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Positive effect of low dose vitamin D supplementation on growth of fetal bones: A randomized prospective study

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ABSTRACT

The effect of vitamin D supplementation on growth of fetal bones during pregnancy is unclear. The aim of this study was to assess the effect of low dose vitamin D supplementation during pregnancy on bony anthropometric aspects of the fetus. In this prospective randomized trial, 140 patients were divided into two equally matched groups according to age, 25(OH)D level, exercise, and dietary intake. Then 1000 IU per day vitamin D supplement was given to the intervention group while the control group received placebo. Then crown-rump length (CRL) and femur length (FL) during the first trimester and humerus and femur lengths as well as their proximal metaphyseal diameter (PMD), midshaft diameter (MSD) and distal metaphyseal diameter (DMD) in the second and third trimester were measured using ultrasonography technique. Finally, no significant difference was observed for CRL ($p = 0.93$). Although FL was not statistically significant in the first trimester ($p = 0.54$), its measurement in the intervention group and the control group in the second (28.87 ± 2.14 vs. 26.89 ± 2.08; $p \leq 0.001$) and the third (65.31 ± 2.17 vs. 62.85 ± 1.94; $p \leq 0.001$) trimesters was significantly different. Femoral PMD, MSD, and DMD measurement increased more in the intervention group in comparison with the control group with P values < 0.05. HL measurement in the intervention group and the control group in the second (28.62 ± 1.94 vs. 27.23 ± 2.08; $p \leq 0.001$) and the third (61.29 ± 2.84 vs. 59.85 ± 1.79; $p \leq 0.001$) trimesters revealed significant differences. Humeral PMD, MSD, and DMD measurement increased in the intervention group in comparison with the control group with P values < 0.001 for all. It is suggested to prescribe low dose vitamin D (1000 IU per day) from early pregnancy with possible increment in length and diameter of femur and humerus bones of the fetus.

1. Introduction

Vitamin D as one of the main elements has a significant role in regulating calcium and phosphorus in the body [1–3]. Vitamin D effect on cell differentiation and maturation [4,5], immunity [6], improved quality of life [7], autoimmune diseases such as thyroiditis, cancers and cardiovascular diseases have been proven [8–10]. Vitamin D can be acquired through diet or sun exposure. Its deficiency is reported to be common amongst people, especially pregnant women [11–13].

One of the challenging issues for the role of vitamin D supplementation during pregnancy is its effect on the newborn anthropometry measurements such as birth weight, head circumference and long bone length [14–17]. Based on the literature review, with regard to the impact of vitamin D on fetus, there are controversies on vitamin D deficiency consequences on the estimated fetal weight [18–21], growth retardation [18–20,22,23], and femur length (FL) [24–26] and crown-rump length (CRL) [27]. Although several observational studies showed the effect of vitamin D during pregnancy on anthropometric fetal features [24,26–28], as far as we know, no randomized clinical trial has been performed to describe vitamin D supplementation effect on the fetal bone growth [29]. High prevalence of vitamin D deficiency in addition to loss of a randomized study motivated us to determine and assess the impact of vitamin D supplementation on fetal anthropometric measurements in this clinical prospective double-blind randomized survey.

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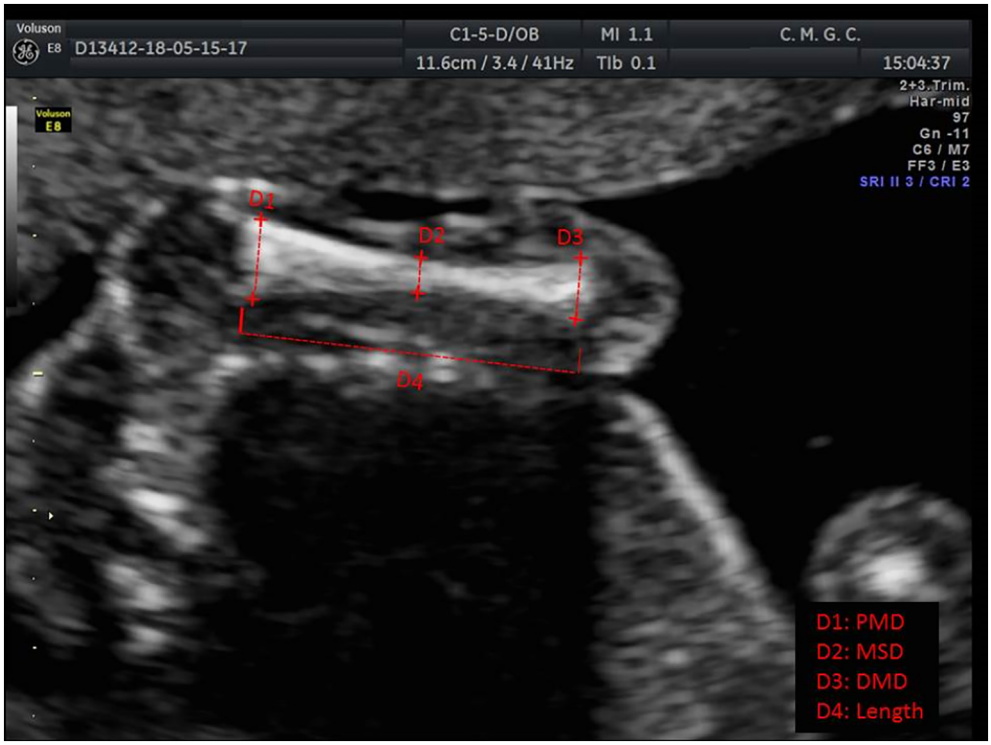

Fig. 1. Sonographic measurement of fetus long bone including D1: proximal metaphyseal diameter (PMD), D2: mid shaft diameter (MSD), D3: distal metaphyseal diameter (DMD) and D4: bone length.

2. Materials and methods

2.1. Study design and data collection

This prospective randomized trial was performed on early pregnant women under antenatal clinic care of Hafez hospital, the main center for perinatology in Shiraz, in the south of Iran from June 2017 to September 2017. Pregnant women were referred to the obstetric clinic after 2 weeks of menstrual retardation as they were previously educated at preconception counselling. Inclusion criteria were 20–35-years-old healthy primigravida pregnant Iranian woman with normal body mass index (BMI), without any comorbidities such as diabetes mellitus, thyroid disease, liver disease, or mental illnesses. Exclusion criteria were smoking, drug abuse, alcohol consumption, multiple pregnancy, congenital anomaly or chromosomal abnormality, and cases that did not accept to participate or did not sign the informed consent form. Moreover, during the survey, complicated pregnancies such as hypertension, preeclampsia, premature rupture of membrane, severe vaginal bleeding, and threatened course of labor were excluded from the study. Informed consent was obtained from all individual participants included in the study. This study was approved by the local Ethics Committee of Shiraz University of Medical Sciences review board (code: IR.SUMS.MED.REC.1396.78). This research was also registered at Iranian Registry of Clinical Trials (code: IRCT 20140317017034N6).

The sample size was set at 120 individuals considering α: 05 and power of 80%. To increase the reliability and power of the study, we initiated the study with 140 pregnant women. Block randomization (size of each block = 4) was performed to divide participants into two groups of 70 each, using random allocation software.

2.2. Sampling and laboratory analysis

First, a 10 cc of peripheral blood sample was taken with routine work-up for pregnancy before any supplementation was used. It was stored at −80 °C after being centrifuged (1000 × *g* for 15 min) until analysis at the end of collecting sonography data from all cases [30]. 25(OH)D level was quantified using Roche-electrochemiluminescence (ECL) [31] technology by the immunoassay analyzer Cobas e 411 (Roche Diagnostics, Mannheim, Germany). The accuracy of the process was monitored by lab quality control staff. The lab technicians were blinded to the group allocation. The inter- and intra-assay coefficients of variation were < 15%. Based on serum 25(OH)D level at the end of the study, three groups were defined; vitamin D deficient (< 20 ng/mL), insufficient (20–30 ng/mL) and sufficient (> 30 ng/mL) in order to confirm intervention and control groups to be matched in the aspect of mean 25(OH)D level and distribution in each group.

2.3. Clinical trial

After randomly dividing the patients into two groups as previously mentioned, the control group received placebo (same color and shape capsules containing starch) while the intervention group received 1000 IU of vitamin D (Jalinous Pharmaceutical Company, Tehran, Iran) daily, starting two weeks after menstrual retardation. The pills were continued till the last sonography at 34 weeks of gestational age. Both study groups received routine prenatal care. Monitoring of vitamin D pills or placebo consumption in both groups was done during each visit for pregnancy care.

2.4. Sonographic study

Synchronous to each trimester sonography, all sonographic data collection was done by an expert sonographer who was blinded to the group allocation. CRL and FL were measured by the standard technique with standard view at 13 weeks of gestational age. Then, at 18 and 34 weeks of gestational age, in addition to measuring humerus length (HL) and FL, proximal metaphyseal diameter (PMD), mid shaft diameter (MSD) and distal metaphyseal diameter (DMD) of humerus and femur (Fig. 1) were measured by the same expert sonographer, using Voluson E8 Sonography machine (General Electronic Healthcare

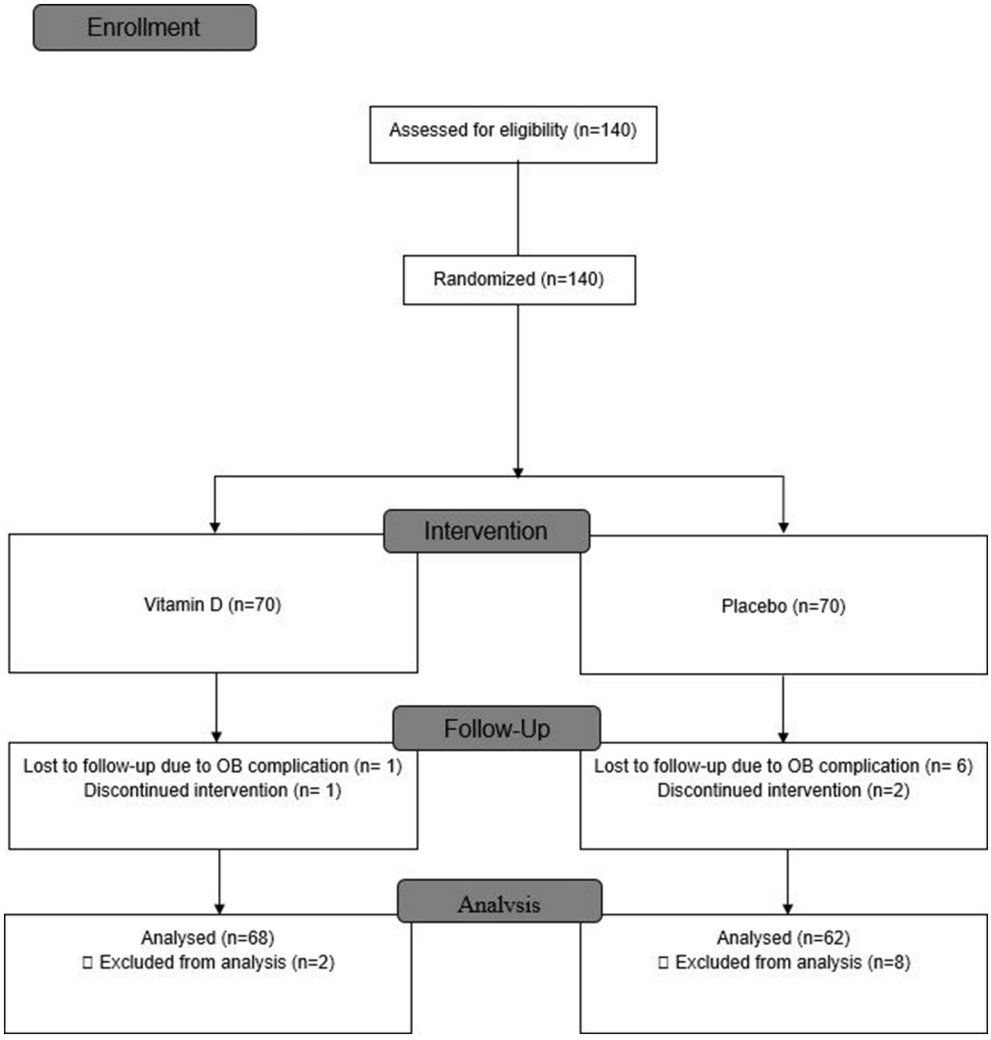

Fig. 2. CONSORT flow diagram for this randomized, double-blinded, placebo controlled clinical trial of the use of Vitamin D in pregnant women.

Table 1
Comparison of demographic characteristics of the two groups.

Characteristics		Intervention group (n = 68)	Control group (n = 62)	P-value
		Mean ± SD	Mean ± SD	
Age		27.0 ± 3.8	26.5 ± 3.2	0.41
25(OH)D level		18.6 ± 9.8	18.6 ± 9.0	0.98
Exercise	No	54 (79.4)	56 (90.3)	0.09
	Yes	14 (20.6)	6 (9.7)	

Technologies, Wisconsin, USA, seral number D 13412). To measure bone features and criteria variables coronal view was used [25,29].

2.5. Statistical analysis

Descriptive statistics for qualitative and quantitative variables were reported as frequency (%) and mean ± SD. To compare groups, Chi-square or independent t-test was performed and paired t-test was used for group comparison. All the statistical analyses were performed in SPSS 19.0 (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered to be statistically significant.

3. Results

Finally, there were 68 pregnant women in the intervention group and 62 in the control group (Fig. 2). During the study, two individuals from the intervention group were excluded (premature labor pain in one and the other one decided not to continue her participation). Also 8 from the control group could not complete the project (2 not willing to continue, 1 abortion, 1 preeclampsia, 2 premature rupture of membrane, 1 insulin-dependent gestational diabetes, and one due to severe vaginal bleeding and abruption of placenta).

Mean age of participants in the intervention group was 27.0 ± 3.8 years and in the control group was 26.5 ± 3.2 years, which was not statistically significant (P = 0.41). Although amount of exercise in the intervention group was higher than control, Chi-square test showed that the observed difference was not statistically significant (20.6% vs. 9.7%, P = 0.09). Moreover, independent t-test revealed that the two groups were the same in term of 25(OH)D level as a whole (P = 0.98) (Table 1). Also these groups were matched based on the number of cases with diagnosis of vitamin D deficiency, vitamin D insufficiency and vitamin D sufficiency by checking the frozen samples at the end of survey (Table 2).

No significant difference was observed for CRL with mean ± standard deviation in the intervention group vs. the control (65.4 ± 5.3 versus 65.3 ± 3.9; P = 0.93).

Fetal FL in the first trimester for the intervention group was 9.92 ± 1.11 vs. 9.80 ± 1.17 for the control, which was not

Table 2
Serum level of 25(OH)D in the two groups.

		Group		p-value
		Intervention	Control	
Sufficient	Count	13	11	0.999
	% within Group	19.1%	17.7%	
Insufficient	Count	9	12	0.475
	% within Group	13.2%	19.4%	
Deficient	Count	46	39	0.585
	% within Group	67.6%	62.9%	

Table 3
Comparison of different variables between the two groups.

Variables	Trimester	Intervention group	Control group	*p*-Value (Independent *t*-test)
		Mean ± SD	Mean ± SD	
CRL	First	65.4 ± 5.3	65.3 ± 3.9	0.93
NT	First	1.6 ± 0.3	1.6 ± 0.3	0.93
FL	First	9.92 ± 1.11	9.80 ± 1.17	0.54
	Second	28.87 ± 2.14	26.89 ± 2.08	< 0.001
	Change	18.94 ± 2.40	17.09 ± 2.69	< 0.001
p-Value (paired *t*-test)		< 0.001	< 0.001	
FL	Second	28.87 ± 2.14	26.89 ± 2.08	< 0.001
	Third	65.31 ± 2.17	62.85 ± 1.94	< 0.001
	Change	36.44 ± 2.76	35.97 ± 3.15	0.36
p-Value (paired *t*-test)		< 0.001	< 0.001	
PMD-FL	Second	4.72 ± 0.67	4.17 ± 0.53	< 0.001
	Third	11.91 ± 1.02	10.00 ± 1.68	< 0.001
	Change	7.19 ± 1.17	5.82 ± 1.70	< 0.001
p-Value (paired *t*-test)		< 0.001	< 0.001	
MSD-FL	Second	2.90 ± 0.48	2.62 ± 0.34	< 0.001
	Third	7.90 ± 1.12	6.56 ± 0.91	< 0.001
	Change	5.00 ± 1.18	3.95 ± 1.01	< 0.001
p-Value (paired *t*-test)		< 0.001	< 0.001	
DMD-FL	Second	4.60 ± 0.64	4.21 ± 0.63	0.001
	Third	10.05 ± 0.86	9.05 ± 1.20	< 0.001
	Change	5.46 ± 1.09	4.85 ± 1.37	0.006
p-Value (paired *t*-test)		< 0.001	< 0.001	
HL	Second	28.62 ± 1.94	27.23 ± 2.08	< 0.001
	Third	61.29 ± 2.84	59.85 ± 1.79	0.001
	Change	32.68 ± 3.00	32.62 ± 2.78	0.91
p-Value (paired *t*-test)		< 0.001	< 0.001	
PMD-HL	Second	4.64 ± 0.64	4.06 ± 0.59	< 0.001
	Third	9.21 ± 0.65	7.92 ± 1.00	< 0.001
	Change	4.57 ± 0.74	3.86 ± 1.08	< 0.001
p-Value (paired *t*-test)		< 0.001	< 0.001	
MSD-HL	Second	2.71 ± 0.41	2.46 ± 0.31	< 0.001
	Third	5.46 ± 0.79	4.52 ± 0.51	< 0.001
	Change	2.75 ± 0.88	2.06 ± 0.57	< 0.001
p-Value (paired *t*-test)		< 0.001	< 0.001	
DMD-HL	Second	4.07 ± 0.45	3.61 ± 0.50	< 0.001
	Third	7.89 ± 0.93	6.81 ± 1.16	< 0.001
	Change	3.82 ± 0.94	3.20 ± 1.30	0.002
p-Value (paired *t*-test)		< 0.001	< 0.001	

statistically significant ($P = 0.54$), but fetal FL of the intervention group at the second ($P < 0.001$) and third trimester ($P < 0.001$) was significantly higher than controls (Table 3). Our results also showed that PMD-FL, MSD-FL and DMD-FL were significantly higher in the intervention group at the 2nd and 3rd trimesters ($P < 0.001$) (Fig. 3).

Fetal HL at the second (28.62 ± 1.94 vs. 27.23 ± 2.08, $P < 0.001$) and third (61.29 ± 2.84 vs. 59.85 ± 1.79, $P < 0.001$) trimesters were significantly higher in the intervention group in comparison with the control (Fig. 4), but the difference was not statistically significant (32.68 ± 3.00 vs. 32.62 ± 2.78, $P = 0.91$). In addition, amounts of growth in PMD-HL, MSD-HL and DMD-HL were significantly higher in the intervention group ($P < 0.001$, Table 3).

4. Discussion

The prevalence of vitamin D deficiency and insufficiency is high in several parts of the world, especially in the Middle Eastern region of Asia [32–34]. Several factors such as genetics, demographics, BMI and skin color, lifestyle variables including smoking, sunscreen usage and clothing, latitude and location of living have an important role in the 25(OH)D level [11,30,35]. The problem of vitamin D deficiency might be aggravated during winter due to reduced sun exposure [36,37]. We selected participants during the summer season with normal BMI from a similar race and ethnicity to overcome the above affecting factors.

Vitamin D deficiency could result in unfavorable pregnancy outcomes amongst mothers including preeclampsia, gestational diabetes mellitus, premature rupture of membrane and premature labor pain with increased rate of cesarean section [1,18,20,36,38–40]. Based on our study, one participant from the case group (1.4%) suffered premature labor pain while 6 individuals (8.5%) had obstetric complications such as abortion, preeclampsia, premature rupture of membrane, insulin-dependent gestational diabetes and severe vaginal bleeding with abruption of placenta. Besides, negative effects of vitamin D deficiency on the fetus were reported such as neural tube defects, brain neurodevelopment, smaller head circumference, intrauterine growth retardation, small for gestational age and macrosomia [5,19,22,38,39,41]. On the other hand, effects of sufficient vitamin D during pregnancy on child intelligence, psychological health and cardiovascular system were reported [4]. Therefore, more randomized control studies are warranted to make clear the effect of vitamin D supplementation on reducing pregnancy complications and neonatal adverse outcome.

Definite recommended dose of vitamin D supplementation during pregnancy is unclear [1]. Some authors recommended 25(OH)D level > 50 nmol/L (20 ng/mL) while others agreed with 75 nmol/L (30 ng/mL) during pregnancy [1,42,43]. Kisa et al. stated that maternal serum 25(OH)D levels < 10 ng/mL is a risk factor for adverse pregnancy outcomes [38]. World Health Organization (WHO) recommends 400–600 IU of vitamin D daily during pregnancy while some investigators believe that it cannot establish the optimal level of 25(OH)D level through circulation [1,44,45]. Dawson-Hughes et al. recommended 1000–1600 IU per day to afford pregnancy demand of vitamin D [42]. Some researchers do not agree to prescribe 2000 IU, since they believe that it does not improve anthropometric measures of a newborn [9,45], Others agreed that high dose of 4000 IU is effective in reducing maternal and neonatal complications of pregnancy [1,44–46]. To perform this study, 1000 IU vitamin D was prescribed with positive effects on both femur and humerus features criteria including length and diameters during the second and third trimester of pregnancy ($P < 0.05$). Although high dose of vitamin D is mentioned to be safe, this randomized control trial study showed effectiveness of low dose of vitamin D on fetal bones to diminish the fear of toxicity and adverse effects of vitamin D. One factor in this outcome might be the season we chose the patients and amount of sun exposure. Hence, the recommended dose of vitamin D supplementation to affect fetal bone might vary according to season, nationality, sun exposure and other variables. More studies should be done to determine the definite dose of vitamin D supplementation effective for fetal bone improvement in other regions in order to develop a national policy.

To clear the correlation between the first trimester measurements and 25(OH)D levels, Fernandez-Alonso et al. in a cohort study measured CRL as a variant of fetal growth [27]. In our randomized control trial study, no correlation was found between the treated patients and non-treated individuals measuring CRL ($P = 0.93$) and the first trimester FL ($p = 0.54$). Lack of correlation mentioned in the aforementioned study and ours, might obscure the real effect of 25(OH)D level or vitamin D supplementation on the variables since the time of initiation of the therapy was two weeks after menstrual retardation, which might be too early to increase maternal serum 25(OH)D level and affect the first

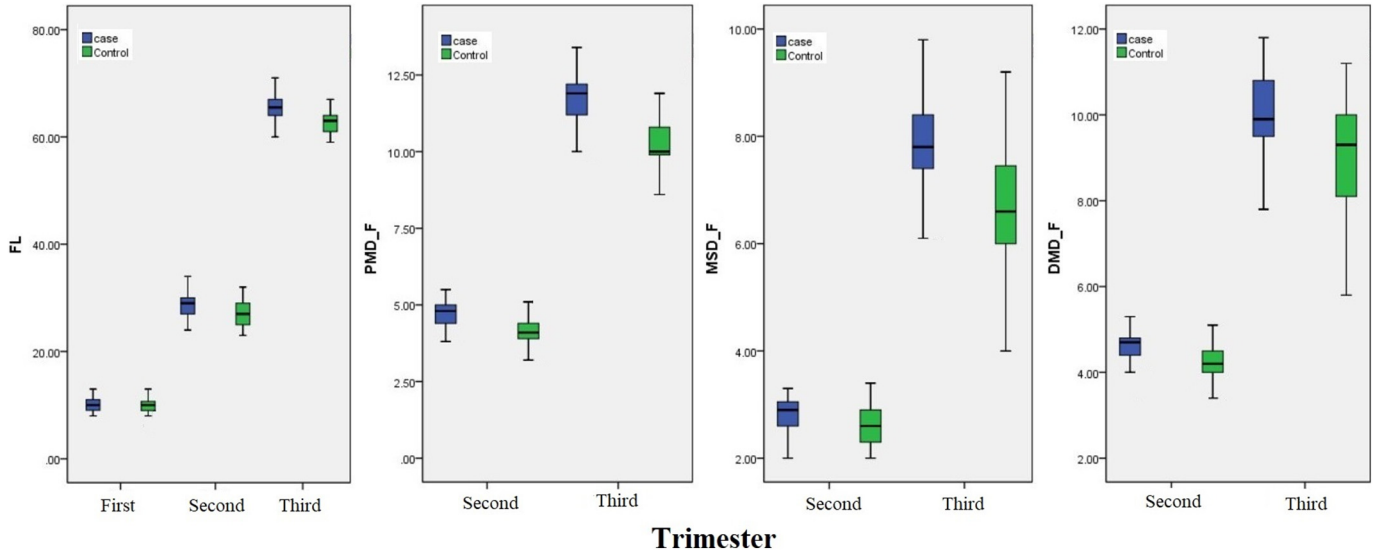

Fig. 3. Femoral length and diameter in both groups in the second and third trimester of pregnancy.

trimester variables. More studies should be conducted to evaluate the effect of starting vitamin D supplementation prior to preconception.

Vitamin D is known as a major factor in the development of musculoskeletal system. Mahon et al. in a cohort study, established the correlation between maternal 25(OH)D level and distal metaphyseal cross-sectional area and splaying index, defined as FL/distal metaphyseal cross-sectional area, but not FL at 19 and 34 weeks of gestational age. They emphasized on the importance of vitamin D effect on bone as early as 19 weeks of gestational age [24]. Relationship between maternal 25(OH)D and PMD was displayed by another observational cohort study [25]. Walsh et al. presented the association between early pregnancy maternal 25(OH)D and FL measured at 20 weeks in the winter group. They reported correlation between maternal 25(OH)D at 28 weeks of gestational age and FL measurements at 34 weeks of pregnancy [47]. In a cross sectional study by Lee et al., no correlation was mentioned between maternal 25(OH)D and bone growth variables such as FL and HL with the exception of growth velocity of biparietal bone diameter [28]. In our study, we found bone features improvement in both femur and humerus length and diameter in the intervention group ($P < 0.001$ for all) after the second trimester, which is not in line with some previous data [27,28]. One of the possible reasons for this contrary may be this hypothesis that vitamin D deficiency is effective first on the diameter of long bones followed by late effect on length of the long bones in fetal period. Therefore, vitamin D supplementation might stimulate both length and diameter as shown in our study ($P < 0.001$).

This study had several strength including being a randomized control trial to assess long bone measurements in both the intervention and control groups at all trimesters of pregnancy at low dose. There were several limitations such as 2D-ultrasonography, which cannot reveal bone hormonal and chemical interactions. Moreover, interfering acoustic shadow for determining boundaries of bones should be considered. We conducted the study during summer season in our country, Iran, so more studies in different seasons and worldwide are warranted to establish the optimal dose of vitamin D to influence fetal long bones, the time to initiate supplementation and other questions that may arise need to be answered.

5. Conclusion

In conclusion, our study revealed that low dose vitamin D supplementation (1000 IU daily) starting from early pregnancy could not

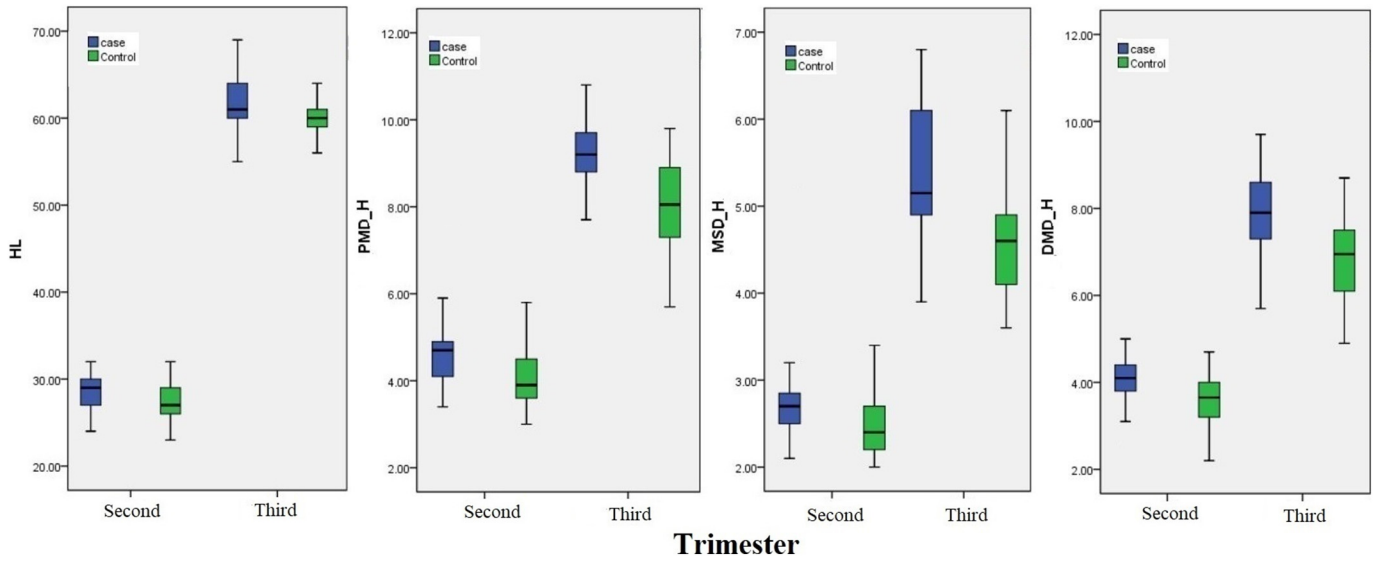

Fig. 4. Humeral length and diameter in both groups in the second and third trimester of pregnancy.

affect CRL and FL in the first trimester, but it was able to improve all features of femur and humerus including length and diameter in the second and third trimesters. Since vitamin D deficiency prevalence is high amongst pregnant women, vitamin D supplementation is recommended from early pregnancy if not before conception to improve bone measures of the fetus. This fact might be important, especially in societies that suffer from stunting or osteoporosis. Further studies are necessary to elucidate the effect of vitamin D supplementation on fetal bone growth in different parts of the world.

Conflict of interest statement

None.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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CRediT authorship contribution statement

Homeira Vafaei: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Visualization, Writing - review & editing. **Nasrin Asadi:** Conceptualization, Investigation, Supervision, Validation, Writing - review & editing. **Maryam Kasraeian:** Conceptualization, Investigation, Supervision, Validation, Writing - review & editing. **Hadi Raeisi Shahraki:** Formal analysis, Methodology, Validation, Writing - original draft. **Khadije Bazrafshan:** Data curation, Project administration, Software, Writing - original draft. **Niloofar Namazi:** Data curation, Formal analysis, Methodology, Software, Visualization, Writing - original draft.

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