Original Article

Could Maternal Vitamin D Concentration Affect Cervical Length in Mid-Pregnancy? A Report From an Iranian Tertiary Center

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Received April 2022; Revised and accepted July 2022

Abstract

Objective: This descriptive-analytic cross-sectional study aimed to figure out whether maternal serum vitamin D concentration correlates with cervical length measurement in mid-gestation or not.

Materials and methods: During Jun-Jan 2021, 213 pregnant women at 18-22 weeks of gestation were investigated. First, demographic features were obtained then, maternal serum vitamin D concentration was measured by enzyme-linked immunoassay and cervical length was measured via transvaginal sonography according to fetal maternal foundation guideline and appropriate statistical test was used to analyze the correlation between maternal vitamin D level and cervical length in mid-pregnancy.

Results: It was shown that 29.6% and 25.4% of participants had vitamin D deficiency and insufficiency, respectively. Spearman's test found no significant correlation between maternal vitamin D level and cervical length in mid-pregnancy. Moreover, cervical length and maternal vitamin D level had no association with maternal BMI.

Conclusion: Although maternal vitamin D level and its sufficiency status was not associated with cervical length in mid- pregnancy, as a trend toward decreased maternal serum vitamin D level by advancing gestational age was observed. It may be concluded that it is prolonged vitamin D deficiency during gestation that may lead to cervical length shortening and subsequent preterm delivery later in gestation.

Keywords: Vitamin D; Cervical Length; Body Mass Index; Deficiency; Pregnancy; Maternal

Introduction

Vitamin D is a unique endogenously synthetized nutrient and also a hormone which plays various important functions in human bodies such as immune system regulation, cell proliferation and musculoskeletal health and integration (1, 2). It has

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been proved that vitamin deficiency could predispose affected patients to various cancers, autoimmune, neurological and/or cardiac diseases and etc. (3). Furthermore, during pregnancy, vitamin D deficiency is associated with serious maternal and neonatal adverse outcomes as pre-eclampsia, preterm labor, gestational diabetes, low birth weight, abnormal fetal thymus growth, poor fetal and/or neonatal skeletal growth and etc. (4-6). In Fact, well-balanced coordination between anti-inflammatory and anti-microbial agents in feto-placental unit is essential to maintain the pregnancy and vitamin D plays a key role in this regard (7). During pregnancy 1a-hydroxylase activity intensifies both in maternal and fetal kidney, placenta and decidual tissue. Decidua can also produce 1, 25(OH) $_2$D and 24, 25(OH) $_2$D and 24, 25(OH) $_2$D is synthesized in placenta too. On the other hand, 1, 25(OH) $_2$D can help endometrial cells to transform into decidual cells (8, 9). Additionally, vitamin D can induce Cathelicidin activation in decidual and trophoblastic cells which is per se an anti-microbial peptide. So, Presence of vitamin D and its receptors in genital tract reveals vitamin D can have some locally-specific actions here (1).

In a large amount of laboratory studies, it has been confirmed vitamin D deficiency is associated with preterm labor and this is due to its role in reduction of bacterial infections in various tissues as maternal, fetal and placental cells (2, 5, 10). Preterm delivery prevalence reaches up to 5-10% and 25% in developed and under-developed countries, respectively (11-15) and many different individual and environmental factors can influence preterm labor occurrence and this has resulted in challenges in predicting and preventing preterm labor (16). In line with these studies, vitamin D supplementation could have reduced preterm labor prevalence (1). Wagner et al found out that preterm labor is approximately 3.8 times higher in pregnant women whose vitamin D concentration was less than 20 ng/dL compared to those with levels greater than 40 ng/dL (17). Shakir et al in 2019 reached a relative risk of 6 for preterm birth in pregnant women with insufficient or deficient vitamin D status at 14th to 20th weeks of gestation (2). Although vitamin D role in decreasing bacterial infection, NK cells and monocytes regulation and increasing tumor necrotizing factor alfa has been strongly established (18), some reports exists declaring decreased prevalence of preterm labor in case of maternal vitamin deficiency (19).

In fact, it is due to increased inflammatory response to clinical or subclinical infections that vitamin D deficiency could lead to higher prevalence of preterm labor (20). Moreover, As cervical length is a well-known predictor for preterm birth, cervical length screening has become one of the most effective screening tests to predict preterm delivery (21, 22). On the other hand, data regarding association of vitamin D deficiency and short cervical length in mid-gestation is lacking in literature. Kook et al in their study of 148 cases of preterm labor and 103 cases of premature preterm rupture of membranes in 2017, found out that vitamin D binding protein (VDBP) increases in cervico-vaginal fluid of those with intra-amniotic infection and this rise was associated with impeding delivery within next 48 hours (23). Yoo et al also reached same results and declared that VDBP, tissue inhibitor of metalloproteinases (TIMP-1), and Dickkopf-related protein 3(DKK3) levels were higher in cervico-vaginal fluids of patients who delivered before 32 weeks of gestation (21). Considering vitamin D anti-inflammatory action in preventing preterm labor prevalence and local function of vitamin D and its binding protein and receptors in genital tract, we aim to design a study to answer this question whether maternal vitamin D deficiency could result in cervical shortening in mid gestation which later may lead to preterm labor or not.

Materials and methods

This study was carried out in compliance with the Helsinki Declaration and the Ethics Committee of Imam Khomeini Hospital Complex, affiliated by Tehran University of Medical Sciences approved the study (number: IR.TUMS.IKHC.REC.1400.116). All the participants assigned an informed consent too.

This cross-sectional study was conducted on 213 pregnant women who referred to tertiary center of Imam Khomeini Hospital Complex, Tehran, Iran to undergo an anomaly scan at the gestational age of 18 to 22 weeks during Dec 2021 to Jan 2022. Inclusion criteria were as follow, maternal age over 18 years old, singleton pregnancy, absence of serious medical comorbidities (diabetes, hypertension, autoimmune or renal diseases, epilepsy, uncontrolled hypothyroidism, early onset preeclampsia or premature rupture of membranes), absence of previous preterm birth or early second trimester loss, spontaneous conception, and patient's tendency for participation. Among these candidates, patients who

showed any signs and/or symptoms of active clinical infections like cystitis, upper respiratory tract infection and etc., those with current use of progesterone in any forms, cigarette smokers and alcohol consumers, and patients with any major structural abnormalities or fetal growth disturbance on the sonographic scan were all excluded.

According to Ha-Na Yoo (21) study in which correlation efficient was estimated 0.366, and considering confidence interval of 95% and study power of 80%, sample size of 50 was calculated (Figure 1).

$$n = \left[\frac{Z_\alpha + Z_\beta}{c}\right]^2$$
$$c = 0.5 * Ln[(1+r)/(1-r)]$$

Figure1: sample size estimation formula

After obtaining demographic information throw reviewing patients 'medical records and individual interviews, as necessary, patients underwent an anomaly scan by a single certified primatologist to eliminate inter-observer variability. Subsequently transvaginal sonography with empty bladder was done by means of Affinity70, PHILIPS sonography machine ((PHILIPS Co., USA), using a 3-6 MHtz vaginal probe. cervical length was measured in its longitudinal axis from internal to the external os, in compliance with fetal maternal foundation recommendations on cervical length measurement (24).

After sonography examination, 3 cc venous blood was collected from each participant and maternal vitamin D serum concentration was measured by using PGI ELISA kits (Padtan Gostar Isar Co., Iran) in the reference laboratory of Imam Khomeini Hospital. Based on the endocrine society practice guideline on vitamin D status, vitamin D deficiency, insufficiency, and sufficiency were defined as vitamin D concentration =< 20ng/dL, within the range of 21-29 ng/dL, and at least 30 ng/dL respectively (25).

All data were analyzed by SPSS software version 23 (IBM, USA). For quantitative and qualitative variables, mean values with standard deviations and frequencies were calculated respectively. To determine correlation between vitamin D concentration and cervical length Spearman test was performed and for further analysis Chi-square and Mann-Whitney or Kruskal-Wallis tests were applied as indicated.

Results

In this cross-sectional study 213 patients were finally included in analysis. Mean values± standard deviations for maternal age, gestational age and maternal body mass index (BMI) were 32.25±5.57 years, 18.76±0.98 weeks and 27.47±4.64 kg/m^2, respectively. Additionally, 112(52.6%) fetuses were male and 101(47.4%) were female. 145(68.1%) mother were at 18th weeks of gestation, 41(19.2%), 10(4.7%) and 17(8%) were at 19th, 20th and 21st weeks of gestation, respectively. Fetal biometric characteristics is illustrated in table 1.

Mean (±standard deviation) maternal vitamin D concentration was 30.14±16.98 ng/dL. Furthermore, Among 213 participants, 63 (29.6%) had vitamin deficiency (values equal or less than 20 ng/dL), 54 (25.4%) had insufficiency (values between 20 and 30 ng/dL), and 96 had sufficient levels (values at least 30 ng/dL). Meanwhile, 105 (49.3%) patients stated that they were using 1000 international unit (IU) vitamin D supplementary tablets daily and 80 patients (37.6%) declared no intake. Moreover, Nine women were consuming vitamin D supplements irregularly (less than 4 times a week), and 19 were taking supplements occasionally during pregnancy (less than once a week). In contrast to vitamin D supplements, majority of patients (62.9%) were taking multi-prenatal tablets daily (containing 200 to 400 IU vitamin D).

Mean (±standard deviation) cervical length was 34.10±3.4mm and no correlation with maternal 25-OH vitamin D level was evident (spearman's rho coefficient: 0.045, p-value: 0.509). Further analysis showed no significant correlation between patients' age, gestational age and BMI and maternal vitamin D concentration neither. Table 2 demonstrated maternal vitamin D concentration in different gestational age groups in this study.

Table 1: Fetal biometric characteristics

	BPD (mm)	HC (mm)	FL (mm)	AC (mm)	EFW (grams)
Mean	42.28	158.10	28.39	134.53	263.53
Median	41.38	155.30	27.93	132.11	245.00
Std. Deviation	3.88	13.59	3.49	15.76	68.30
Minimum	34.53	128.13	21.88	28.14	139.75
Maximum	53.95	202.57	42.10	190.93	565.00

Table 2: Maternal vitamin D concentration in different gestational age groups

Gestational Age	Mean maternal vitamin concentration (ng/dL)	n	Std. Deviation	Median
18th week	31.60	145	17.3	28.90
19th week	29.29	41	16.3	24.00
20th week	26.36	10	10.2	27.50
21th week	25.42	17	17.9	18.00
Total	30.41	213	16.9	28.00

Kruskal-Wallis test was also applied to analyze correlation between maternal vitamin D status defined as sufficient, insufficient or deficient and mid-trimester cervical length measures and no statistically significant correlation was found. Similarly, Mann-Whitney test could not indicate any correlation between maternal vitamin D status, and cervical length when levels of 10, 20 or 30 ng/dL were considered as cut points too.

Moreover statistical analysis failed to show any significant correlation between maternal BMI and cervical length measurement in mid-gestation.

Discussion

This descriptive-analytic cross-sectional study, which was carried on 213 participants in Dec 2021 to Jan 2022, was designed to assess whether Vitamin D deficiency, a well-known risk factor for preterm labor, is associated with cervical length in mid-trimester of pregnancy. In fact, vitamin D insufficiency and/or deficiency are increasingly being considered as a health problem worldwide and although the elders are the most prominent population affected, pregnant women are also particularly affected, (4, 20). In line with, in this current study, only 45.1% of mothers had sufficient vitamin D status. As well, vitamin D deficiency is associated with adverse events in pregnancy, hence, many organizations recommend vitamin D supplementation intake prenatally and during pregnancy. (4, 10). Unfortunately despite national guidelines recommending consumption of 1000 IU vitamin D tablets during pregnancy (26), only 49.3% of study population was taking supplements regularly. Hence, it seems that we need to seek a solution to enhance our patients' inherence to be able to maintain sufficient vitamin D concentration and subsequently prevent related adverse outcomes. Moreover, It has also been shown that supplementation with vitamin D reduces inflammatory markers and preterm labor incidence rate (2, 4, 7) Although this correlation has been well studied, available evidences has not evaluated the correlation between vitamin D levels with cervical length as a predictor of preterm labor. In Eltemamy et al. study in 2021, 188 pregnant women at gestational age of 20 to 24th weeks were assessed and followed till delivery. They found out that in patients who delivered preterm mid-trimester cervical length was significantly shorter but mean maternal serum vitamin D concentration did not differ between who delivered preterm and those delivered at term (27). But, the researchers did not investigate the association between cervical length and maternal vitamin D level.

In contrast to them, this study was aimed to assess this potential association but our results showed that despite both short cervical length and Vitamin D deficiency are directly contributable to preterm labor, there is no significant correlation between maternal vitamin D level and mid-trimester cervical length measurement. Moreover, current data revealed that neither vitamin D deficiency nor insufficiency is correlated with cervical length measurement and when even serum levels of 10 or 20 ng/dL were considered as thresholds no significant correlation was reached. It is worth mentioning that the main limitation of current study is its design and as it was not a prospective cohort one to follow the participant till delivery time, prognostic value of cervical length or maternal vitamin D level could not be investigated.

Interestingly, maternal BMI was not correlated with cervical length in mid gestation too. This finding is in contrast to some previous studies which reached a statistically significant positive correlation (27-31). Although this remains unexplained as similar or even greater sample size was studied here but some differences in study design, population study, and ethnical or geographical heterogeneity may justify this. As an example Q Jalal measured BMI at the first trimester and cervical length were obtained in mid-gestation (29).

In summary despite of not finding a statistically significant correlation between cervical length and maternal vitamin D concentration in mid-gestation, as there was a statistically non-significant trend toward decreased maternal vitamin D concentration as

gestational age increased, it seems that prolonged vitamin D deficiency during pregnancy may lead to cervical shortening in late second trimester or third trimester and further prospective studies are needed to confirm this hypothesis. Additionally as majority of the participants (54.9%) have serum vitamin D levels below 30 ng/dL it may contribute to not finding a statistically significant correlation between vitamin D level and mid-gestation cervical length. So, larger sample size studies on more heterogonous population, in regard to serum vitamin D concentration, are essential to address this issue.

Conclusion

Although maternal vitamin D level and its sufficiency status was not associated with cervical length in mid-pregnancy, as a trend toward decreased maternal serum vitamin D was observed, it seems that prolonged vitamin D deficiency may be necessary for inducing cervical length shortening and subsequent preterm delivery.

Conflict of Interests

Authors declare no conflict of interests.

Acknowledgments

All the authors kindly appreciate their participants for their cooperation and also Dr. Mamak Shariat who helped us in data analysis and interpretation.

Tehran University of Medical Sciences support this project financially under research grant number of 132/9075.

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Citation: Shabani A, Abiri A, Ghalandarpoor-Attar SN, Hadizadeh A, Ghalandarpoor-Attar SM. **Could Maternal Vitamin D Concentration Affect Cervical Length in Mid-Pregnancy? A Report From an Iranian Tertiary Center.** J Family Reprod Health 2022; 16(4): 290-5.