%) pregnant mothers. Birth weight of only 63 deliveries could be recorded. The proportion of LBW deliveries was 18/63 (29%). There was no difference in the birth weight and periodontal status of women supplemented with vitamin D compared to placebo.

Conclusions: About 6 months oral supplementation of vitamin D to pregnant women did not bring any improvement in their periodontal status. Similarly, no significant improvement was observed in the birth weight.

Key Words: Low birth weight, periodontal health, pregnancy outcome, vitamin D

Introduction

Studies suggest that periodontal disease is one of the known sources of persistent bacterial infection and it has the potential to induce systemic inflammatory responses that increase the risk of adverse pregnancy outcomes.[1-3] However, the results of the studies done on this relationship have been mixed. A systemic review[4] done on 25 studies showed that 18 studies suggested an association between periodontal disease and increased the risk of adverse pregnancy outcome (odds ratio [OR] 1.10-20.0), while seven studies found no such association (OR 0.78-2.54). Similarly, a clinical trial[5] inferred that although periodontal treatment during pregnancy is not hazardous to the expecting mother but it does not prevent any adverse outcomes such as preterm birth, fetal growth restriction, or preeclampsia. The European Federation of Periodontology and American Academy of Periodontology jointly validated the current scientific evidence from randomized controlled trials that does not recommend any periodontal therapy for improving pregnancy outcomes.[6] However, it was observed that periodontal therapy in pregnancy certainly leads to improved maternal periodontal conditions.[7]

Vitamin D and its active metabolite 1,25-dihydroxyvitamin D (1,25(OH)2D) have classical actions on calcium balance and bone metabolism. Without sufficient vitamin D, the intestine cannot absorb calcium and phosphate adequately, which leads to secondary hyperparathyroidism and a lack of new bone mineralization (known as rickets in children and osteomalacia in adults).[8] Vitamin D metabolites have shown to alter the inflammatory response[9] and have also demonstrated antimicrobial effects,[10] and thus vitamin D supplementation is likely to affect the periodontal status. A study[11] of vitamin D and calcium supplementation on periodontal health of otherwise healthy subjects showed that the supplement takers had better periodontal outcomes. Therefore, we hypothesized that if pregnant women are supplemented with vitamin D, their periodontal health is likely to improve.

A recent systematic review and meta-analysis on 24 studies[12] done on pregnant women with 25(OH)D levels <20 ng/mL clearly demonstrated an increased risk of preeclampsia (OR

1.50-2.90), gestational diabetes mellitus (OR 1.12-1.70), preterm birth (OR 1.08-2.31), and small for gestational age (OR 1.08-2.15).

Vitamin D insufficiency has been increasingly recognized as a public health concern and its supplementation to pregnant women has been considered as an emerging intervention to prevent adverse pregnancy outcomes.[13] Thus, we speculated that vitamin D supplementation among pregnant may not only improve maternal periodontal health but also reduce the incidence of (LBW) babies.

Objectives

1. To determine if vitamin D supplementation to pregnant women reduces the incidence of LBW in neonates.
2. To determine if oral vitamin D supplementation improves periodontal probing depth (PD) and clinical attachment loss (AL) among pregnant women.

Materials and Methods

It was a community based, double-blinded, block-randomized, placebo-controlled trial conducted at district Jhelum, Pakistan. A list of the pregnant women was generated with the help of lady health visitors (LHVs) posted in the rural area. A non-probability, convenience sampling was done to recruit the participants. Pregnant females with ultrasound confirmed gestational age of 12-16 weeks were asked to participate in the study. The laboratory phase was done at the Aga Khan University Hospital, Karachi, Pakistan. The study was approved by AKU ERC (ref# 147-Ped-ERC-2010) and was registered at the clinicaltrials.gov with identifier# NCT01422122. Written informed consent was obtained from the study participants in the local language. Physical examination, blood pressure reading and anthropometric measurements of the pregnant women were done. The participants with pre-existing Type 1 or II diabetes or known high level of vitamin D or those with more than eight permanent teeth missing were excluded.

Sample size

The sample size was calculated for both objectives. For periodontal outcome, the sample size necessary to detect a reduction in PD anticipated with vitamin D supplementation to pregnant was 31 (difference: 0.5 mm, standard deviation [SD]: 0.7, level of significance: 0.05, power: 0.80). Since there were two groups, i.e. intervention versus placebo so 62 participants were needed. For birth outcome, the sample size necessary to detect a 100 g change in birth weight anticipated with supplementation of vitamin D among pregnant, (mean birth weight 3100 g, SD: 150 g, difference: 100 g, level of significance: 0.05, power: 0.80), the required number was 36 in each group. We inflated the sample by 20% to adjust for the dropouts and hence inducted 86 females assuming to get the 43 expecting mother in each arm. The latter sample size could answer both research questions.

Intervention group comprised of pregnant women who received oral dose of 4000 IU of vitamin D daily for approximately 6 months (starting from 12-16 weeks until the end of the pregnancy). The control group comprised of pregnant women who received placebo for the same duration. The study drug and placebo were prepared by the pharmacy division at the Aga Khan University. It's an ISO certified department for the drug preparation and delivery. Both study drugs (vitamin D and placebo) were supplied in identical syrup bottles labeled either X or Y.

Community health workers (CHWs) were responsible for the delivery of supplementation to the study participants. The CHWs were assigned to visit study participants, on a fortnightly basis. The first supplementation was provided by the physician at the time of recruitment; later on, the CHWs continued to replenish the supply fortnightly.

The investigators, study staff, and the participants were blinded about the group allocation. Allocation codes for vitamin D and placebo were kept in a sealed envelope in a locked cabinet at the Aga Khan University until the completion of the study.

The study participants were randomized in blocks. The block randomization was originally made for another study (aimed at different outcomes where over 400 pregnant women participated), but this study focuses on the birth weight and the periodontal outcomes only. The present protocol was conceived, approved, and started at a later date; thus a big number of potentially eligible pregnant women had already passed their 16th week of pregnancy and consequently were not eligible in this study. Moreover, a number of eligible females in the randomized blocks did not consent for participation; therefore, the 86 participants originally inducted in this study with an assumption of having roughly equal numbers in the two study arm were found to be distributed unequally. This was revealed at the end of the study when randomization codes were opened. We ended up with 36 participants in the vitamin D (intervention) group and 49 in the placebo. However, as mentioned earlier, the minimum required sample for this study was 36 participants per group; therefore, both the research questions could still be addressed.

To nullify any potential effect of oral hygiene practice on periodontal status and birth outcome, both study groups were given similar standardized oral hygiene education.

Data collection

The interviews took place at rural data collection centers and at women's residence in district Jhelum. All study participants underwent clinical dental examination on a mobile dental unit. The periodontal examination at the baseline (12-16th week of pregnancy) included full-mouth examination performed by single trained dentist (FRK*).

For case definitions: We labeled a baby as LBW, if it weighs <2500 g at birth. For hypovitaminosis D among women, serum level <19.9 ng/mL was used as the cutoff. For the definition of periodontal disease, at least two sites with AL of ≥2 mm or PD ≥3 mm was employed.

All periodontal measurements were made with the University of Michigan periodontal probe. Bleeding on probing (BoP) was recorded as yes or no. Recordings were taken from four sites per tooth. The endpoint periodontal examination was done after 2-3 weeks of the delivery the baby. To calculate the method errors, 5% baseline and 5% end-line examinations were repeated for PD parameter and yielded a good reliability of 0.80 and 0.76, respectively.

Blood samples (10 ml) were obtained at the baseline and at the end for vitamin D levels (analyzed on DiaSorin-LIASON Inc, kit). This is based on a direct competitive chemiluminescenec immunoassay.

Data analysis

Data were analyzed on SPSS 19.0. The frequency distribution of all the categorical variables and means and SD of continuous variables were computed. Independent sample t-test was applied to compare the means of continuous variables (age, body mass index [BMI], vitamin D, PD, AL, and Vitamin D). Repeated measures ANOVA were applied to see before-after difference in the vitamin D level. For birth outcome: OR was applied by keeping the LBW (<2500 g) as an outcome variable while age, vitamin D status, education status, parity status, baseline PD, baseline AL, BoP and decayed missing and filled (DMFT), etc., were kept as binary independent variables. For periodontal outcome: OR was applied by keeping the periodontal disease (PD ≥3 mm) as an outcome while treating previously described exposures as binary independent variables. Only statistically significant variables were planned to be taken to the two logistic regression models (one each for periodontal disease and LBW). The level of statistical significance was kept at 0.05.

Results

There were 85 pregnant women in the final analysis of which 36 received 6 months of oral vitamin D supplementation. There were no smokers or diabetics in the studied sample. None of the participants has ever received any periodontal surgery or dental implant or visited a dentist in last 6 months.

The participants in the two groups were similar in age, BMI, hemoglobin concentration, education status, parity status, oral hygiene practice, number of teeth, DMFT count, BoP, PD, and AL (Table 1).

Since the study was conducted at a rural community setting, so we could only get a record of birth weight from 63/85 (74.1%) participants. Of these, 27 mothers received intervention and 36 were on placebo. The proportion of LBW babies (<2500 g) was 18/65 (28.6%). The mean birth weight of babies delivered by vitamin D supplemented mothers was not significantly different than the ones who took placebo. The PD and AL did improve mildly in the vitamin D supplementation group, but the difference between the study groups at endpoint remained statistically insignificant (Table 2).

Table 1: Characteristics of study participants at baseline.

Characteristics	Mean±SD		*P* value
	Vitamin D (*n*=36)	Placebo (*n*=49)	
Age (years)	26.22±4.2	27.02±4.8	0.43
BMI	23.72±2.1	24.96±2.6	0.19
Hb (g/dL)	8.6±1.45	8.9±2.1	0.18
Number of teeth	29.7±3.7	30.2±1.7	0.47
PD (mm)	1.83±0.5	1.81±0.6	0.87
AL (mm)	1.2±0.9	1.0±0.85	0.30
Vitamin D level (ng/mL)	12.91±6.3	12.74±5.3	0.90
Vitamin D status	***n* (%)**	***n* (%)**	***P* value**
<19.9 ng/mL	26 (81.2)	36 (89.2)	0.49
≥20 ng/mL	6 (18.8)	4 (10.8)	
Parity status			
0	10 (27.8)	11 (22.4)	0.31
1-2	17 (47.2)	18 (36.7)	
>3	9 (25.0)	20 (40.9)	
Education status			
≤5 years of schooling	26 (72.2)	28 (57.1)	0.16
≥6 years of schooling	10 (27.8)	21 (42.9)	
Oral hygiene practice			
Brush	24 (66.7)	32 (65.3)	0.89
Traditional[¥]	12 (33.3)	17 (34.7)	
DMFT categories			
Low: 0-3	26 (72.2)	29 (59.2)	0.21
High: 4 and above	10 (27.8)	20 (40.8)	
BoP			
Yes	18 (50)	25 (51)	>0.99
No	18 (50)	24 (49)	

Differences between the two groups were assessed using independent samples *t*-test or Chi-square (or Fisher exact) tests. Level of statistical significance was kept at 0.05. Intervention group was supplemented with oral vitamin D 4000 IU/day for 6 months. [¥]Traditional methods refer to using Miswak (neemwood tree bark). BMI: Body mass index, Hb: Hemoglobin, PD: Probing depth, AL: Attachment loss, SD: Standard deviation, DMFT: Decayed missing and filled, BoP: Bleeding on probing

Table 2: Comparison of two groups at the end-point for birth weight and periodontal outcomes.

Variable	Mean±SD		*P* value
	Vitamin D (*n*=36)	Placebo (*n*=49)	
Birth weight in kg	2.80±0.52 (*n*=27)	2.98±0.73 (*n*=36)	0.26
PD in mm	1.72±0.52 (*n*=36)	1.76±0.60 (*n*=49)	0.79
AL in mm	0.86±0.80 (*n*=36)	0.98±0.91 (*n*=49)	0.35
Vitamin D levels in ng/mL	15.36±7.61 (*n*=32)	11.36±4.71 (*n*=40)	<0.01*

Independent sample *t*-test was applied at α 0.05. There were 36 and 49 participants in intervention in placebo, respectively. However, birth weight of only 63 mothers was measured. Vitamin D measurements were done in 72 participants only. *Denotes statistical significance. PD: Probing depth, AL: Attachment loss

None of the dependent variables among the given list was statistically associated with LBW in neonates (Table 3).

Of 85 mothers examined for periodontal status, only 5 had periodontal disease (PD >3 mm). Except for the presence of baseline periodontal disease (PD >3 mm), no statistically significant association with the periodontal disease was observed for any variable at the endpoint (Table 4).

The mean vitamin D levels in the intervention and placebo at baseline were 12.9±6.3 ng/mL and 12.7±5.3 ng/mL, respectively. The vitamin D levels in the intervention group improved with oral supplementation, reaching to 15.63 ± 7.6 ng/mL at endpoint, but this improvement was not enough to reach a sufficient plasma level of this micronutrient (cutoff of 20.0 ng/mL). The placebo group showed a mild decline in their vitamin D level. It dropped to 11.3 ± 4.7 ng/mL at the endpoint. This resulted in a statistically significant difference ($P < 0.01$) in the mean vitamin D levels of the two study groups at the endpoint (Figure 1).

The study flow diagram is shown in Figure 2 and the speculated relationship between variables is shown in Figure 3.

Discussion

Vitamin D is an essential fat-soluble vitamin and a key modulator of calcium metabolism. Because calcium demands increase in the third trimester of pregnancy, vitamin D level becomes crucial for maternal health, fetal skeletal growth, and optimal maternal and fetal outcomes. Around 5-50% of pregnant women and 10-56% of breastfed infants are vitamin D deficient. Adverse pregnancy outcomes such as preeclampsia, LBW, neonatal hypocalcemia, poor postnatal growth, bone fragility, and increased incidence of autoimmune diseases have been linked to low vitamin D levels during pregnancy and infancy.[14]

Recent evidence supports a role of maternal vitamin D status, particularly early in pregnancy, in modulating the risk of pregnancy complications and in sustaining fetal growth, bone development, and immune maturation.[15] As most of the studies were observational in nature, there was a need of randomized controlled trials to investigate the effects of vitamin D during pregnancy to study birth weight and other associated outcomes. Similarly, periodontal disease and low birth with have been extensively studied (mostly with case-control and cohort study designs) but due to the heterogeneity of the

Table 3: Variables associated with low birth weight in the study participants.

Variable	Categories	*n*	Low birth weight frequency (%)	Odds ratio (95% CI)
All		62	18 (29.0)	
Age in years	≤26	32	6 (18.75)	1.0
	≥26.1	30	12 (40.0)	1.24 (0.38-4.13)
	P value			0.77
Vitamin D at baseline (ng/mL)	≥20	9	2 (22.2)	1.0
	≤19.9	53	16 (30.1)	0.50 (0.08-2.93)
	P value			0.60
Education	No schooling	22	7 (31.8)	1.14 (0.29-2.55)
	Some education	40	11 (27.5)	1.0
	P value			0.78
Study group	Intervention	27	7 (25.9)	1.0
	Placebo	35	11 (31.4)	1.21 (0.41-3.54)
	P value			0.79
Parity status	≤2	43	12 (27.9)	1.0
	≥3	19	6 (31.6)	0.1.13 (0.37-3.47)
	P value			>0.99
Bleeding on probing	Absent	29	6 (20.6)	1.0
	Present	33	12 (36.4)	1.76 (0.58-5.28)
	P value			0.42
Probing depth at baseline (mm)	≤3	55	14 (25.4)	1.0
	≥3.1	7	4 (57.1)	2.24 (0.57-8.76)
	P value			0.26
Attachment loss at baseline (mm)	<2	44	13 (29.5)	1.0
	≥2	18	5 (27.8)	0.94 (0.30-3.02)
	P value			>0.99
DMFT	<4	55	16 (29.1)	1.0
	>4.1	7	2 (28.5)	0.98 (0.19-5.20)
	P value			>0.99
Oral hygiene	High plaque	22	6 (27.3)	1.10 (0.37-3.34)
	No plaque	40	12 (30.0)	1.0
	P value			>0.99

Odds ratio was calculated for strength of association between variables. Outcome variable: Low birth weight (≤2500 g). CI: Confidence interval, DMFT: Decayed missing and filled

Table 4: Variables associated with the periodontal disease in the study participants.

Variable	Categories	*n*	Periodontal disease frequency (%)	Odds ratio (95% CI)
All		85	5 (5.9)	
Age in years	≤26	43	1 (2.3)	1.0
	≥26.1	42	4 (9.5)	4.10 (0.47-41.34)
	P value			0.36
Vitamin D at baseline (ng/mL)	≥20	11	0 (0)	1.0
	≤19.9	61	5 (7.8)	1.96 (0.10-38.0)
	P value			>0.99
Education	No schooling	32	2 (6.2)	0.90 (0.14-5.72)
	Some education	53	3 (5.7)	1.0
	P value			>0.99
Study group	Intervention	36	2 (5.5)	1.10 (0.18-6.94)
	Placebo	49	3 (6.1)	1.0
	P value			>0.99
Parity status	≤2	56	1 (1.8)	1.0
	≥3	29	4 (13.8)	7.72 (0.83-72.4)
	P value			0.06
Bleeding on probing	present	43	4 (9.3)	0.26 (0.03-2.39)
	absent	42	1 (2.3)	1.0
	P value			0.36
Probing depth at baseline (mm)	≤3	76	2 (2.6)	1.0
	≥3.1	9	3 (33.34)	12.7 (1.86-86.3)
	P value			0.01*
Attachment loss at baseline (mm)	<2	58	2 (3.6)	1.0
	≥2	27	3 (11.1)	3.22 (0.50-20.4)
	P value			0.32
DMFT	<4	73	3 (4.1)	1.0
	>4.1	12	2 (16.7)	4.06 (0.61-26.9)
	P value			0.17
Oral hygiene	No plaque	30	1 (3.3)	1.0
	Plaque present	55	4 (7.3)	2.18 (0.23-20.4)
	P value			0.65

Odds ratio was calculated for strength of association between variables. Outcome variable: Presence of periodontal disease (PD>3 mm). *Denotes statistical significance. CI: Confidence interval, DMFT: Decayed missing and filled

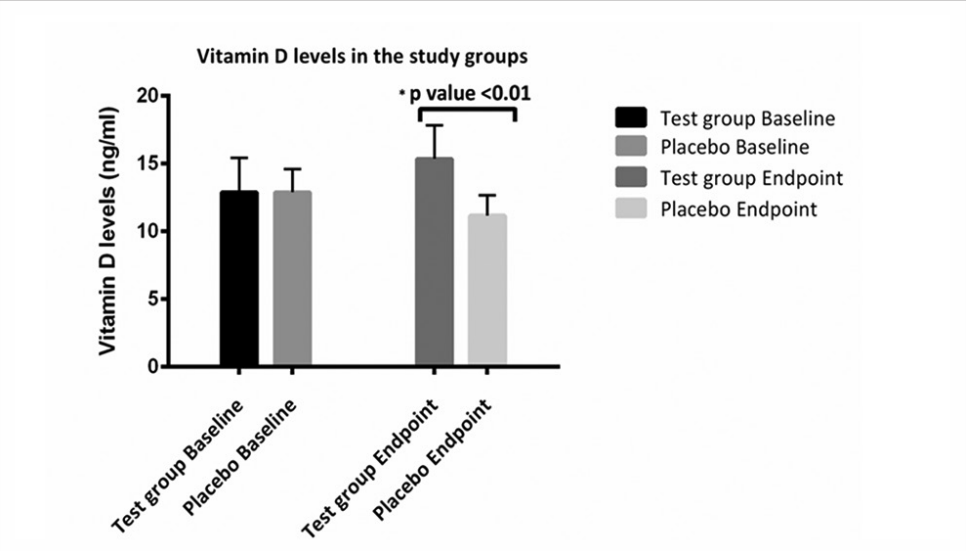

Figure 1: Comparison of serum vitamin D concentration among study groups. Repeated measures ANOVA was applied. Level of significance was kept at 0.05.

data and scarcity of clinical trials, any reported association between periodontal disease and LBW is recommended to be interpreted with caution.[16-18]

Boggess *et al.*[19] suggested that vitamin D supplementation should be offered to pregnant females for improvement in their periodontal status. We used a randomized clinical trial

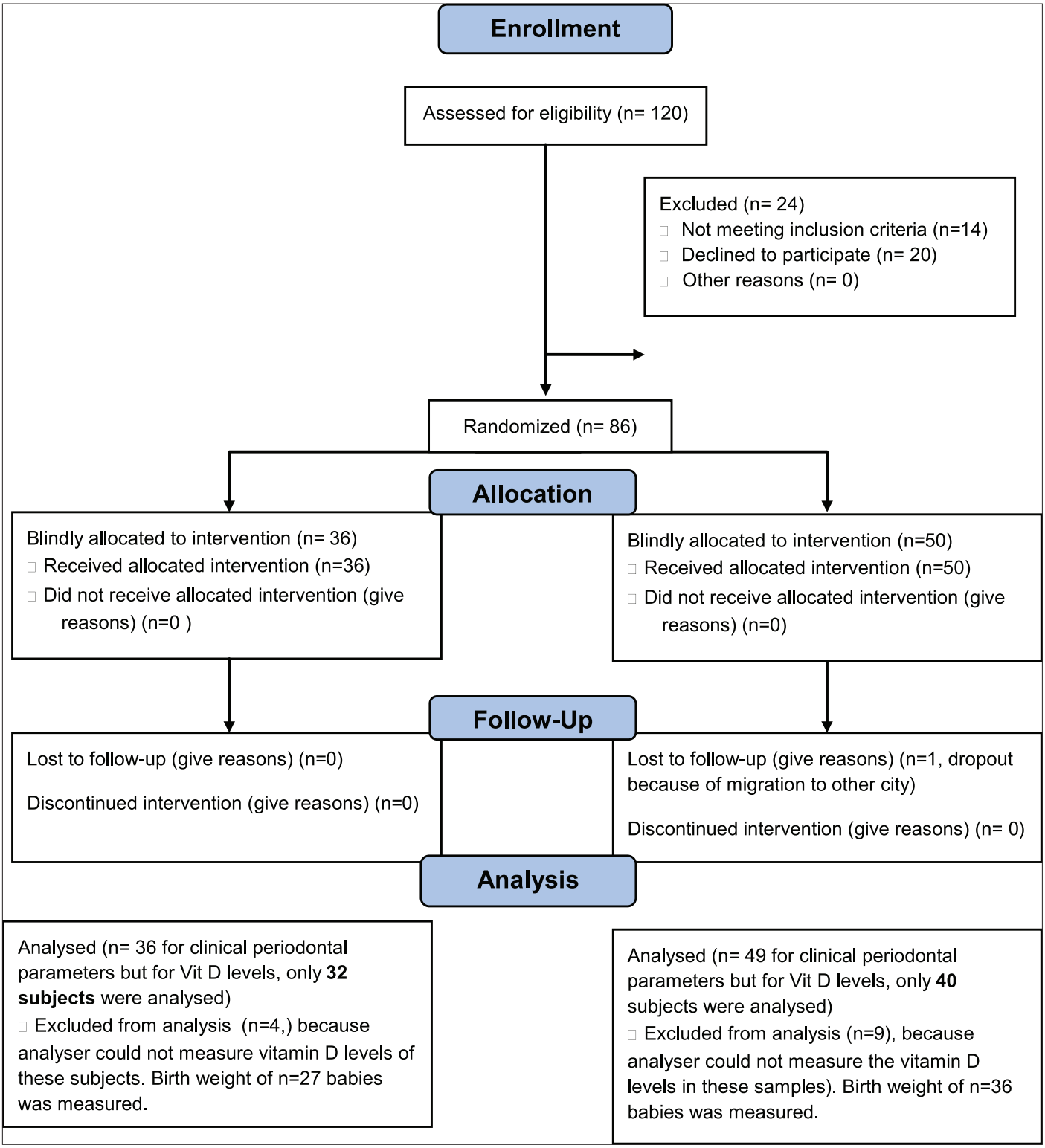

Figure 2: Study flow diagram: A randomized controlled trial of oral vitamin D supplementation in pregnancy to improve periodontal health and birth weight.

design to determine if vitamin D supplements can improve periodontal health among pregnant and can also bring any improvement in birth weight (Figure 3) We did not find any significant protective relationship of vitamin D intake on the parameters of periodontal health.

Our findings are different from the earlier work that shows an inverse relationship of vitamin D level with periodontal disease as reported by Bashutski *et al.*[20] Similarly, Miley *et al.*[11] have shown that the use of calcium (1000 mg/day) and vitamin D (400 IU/day) oral supplements was associated

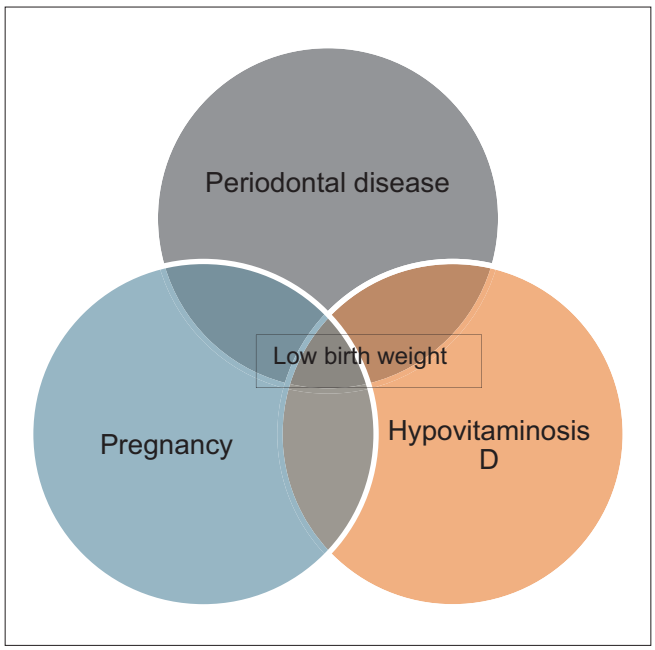

Figure 3: The relationship between low vitamin D and periodontal disease among pregnant and how these factors relate to low birth weight.

with lower PDs compared to the controls. The follow-up of this research was done by Garcia *et al.*[21] who also reported that vitamin D supplement users have less periodontal disease than non-users, although the differences were not statistically significant ($P > 0.05$) at 1-year time point. The major limitation in drawing a relationship of vitamin D and periodontal health from earlier studies[19-21] is the fact that those were mainly observational in nature. Although, Krall *et al.*[22] carry out a randomized, placebo-controlled trial on 82 participants who received calcium and 700 IU of vitamin D per day for 3 years but their main outcome was not periodontal disease. They did collect some data on the periodontal disease at the endpoint, but unfortunately, the corresponding data at the baseline were not available. Using the NHANES analyses; Dietrich et al.[23] reported that higher serum 25(OH)D levels correlated with less AL.

Krall *et al.*[22] and Hildebolt[24] have suggested the need of randomized controlled trial (RCT) to study the association of vitamin D and periodontal disease. However, the findings of the present trial do not show this association (Table 4). Our results are more in agreement with Liu et al.[25] who found that plasma vitamin D levels had no association with periodontal health. An interesting finding was reported by Millen *et al.*[26] that vitamin D status was inversely associated with PD and BoP but bears no association with periodontal disease based on the alveolar crest height. It appears that changes in the periodontal tissues by vitamin D supplementation is attributed more to its anti-inflammatory effects rather than it acting through the bone mineral density.[21,23,27]

The strength of our study is that we addressed the vitamin D and periodontal disease association in a RCT design and used a cohort of pregnant females that has not been studied before. Moreover, any potential effect of oral hygiene practice on the periodontal status and birth outcome was nullified by giving similar standardized oral hygiene education to both study groups.

However, there are a number of limitations in the present study. First, in spite of adhering to a vigilant case definition (PD > 3 mm), we manage to recruit only 9 periodontitis participants. This is mainly because the sample selection was not based on the periodontal status of women. All the participants were otherwise healthy pregnant females. Second, we did not carry out an interim analysis of vitamin D during the course of pregnancy (Figure 2).

Despite oral vitamin D supplementation in a dose of 4000 IU per day for nearly 6 months, 92% of pregnant females remained deficient in this micronutrient (Figure 1). An interim analysis could have identified the problem and appropriate rectifying measures could have been taken. A probable explanation of not reaching the sufficient level may be attributed to either poor compliance among trial participants (despite fortnightly visit of LHVs at the participants household) or probably the drug decomposition (as it was in a syrup form) has taken place in the hot weather as refrigerators are not available in the majority of households in the rural district of Pakistan where this trial was undertaken. The pharmacy services were unable to formulate the intervention drug and placebo in the pill form.

The third limitation was not employing radiographs to quantify alveolar crest levels. This is attributed to the logistics of assigning a radiographic unit in a rural community; moreover, it's observed that pregnant women, in general, are reluctant to undergo any radiographic examination unless indicated for their health care. It can also be argued that lack of ethnic diversity in our sample limits our ability to generalize these results to a broader population of pregnant females.

Endogenous production stemming from sunlight exposure is a major source of serum vitamin D. A lack of information regarding sun exposure is another limitation of our study. However, for pregnant females in Pakistan, who probably get less sun exposure (for the cultural practice of covering their body and face) or are less efficient in generating endogenous vitamin D, the dietary and supplemental sources of vitamin D take on a greater importance.

Pregnant females are known to be at a higher risk for acquiring vitamin D deficiency.[28] It is reported that nearly 46% of the pregnant females in Karachi (largest city of Pakistan with estimated population of 20 million) are vitamin D deficient.[29] These numbers are alarming and have important public health

implications. Irrespective of any periodontal benefits, vitamin D supplementations among pregnant females (especially to those who are deficient in this micronutrient) have its own merits in terms of improving the general bone health.

Conclusions

About 6 months oral supplementation of vitamin D to pregnant women did not bring any improvement in their periodontal status. Similarly, no significant improvement was observed in the birth weight.

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