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Quantitative estimation of serum 25(OH) D and associated risk factors of vitamin D deficiency among pregnant women attending a tertiary care hospital in Udaipur, Rajasthan

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Abstract: *Introduction:* Insufficiency of vitamin D is now known globally and is exceptionally common in temperate and tropical countries. Due to its several negative maternal and fetal health consequences, vitamin D insufficiency in pregnancy is a growing concern worldwide. *Methods:* A cross-sectional study was conducted at a tertiary medical hospital for one year. A sample of 280 pregnant women attending antenatal clinic (ANC) were enrolled using a random sampling technique. A standard questionnaire was designed to collect socio-demographic details and the sun exposure of participants. To analyse dietary habits and consumption of vitamin D-rich foods, a food frequency questionnaire (FFQ) was used. Serum samples were obtained and analyzed for and calcium levels. *Results:* High prevalence of maternal vitamin D insufficiency 122 (43.57%) and deficiency 92 (32.85%) observed. Most 263 (94%) participants had adequate serum calcium levels (> 8.5 mg/dL). Younger participants (< 25 yrs.) had low mean vitamin D (17.61±13.89 ng/mL), adequate calcium (9.52±0.70 mg/dL) levels. Rural residence, multigravida, advanced trimester, poor education, joint family, unemployment, and lower socioeconomic status were found associated with vitamin D insufficiency. *Conclusion:* In the present study a high prevalence of maternal vitamin D deficiency was observed. The burden of Vitamin D inadequacy (76.39%) reflects poor nutritional status and health risks for mothers and fetuses. Sociodemographic factors (Residence, Education, Family, and Socioeconomic status) were linked to this micronutrient deficiency. There is a need for further community-based nutritional research on sociodemographic, biological, and nutritional determinants for in-depth understanding.

Keywords: Vitamin D, Pregnancy, Nutrition, Women, Socio-demographic, India.

Key message: There is a high deficiency of vitamin D among pregnant women which is inherently linked to their socio-demographic, nutritional and clinico-behavioral status. Considering high nutritional demand a vitamin D fortification and supplementation policy can be recommended for them.

Introduction

Insufficiency of vitamin D is now globally recognized. Population-based studies have regularly detailed that it is exceptionally common in temperate and tropical climate nations [1]. Approximately one billion individuals in all age groups, around the world have vitamin D insufficiency or deficiency [2]. Specifically, among pregnant women there is a growing concern due to the extremely higher incidence of vitamin D inadequacy which has several negative consequences for them [3].

Data from a narrative review suggested an 18% -84% prevalence of deficiency in vitamin D and contributing variables such as latitude, region, ethnicity, skin color, seasonal and dietary intake [4-5]. Vitamin D is a fat-soluble steroid and is regarded as an extremely crucial and essential prohormone [6]. It is actively engaged in the homeostasis of calcium and bone mineralization, but recent studies have specifically stated many other benefits of vitamin D beyond healthy bones [7].

Maternal and fetal requirements accelerate during the pregnancy and lead to an increased risk of vitamin D deficiency [8]. Maternal 25(OH) D transferred across the placenta and fetal cord blood concentration linked with the mother's concentration of 25(OH) D. Mother and fetal cord circulation levels 25(OH)D have such a strong association whereby maternal vitamin D deficiency is often replicated with detrimental effects in their neonates [9]. Therefore, the relevance of hypovitaminosis D on the health of pregnant women, fetuses, children, and adults is of concern. Studies demonstrated maternal complications, such as gestational diabetes mellitus, preeclampsia, infections, cesarean section, and recurrent loss of pregnancy [10-11].

We generally obtain vitamin D from the two primary sources: skin synthesis from the sunlight is cholecalciferol D3 and dietary intake provides ergocalciferol D2. The status of vitamin D in our body is manipulated by plenty of factors ranging from seasons, time of body exposure to sunlight, latitude and altitude of that geographic region, air pollution levels, pigmentation and ethnic background, use of sunscreen, passing through glass, aging, and environmental pollution [12]. Besides these attributes, socioeconomic status, education, lifestyle, and culture are indeed crucial predictors of vitamin D status, since they affect diet and sun exposure [13].

India is located between 8°N-38°N and plenty of sunshine is found all year round therefore people in India should have an adequate vitamin D status. However, data from epidemiological studies have revealed contrary results with prevalence of vitamin D deficiency ranging upto 75% in all age groups. Even now data referring to vitamin D insufficiency among pregnant women from tribal areas of Rajasthan are sketchy. The present study aims to assess serum 25(OH) D levels in all three trimesters of pregnancy and to recognize aspects associated with insufficiency of vitamin D.

Material and Methods

For this observational (cross-sectional) analysis, we randomly selected 280 healthy pregnant women from the first, second, and third trimesters. From January 2017 until January 2018, data were collected for 1 year period. The study included all women with Singleton pregnancy, ranging in age 18-35 yrs, booked pregnancy, primi, and multigravida.

Pregnant women with chronic or metabolic disorders such as diabetes, hypertension, thyroid, parathyroid, gastrointestinal, liver, and renal disease) as well as those receiving antitubercular, antiepileptic medication were excluded. The inclusion requirements were met by a total of 280 participants from a tertiary medical teaching hospital located in a tribal district of Udaipur, India.

At the time of recruitment, a serum sample of eligible pregnant women was taken for estimation of serum 25(OH) D. After collection serum sample was centrifuged and stored at minus 20 degrees Celsius.

The Callbiotech, Inc.25-hydroxy (25-OH) vitamin D ELISA kit was used for serum 25(OH) D level assessment as per kit instruction. 25-OH Vitamin D kit is a solid Elisa phase, based on the competitive binding principle. The sensitivity of this test kit is 0.67ng/ml. Vitamin D deficiency was defined as serum 25(OH) D levels < 10ng/ml, insufficient level (10-30ng/ml) and sufficient level was (30-100ng/ml).

Serum calcium estimation was done on a fully automated COBAS 6000 modular Roche analyzers. Calcium Ions from violet complex with O-cresolphthelein complexone in an alkaline medium. Absorbent is measured at 570nm. The intensity of the color is directly proportional to the concentration of calcium ions. The value was expressed in mg/dl.

Anthropometrics included weight (kg) and height (cm), Weight and height were recorded using an Asian standard scale to assess Body mass index (BMI). All participants responded using a structured questionnaire to gather information such as: name, age, socio-demographic details, gravida, medical history, medication, income, area of residence, religion, and sun exposure. The study participant's socioeconomic status was calculated according to the Kuppuswamy scale [14]. Data were also obtained concerns regarding the education (higher and lower) and occupation (employed or unemployed) of pregnant women and their husbands.

A detailed sun exposure questionnaire was used to measure their normal time under the sun, how often and hours they spend under the sun, what kinds of activities individuals do under the sun as well as how much skin they were exposed to sunlight. We used the “Wallace rule of nine” chart to determine the percentage of total surface body area exposed to the sun.

The chart divides the body into sections that represent body parts into a percentage. This chart is for adults only. Sunshine exposure was calculated as – Sunshine exposure (%) = Hours of exposure/day × percentage of body surface area exposed [15]. In this study, a food frequency questionnaire (FFQ) relevant to vitamin D and calcium intake over a 7-day timeframe was developed and quantified. Data regarding supplements intake of calcium and vitamin D (yes/no) were collected, including for how much time (months) they were taking it.

Prior informed consent was obtained from study participants and confidentiality of data at all stages of the study was maintained. The study was approved by the institutional ethical committee (Reference no: GU/HREC/EC/2017/ 1512). The data were first cross-verified and analyzed using a chi-squared test for qualitative and student's t-test (unpaired) or ANOVA test for quantitative data. The odds ratio with a 95% confidence interval was used to quantify the risk factors. P-value less than 0.05 were considered statistically significant.

Results

In the present study majority of pregnant women, 66.4% were of > 25 years of age. The percentage of participants in their primi and multigravida were 43% and 57%. Most participants were in the third trimester 50% and 31% from the second trimester of their pregnancy. Most participants hailed from urban 52% areas belonging to the Hindu religion 91% and joint families 75%. Most participants (58%) had more than 5 members in the family and most had received lower education 58%.

Most of the participant’s husband had higher education (77%) were skilled workers (87%). Most respondents were unemployed (housewives) 81% and only 19% were employed. The majority of respondents (60%) had an income of less than 20,000 rupees per month. Most participants were from upper-middle (46%) and middle (27%) socioeconomic classes.

The mean vitamin D level of the study participant was 21.71±15.57 ng/mL with a range of 4 to 68 ng/mL. Notably, 77.43 % of the study population of the present study were found with insufficient serum levels of vitamin D [5(OH) D] of which 32.85 % were in deficient level [Table 1].

We found that women >25 years, multigravida, and those in the 3rd trimester of pregnancy had significantly lower mean serum 25(OH)D levels. There were no significant differences in women's vitamin D profile compared to their place of residence and religion. Pregnant women hailing from joint families and more than five family members reported significantly lower levels of mean serum 25(OH) D. Vitamin D levels were also linked to the education of pregnant women and their husbands.

We observed significant differences in mean serum 25(OH) D levels of low educated women, as well as in those women whose husbands had a low educational level. Housewives or unemployed women had a low level of mean serum 25(OH) D compared to employed women. However, wives with spouses having below skilled occupation had significantly low mean serum vitamin D level. Results also demonstrated that pregnant women with low family income and from lower socio-economic classes had significantly lower levels of mean serum vitamin D [Table 2].

Table-1: Comparative assessment of various socio-demographic factors to vitamin D insufficiency among pregnant women

Variable		N (%)	Vitamin D level, Mean± SD (ng/mL)	T, P-value
1. Age:	<25 years	94 (33.52%)	29.82±15.61	16.61, <0.001
	≥25 years	186 (66.42%)	17.61±13	
2. Gravida:	Primi	121(43%)	30.78±16.64	9.85, <0.001
	Multi	159(57%)	14.81±10.35	
3. Trimester:	1	53 (19%)	35.02±17.42	39.55, <0.001
	2	88 (31%)	23.51±15.33	
	3	139 (50%)	15.49±10.90	
4. Residence:	Urban	145 (52%)	23.58±16.53	4.40, 0.037
	Rural	135 (48%)	19.69±14.27	
5. Religion:	Hindu	256 (91%)	22.15±15.81	2.44, 0.1119 (NS)
	Muslim	24 (9%)	16.96±11.96	
6. Family:	Nuclear	71 (25%)	32.46±17.59	53.86, <0.001
	Joint	209 (75%)	18.06±12.97	
7. Family members:	5 or less (≤5)	118 (42%)	30.00±16.83	8.52, <0.001
	More than (>5)	162 (58%)	15.67±11.28	
8. Education (Self):	≥12th or higher	120 (43%)	26.15±17.56	4.25, < 0.001
	≤10th or lower	160 (58%)	18.38±12.98	
9. Education (Husband):	≥12th or higher	159 (77%)	24.12±17.18	3.01, 0.003
	≤10th or lower	121 (23%)	18.53±12.55	
10. Occupation (Self):	Unemployed	228 (81%)	20.21±14.62	-3.43, 0.044
	Employed	52 (19%)	28.27±17.92	
11. Occupation (Husband):	Skilled and above	243 (87%)	24.95±16.52	4.90, < 0.001
	Below skilled	37 (13%)	15.77±11.58	
12. Monthly income:	≤ 20,000	169(60%)	17.73±12.64	-5.55, < 0.001
	>20,000	111(40%)	27.77±7.59	
13. Socio-economic class:	Upper	37 (13%)	32.66±19.60	10.72, < 0.001
	Upper middle	130 (46%)	22.71±14.98	
	Lower middle	75 (27%)	17.79±13.31	
	Upper lower	38 (14%)	15.35±11.30	

Table-2: Comparative assessment of various dietary factors to vitamin D insufficiency among pregnant women

Variable		Vitamin D level, Mean± SD (ng/mL)	n (%)	T,P-value
1. Supplements intake:	Present	179 (64%)	19.95±13.65	23.68, <0.001
	Absent	101 (36%)	24.83±18.15	
2. Dietary Pattern:	Vegetarian	197 (70%)	16.55±11.42	48.99, <0.001
	Non-vegetarian	71 (25%)	33.89±17.62	
	Eggetarian	12 (4%)	34.34±15.60	
3. Consumption of milk products:	Never	193 (69%)	16.72±12.78	45.77, <0.001
	Daily	87 (31%)	33.59±16.04	
4. Fish consumption:	Never	221 (78.92%)	17.64±12.36	49.90, <0.001
	Occasionally	59 (21.07%)	37.14±16.76	
5. Egg consumption:	Never	197 (70%)	16.74±11.47	45.77, <0.001
	Occasionally	83 (30%)	33.61±17.53	
6. Meat consumption:	Never	209 (74.64%)	17.62±12.37	38.85, <0.001
	Occasionally	71 (25.35%)	33.89±17.62	

Table-3: Comparative assessment of various clinic behavioral factors to vitamin D insufficiency among pregnant women			
Variable	Vitamin D level, Mean± SD (ng/mL) n (%)		T, P-value
1. BMI Classification: Underweight < 18.5	32 (11%)	27.54±15.86	3.53, 0.030
Normal 18.5-22.9	154 (54%)	19.88±15.90	
Over-weight >23	99 (35%)	22.68±14.37	
2. Sun Exposure: ≤1 hour	208(%)	17.89±12.63	42.81, <0.001
> 1 hour	72(%)	29.03±17.95	
3. Total sun exposure: ≤ 20%	184(66%)	17.89±12.63	-6.03, < 0.001
>20%	96(34%)	29.03±17.95	
4. Hypertension: Normotensive	259 (93%)	21.86±15.24	
Hypertensive	21 (7%)	19.80±19.51	0.58, 0.561 (NS)
5. Backache: Yes	259 (92.5%)	21.40±15.73	-0.50, 0.617 (NS)
No	21 (7.5%)	22.41±15.28	
6. Joint ache (any): Yes	212 (75.71%)	21.87±15.29	0.30, 0.758 (NS)
No	68 (24.28%)	21.20±16.51	
7. Muscular pain: Yes	253 (90.35%)	22.26±15.88	1.83, 0.068 (NS)
No	27 (9.64%)	16.51±11.27	

The dietary factors of the present study showed that women who were taking calcium supplementation, eating a vegetarian diet, never consumed milk, fish, eggs, and meat had significantly low mean serum 25(OH) D levels [Table 3].

Results of the present study showed there weren't any significant differences in the vitamin D profile of women compared to their BMI, hypertension, and pain in the back, joint, and muscle. Women who had higher BMI usually spent less than one hour in sunlight and those who had total sun exposure less than 20% had significant low mean serum vitamin D levels.

Discussion

The pandemic of vitamin D deficiency encompasses all ethnicities and age groups across the world [16]. Across several studies, the prevalence of vitamin D deficiency among pregnant women is globally mentioned [17]. Tropical regions also seem to have an elevated prevalence of low 25(OH) Dlevels [18]. A study from the eastern part of India (n=177) reported 84.18 % of vitamin D deficiency in the second trimester, caesarean section deliveries, babies with low birth weight, with preeclampsia attributed to low vitamin D levels [19]. The vitamin D deficiency was identified in 93.5% (n=418) of pregnant women in New Delhi and the mean serum level of 25(OH) D in the severe deficiency category was 7.10 ± 1.49 ng/ml [20].

Aging in pregnant women substantially integrated with deficiency of vitamin D. It is a potential risk factor for pregnant women during the second trimester of gestation and over 30 years of age [21]. Likewise, our data showed a significant association with aging and vitamin D deficiency. Pregnant women over 25 years of age had a lower level of serum vitamin D compared with those below 25 years of age.

In our analysis, we observed with advancing gestation (trimester) there is a gradual decline in the level of vitamin D. However, 25(OH) D levels in late pregnancy reduced significantly and the variation could be due to the fat mass associated with 25(OH) D levels during pregnancy [22]. Parity has a significant correlation with vitamin D levels. Repetitious and untidy pregnancy can exacerbate Vitamin D deficiency. While comparing the insufficiency of vitamin D between primigravida and multigravida we found that multigravida women had 94.3% insufficiency and significantly lower vitamin D level. We also observed that parity (no. of children) significantly correlated to their vitamin D levels.

Women with more than two children had lower levels of vitamin D. The reason could be that majority of our study participants were from a lower-middle-class background. Such large families with less incomes have poor nutritional status among members which is manifested biologically in the pregnant women with higher nutritional demand. The joint families and more than 7 family members were also significant predictors of vitamin D insufficiency in our research.

Good education often trends to an improved life quality and standards with enriched diets. The present study expectedly demonstrated that both education of pregnant women and husbands were associated with significantly lower levels of vitamin D. Poorly educated pregnant women were found more deficient in vitamin D (86.3%) compared to more educated ones (58.3%). The educational level and socioeconomic status are effective tools for evaluating a host of social and environmental exposures in pregnant women responsible for low vitamin D status [23].

Our study results indicate that lack of education, knowledge, and awareness of food sources of vitamin D contribute to a deficiency state among pregnant women. Nutrition is inherently related to employment as well as socioeconomic status. The unemployed participants were found to have lower levels of vitamin D deficiency compared to employed women [24]. Pregnant housewives living in poorly educated communities have been reported vulnerable to vitamin D deficiency respectively, due to lack of awareness in this specific group [25].

LSES (low socioeconomic status) was indeed a significant risk factor for the deficiency of vitamin D during pregnancy. Most LSES women were housewives (unemployed) spending most time in indoor activity thereby getting low exposure to sunlight [26]. Residence and religion were not found to be risk factors for lower vitamin D levels in our study.

In our study, an estimated 64% of women were taking supplements with calcium and vitamin D, but only 6% had taken supplements for 3 months or more period. During pregnancy, the universal recommended dietary intake of vitamin D is 400 IU /d. In the sample of participants studied here, the consumption of vitamin D via diet was reportedly poor, with very few subjects consuming milk daily. Milk is a beneficial food resource for vitamin D and poor milk intake during pregnancy can substantially reduce daily vitamin D consumption. Intake of fish, eggs, and other non-vegetarian diets also directly impact the vitamin D levels of pregnant women [21, 27].

Our study also clearly indicated that limited consumption of dairy products and a non-supplemented vegetarian diet can significantly increase the incidence of vitamin D deficiency. Consumption might not be the only attribute of nutrition but perhaps the quantity and frequency of consumption invariably affect pregnant women's vitamin D levels. Suggesting a nationwide food fortification of vitamin D according to RDA would therefore be convenient. Skin synthesis from sun exposure is the key source of vitamin D, while dietary sources tend to be poor despite food fortification [28].

In this study, inadequate exposure to sunlight was observed to be a causative factor for insufficient maternal vitamin D levels. Thus, spending less time outdoors and restricting sunlight is a massive predictor of India's vitamin D deficiency epidemic, which as a tropical country receives sufficient sunlight. Achieving serum 25(OH)D at 20ng / mL if the sun exposure of our body, such as arms, hands, and face, indicating 22% of the body surface area, is adequate for the elderly, especially those living in a temperate environment [12]. Pregnant women with limited areas of skin exposed to sunlight, including the face and hands, had low levels of vitamin D contrasting to those with a wider area of skin exposed to the sun. In our study, vitamin D levels had no significant difference with BMI, hypertension and back, joints, and muscle pain in pregnant women.

Limitations of the study: Among the limitations of this study are that the participants' enrollment was restricted to one district of Southern Rajasthan. Additionally, clinical complaints reported weren't examined or corroborated with medical records. Besides, it's a cross-sectional study and does not allow us to establish a cause-effect relationship.

Conclusion

Vitamin D deficiency was prevalent among pregnant women in the study district of Southern Rajasthan. Various socio-demographic, clinical-behavioral and dietary factors have been found linked to the deficiency state. To address the current situation, multiple steps are required to be taken. For such women, nutritional education, supplementation and behavioral strategies are required for ensuring enough sun exposure for adequate vitamin D synthesis. Also, effective national nutrition policy initiatives and awareness of micronutrient deficiencies during pregnancy state must be done for public on a wider scale.

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