


Comparison of Serum Vitamin D Levels with and without Bacterial Vaginosis among Women in First Trimester of Pregnancy in a Tertiary Care Hospital

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Received on: 15 March 2023; Accepted on: 08 May 2023; Published on: 31 July 2023

ABSTRACT

Aim: To compare vitamin D levels in the serum of women in the first three months of gestation with bacterial vaginosis (BV) occurrence in a tertiary care hospital.

Materials and methods: Bacterial vaginosis diagnosis was done using the Nugent scoring system. It has been considered as one of the best standards to diagnose BV. Vitamin D analysis was done using ELISA method.

Results: About 66.7% of pregnant women positive for BV in the first trimester of pregnancy were with vitamin D deficiency, 22% of women who had a normal vaginal flora had sufficient measures of vitamin D in the serum. In this study, the subnormal and deficient measures of vitamin D had a good and significant association in women with BV with a *p*-value of 0.007.

Conclusion: In women in the first trimester of pregnancy, BV has significant association with deficient measures of vitamin D in the serum.

Clinical significance: Bacterial vaginosis presenting as vaginal discharge is mainly caused by increase in the levels of anaerobic bacteria. There is a balance between *Lactobacillus* species, which is present in abundance and maintains the normal pH of the vaginal fluid with anaerobic bacteria, which is altered in BV. It is said to be a polymicrobial clinical syndrome that can lead to preterm birth and its related complications. In developing countries, public health diseases are given importance and one among them is vitamin D deficiency. Interestingly, deficient measures of vitamin D have been found to be increasingly linked to the symptomatic presentation of several medical and immunological conditions that can lead to hypertension, diabetes in pregnancy, and can also lead to preterm labor. In this study, we tried to see if there is any relationship between BV and deficiency of the levels of serum vitamin D in the first trimester of pregnancy. Diagnosing BV and vitamin D deficiency in the first trimester gives us ample opportunity to treat and reduce the obstetric complications.

Keywords: Bacterial vaginosis, First trimester, Pregnancy, Vitamin D levels.

Journal of South Asian Federation of Obstetrics and Gynaecology (2023); 10.5005/jp-journals-10006-2262

INTRODUCTION

Among various infections of the vagina in the reproductive age-group, bacterial vaginosis (BV) is a frequently diagnosed infection that is of concern, especially when encountered in pregnancy. It is a clinical polymicrobial syndrome, mainly due to the imbalance of vaginal flora, wherein the *Lactobacillus* species that produces hydrogen peroxide is reduced and there is high concentration of anaerobic bacteria.^{1,2} Centers for Disease Control estimated that approximately 1 million pregnant women can contract BV every year. It is the most common diagnosis made when women complain of vaginal discharge. When it occurs during pregnancy, it is said to be associated with adverse outcomes, especially preterm labor and its related complications.³ There is an increased risk during pregnancy because of the hormonal changes of pregnancy that increases the susceptibility to contract BV.

On the other hand, in the developing countries, among the community health problems, deficiency of the levels of vitamin D in the serum is linked with several medical conditions. There are multiple studies that have reported adverse obstetric and perinatal complications associated with vitamin D deficiency, which includes hypertensive disorders of pregnancy, preterm labor, gestational diabetes, Small for gestational age (SGA) babies, and increased incidence of cesarean section.⁴ The bad obstetric outcomes that can occur during the pregnancy of a patient with BV and vitamin D

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How to cite this article: Mahesh N, Navikala K, Beena, *et al.* Comparison of Serum Vitamin D Levels with and without Bacterial Vaginosis among Women in First Trimester of Pregnancy in a Tertiary Care Hospital. *J South Asian Feder Obst Gynae* 2023;15(3):287–291.

Source of support Nil

Conflict of interest: None

deficiency are the background objective to understand the association between the two.

Maternal morbidity and mortality are one of the indicators of women's health in a country. Reducing maternal morbidity and mortality is one of the sustainable health goals that our country aims at. The association of BV with vitamin D status can be assessed with this study. Also, the susceptibility to BV can be reduced by the

immunomodulatory property of vitamin D, which also influences the immune cycles. Treating the vitamin D deficiency can also be promoted to reduce vaginal infections and in turn reduce adverse obstetric outcomes. Reduction of BV reduces preterm labor, which in turn can reduce neonatal and infant mortality. The need for the study is hence established.

OBJECTIVES

- To ascertain the levels of vitamin D and diagnose BV in pregnant women in their first trimester visiting a tertiary care hospital.
- To look for the association between them.

MATERIALS AND METHODS

This study is done in the Department of Obstetrics and Gynecology of Tertiary Medical College Hospitals in South India, Karnataka, over a period of three months. It is a cross-sectional observational type of study with a sample size of 90. The sample size was decided by analyzing the results of the previously conducted study done by Lisa M Bodnar and others, wherein, it was observed that in women with and without BV, the geometric mean concentration of vitamin D was 29.5 [95% CI: (27.1, 32.0) nmol/L] and 40.1 [95% CI: (37.0, 43.5) nmol/L], respectively. In this study, expecting a similar outcome, with a power of 80%, confidence interval of 95%, and an effective size of 0.74, at least 29 subjects were necessary under each group.

In order to get 29 BV-positive patients, it was essential to screen at least 87 subjects, since the above study has shown that 1 in 3 women in reproductive age-group had BV.

All women in their first three months of pregnancy within the ages of 18–45 years visiting the OBG Department both OPD and IPD of the tertiary hospitals attached to the medical college were included in this study. Women formerly on vitamin D supplementation and women who did not give consent were excluded.

Institutional Ethics Committee approval was taken to conduct this study. Women in the first trimester of pregnancy visiting the tertiary hospitals were recruited after taking informed consent. Women who consented were asked to give details pertaining to their sociodemographic characteristics, clinical symptoms related to BV, gestational age of pregnancy, and medical and reproductive history. Subsequently, pregnant women underwent vaginal swab test and were asked to give their blood sample in order to estimate the vitamin D levels.

Bacterial vaginosis diagnosis was done using the Nugent Scoring. This scoring system is the gold standard to diagnose BV. The vaginal smears were examined microscopically in oil immersion. A 10 minimum high-power fields were examined for the three main bacteria – *Lactobacillus*, *Gardnerella*, and curved Gram-negative bacilli. Scoring was done based on the bacterial count per oil immersion field. The sum total of the three category scores is considered to be the final score. This score ranges from 0 to 10. Diagnosis of BV based on the Nugent score:

- 0–3: negative.
- 4–6: intermediate.
- 7 and above: positive.

Vitamin D analysis was done using ELISA method. About 5 mL of blood was collected with due aseptic precautions in plain vacutainer tubes. The samples were stored at –80°C and the test was conducted using ELISA kit procured from Welden Biotech Ltd. and

Table 1: Personal and medical characteristics of first trimester pregnant women

Personal characteristics	Number	Percentage
Age-group		
21–25	11	12.2
26–35	66	73
36–40	11	12.2
Obstetric details		
Primigravida	47	52.2
Multi with previous live birth	30	69.7
With previous 1 miscarriage	20	46.5
With previous 2 miscarriages	4	9.3
With history of RPL	3	6.9
Medical history		
Hypothyroidism	39	43.3
Diabetes mellitus	8	8.9
Hypertension	4	4.4

Table 2: Prevalence of bacterial vaginosis and vitamin D levels among pregnant women

	Result	No %	Total
Bacterial vaginosis	Positive	15 (17%)	90
	Intermediate	34 (37.5%)	
	Negative	41 (45.5%)	
Vitamin D	Deficiency	28 (31%)	90
	Insufficiency	50 (55%)	
	Sufficiency	12 (13%)	

processed in Central Research Laboratory of the tertiary hospital. Reference range for categorization of vitamin D status:

- Less than 10 ng/mL: deficient.
- 10–30 ng/mL: insufficient.
- More than 30 ng/mL: sufficient.

Analysis of the data obtained was done using the SPSS software version 20, which gives descriptive and inferential statistics.

The relationship between the two variables was determined using the Pearson’s Chi-square test. The hypothesis about the variables was said to be statistically significant when the *p*-value was found to be less than 0.05.

RESULTS

Pregnancy details of the women considered for the study: 73% (66) of women were in the age-group of 26–35 years. About 12% (11) women each belonged in the age-groups of 21–25 and 36–40 years. About 22% (20) women had a history of 1 previous miscarriage and 4% (4) women had a history of previous 2 miscarriages. About 33% (30) women had previously at least one live birth, as shown in Table 1.

About 45.5% (41) had a normal vaginal flora and approximately 17% (15) of the women were diagnosed to have BV. The remaining 37.5% (34) of women were intermediate for BV. About 98.9% (89) of the women were asymptomatic. Only about 13% (12) of the pregnant women had adequate serum vitamin D levels. About 55% (50) had insufficient levels and about 31% (28) were having vitamin D deficiency (Table 2).



Table 3: Nugent score and serum vitamin D level relationship

		Nugent score * Vitamin D (ng/mL) cross tabulation				
		Vitamin D (ng/mL)			Total	
		Sufficient	Insufficient	Deficient		
Nugent score	Negative	Count	9	23	9	41/45.5%
		% within Nugent score	22.0%	56.1%	22.0%	100.0%
	Intermediate	Count	3	22	9	34/37.5%
		% within Nugent score	8.8%	64.7%	26.5%	100.0%
	Positive	Count	0	5	10	15/17%
		% within Nugent score	0.0%	33.3%	66.7%	100.0%
Total	Count	12	50	28	90	
	% within Nugent score	13.3%	55.6%	31.1%	100.0%	

Chi-square tests			
	Value	df	Asymptomatic. sig. (2-sided)
Pearson Chi-square	14.087 ^a	4	0.007
Likelihood ratio	14.602	4	0.006
Linear-by-linear association	10.562	1	0.001
No. of valid cases	90		

^aThree cells (33.3%) have expected count less than 5. The minimum expected count is 2.00

About 66% (10) of patients in the first 3 months of pregnancy who turned to be positive for BV were with vitamin D deficiency, 22% (9) who had normal vaginal flora had adequate vitamin D levels. None of the women who had BV had sufficient vitamin D levels. About 22% (9) of women with vitamin D deficiency had normal vaginal flora and were negative for BV. About 64.7% (22) of women with intermediate score for BV had insufficient levels of vitamin D. It was noted that there was a reduction in the prevalence of BV as the vitamin D status of the pregnant women improved (Table 3).

According to our study, *p*-value for the association of BV with vitamin D deficiency is 0.007, which is a significant association. Even the likelihood ratio and linear-by-linear association calculation showed significant association of BV in the first trimester of pregnancy with vitamin D deficiency.

DISCUSSION

This was a sample population study that was conducted in a tertiary hospital, wherein it was demonstrated that a significant association existed between deficiency of vitamin D and BV. Approximately one-third of women in the reproductive age-group were known to be affected by BV.⁵ In one study, BV is said to be prevalent in about 16–69% in women of reproductive age.⁶

It can also be associated with a variety of other gynecological disorders. The common symptom associated with this condition is foul-smelling vaginal discharge that has a characteristic fishy odor due to volatilization of the metabolic bacterial products in the vaginal fluid.⁷ Women may also complain of vulval itching with burning sensation.⁸

The risk for developing BV has various inherent factors that cannot be corrected and several variable factors that can be modified to improve the outcomes. Some of the factors that influence the development of BV are socioeconomic conditions, immune status of the body, nutritional intake, and history of previous infections, including sexually transmitted infections.⁹

Eradication of BV among the high-risk pregnant women has shown a reduction in preterm labor in randomized control trials. Hence, treatment of BV in pregnancy has remained to be a major priority in pregnancy management for the obstetricians.¹⁰

Vitamin D is not freely available and has to be synthesized endogenously by exposing the skin to sunlight. This is the main source, diet and supplements are the other limited sources. Vitamin D levels in the blood are influenced by skin pigmentation (due to the presence of melanin that has ethnic and geographical variations) and also with the use of emollients like sunscreen.

Lisa M Bodnar et al. study showed that 57% of women with decreased values of vitamin D in the serum had BV in their first three months of pregnancy. This study also showed that the relationship of the prevalence of BV with vitamin D levels was dose-dependent.¹⁰ Our study correlated with the results of this study. Another study, which was conducted by Hensel et al., also showed that deficiency of vitamin D was found to be closely and significantly related with occurrence of BV in pregnant women and AOR was about 2.87, with 95% CI between 1.13 and 7.28. Risk factors that can lead to development of BV are said to be linked to the status of vitamin D in pregnancy. While considering the modifiable risk factors for preventing obstetric complications, it was identified that supplementation of vitamin D in pregnant women with vitamin D deficiency had a significant role not only as a preventive measure, but also as a therapeutic action with good results.¹¹ Sadiya et al. conducted a study, whereby they found 49.3% association in pregnant women in the first three months of pregnancy between the deficiency of vitamin D and BV¹² (Table 4).

Several studies also have concluded that there is an inseparable association between BV and the levels of vitamin D in the women during their first three months of pregnancy. Wagner et al. analyzed to see if treatment of deficiency of vitamin D was found to be better to help to reduce the occurrence of asymptomatic BV, and they concluded that vitamin D as a therapeutic drug, did help in the same.¹³ There are studies that have shown independent

Table 4: Comparison of our study with other studies

Author	Number of subjects	BV positive	Vitamin D deficiency	Association between vitamin D deficiency and BV
Our study	90	17%	31%	66%
Lisa M Bodnar (2009)	469	41%	52%	57%
Hensel et al. (2011)	440	29%	AOR -1.01; 95% CI, 0.91-1.13	AOR -2.87; 95% CI, 1.13-7.28
Sadiya et al. (2019)	100	53%	67%	49.3%

AOR, adjusted odds ratio; CI, confidence interval

relationship between deficiency of the levels of vitamin D and the development of BV in pregnancy, inspite of adequate exposure to sunlight.^{14,15} The cause identified for this association is reduced levels of metabolites like defensins and cathelicidins, in these deficient women, which have antibacterial properties.¹⁶

If the hypothesis of this association is confirmed, it can open up the possibilities to identify the status of the levels of vitamin D during early gestation and gives opportunity to help treat this deficient state in order to reduce the maternal and perinatal morbidity. In our study, a significant relationship has been identified with occurrence of BV with deficiency of vitamin D levels, which has opened up the hypothesis, whereby the risk of BV can be reduced by checking the level of vitamin D in the serum and identifying the deficient state. In the first three months of pregnancy, fetal development occurs. Vitamin D is transferred to the fetus for this process by the transplacental route. Due to this transplacental transfer, some women develop vitamin D insufficiency, sometimes even deficiency. It has also been seen that other factors can influence the levels of vitamin D, including reduced intake of fortified food, inadequate exposure to the sun, lifestyle, and seasonal and ethnical variations.¹⁷⁻¹⁹ In our study, about 66% of women with BV had deficiency of vitamin D, 22% had normal levels of the vitamin and no evidence of BV, and about 33% of women who were found to have insufficient levels of the vitamin had BV. One of the explanations given for this association can be the immunomodulatory action of vitamin D.²⁰ Deficiency of the levels of vitamin D among pregnant women was reported in studies by Bodnar LM et al. and Jefferson KK et al., which was associated with BV, which was in agreement with our study.^{10,21}

CONCLUSION

In our study, significant association between the occurrence of BV and vitamin D deficiency was seen among women in the first trimester of pregnancy. Healthcare professionals should suggest the pregnant women to check their vitamin D status and get screened for BV in early pregnancy and help the women to correct the vitamin D levels and prevent BV to reduce the adverse effects on pregnancy. However, to reduce the occurrence of BV, one should not entirely depend only on vitamin D supplementation.

Ethical Approval

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008(5).

Informed consent was obtained from all the patients for being included in the study.

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