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Low serum 25(OH) D concentration and its correlation with consumption of vitamin d rich foods among pregnant women in India

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Abstract
Introduction: Research indicates that serum 25(OH) D insufficiency is widespread across all age groups with adverse health effects, but situations get worse when we talk about deficiency in pregnancy. Data from worldwide studies showed high prevalence of vitamin D deficiency ranged from 15 – 80%. Mother and fetus nutritional demands and metabolism rise exponentially during pregnancy, hence low dietary intake of vitamin D during pregnancy may bring adverse health effects. Methods: Observational study conducted in tertiary medical hospital. Total of 280 healthy pregnant women visiting antenatal clinics were randomly selected. For collecting socio-demographic details structured questionnaire was used. FFQ was used to obtain dietary intake of vitamin D rich foods. Serum samples were obtained and analyzed for serum 25(OH) D concentration. Results: High prevalence of vitamin D deficiency (32.85%) and insufficiency (43.57%) found. Most (70%) participants were vegetarians and had low mean serum 25(OH) D (16.55±11.42ng/mL) level. Discussion: Low consumption of milk and milk products, fish, meat and egg associated with serum 25(OH) D inadequacy. Burden of serum 25(OH) D inadequacy reflects poor nutritional status and health risks for mothers and fetuses. Low socioeconomic status, hike in food sources price, lack of awareness, food fads, no use of supplements were linked to micronutrient deficiency. There is a need for further research on community-based nutritional status and dietary intake assessment for more in depth understanding. Nutritional programs and policies need to be revised for vitamin D deficiency in pregnancy.

Keywords: serum 25(OH) D, pregnancy, nutrition, dietary intake, India

1. Introduction

Vitamin D deficiency is widely characterized as a pandemic and continues to be a great public health problem in many countries [1]. Higher incidence of vitamin D deficiency among pregnant women was observed, even in sunny regions [2]. The possible cause of the vitamin D deficiency is the lack of awareness that the main source of vitamin D for most humans is moderate sun exposure [3]. This pandemic of hypovitaminosis D can be linked predominantly to lifestyle and environmental factors that restrict sunlight exposure needed ultraviolet-B (UVB)-induced synthesis of vitamin D in skin [4]. Hypovitaminosis D is an independent potential risk for the mortality in overall population [5]. Evolving evidence demonstrates the potential role of vitamin D beyond bone health are: against cancer, cardiovascular disease, autoimmune diseases, depression and type 2 diabetes [6]. Research indicates that vitamin D insufficiency is widespread across all age groups with adverse health effects, despite adequate sun exposure and tropical environment but situations get worse when we talk about deficiency in pregnancy [7, 8, 9]. Data from worldwide studies showed high prevalence of vitamin D deficiency ranged from 15 – 80% [10, 11]. Indian data on the prevalence of hypovitaminosis D during pregnancy are limited [12]. Observation - based studies globally, have shown a positive correlation between vitamin D status and negative outcomes of pregnancy, such as hypertension, abortions, gestational diabetes mellitus, low birth weight, premature birth and cesarean section [13, 14]. Mother and fetus nutritional demands and metabolism rise exponentially during pregnancy and threaten a deficiency in vitamin D [15]. Infants of deprived mothers subsequently become severely deficient compared to satisfactorily nourished mothers [16]. Vitamin D, also known as calciferol, derived form of cholesterol and fat-soluble steroid. The biological role of vitamin D in the maintenance of calcium and phosphorus homeostasis and facilitating bone mineralization is well documented [17]. Deficiency of maternal vitamin D analyzed by circulating levels of 25-hydroxyvitamin D [25(OH) D] in serum, which is the finest diagnostic tool of vitamin D status [18]. Vitamin D is categorized into two types: Ergocalciferol (vitamin D2) and Chlecalciferol (vitamin D3). Vitamin D2 found in yeast, plants and particularly fungi, whereas vitamin D3 is obtained from animal food items and can be generated in animal and human skin with the use of ultraviolet light (UV) exposure. The principal sources of vitamin D are sunlight exposure, consumption of vitamin D and calcium rich food, vitamin D supplements, and food intake fortified with vitamin D [19]. Vitamin D deficiency is widespread in developing countries and situations of severe hypovitaminosis D are quite frequent in Middle Eastern and Asia-Pacific countries. Attributes related to vitamin D deficiencies include socioeconomic status, lifestyle, clothing styles, consumption of vitamin D supplements, activity level, obesity, use of sunscreens, seasons, status of employment, consumption of vitamin D rich food, location of residence and skin color. Tropical and subtropical areas have varying sunlight exposures, as well as varying altitudes from where people live [20]. Furthermore, data on vitamin D levels among pregnant women, at both dietary and 25-hydorxyvitamin D levels, is confined in India especially in a sunny region like Rajasthan. The aim of this study is to find out the status of serum 25(OH) D levels and its association with lifestyle and consumption of vitamin D rich food during pregnancy in Southern Rajasthan, India.

2. Material and Method

An observational study was conducted on a total of 280 healthy pregnant women in the age group of 18–35 years. The participants visited antenatal services of a tertiary care hospital were enrolled for the study. Approval from the institutional ethical committee and informed consent of each participant was obtained before study. Pregnant women were randomly selected from all three trimesters and primi and multi gravida. Inclusion criteria adopted was healthy subject with singleton pregnancy and exclusion criteria was unbooked pregnancy, pregnant women without any history of chronic disease (renal and liver disease, diabetes, hypertension thyroid, parathyroid, gastrointestinal). Structured questionnaire was used to obtain information regarding name, age socio- demographic details of pregnant women. Food frequency questionnaire (FFQ) was used to evaluate vitamin D rich food consumption. A Food Frequency Questionnaire (FFQ) is a frequently eaten food classified among the 9 main food groups. Under each of these food groups items rich in vitamin D foods explained to the subject to recognize and remember the pattern of food items intake per day. The information can represent either the usual frequency of consumption only or the total quantity, depending on the portion size. Frequency of consumption will be asked in no. of days per week, rarely never. Non-fasting blood samples of pregnant women was taken at time of recruitment for the estimation for serum 25(OH) D level. After collection serum sample was centrifuged and stored at minus 20 degree Celsius. The Callbiotech, Inc.25-hydroxy (25-OH) vitamin D ELISA kit used for the estimation of serum 25(OH) D level according to kit instruction. The 25-OH Vitamin D is a solid phase Elisa based on the principal of competitive binding. The sensitivity of this test kit is 0.67ng/ml. Vitamin D deficient was defined as serum 25(OH) D levels < 10ng/ml, insufficient level (10-30ng/ml) and sufficient level was (30-100ng/ml). The data were first cross-verified and entered in MS-EXCEL software, Microsoft Inc. and then analyzed using SPSS-PC- 21 version software, IBM Corp.

3. Result

In the present study, Majority of respondents 66.4% (n=186) are of > 25 years of age and 57% (n= 159) were multigravida and 50% (n=139) from third trimester of their pregnancy. The percentage of study participants from urban and rural areas was 52% (n=145) and 48% (n=135). It was observed that 32.85% pregnant women were vitamin D deficient, 43.70% were insufficient and 23.45% had sufficient level. It was found that pregnant women from the lower socioeconomic class had the lowest serum 25(OH) D levels as compared to women from upper and middle socioeconomic class. Analysis of dietary habits suggests that among all 70% respondents were vegetarians, 25% non-vegetarians and 4 % of them were eggitarians. The mean SD of vitamin D levels of vegetarians, non-vegetarians and eggitarians was 16.55±11.42 ng/ml, 33.89±17.62 ng/ml and 34.34±15.60ng/ml respectively. The prevalence of vitamin D insufficiency (91.9%) was highest among vegetarian participant. On data analysis, it was observed variation among the group was statistically significant (p <0.001).

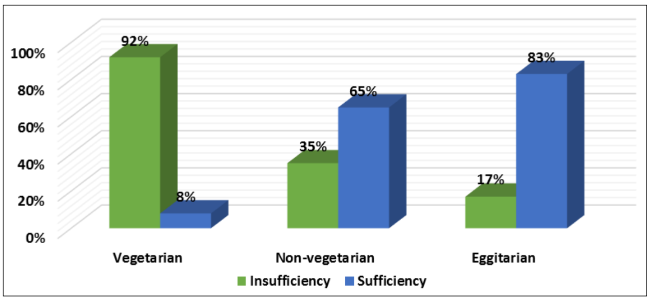

Fig 1: Comparative assessment of dietary pattern in relation to serum 25(OH) D insufficiency

Figure 1 suggests that women having a vegetarian diet had a higher prevalence (91.9%) of serum 25(OH)D insufficiency as compared to women with non-vegetarian diet (35.2%) and lowest in women having eggs in the diet (16.7%).On data analysis, it was observed variation among the group was statistically significant (p <0.001). Frequency of consumption vitamin D rich foods shows that only (28%) pregnant women drink milk daily. Majority of study population consumed meat once in 15 days (22%). Fish consumption among respondents was found very low and most of them (11%) eats once in a month. Among all the frequency of consumption egg was high. Although only (3%) of respondents were having egg 5 times in a week. Only (4%) of study population consume mushroom occasionally. (Table 1).

Table 1: Frequency of consumption vitamin D rich food by respondents

Frequency of consumption	Milk and milk products N (%)	Meat N (%)	Fish N (%)	Egg N (%)	Mushroom N (%)
Daily	78 (28%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
6/week	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
5/week	0 (0%)	0 (0%)	0 (0%)	7 (3%)	0 (0%)
4/week	7 (3%)	0 (0%)	0 (0%)	9 (3%)	0 (0%)
3/week	0 (0%)	0 (0%)	0 (0%)	8 (3%)	0 (0%)
2/week	2 (1%)	1 (0%)	0 (0%)	6 (2%)	0 (0%)
1/week	0 (0%)	8 (3%)	1 (0.4%)	37 (13%)	0 (0%)
1/15 days	0 (0%)	62 (22%)	3 (1%)	15 (5%)	0 (0%)
1/month	0 (0%)	0 (0%)	30 (11%)	0 (0%)	0 (0%)
Occasionally	0 (0%)	0 (0%)	25 (9%)	1 (0.4%)	10 (4.0%)
Never	193 (69%)	209(75%)	221 (79%)	197 (70%)	270 (96%)

It is important to note that regardless the frequency of consumption from daily to never we divided participants into three subgroup according to their intake. The percentage of participants in each group represented in Figure 2. The frequency of consumption vitamin D rich foods among pregnant women. Each column represents vitamin D rich foods and different colors are used to identify frequency of consumption as never, daily and occasionally. The numbers in each column indicate percentage of pregnant women according to their consumption.

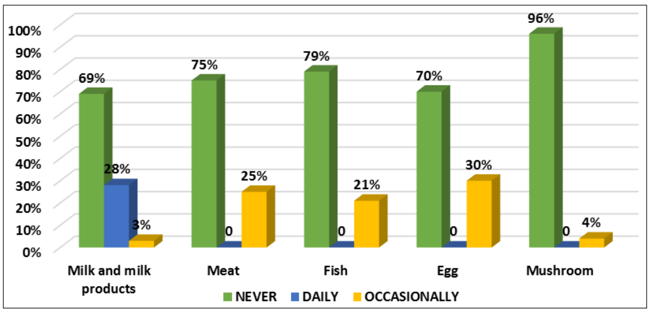

Fig 2: Frequency of consumption vitamin D rich food

Comparative assessment of consumption of vitamin D rich food in relation to serum 25(OH) d insufficiency found that those respondents who never consumed milk had higher percentage of insufficiency (87%) when compared to those who were taking daily. Figure 2 represented graphical illustration to understand insufficiency and sufficiency of serum 25(OH) D concentration associated with intake of each vitamin D rich food. Respondents who are vegetarian and never consumed meat and fish had higher percentage of insufficiency (87.5%) and (86.4%) respectively. The mean vitamin D levels of meat (33.89 ±17.62) and fish (37.14 ±16.76) eaters are better, when compared to those who never consumed them. Among all, those who added eggs frequently in their diet had low percentage of insufficiency (33.7%) when compared to those who never consumed eggs in diet (91.37%). There were (73.3%) of vitamin D insufficiency found in those who never consume mushroom. We observed a significant difference ($p<0.001$) in the terms of insufficiency in relation with vitamin D rich food consumption, as well as we got same significant results in the mean ±SD vitamin D level of pregnant women with their dietary intake (never, daily, occasionally). (Table 2)

Table 2: Comparative assessment of vitamin D insufficiency and mean vitamin D level of pregnant women in association with consumption of vitamin D rich foods

Frequency of consumption	Never		Daily		Occasionally		
Vitamin D rich food	Insufficiency	Sufficiency	Insufficiency	Sufficiency	Insufficiency	Sufficiency	p value*
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	
Milk and milk products	168	25	31	47	-	-	t=67.56,
	-87.50%	-12.95%	-39.70%	-60.30%			p <0.001
Meat	183	26	-	-	25	46	t=76.11
	-87.50%	-12.50%			-35.20%	-64.80%	,p <0.001
Fish	190	30	-	-	17	42	t= 81.01,
	-86.40%	-13.60%			-28.80%	-71.20%	p <0.001
Egg	180	17	-	-	28	55	t=101.59,
	-91.37%	-8.70%			-33.70%	-66.30%	p <0.001
Mushroom	198	72	-	-	10	0	t=3.59,
	-73.30%	-26.70%			-100.70%	0.00%	p 0.058

*Chi-square test

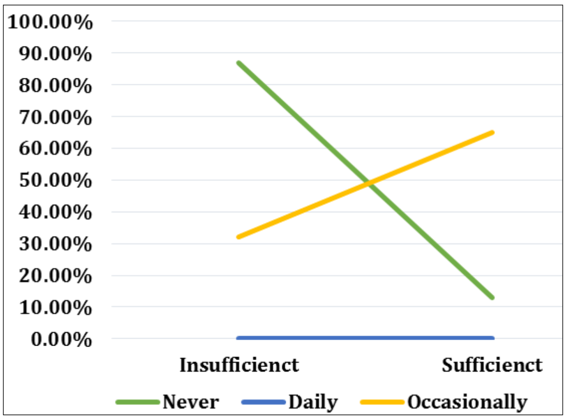

Fig 3: Comparative assessment of consumption of meat in relation to D serum 25(OH) D insufficiency

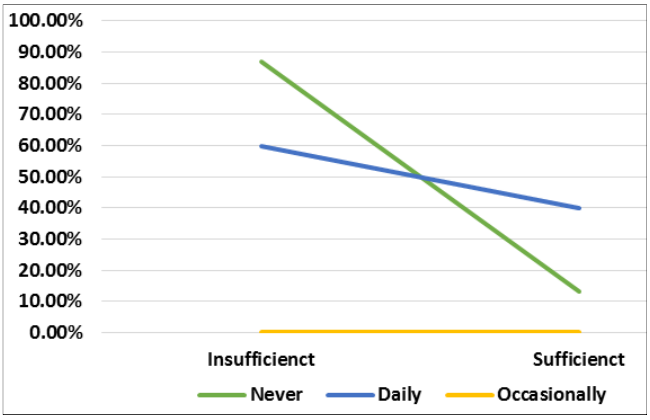

Fig 4: Comparative assessment of consumption of milk and milk products in relation to D serum 25(OH) D insufficiency

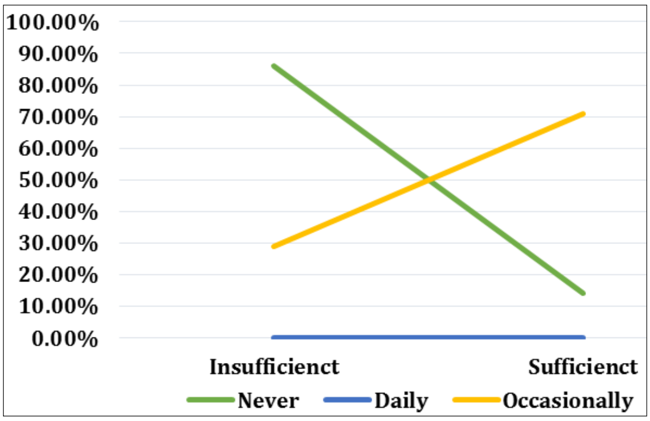

Fig 5: Comparative assessment of consumption of Fish in relation to D serum 25(OH) D insufficiency

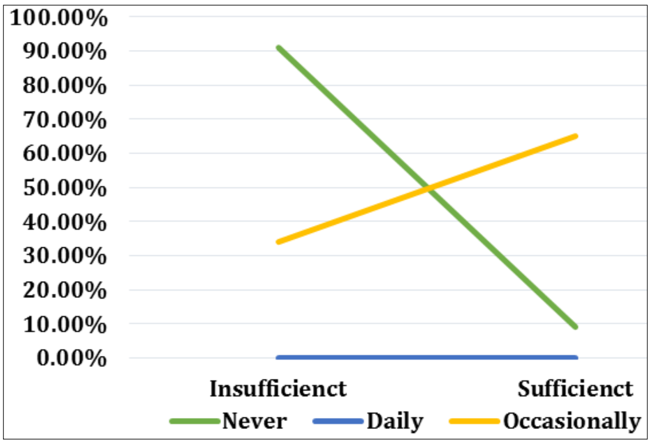

Fig 6: Comparative assessment of consumption of egg in relation to D serum 25(OH) D insufficiency

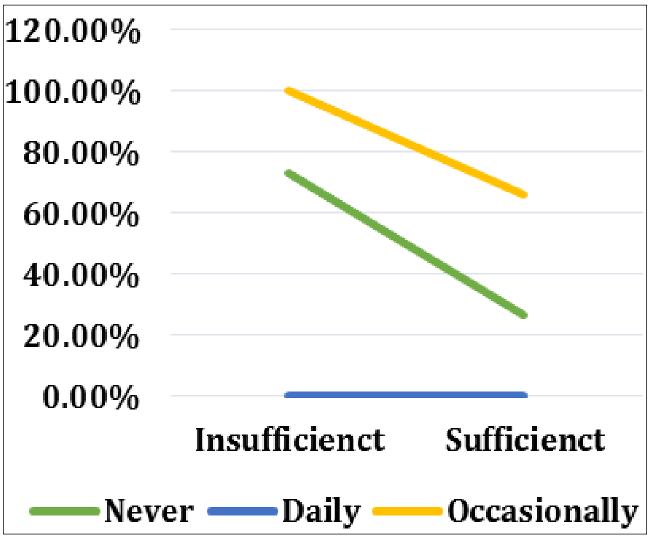

Fig 7: Comparative assessment of consumption of Mushroom in relation to D serum 25(OH) D insufficiency

Figure 3-7 Comparative assessment of consumption of vitamin D rich foods in relation to serum 25(OH) D insufficiency. Column represent percentage of insufficiency and sufficiency of serum 25(OH) D concentration among pregnant women. Different colors of lines indicates frequency of never, daily and occasionally consumption.

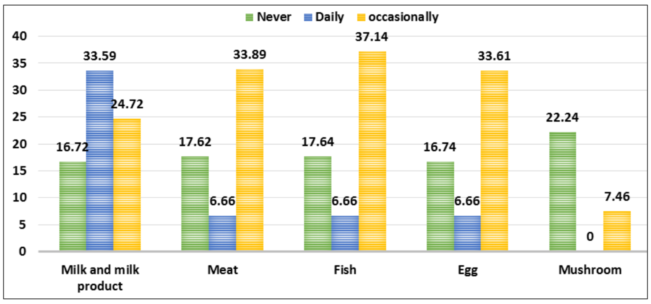

Fig 8: Classification of vitamin D rich food consumption by pregnant women and their mean serum 25(OH) D concentration.

Discussion

Several researches in the tropical countries have documented high prevalence of vitamin D deficiency and insufficiency [22, 23, 24]. Despite India being a tremendous tropical nation and having sufficient sunlight widely available constantly all through the year, vitamin D deficiency has been reported up to 96 % of pregnant women from India [25, 26, 27, 28]. This study reported a high prevalence of the deficiency and insufficiency of vitamin D among pregnant women of south Rajasthan. In this study more than 75 percent of pregnant women were whether insufficient or inadequate in vitamin D. Rajasthan is India's largest state, situated at latitude 27.0238 ° N, 74.2179 ° E. Even when accessibility to the sunlight is abundant, the prevalence of vitamin D deficiency among its population is high. Limited data on vitamin D status among pregnant of Rajasthan women are available. A recent local study reported that the mean serum concentration of 150 pregnant women in their first, second and third trimester was 28.84±9.66 ng/ml, 22.76±6.30 ng/ml and 17.25±4.25 ng/ml respectively, which is comparatively low from the non-pregnant women [29]. The state of vitamin D in our body is affected by several factors such as different seasons, time of body exposure to sunlight, latitude and altitude of that specific region, air pollution levels, skin pigmentation and color, use of sunscreen, exposure through glass, aging, extensive clothing cover, socio economic status, obesity and low intake of vitamin D rich food [30, 31]. Low dietary intake of vitamin D rich food one of the major risk factor which lowers the serum 25(OH) D levels during pregnancy.

In this study, pregnant women with higher frequency of vitamin D rich food intakes seem to be more likely to have reduced risks of vitamin D deficiency. Vitamin D2 and vitamin D3 are the two principal nutritional form. Ergocalciferol (vitamin D2) is mainly human-made and added to foods, while cholecalciferol (vitamin D3) is synthesized from 7-dehydrocholesterol in human skin and is often absorbed via the consumption of animal-based foods in diets [32]. Vitamin D3 and vitamin D2 are both commercially synthesized and contained in dietary supplements or fortification [33]. Yeast and plants are sources of D2 (ergocalciferol) and fatty fish and eggs, vitamin D fortified milk or margarine are sources of D3 (cholecalciferol) [34]. The side chain structure is the only difference in D2 and D3 forms. Both forms have been documented to have similar responses in the body when triggered, and have the ability to cure vitamin D – deficiency is the same [35].

Current study reported higher insufficiency of vitamin D among those who were not consuming vitamin D rich food or the frequency of intake is low. This finding is in agreement with Woon [24] and Shiraishi [36] studies that found higher intake of vitamin D contributed significantly to higher serum concentration of 25(OH) D among pregnant women. This may be due to a high intake of food containing vitamin D.

Accessible literature indicates that serum levels of 25OHD increase in response to rising intake of vitamin D. Factors that may influence the association among intake of vitamin D and levels of serum 25OHD are not adequately clear, and the accuracy of such indicators may be less than optimal [37]. The recommended dietary allowances of vitamin D in pregnancy are varies worldwide. National Institute for Health and Care Excellence (NICE) guidelines, UK [38] suggests 400 IU/per day while Institute of Medicines (IOM) recommend 600 IU per/day [39]. Endocrine Society, Canadian Pediatric Society and American Congress of Obstetricians & Gynecologists (ACOG) revised the recommendations and they suggests overall intake between 1000 - 2000 IU per/day [40, 41, 42]. The guidelines for vitamin D supplementation in India is lacking and food consumption of vitamin rich foods low too. USA and Canada's vitamin D RDA has been updated but India's remains the same. Indian Council of Medical Research (ICMR) suggests 400 IU per/day [43]. The study indicates that the serum 25OHD concentrations increased by 1 to 2 nmol / L for each additional 100 IU of vitamin D3 [44].

In this study participants were divided into three groups according to their frequency of consumption viz, never, daily, occasionally. The mean serum concentration of 25(OH) D of those participants who were consuming milk and milk products daily had higher levels when compare to those who never consumed milk. Yang *et al*. [45] demonstrated that milk and dairy products are the major sources of food that contribute to the intake of vitamin D among pregnant women in their study. Hiking milk prices in India could be a reason for under- and middle-class families not being accessible. People have a supply of milk in a rural area, but they do not consume it on their own while selling it for income. Many large families cannot afford milk for everyone in family. We found significant association between consuming milk and milk products and low serum 25(OH) D levels. Similar results found by another study of India, that 82% pregnant women did not consume milk or dairy product in their diet. Among them, 32.5% were deficient in vitamin D and the correlation between inadequate dietary consumption of milk and dairy products and low levels of vitamin D (p value 0.005) was statistically significant [46].

The dietary habit preferred by pregnant women in our study has been a vegetarian diet. In certain culture there is connection between non-vegetarian food and religious beliefs. 25% of our study population was non-vegetarian and consumed preferably chicken and mutton. Consumption is not the only measure of nutrition but the amount and frequency of consumption also affect pregnant women's serum vitamin D levels. Pregnant women who consumed meat frequently had a higher mean level of vitamin D compare to group with less frequent consumption. We observed a significant difference between all three subgroups and meat consumption was correlated significantly with insufficiency of vitamin D. Dent *et al*. [47] study analysis showed that throughout pregnancy the serum vitamin D levels in the vegetarian Asians were lower when compared to non-vegetarian Asians.

Although fish is the richest source of vitamin D, but in our study frequency of fish consumption is low. The mean vitamin D level of those who consumed fish was higher and statistical analysis shows positive correlation. A study from Japan demonstrated that fish consumption elevates serum 25(OH) D levels among pregnant women [48]. A research by Egypt [49] concluded in their analysis that fish consumption was significantly correlated with pregnant women's vitamin D levels but no correlation of eggs and dairy products was identified with insufficiency. In our findings, we found an association of egg consumption with serum 25(OH) D levels and results are extremely supported by the research of Wang C *et al*. [50] they concluded that pregnant women who consumed eggs daily or very frequently in their diets had an adequate level of vitamin D. People are still not aware of mushrooms as a vegetable in rural areas, while in urban areas people still eat mushrooms and are not aware of their nutritional value. Urban pregnant women eat mushrooms in fast food but the volume has been very limited and has no nutritional value, though in our study mushroom consumption was not correlated with serum 25(OH0D level.

Conclusion

While living in a nation with high sun exposure in, pregnant women showed a high prevalence of serum 25(OH) D deficiency associated with vegetarian diet, low consumption of vitamin D-rich foods, and no prior pregnancy vitamin D supplements. The identification and prevention recommendations and methods need to be updated in India. Vitamin D insufficiency indeed be created during pregnancy, taking into account the community-based nutritional assessment required with the programs and policies.

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