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PLASMA VITAMIN D STATUS AND ASSOCIATED FACTORS AMONG PREGNANT WOMEN OF PESHAWAR, KHYBER PAKHTUNKHAWA, PAKISTAN: A PILOT STUDY

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ABSTRACT

OBJECTIVE: To assess plasma vitamin D status and its association with dietary intake and body mass index (BMI) in primary gravida women.

METHODS: This cross-sectional study was carried out in the Department of Gynecology, Lady Reading Hospital (LRH) and Institute of Basic Medical Sciences, Khyber Medical University, Peshawar from January to March 2016. Primary gravida women (n=88) in their first trimester attending antenatal clinic at LRH were included. Socio-demographic and anthropometric measures were recorded using standard methods. Nutritional intake was assessed using 24-hours dietary recall. Blood samples were collected to assess plasma vitamin D status through ELISA.

RESULTS: Median plasma 25-OH vitamin D levels were 41.71 ηg/mL (IQR=17.29). Only 8 (9.09%) women had vitamin D deficiency (<15 ηg/mL), 10 (11.36%) had insufficiency (15-30 ηg/mL) and 70 (79.54%) had adequate (>30 ηg/mL) levels of plasma vitamin D. Dietary intake of vitamin A and thiamine were significantly different between the three groups (sufficient/insufficient/deficient). No significant correlation was found between plasma vitamin D and anthropometric and dietary variables. Only socioeconomic status (p=0.03) was significantly associated with plasma vitamin D status.

CONCLUSION: In our study, plasma vitamin D status was not significantly associated with dietary intake and BMI in primary gravida women. However, due to relatively small sample size, results may be taken with caution and large-scale study is recommended to establish the relationship of plasma vitamin D status with dietary intake and BMI in antenatal women.

KEYWORDS: Vitamin D (MeSH); Body Mass Index (MeSH); Dietary intake (Non-MeSH); Pregnancy (MeSH); Gravidity (MeSH); Diet Record (MeSH); Adult (MeSH); Female (MeSH).; Blood Glucose (MeSH); Insulin (MeSH); Glutathione Peroxidase (MeSH).

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INTRODUCTION

Vitamin D (calciferol) is a fat-soluble vitamin found in human body in either the form of ergocalciferol (vitamin D_2) or cholecalciferol (vitamin D_3). Vitamin D is unique vitamin in a sense that it's synthesis in the body occurs through exposure of the skin to ultraviolet-B (UV-B) radiations of the sun. These radiations penetrate the skin and convert 7-dehydrocholesterol into pre-vitamin D_3 that is subsequently converted into vitamin D_3 in the liver.[1] Food sources of vitamin D are very few such as oily fish, cod liver oil, butter and eggs.[2] The recommended dietary allowance of vitamin D is 600 IU/day for adult male and female.[2]

Vitamin D is classically known for its role in bone mineralization through absorption and homeostasis of calcium and phosphorus.[3] In the body, it does so by increasing calcium channels and the expression of calcium binding protein in the small intestine.[4] Vitamin D also increases cell maturation and apoptosis and decreases cell proliferation.[5] Furthermore, it also regulates insulin secretion and protect against renal diseases by suppressing inflammation, inhibiting rennin-angiotensin-aldosterone system and restoring glomerular filtration barrier.[6] Deficiency of vitamin D mainly causes rickets in children and osteomalacia in adults.[7] Vitamin D deficiency has been implicated in obesity, decreased insulin sensitivity, increased risk for developing the metabolic syndrome, nonspecific musculoskeletal pain, autoimmune diseases, cardiovascular disease and cancer.[2] Furthermore, deficiency of vitamin D during pregnancy can also result can increase the risk for preeclampsia in women[8,9] and rickets in infants.[10,11]

Vitamin D deficiency is a major public health problem in many countries of the world. It is estimated that approximately 15% of the global population (around one billion people) have vitamin D deficiency (<20 ηg/mL) or insufficiency (20 - 30 ηg/mL).[12] The problem is more evident and issue of public health concerns in low and middle income countries (LMIC)[13] including South Asian countries such as India,[14] Bangladesh[15] and Afghanistan.[16] Similarly, according to National Nutrition Survey of Pakistan (NNS 2011), approximately 68.8% women were found to be vitamin D deficient (<30ηg/mL).[17] These figures were also reflected at provincial levels in Khyber Pakhtunkhwa where prevalence of vitamin D deficiency is approximately 64% in women. Other studies from Pakistan have also reported vitamin D deficiency in different populations.[18,19] Previously, studies in different populations revealed that plasma vitamin D status during pregnancy also depends on nutritional intake/status,[20] multiparity[21] and stage of pregnancy.[22] However, to the best of our knowledge, no such studies are conducted in our local population so far. Therefore, this study aimed to investigate plasma vitamin D status and its

TABLE I: BASIC DEMOGRAPHIC AND ANTHROPOMETRIC DATA OF THE STUDY PARTICIPANTS (n=88)

Variable	Median	Q1, Q3	IQR
Age (years)	20.00	18.0, 23.0	5.000
Years of Marriage	0.850	0.5, 1.900	1.400
Family Size (n)	8.000	6.0, 12.00	6.000
Systolic BP (mm Hg)	110.00	100.0, 110.00	10.00
Diastolic BP (mm Hg)	70.00	60.0, 80.00	20.00
Height (cm)	155.15	151.9, 159.02	7.17
Height (m)	1.5530	1.5, 1.5902	0.0702
Weight (kg)	54.500	48.9, 61.375	12.450
Body Mass Index (kg/m^2)	22.221	20.5, 24.752	4.277

n = number; Q = Quartile

TABLE II: BASIC DEMOGRAPHIC AND SOCIOECONOMIC STATUS DATA OF STUDY PARTICIPANTS

Variable		Frequency	Percentage
Socioeconomic status	High	1	1.14
	Middle	31	35.23
	Low	56	63.64
Education	Higher Education	11	12.50
	Higher Secondary	11	12.50
	Primary Education	25	28.41
	Uneducated	41	46.59
Working status	House wife	83	94.32
	Working	5	5.68

TABLE III: DIETARY INTAKE OF STUDY PARTICIPANTS MEASURED BY 24-HOURS DIETARY RECALL METHOD

Variable	Median	Q1, Q3	IQR
Energy (kJ)	4354	3188, 5613	2425
kcal (kc)	1032.5	753.5, 1338.5	585.0
Fat (g)	40.45	23.48, 53.27	29.80
Protein (g)	34.70	22.88, 50.40	27.52
Carbohydrate (g)	146.00	90.95, 187.80	96.85
Thiamine (mg)	0.7050	0.4550, 0.9250	0.4700
Riboflavin (mg)	0.5350	0.3100, 0.7850	0.4750
Niacin (mg)	16.350	9.450, 21.600	12.150
Vitamin B6 (mg)	0.7250	0.4225, 1.0375	0.6150
Vitamin B12 (μg)	0.745	0.000, 1.555	1.555
Folic acid (μg)	99.50	60.00, 135.00	75.00
Vitamin C (mg)	23.30	8.90, 64.45	55.55
Vitamin E (mg)	3.785	2.145, 5.607	3.462
Vitamin D (μg)	0.4550	0.1300, 0.9800	0.8500
Vitamin A (μg)	241	126, 439	312

relationship with dietary intake and anthropometric parameters in first trimester of primary gravida women of Peshawar, Pakistan.

METHODS

This single center, cross-sectional study was carried out in the Department of Gynecology, Lady Reading Hospital and Institute of Basic Medical Sciences, Khyber Medical University, Peshawar from January to March 2016. Women (n=88) who were apparently healthy, aged 18–40 years, in the first trimester, and having their first pregnancy were included in the study. Those having previous pregnancies (multigravida), severe hyperemesis gravidarum (severe episodes of vomiting), acute or chronic medical condition and those who used antibiotics in the past one month or nutritional supplements in the past 3 months were excluded. The Ethical Review Committee of Khyber Medical University, Peshawar, approved the study.

Socio-demographic characteristics such as age, socioeconomic status and family size of all the participants were recorded. Anthropometric measurements were recorded following standard methods. Height was measured to the nearest 0.1 cm by using wall mounted Stadiometer (Seca, UK) while the subjects were standing in the Frankfurt plane position. Weight was recorded in kilograms to the nearest 0.1 kg using calibrated electronic scale (Seca, UK). Body mass index (BMI) was computed as the fraction of weight to the squared height (kg/m^2).

Nutritional intake of the participants was assessed by a trained nutritionist using 24-hours dietary recall. The 24-hours dietary recall was conducted in the form of an in-depth interview using a standardized four stage protocol.[23] Each participant was asked to provide detailed information about foods and beverages consumed in the past 24 hours. This included the time of day and source of food, portion size of each food and beverage and preparation method were also recorded. Food models and pictures were used to help participants judge and report portion size and improve accuracy.

Blood sample from each participant was collected by a trained phlebotomist in EDTA tubes. At least 10 mL of blood was collected from each participant in aseptic manner. The samples were then centrifuged for 10 minutes at 4,000 rpm and stored at -80°C till further analysis.

Plasma vitamin D was analyzed through 25-OH vitamin D Diasorin radioimmunoassay ELISA kit (Euroimmun, Germany) following manufacturer instructions. Briefly, stored samples were thawed and mixed thoroughly at room temperature. Around 100μL of the samples and biotinylated working were added to each well of ELISA plate followed incubation for 2 hours at 37°C. The wells were washed three times with washing buffer followed by addition of chromogenic substrate and incubation. After 30 minutes, the optical density was measured at 450 nm wavelength through reader microplate reader (Biotek® Elx800). Levels were defined as normal (>30ηg/mL), insufficient (15-30 ηg/mL) or deficient (<15 ηg/mL) as per standard

TABLE IV: PLASMA 25-OH VITAMIN D STATUS: DEFICIENCY/INSUFFICIENCY/ADEQUACY

Plasma vitamin D levels	Frequency	Percentage
Deficient (<15 ηg/mL)	8	9.09
Insufficient (15-30 ηg/mL)	10	11.3
Adequate (>30 ηg/mL)	70	79.5
Total	**88**	**100.0**

TABLE V: CORRELATION OF PLASMA VITAMIN D WITH BMI, SYSTOLIC BP, DIASTOLIC BP, HEIGHT, WEIGHT, DIETARY VITAMIN D, FAT AND CARBOHYDRATES

Variable	R Value	P Value
Body Mass Index (kg/m^2)	0.06	0.54
Systolic BP (mm Hg)	-0.01	0.95
Diastolic BP (mm Hg)	0.08	0.43
Height (cm)	0.19	0.06
Weight (kg)	0.01	0.96
Dietary vitamin D (ηg)	-0.01	0.95
Protein (g)	0.06	0.58
Fat (g)	-0.01	0.90
Carbohydrate (g)	0.08	0.42

TABLE VI: COMPARISON OF DEMOGRAPHIC, SOCIOECONOMIC, ANTHROPOMETRIC, AND DIETARY DATA BETWEEN PARTICIPANTS WITH LOW (<30 ηG/ML) AND NORMAL (>30 ηG/ML) VITAMIN D LEVELS

Variable	Low Median (average Rank) (<30 ηg/mL)	Normal Median (average Rank) (>30 ηg/mL)	p-value
Age (years)	21.00 (44.3)	20.00 (44.6)	0.96
Education	1.000 (47.4)	1.000 (43.8)	0.56
Years of marriage	0.7000 (42.1)	0.9000 (45.1)	0.65
Family Size (n)	7.500 (41.4)	8.000 (45.3)	0.56
Socioeconomic Status	1.000 (35.8)	1.000 (46.8)	0.05*
Working/ House wife	1.000 (44.4)	1.000 (44.5)	0.97
Systolic BP (mm Hg)	110.0 (44.1)	110.0 (44.6)	0.93
Diastolic BP (mm Hg)	70.00 (42.2)	70.00 (45.1)	0.64
Height (cm)	156.7 (51.8)	154.9 (42.6)	0.17
Weight (kg)	56.45 (47.0)	53.95 (43.9)	0.64
BMI (kg/m^2)	22.26 (45.1)	22.15 (44.4)	0.91
Vitamin D intake (ηg)	260.0 (39.5)	495.0 (45.8)	0.35
Energy intake (kJ)	4147 (40.3)	4392 (45.6)	0.43
Fat (g)	41.60 (42.8)	40.45 (44.9)	0.75
Protein (g)	33.20 (40.3)	35.75 (45.6)	0.42
Carbohydrate (g)	99.55 (39.1)	147.40 (45.9)	0.31
Thiamine (mg)	0.4900 (38.1)	0.7600 (46.1)	0.23
Riboflavin (mg)	0.5400 (41.5)	0.5350 (45.3)	0.58
Niacin (mg)	14.45 (36.9)	16.40 (46.5)	0.15
Vitamin B6 (mg)	0.5650 (38.5)	0.7600 (46.0)	0.26
Vitamin B12 (μg)	0.8700 (44.6)	0.7250 (44.5)	0.98
Folate (μg)	79.00 (39.8)	101.50 (45.7)	0.38
Vitamin C (mg)	21.40 (39.4)	24.75 (45.8)	0.34
Vitamin E (mg)	3.295 (36.9)	3.785 (46.4)	0.15
Vitamin D (μg)	0.2600 (39.5)	0.4950 (45.8)	0.35
Vitamin A (μg)	194.0 (37.7)	266.5 (46.3)	0.20

*p-value based on Kruskall-Wallis test

criteria.[24]

Data was analyzed using Minitab® software Version 17 (Minitab, Inc.). Anderson Darling Normality test was applied to check normality. Data was represented as median and interquartile range. Mann-Whitney U test was used to estimate the difference between two variables. Spearman Rank correlation was used to measure correlation of plasma vitamin D with socio-demographic and anthropometric variables. Kruskall-Wallis test was applied to determine the difference between sufficient, insufficient, and deficient plasma vitamin D levels. Binary logistic regression analysis was used to determine the association of plasma 25-OH vitamin D (normal/low levels) with age, socioeconomic status, dietary intake and BMI. Results were considered significant where p value was <0.05.

RESULTS

Data regarding demographic, anthropometry and socioeconomic status was collected from all the participants and presented in Table I and II. Median (interquartile range; IQR) age of participants was 20.00 years (IQR = 5.00) and the range was 16-30 years. Median (IQR) height of participants was 155 cm (IQR = 7.17) and weight 54.50 kg (IQR = 12.450). The median BMI was 22.22 kg/m^2 (4.27) and were normotensive (Table I). Most of the participants (n=56, 63.64%) were from lower income group, uneducated (n=41, 46.59%) and were house-wives (n=83, 94.32%) (Table II).

Median energy intake was significantly lower than the recommended intake for Pakistani women at this age. The median energy intake of the participants was 4354 (2425) kJ (Table III).

Based on the criteria, of all the participants (n=88), 8(%) (Table IV) were vitamin D deficient and 10(%) (Table II) had insufficiency. Rest of the participants [n= 79,54%] (Table II) had normal vitamin D status.

Plasma vitamin D was not correlated with any of the anthropometric and dietary variable. Only height showed some tendency of positive correlation (R=0.19, p=0.06) (Table V).

When participants were grouped into two groups based on whether they had normal vitamin D levels (≥30 ηg/mL) or low vitamin D levels (<30 ηg/mL), only

socioeconomic status was marginally significantly different between the two groups (p=0.05, Kruskal-Wallis test) (Table IV).

DISCUSSION

Deficiency of vitamin D, the main regulator of the bone mineralization and calcium homeostasis in body is a global public health especially in pregnant women and children. According to estimates, 15–84% of pregnant women are deficient in vitamin D ("serum 25(OH)D <25 ηmol/L") thereby increasing the risk of osteomalacia,[25] intrauterine growth retardation and low birth weight in neonates.[26] In this pilot study, we have quantitatively assessed vitamins D status of pregnant women in Peshawar and its relationship with dietary intake and socio-demographic and anthropometric characteristics. We have found that majority of pregnant women in the study sample have normal plasma vitamin D status. Furthermore, except socioeconomic status, no significant association was observed between plasma vitamin D status and with dietary intake or BMI. Our results are in conflict with study conducted in India[27] and other cities of Pakistan (Karachi & Lahore) who reported that 50 - 98% of pregnant women are vitamin D deficient.[28,29] The conflicting results might be due to the fact that majority of our participants were young, housewives and residing in rural areas. In rural areas of KP, people live in open houses and during daily activities, the women are exposed to sunlight and thus adequate vitamin D is produced in the body. The importance of sunlight exposure in vitamin D synthesis is also confirmed by various studies.[30] Furthermore, gestational age of the participants and lack of fast foods and carbonated drinks in their diet which negatively impact vitamin D status can be a contributing factor.[31]

In our study, we have observed no significant association between plasma vitamin D status and dietary intake although a study conducted in Karachi on healthy population, showed a significant relationship of vitamin D with dietary intake.[32] The reason might be that we have collected a dietary intake of only 24 hours which is recall biased and also does not reflect their past dietary intake. Socioeconomic status (p=0.05) was significantly different in low (<30 g/mL) and normal (>30 g/mL) sub-groups of vitamin D status. Moreover, it was significantly associated (p=0.03) with plasma vitamin D status. Similar result found by a study that high socioeconomic status and working indoor jobs of females are significantly associated with lower vitamin D status.[30]

Our study is unique that we have recruited apparently healthy primary gravida women in their first trimester of pregnancy. However, due to relatively small sample size, we could not observe any significant association with other study variables has certain limitations. Our sample size was relatively small thus did not show any significant relationship between the variables. Therefore, further quantitative studies with a large sample size are required to establish the relationship of plasma vitamin D status with dietary intake and body mass index in antenatal women.

CONCLUSION

In our study, plasma vitamin D status was not significantly associated with dietary intake and BMI in primary gravida women.

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AUTHORS' CONTRIBUTIONS

Following authors have made substantial contributions to the manuscript as under:

BS: Acquisition, analysis and interpretation of data, drafting the manuscript, final approval of the version to be published

MS: Study design, analysis and interpretation of data, drafting the manuscript, critical review, final approval of the version to be published

MJK: Conception and study design, Acquisition, analysis and interpretation of data, drafting the manuscript, final approval of the version to be published

SK & HB: Acquisition of data, drafting the manuscript, final approval of the version to be published

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity ofany part of the work are appropriately investigated and resolved.

CONFLICT OF INTEREST

Authors declared no conflict of interest

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