OPEN ACCESS

Scholars International Journal of Obstetrics and Gynecology

Abbreviated Key Title: Sch Int J Obstet Gynec
ISSN 2616-8235 (Print) |ISSN 2617-3492 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article

Comparison of Vitamin D Status between Healthy Pregnant Women and Women with Recurrent Early Spontaneous Pregnancy Loss

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DOI: 10.36348/sijog.2023.v06i09.003 | **Received:** 20.08.2023 | **Accepted:** 16.09.2023 | **Published:** 28.09.2023

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Abstract

***Introduction*:** Recurrent early spontaneous pregnancy loss is the most common negative outcome of pregnancy and identification of modifiable risk factors is potentially of great importance for public health. The immunological factors may play a role in failure of pregnancy in women with recurrent pregnancy loss. The role of this vitamin D has also been proved in the immune system. ***Aim of the Study*:** This study aim to determine serum vitamin D status among women with recurrent early spontaneous pregnancy loss and to compare the vitamin D level among them with healthy pregnant women. ***Methods*:** This cross sectional comparative study was done at the out patient department of obstetrics and Gynecology in Bangabandhu Sheikh Mujib Medical University during September 2018 to August 2019. The study comprises of 70 women carrying singleton fetus, gestational age between 8 to 12 weeks, in the age range of 18 to 35 years. The respondents were divided into two groups, 35 pregnant women in their early pregnancy loss with previous history of two or more early spontaneous pregnancy loss were considered as group I and 35 women in their early live pregnancy with previous history of one or more successful pregnancy and no history of any pregnancy loss were considered as group II. Fasting serum vitamin D level was measure by CMIA. ***Results*:** This study found that most the respondents were vitamin D deficient in both groups. In group I 17(48.6%) and in group II 10(28.6%) were vitamin D severe deficient. Again in group I 18(51.4%) and 24(68.6%) in group II were vitamin D deficient. The mean vitamin D level was 9.90±2.28 ng/ml in group I and 11.43±3.96 ng/ml in group II. The differences was statistically not significant (P>0.05) between two groups. There is a negative correlation (r-0.235; p 0.175) between serum vitamin D level and number of pregnancy loss. Which indicate increase number of pregnancy loss associated with decrease level of vitamin D. ***Conclusion*:** Vitamin D deficient in both groups, however the vitamin D level was comparatively low in women with recurrent early spontaneous pregnancy loss but the differences was not significant between two groups.

Keywords: Vitamin D Status, Pregnant Women, Recurrent Early Spontaneous, Pregnancy Loss.

INTRODUCTION

Vitamin D deficiency is a strong predictor of early pregnancy loss and that correction of Vitamin D status among pregnant women may substantially reduce the frequency of spontaneous abortion. Pregnancy loss (PL) is an adverse outcome of pregnancy in which conception does not result in a live-born child. Of clinically recognized pregnancies, 10- 15% terminates with spontaneous loss. Early pregnancy loss (spontaneous miscarriage) is defined as the spontaneous termination of pregnancy before 12 gestational weeks [1]. The great majority of pregnancy losses occur early, before 12 weeks' gestation; hence, the pathophysiology

is enough to be major concern. Multiple causes of recurrent pregnancy loss are charged alone or in combination with each other [2], including chromosomal anomalies, hormonal problems, uterine abnormalities, infections, uncontrolled DM, autoimmune disorders and thrombophilias and up to 50% of cases of recurrent pregnancy loss will not have a clearly defined etiology Practice Committee of the American Society for Reproductive Medicine [3]. Vitamin D deficiency is a common problem in reproductive aged women and its prevalence may be increasing [4]. However, the effect of severe vitamin D deficiency and insufficiency on maternal and fetal outcomes during pregnancy is less clear. Recent observational and randomized control trials have attempted to investigate this issue but there remains limited guidance on the management of vitamin D deficiency during pregnancy. Vitamin D deficiency and insufficiency have been associated with a variety of adverse maternal and fetal outcomes, ranging from preeclampsia, gestational diabetes, preterm delivery, intrauterine growth restriction, bacterial vaginosis, spontaneous abortion [5]. Vitamin D deficiency is a common problem in reproductive aged women in the United States. The effect of vitamin D deficiency in pregnancy is unknown but has been associated with adverse pregnancy outcomes. Recently published criteria from the Institute of Medicine are used to categorize vitamin D status by plasma 25-OH vitamin D concentrations: severe deficiency (<10 ng/ml [25nmol/L]); deficiency (<20 ng/ml [50nmol/L]); insufficiency (21-29 ng/ml [51-74 nmol/L]); and sufficiency (230 ng/ml [75nmol/L] [6]. There is some evidence suggesting that vitamin D modulates human reproductive processes [7]. Vitamin D deficiency is a common problem in childbearing age women and pregnant women in China [8, 9]. In addition, the vitamin D metabolic pathway involves multiple enzymatic reactions. Vitamin D is metabolized in the liver to the form 25 (OH)D which is used to determine a patient's vitamin D status; 25 (OH)D is metabolized in the kidneys by 25-hydroxyvitamin D-1 alpha hydroxylase (CYP27B1) to its active form, 1,25-(OH)2D [10]. The increased synthesis of 1, 25-(OH) 2D is linked to higher CYP27B1 activity in maternal kidney, placental trophoblasts and Deciduas [11]. Vitamin D deficiency is a growing topic of interest around the world. The prevalence of sub-optimal vitamin D concentrations in patient plasma emphasizes the need for continued research on hypovitaminosis D in pregnancy and reproductive health. Therefore, the present study is aimed to determine the association between serum vitamin D levels and pregnancy outcome and also to assess the role of serum vitamin D levels in the immediate diagnosis of pregnancy loss.

MATERIALS AND METHODS

Study Design: A cross sectional comparative study.

Place of Study: This study was carried out in the Department of Obstetrics and Gynaecology, BSMMU, Dhaka.

Duration of Study: 1 year (September 2018 to August 2019). But the patients enrollment was started after IRB clearance (15th January 2019).

Study Population: Pregnant women with gestational age between 8 to 12 weeks.

Sample Size (n):

To calculate sample size for two means by the following formula was followed:

Sample size (n) = $\frac{(Z_a+Z_b)^2 \times (\sigma_1^2+\sigma_2^2)}{(\mu_1-\mu_2)^2}$ [12].

Estimated sample size 32 in each group

But to increase the power of the study more sample as much as possible was taken. We took 35 sample size in each group.

Therefore, total sample size was 70 (35×2).

All values were taken from Hou *et al.,* [13].

Inclusion Criteria:

1. Maternal age between 18 to 35 years
2. Gestational age between 8 and 12 week
3. Women carrying singleton fetus

Exclusion Criteria:

1. Multiple pregnancy
2. Pregnant women with prior treatment with Vitamin D
3. Diabetes mellitus
4. Thyroid disorder
5. Auto immune diseases (SLE, Antiphospholipid antibody syndrom)
6. Renal, trophoblastic and thrombophilic diseases
7. Known paternal chromosomal abnormality
8. Fibromyoma with pregnancy
9. Pregnant women with vaginal bleeding due to local causes- cervical polyp, cervical cancer, local trauma
10. Assisted pregnancy and ectopic pregnancy

Sample Selection

I attained all antenatal cases in the OPD of Obstetrics and Gynaecology Department of BSMMU, Dhaka, Bangladesh. The subject who were in 1" trimester of pregnancy (8 to 12 weeks) and agreed to attain and fulfill the inclusion and excluding the exclusion criteria recruited for the study. They were allocated into two groups of which 35 women in their early pregnancy (which documented by USG as missed or incomplete abortion) with history of two or more early spontaneous pregnancy loss as group I and 35 women in their early live pregnancy (documented by ultrasonography) with previous history of one or more successful pregnancy and no history of any pregnancy loss as group II.

Study Procedure

This cross-sectional comparative study was conducted in Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka. A total 70 women carrying singleton fetus, gestational age between 8 to 12 weeks in the age range between 18 to 35 years, attending the OPD of obstetrics and Gynecology Department, BSMMU were recruited for the study. After fulfilling the inclusion and excluding the exclusion criteria, subjects were enrolled with unique ID. Subjects were briefed about the objectives of the study, risk and benefits for participating in the study and confidentiality. Patient were allocated into two groups of which 35 women in their early pregnancy (which documented by USG as missed or incomplete abortion) with history of two or more early spontaneous pregnancy loss as group I and 35 women in their early live pregnancy (documented by ultrasonography) with previous history of one or more successful pregnancy and no history of any pregnancy loss as group II. Written inform consent was obtained from each subject. Detailed history was taken about their educational status, area of residence, monthly family income, regarding personal history exposure to sunlight, during sun exposure average surface area of the body covered with clothes or not and asked about consumption of milk, egg and small or oily fish. The body weight was measured on footed with minimum clothing. The average weight (0.5 kg) of the clothes was later subtracted from the measured weight. The height was measured keeping the women standing on level ground without footwear, against a wall by using measuring tape to the nearest of 1 cm. Weight and height was obtained to calculate BMI (kg/m^2). Fasting blood sample was collected for measurement of serum 25(OH) D Level.

Statistical Analysis of Data

All the relevant collected data was compiled on a master chart first. Then organized by using scientific calculator and standard statistical formula. Percentages were calculated to find out the proportion of the findings. Further statistical analysis of the results was done by Microsoft excel. Quantitative variables were expressed as frequency, percentage and mean ± SD and qualitative data was expressed as percentage. The p value of categorical variables was determined by chi square test whereas the same value of quantitative variables was determined by student's t test and ANOVA test. Statistical analysis of relationship between serum vitamin D and total number of pregnancy loss was done by Pearson's correlation. P-value <0.05 was considered statistically significant.

RESULTS

Table I: Distribution of the study subjects by demographic variable (n=70)

Demographic variable	Group I (n=35)		Group II (n=35)		p value
	n	%	n	%	
Age (in years)					
20-25	12	34.3	13	37.1	
26-30	15	42.9	19	54.3	
>30	8	22.9	3	8.6	
Mean SD	26.83 ±4.32		26.06±3.54		[a]0.419[ns]
Rang (min,max)	20,33		20,33		
Area of residence					
Urban	29	82.9	31	88.6	[b]0.495[ns]
Rural	6	17.1	4	11.4	
Education					
Illiterate	4	11.4	1	2.9	[b]0.333[ns]
<5years	5	14.3	4	11.4	
6-10years	13	37.1	12	34.3	
10-12yrars	4	11.4	10	28.6	
>12years	9	25.7	8	22.9	
Monthly family Income (taka)					
5,000-10,000	8	22.9	3	8.6	[b]0.051[ns]
10,000-20,000	6	17.1	16	45.7	
20,000-30,000	7	20.0	4	11.4	
>30,000	14	40.0	12	34.3	
Occupation status					
House wife	28	80.0	29	82.9	[b]0.151[ns]
Service	2	5.7	5	14.3	
Business	1	2.9	1	2.9	
Other	4	11.4	0	0.0	

ns-not significant, [a]p value reached from Unpaired t-test, [a]p value reached from Chi-square test

Table I shows 15(42.9%) subjects belong to age 26-30 years in group I and 19(54.3%) in group I and 26.06±3.54 years in in group II. The mean age was 26.83±4.32 years group II. Most of the subjects come from urban area. 13(37.1%) subject's educational status had 6-10 years in group I and 12(34.3%) in group II. Most of the subjects were housewife in both groups, where 28(80%) in group I and 29(82.9%) in group II. k was observed that 14(40.0%) subject's monthly income had >30000 taka in group and 12(34.3 %) in group II. The differences were statistically not significant (p>0.05) between two groups.

Table II: Distribution of the study subjects by personal history (n=70)

Personal history	Group I (n=35)		Group II (n=35)		p value
	n	%	n	n%	
Sunlight exposure					
<3 hour	22	62.9	19	54.3	0.467^{ns}
½-1 hour	13	37.1	16	45.7	
Area of skin expose to sunlight					
Face and hands (5% BSA)	32	91.4	33	94.3	0.324^{ns}
Face, arms and hands (10% BSA)	3	8.6	2	5.7	

Ns= not significant, p value reached from Chi-square test

Table II shows the distribution of the study subjects by sunlight exposure. It was observed that 22(62.9%) subjects sunlight exposure were <½ hour in group I and 19(54.3%) in group II. Majority 32(91.4%) subjects had most of the surface area of the body covered with cloths in Group I and 33(94.3%) in group II. The differences was statistically not significant (p>0.05) between two groups.

Table III: Food frequency of the study subjects (n=70)

Dietary pattern	Group I (n=35)		Group II (n=35)	
	n	**%**	**n**	**n%**
Daily intake of egg with egg yok				
Yes	25	71.4	32	65.7
No	10	28.6	12	34.3
Milk consumption 4 times a week (150 ml)				
Yes	13	37.1	11	31.4
No	22	62.9	24	68.6
Daily intake of fish (30 gm)				
Yes	18	51.4	16	45.7
No	17	48.6	19	54.3

Table III shows the food frequency of the study subjects. It was observed that 25(71.4%) participants had daily intake of egg in group I and 23(65.7%) in group II. In group I 13 (37.1%) participants had milk consumption 4 times a week (150 ml) and 11(31.4%) in group II. 18(51.4%) participants had daily intake of fish in group I and 16(45.7%) in group II.

Table IV: Distribution of the study subjects by physical examination (n=70)

Physical examination	Group I (n=35)	Group II (n=35)	p value
	Mean±SD	**Mean±SD**	
Height (cm)	152.91±4.49	153.43 ±5.24	0.657^{ns}
Weight (kg)	56.80±9.42	56.28±7.27	0.797^{ns}
BMI (kg/m²)	24.37±3.87	24.23±2.99	0.866^{ns}

ns-not significant, p value reached from Unpaired t-test

The mean height was 152.91±4.49 (cm) in group I and 153.43±5.24 (cm) in group. The mean weight was 56.80±9.42 (kg) in group I and 56.28±7.24 (kg) in group II. The mean BMI was 24.37±3.87 (kg/m²) in group I and 24.23±2.99 (kg/m²) in group II. The differences was statistically not significant (p>0.05) between two groups (table-IV).

Table V: Distribution of the study subjects by vitamin D level ng/ml (n=70)

Vitamin D level ng/ml	Group II (n=35)		Group II (n=35)		P value
	n	**%**	**n**	**%**	
Sufficiency	0	0.0	0	0.0	
Insufficiency	0	0.0	1	2.9	^{a}0.314^{ns}
Deficiency	18	51.4	24	68.6	^{a}0.143^{ns}

Vitamin D level ng/ml	Group II (n=35)		Group II (n=35)		P value
	n	%	n	%	
Severe deficiency	17	48.6	10	28.6	[a]0.085[ns]
Mean±SD	9.90±2.28		11.43±3.96		[b]0.52[ns]
Rang (min,max)	5.2,14.5		5.6,21.7		

ns-not significant, [a]p value reached from Chi-square test, [b]p value reached from Unpaired t-test

Table V shows the distribution of the study subjects by vitamin D level. It was observed that 18(51.4%) subjects had deficiency in group I and 24(68.6%) in group II. Severe deficiency: was 17(48.6%) in group I and 10(28.6%) in group II. The mean vitamin D level was 9.90±2.28 ng/ml in group I and 11.43±3.96 ng/ml in group II. The differences was statistically not significant (p>0.05) between two groups.

Table VI: Relationship between occupation with vitamin D level of the study subjects (n=70)

Occupation status	Vitamin D level (ng/ml)			P value
	n	Mean±SD	Rang (min-max)	
House wife	57	10.81±2.75	5.2-21.7	0.686[ns]
Service	7	9.50±2.40	7.6-14.5	
Business	2	10.80±3.39	8.4-13.2	
Othe	4	10.67±1.45	9.5-12.5	

ns-not significant, p value reached from ANOVA test

The mean vitamin D level was 10.81±2.75 ng/ml in house wife, 9.50±2.40 ng/ml in service, 10.80±3.39 in business and 10.67±1.45 ng/ml in others. The difference was statistically not significant (p>0.05) between occupation and vitamin D level (Table-VI).

Table VII: Relationship between sunlight exposure with vitamin D level of the study subjects (n=70)

Sunlight exposure	Vitamin D level (ng/ml)			P value
	n	Mean±SD	Range (min-max)	
<½ hour	41	10.34±2.73	5.2-15.1	0.196[ns]
½ -1 hour	29	11.18±2.54	7.6-21.7	

ns-not significant, p value reached from Unpaired t-test

The mean vitamin D level was 10.34±2.73 ng/ml in sunlight exposure <<½ hour and 11.18±2.54 ng/ml in sunlight exposure ½ -1 hour. The difference was statistically not significant (p>0.05) between sunlight exposure and vitamin D level (Table-VII).

Table VIII: Distribution of vitamin D level and number of pregnancy loss (n=35)

Number of pregnancy loss	N	Vitamin D level ng/ml Mean±SD	P value
Two pregnancy loss	29	10.13±0.40	0.147[ns]
Three pregnancy loss	4	9.65 ±0.94	
Four pregnancy loss	2	7.0 ±1.4	

ns=not significant, p value reached from ANOVA test

The mean vitamin D level was 10.13±0.40 ng/ml in wonmen with two pregnancy loss, 9.65±0.94 ng/ml in three pregnancy loss and 7.0±1.4 ng/ml in four pregnancy loss. The difference was statistically not significant (p>0.05) among vitamin D level and number of pregnancy loss (Table-VIII).

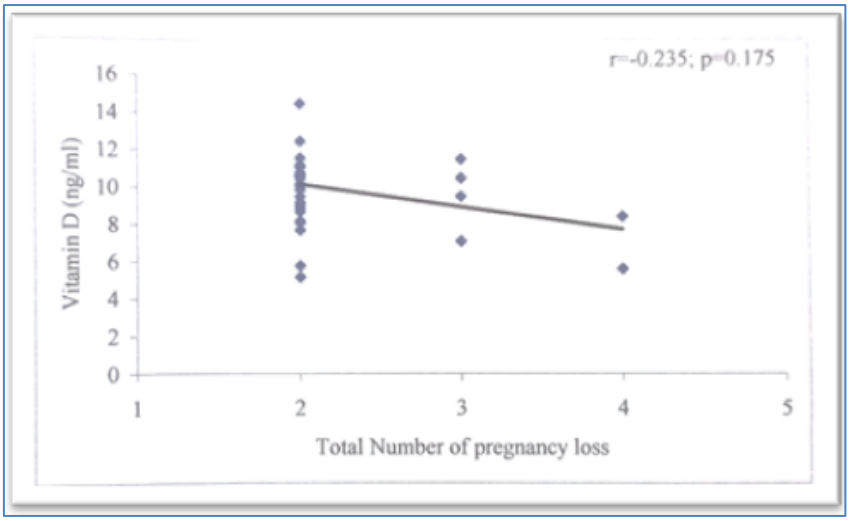

Figure 1: Scatter diagram showing negative pearson's correlation (r=-0.235; p=0.175) between serum vitamin D level and number of pregnancy loss.

DISCUSSION

In this study it was observed that most of the subjects were vitamin D deficient in both groups. In group 1 17(48.6%) and in group II 10(28.6%) were vitamin D severe deficient Again in group 1 18(51.4%) and 24(68.8%) in group II were vitamin D deficient. The mean vitamin D level was 9.90±2.28 ng/ml in group 1 and 11.42±3.96 ng/ml in group II. The mean vitamin D level differences was comparatively low in group 1 but the difference was not significant (p>0.05) between two groups. This finding are in agreement with other study findings Moller *et al.*, [14]. Flood- Nichols *et al.*, [15], did not find an association between vitamin D deficiency and the clinical outcome of miscarriage in the first-trimester pregnancy. Damiati *et al.*, [16], study showed Vitamin D concentrations were deficient in both groups for women with and without a history of miscarriages, respectively. In this current study, it was observed that the mean vitamin D level was 10.13±0.40 ng ml in women with two pregnancy loss, 9.65±0.94 ng/ml in three pregnancy loss and 7.0+1.4 ng/ml in four pregnancy loss. The difference was statistically not significant (p>0.05) among vitamin D level and number of pregnancy loss. In this study, it was observed that there is a negative not significant correlation (r-0.235; p-0.175) between serum vitamin D level and total number of pregnancy loss. Based on the results Ghaedi *et al.*, [17], the median serum level of vitamin D was 9.20 ng/ml in the women with four or more abortions, 10.85 ng/ml in those with three abortions, and 11.50 ng/ml in the women with two abortions. Thus, the women with higher number of abortions had lower vitamin D serum levels. In this current study, it was observed that 22(62.9%) subjects sunlight exposure were <½ hour in group I and 19(54.3%) in group 11. The differences was statistically not significant (p>0.05) between two groups. This present study, it was observed that maximum surface area of there body covered with clothes in both groups which was 32(91.4%) in group I and 33(94.3%) in group II. This might be a reason for vitamin D deficiency in both groups and also due to changing life styles, like many women stay at home which is almost closed sunlight and urban population is now increasingly living in densely populated apartment blocks with very little natural light. Guzel *et al.*, [18], compared serum vitamin D levels in Turkish women who did and did not were traditional Islamic dressing (Veils), mean 25(OH)D level was significantly lower among veiled compared to unveiled women. In this study, it was observed that 25(71.4%) participants had daily intake of egg in group 1 and 23(65.7%) in group II. More than one third 13(37.1%) had milk consumption 4 times a week (150 ml) in group I and 11(31.4%) in group II. More than half 18(51.4%) participants had daily intake of fish in group I and 16(45.7%) in group II. Williams *et al.*, [19], that the etiology hypovitaminosis D is likely multifactorial because it has been associated with several dietary factors as well as decreased sunlight exposure and poor vitamin D intake. In this present study, it was observed that the mean vitamin D level was 10.81±2.75 ng/ml in house wife, 9.50±2.40 ng/ml in service, 10.80±3.39 in business and 10.67±1.45 ng/ml in others. The difference was statistically not significant (p>0.05) between occupation and vitamin D. In this current study, it was observed that the mean vitamin D level was 10.34±2.73 ng/ml in sunlight exposure < hour and 11.18±2.54 ng/ml in sunlight exposure ½-1 hour. The difference was statistically not significant (p>0.05) between sunlight exposure and vitamin D. However the vitamin D level was comparatively low in sunlight exposure <<½ hour, The discrepancy with the present study may be explained in part by differences in our study population relative to other published studies, including difference in parity, maternal age at dietary intake, and/or gestational age of specimen collection [20, 21]. Although vitamin D has a biologically possible role in all the aforementioned disorders, this was not demonstrated in this study population as vitamin D deficiency did not increase the odds for recurrent early spontaneous pregnancy loss but showed deficiency in this study subjects and also showed

that increase number of pregnancy loss associated with decrease level of vitamin D. Study-specific parameters such as maternal demographics and parity may account for some of these differences in this study subjects relative to published studies. It suggested that decreased serum vitamin D levels among those women with the failed clinical pregnancies history may predispose to increased risk for PL. From the findings of this study, we need to screen vitamin D deficiency who are at risk based on diet, sun exposure, age and lifestyle factors, as features of hypovitaminosis D are mostly reversible with proper replacement.

CONCLUSION

Vitamin D deficient in both groups, however the vitamin D level was comparatively low in women with recurrent early spontaneous pregnancy loss but the differences was not significant between two groups.

LIMITATIONS

1. The study population was selected from one selected hospital in Dhaka city, so that the results of the study may not be reflect the exact picture of the country.
2. The present study was conducted at a very short period of time.
3. Small sample size was also a limitation of the present study.
4. Samples were taken by purposive method in which question of personal biasness might arise.

RECOMMENDATIONS

A Large scale, multicentral national study for long period should be carried out to determine the cut off value of serum vitamin D level in pregnant women with risk of pregnancy loss. We can consider serum vitamin D as a screening test in preconceptional period.

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