ORIGINAL ARTICLE
IN OBSTRETICS

Impact of diet on vitamin D status in a Sri Lanka-based sample of pregnant women

Kaneshapillai Anusha[1], Liyanage Guwani[2], Hettiaratchi Usha[3], Gunasekera Dulanie[4]

Affiliations:

[1] Bsc, Research Assistant and Clinical Nutritionist, Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka
[2] M.D., Senior Lecturer, Department of Paediatrics, Faculty of Medical Sciences, University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka
[3] PhD, Senior Lecturer, Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka
[4] M.D., Professor, Department of Paediatrics, Faculty of Medical Sciences, University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka.

Corresponding author:

Ms. Kaneshapillai Anusha, Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka.
E-mail: anushakanesh@yahoo.com

Abstract

Introduction: Vitamin D deficiency is common during pregnancy in Asian countries. However, there is little knowledge about vitamin D status of pregnant mothers and, therefore, supplements are not routinely provided in public clinics and government hospitals in Sri Lanka. Therefore, aim of this study was to assess vitamin D status and adequacy of vitamin D intake in a sample of pregnant mothers.
Methods: This was a secondary analysis of existing data from a prospective cohort study. A convenience sample of 89 healthy and non-vitamin D supplemented Sri Lanka-based pregnant mothers was recruited during the third trimester of their pregnancy. Dietary vitamin D intake was assessed through a food frequency questionnaire, while serum was analysed for vitamin D, parathyroid hormone (PTH) and other markers of bone biochemistry.
Results: In our sample, average daily dietary intake of vitamin D was 1,289.4 ± 1,225.6 IU/day (range: 56 - 5400 IU). A significant proportion of mothers (45%) consumed < 600 IU of vitamin D per day. More than half of our sample (56.9%) received vitamin D though fortified milk powder and 36% from fish consumption. Most of mothers (69%) consumed small fish and none of them received vitamin D supplementation. There was a significant positive correlation between dietary vitamin D and serum 25-hydroxyvitamin D (25(OH) D) (r = 0.355, P < 0.01). 12.4%, 50.6% and 37% of the mothers were vitamin D deficient, insufficient and sufficient, respectively. We showed a significant difference in levels of dietary vitamin D intake between serum 25(OH)D deficient/insufficient (dietary vitamin D: 1,083.6 ± 1,026.4 IU/day) and 25(OH)D sufficient (dietary vitamin D: 1,638.5 ± 1,456.1 IU/day) groups.
Discussion and Conclusion: Dietary intake of vitamin D was inadequate in Sri Lankan non-vitamin D supplemented mothers. Further evaluation of vitamin D status and requirement for supplementation in a nationally representative sample is essential.

KEY WORDS: Bone; calcium; diet; parathyroid hormone; pregnancy; vitamin D; Sri Lanka.

Riassunto

Introduzione: Il deficit di vitamina D durante la gravidanza è frequente nei Paesi dell'Asia. Tuttavia, c'è poca conoscenza sui livelli di vitamina D nelle donne in gravidanza e pertanto non vengono regolarmente forniti integratori alimentari nelle cliniche pubbliche e negli ospedali governativi dello Sri Lanka. Per tale motivo, questo studio ha l'obiettivo di valutare lo status relativo alla vitamina D ed all'adeguatezza dell'assunzione della vitamina D in un campione di donne in gravidanza.
Metodi: E' stata effettuata un analisi secondaria dei dati esistenti da uno studio di coorte prospettivo. Un campione di convenienza di 89 madri gravide dello Sri Lanka in buona salute e non sottoposte a supplementazione con vitamina D è stato arruolato durante il terzo trimestre di gravidanza. L'assunzione di vitamina D attraverso la dieta è stata valutata con un questionario riguardante la frequenza di assunzione del cibo, mentre sono state effettuate indagini sierologiche per il dosaggio della vitamina D, del paratormone e degli altri marcatori biochimici del metabolismo osseo.
Risultati: Nel nostro campione, l'assunzione dietetica giornaliera media di vitamina D è stata pari a 1,289.4 ± 1,225.6 UI/die (range: 56 - 5400 UI). Una proporzione significativa di madri (45%) consumava < 600 UI di vitamina D al giorno. Più di metà del nostro campione (56.9%) riceveva la vitamina D attraverso il latte in polvere arricchito ed il 36% dal consumo di pesce. Molte delle madri (69%) consumavano pesce di piccola taglia e nessuna di loro riceveva supplementazione di vitamina D. E' risultata una correlazione positiva significativa tra la vitamina D introdotta con la dieta ed i livelli ematici di 25-idrossivitamina D (25(OH)D) (r = 0.355, P < 0.01). Il 12,4%, 50,6% and 37% delle madri è risultata avere livelli di vitamina D scarso, insufficiente e sufficiente, rispettivamente. Abbiamo evidenziato una differenza significativa nei livelli di vitamina D introdotti con la dieta tra il gruppo con livelli ematici di 25(OH)D di tipo "scarso/insufficiente" (livello di vitamina D alimentare: 1,083.6 ± 1,026.4 UI/die) ed il gruppo con livelli ematici di 25(OH)D "sufficiente" (livello di vitamina D alimentare: 1,638.5 ± 1,456.1 UI/die).
Discussione e Conclusione: L'introduzione alimentare di vitamina D è risultata inadeguata nelle madri srilankesi non supplementate con vitamina D. E' essenziale un ulteriore valutazione relativa allo stato della vitamina D ed ai requisiti per la supplementazione in un campione rappresentativo sul piano nazionale.

TAKE-HOME MESSAGE

In this Sri-Lanka based study, dietary vitamin D intake of pregnant mothers is below the recommended daily allowance and this could have contributed to the presence of low serum vitamin D levels among non-vitamin D supplemented pregnant women of our sample.

Competing interests - none declared.

Cite this article as: Anusha K, Guwani L, Usha H. Dulanie G. Impact of diet on vitamin D status in a Sri Lanka-based sample of pregnant women. J Health Soc Sci. 2018;3(1):75-84

DOI 10.19204/2018/mpct7

Received: 06/01/2018 Accepted: 14/02/2018 Published: 15/03/2018

INTRODUCTION

Vitamin D deficiency and insufficiency are common worldwide [1, 2, 3]. Studies conducted in many countries lying in the tropics and subtropics have shown low vitamin D status during pregnancy and childhood periods [1]. Overcrowding, atmospheric pollution, low availability or low affordability of food containing vitamin D, and dress customs that limit skin exposure are main factors responsible for vitamin D deficiency [4]. Some studies have revealed vitamin D deficiency is common in pregnant and breastfeeding mothers [2, 5, 6]. High 1,25-dehydroxy vitamin D levels during the second and third trimesters and a high foetal demand for calcium indicate that requirement for vitamin D during pregnancy is high [7]. Adverse maternal outcomes of low vitamin D are documented [8–10]. Pregnancy-induced hypertension, recurrent miscarriages, gestational diabetes mellitus, premature delivery, and postpartum depression are some of them. A myriad of other metabolic/non-skeletal outcomes of vitamin D deficiency have been identified, including a link between vitamin D deficiency and susceptibility to infection, suggesting a role of vitamin D in immune-modulatory and anti-infective activities. In addition, certain studies have shown that vitamin D deficiency can affect the health of offspring leading to poor rates of skeletal growth and rickets [11].

A certain number of studies in the South-East Asian Region have evaluated the prevalence of vitamin D deficiency among pregnant women using a variety of different cut-off levels for serum 25-hydroxyvitamin D (25(OH)D) [2, 12]. However, there is no consensus on optimum serum 25-(OH)D during pregnancy. The Institute of Medicine (IOM) of the US National Academy of Sciences has recommended a cut off level for 25-(OH)D sufficiency as > 20 ng/L, while the risk of vitamin D deficiency is as < 10 ng/L in the general population [13].

Daily requirement of vitamin D and calcium during pregnancy is controversial too. A randomized controlled trial showed that vitamin D supplementation in pregnancy could up to a safe dose of 2000-4000 IU/day [14–16]. A number of reports have shown that a 4,000 IU/day of vitamin D supplement is more effective in achieving satisfactory serum concentrations [15, 16]. IOM recommends safe upper limit of vitamin D and calcium in the diet as 4,000 IU and 2,500 mg, respectively [13]. World Health Organisation recommends 1,500-2,000 mg of elemental calcium/day as supplementation [17]. However, the optimal dose of vitamin D and calcium for pregnant women is controversial and debatable [18].

UVB sunlight exposure and diet have been reported as the main source of vitamin D for the majority of the population [19]. In developing countries many pregnant women have inadequate vitamins D levels depending on darker pigmentation with low sun exposure during the winter and limited dietary intake of vitamin D due to lack of availability of fortified foods. This condition is aggravated by an infrequent and inadequate use of antenatal care in rural regions [20].

Vitamin D occurs naturally in oily fish, egg yolk and fortified food products such as milk, margarine, vegetable oils and ready-to-eat breakfast cereals. Most of these food items are less affordable to lower socioeconomic class, in Sri Lanka. Further, vitamin D supplements are not routinely provided in our public hospital and clinics. Therefore, aim of this study was to assess vitamin D status and adequacy of vitamin D intake through diet among pregnant mothers of Colombo, the capital of Sri Lanka.

METHODS

Our study was based on a secondary analysis of existing data, which was drawn from a prospective cohort study investigating the effects of maternal vitamin D status in pregnancy and breastfeeding on infant growth. This research was approved by Ethics Committee of Faculty of Medical Sciences, University of Sri Jayewardenepura. Pregnant mothers in their third trimester were recruited from the obstetric department of Colombo South Teaching Hospital, Colombo City. Exclusion criteria were mothers who have been receiving treatment with vitamin D supplements, mothers with multiple pregnancy or affected by serious medical disorders or disability that were no associated with pregnancy-related conditions and could be caused by metabolic bone diseases. A trained investigator did a brief clinical examination of all eligible mothers. Convenient sampling technique was employed and all eligible mothers were invited to participate in the study. They were divided in small groups and were accurately explained the study procedure. Once the informed written consent was obtained, we collected data on demography, obstetric history, general health and past medical and surgical conditions, medications and nutritional supplements through a pre-tested interviewer administered questionnaire. Gestational age was based on last menstrual period and ultrasonography findings. In this study, socio-economic status (SES), which is a mixed of education, income and occupation, was based on the gross household income that is the most common measure of income used in calculations of SES. We obtained low, medium and high-income categories, dividing them into tertiles, depending on the distribution of the sample.

A food frequency questionnaire (FFQ) was designed and pretested to collect some details on mothers' diet. It was based on a 7 - day estimated food record method, by which a quantification of foods items and beverages were estimated rather than weighed. This was carried out using household measures such as cups or spoons and food photographs. Afterly, estimates were converted into weights (grams). A trained investigator administered the FFQ. Details were obtained on food brands, vitamin and mineral supplementation received. Dietary details were verified with each participant for accuracy and completeness. Estimation of dietary intake of vitamin D and calcium were performed with Nutrisurvey 2007 modified for Sri Lankan food items and recipes [21, 22]. This is a software considering cooking procedures and locally available brands. Vitamin D content was measured in micrograms and converted to International Units (IU = micro grams/0.025). Dietary calcium levels were directly calculated in milligrams.

Blood samples were collected and serum was stored at - 20°C until analysis. 25(OH)D was measured by VIDAS® 25 OH Vitamin D Total, in serum using the Enzyme Linked Fluorescent Assay (ELFA). It is very well correlated with the Liquid Chromatography/Mass Spectrometry reference method with cross reactivity of 100% with 25(OH)D3 and 91% with 25(OH)D2. For analysis of calcium, inorganic phosphorus (IPh) and alkaline phosphatase (ALP), colourimetric method was employed. DRG (EIA-3645), Intact-PTH ELISA was used for quantitative determination of intact-PTH in serum.

SPSS version-15 was used for statistical analysis. Results were presented for serum 25(OH)D, PTH, calcium and alkaline phosphatase as means and standard deviations. Serum concentrations of 25(OH)D of > 20 ng/L (sufficient), 10 – 20 ng/L (insufficient) and < 10ng/L (deficient) were taken according to IOM classification [13].

Data were log transformed where necessary and presented as mean ± SD. Spearman correlation was used to study the relationship between maternal dietary vitamin D intake and bone biochemistry. Serum 25(OH)D levels were dichotomized as 'deficient/insufficient' or 'sufficient' and comparisons were done by independent sample t-test. Chi square test was performed to study the associations between socio demographic characteristics and vitamin D levels. The statistical significance cut-off was set at $P < 0.05$.

RESULTS

Results of 89 pregnant mothers were analysed. Mean age was 29 ± 6 years. As shown in Table 1, nearly half of them (52%) had only primary education. Most of them (79.8%) were housewives and fell into lower socio-economic class (61%). Almost all mothers from this study population came from urban areas (93%). No significant relationship between all socio demographic characteristics and serum vitamin D levels was showed ($P > 0.05$). The percentage of pregnant women with sufficient vitamin D level did not differ by ethnicity, ($\chi 2$ $(3, n = 89) = 1.20, P = .273$), socio-economic status ($\chi 2$ (2, n = 89) = 0.37, P = .695), area of residence ($\chi 2$ (1, n = 89) = 0.28, P = .435), profession ($\chi 2$ (3, n = 89) = 0.03, P = .859), and educational level ($\chi 2$ (2, n = 89) = 0.82, P = .367).

Average total daily intake of vitamin D was around 1,289.4 ± 1,225.6 IU/day (range: 56 - 5400 IU). A significant proportion of mothers (45%) consumed < 600 IU of vitamin D per day. More than half of our sample (56.9%) received vitamin D though fortified milk powder and 36% from fish consumption. Most of mothers (69%) consumed small fish and none of them received vitamin D supplementation. All mothers were prescribed calcium lactate as supplementation (300 mg/day) from the booking visit to 12 weeks of gestation onwards. However, 2.2% of mothers did not take calcium, 5.6% of the subjects consumed only half the dose that was prescribed (150 mg/day). Majority of them (92.1%) adhered to the prescribed dose. Daily dietary intake of calcium was 582.0 ± 384.0 mg/day (range: 24.8 – 2,060 mg). The average intake of total calcium intake (dietary calcium intake and supplementation together) was 870.2 ± 394.0 mg/day (range: 24.8 – 2360 mg/day). Only 6.7% of the subjects consumed calcium above the recommended levels (> 1500 mg/day).

Mean serum values for Vitamin D and bone biochemistry of the subjects are shown in Table 2. Of them, 12.4%, 50.6% and 37% of the population were vitamin D deficient, insufficient and sufficient, respectively. Prevalence of vitamin D deficient/insufficient group (63%) was higher than sufficient group (37%). However, serum parathyroid hormone (PTH) level (reference range 10.4 - 66.5 pg/mL) was above the cut-off only for a small proportion of the sample (4.5%). Serum calcium was within the normal range in all of them. A high level of alkaline phosphatase was found in 13.5% of the study population (> 240 IU/L) where the cut-off level is considered as > 240 IU/L [23].

Relationship between vitamin D and bone biochemistry is shown in Table 3. Serum vitamin D and PTH showed a weak negative correlation. However, there was no significant correlation between vitamin D and alkaline phosphatase or calcium.

We analysed the relationship between dietary vitamin D intake and serum 25(OH)D levels. There was a significant positive correlation between dietary vitamin D and serum 25(OH)D (r = 0.355, P < 0.01). As shown in Table 4, there was a significant difference in levels of dietary vitamin D intake (t (87) = 2.10, P = 0.038) between serum 25(OH)D 'deficient/insufficient' (M = 14.5, SD = 3.90 ng/mL) group, which introduces dietary vitamin D levels as 1,083.6 ± 1,026.4 IU/day, and 25(OH)D 'sufficient' group (M = 26.0, SD = 5.5 ng/mL), which reaches dietary vitamin D levels as 1,638.5 ± 1,456.1 IU/day.

DISCUSSION

To our knowledge, this is the first study in Sri Lanka revealing the presence of vitamin D deficiency among pregnant women. Vitamin D deficiency/insufficiency was significantly high in this sample and metabolic bone stress was demonstrated by a significant negative correlation of vitamin D to PTH. However, except for 3 subjects with insufficiency/deficiency of vitamin D, in most of our participants PTH level was below the cut off value. Therefore, our sample of pregnant mothers with hypovitaminosis D did not show the expected rise of PTH. This has implications to define the optimum level of vitamin D in serum. Further studies should elucidate this research query. Although majority of our participants had low 25(OH)D level, only 13.5% reported high ALP levels. However, ALP is not a reliable marker of osteomalacia in any age group. In addition, there is placental production of ALP in pregnancy [18].

Estimated food records through a food frequency questionnaire revealed that intake of calcium and vitamin D in diet were not optimal in most of the mothers surveyed. Natural sources of vitamin D are primarily oily fish, meat (beef and pork) and egg yolk. Oily fish is a common feature of traditional Sri Lankan diet. Thora (spanish mackerel), Balaya (tuna), Thalapath (sword fish), Salaya/Sudaya (sardinella), Hurulla (trenched sardinella) and Mora (shark fish) are common types of oily

Table 1. Socio demographic characteristics of the study population (n = 89).

Characteristics	*n (%)*
Ethnicity	
Sinhalese	70 (78.7)
Tamils	9 (10.1)
Moors	7 (7.9)
Burghers	3 (3.4)
Socio-economic status	
High	8 (9)
Medium	27 (30)
Low	54 (61)
Area of residence	
Urban areas	83 (93)
Rural areas	6 (7)
Profession	
House wives	71 (79.8)
Unskilled workers	1 (1.1)
Skilled workers	7 (7.9)
Professional workers	10 (11.2)
Educational level	
Primary	46 (51.7)
Secondary	36 (40.4)
Tertiary	7 (7.9)

Table 2. Vitamin D status and bone biochemistry of the study participants (n = 89).

Parameter	**Mean (SD)**
25 (OH)D (ng/mL)	18.7 (7.2)
Serum corrected calcium (mmol/L)	2.3 (0.2)
Alkaline phosphatase (IU/L)	180.8 (53.2)
Parathyroid hormone (pg/mL)	24.3 (22.8)
Inorganic phosphorus (mmol/L)	1.3 (0.2)

fish in Sri Lanka [24]. However, in Sri Lanka sword and shark fish are not recommended during pregnancy due to high levels of mercury in their tissues. Majority of mothers consumed small fish due to its high availability and low cost. Large fishes such as Spanish mackerel and sword fish are less affordable to lower socioeconomic class. Although it is relatively expensive in Sri Lanka, they consumed vitamin D mostly through fortified powder milk. In our study, we showed that vitamin D deficiency/insufficiency is not uncommon in pregnancy and dietary vitamin D intake is significantly low in mothers with low serum 25(OH)D levels. Since there are no previous studies reporting vitamin D deficiency and there is controversy regarding supplementation, public clinics and hospitals in Sri Lanka have not included vitamin D supplementation as a part of antenatal care programme. Further, most of the vitamin D containing food items is less affordable to many mothers during pregnancy and lactation. Therefore, vitamin D supplementation during pregnancy could be suggested as an intervention to protect against adverse effects on both women and foetuses. However, whether supplementation during pregnancy safely improves maternal and neonatal outcomes is still questionable since there are not adequate high-quality studies

Table 3. Relationship between vitamin D status and bone biochemical parameters.

	Bone biochemical parameters			
	PTH	**Calcium**	**ALP**	**IPh**
Serum 25(OH)D	-0.296 (P = 0.005)*	0.002 (P = 0.983)	-0.168 (P = 0.128)	0.189 (P = 0.077)

Significance level at $P < 0.05$

Table 4. Results of t-test for dietary vitamin D intake among dichotomized serum vitamin D groups (n = 89).

	Serum vitamin D groups									
	Deficient/Insufficient			Sufficient						
	M	*SD*	*n*	*M*	*SD*	*n*	95% CI for Mean Difference		*t*	*df*
							lower	upper		
Dietary intake of vitamin D	1,083.6	1,026.4	56	1,638.5	1,456.1	33	-1079.4	-30.4	2.10*	87

M = mean; SD = standard deviation; df = degree of freedom; t = t-test value
*$P < 0.05$

confirming its usefulness and safety. A recent Cochrane review has concluded that there is not enough evidence available to recommend the potential use of vitamin D in routine antenatal care [25]. Therefore, it is clear that further trials studies are required to evaluate usefulness of vitamin D supplementation in pregnancy. Finally, our study did not show any association between socio-demographic characteristics and serum vitamin D levels. Our sample was constituted by Sinhalese housewives pregnant women, who came from urban areas. Only nearly half of our sample reported low educational and socio-economic levels [26, 27]. Conversely, past studies showed a close relationship between low formal education and vitamin D deficiency in pregnant women from developing countries, suggesting also an association of vitamin D sufficiency or insufficiency with socio-economic status of the population [28].

There were also some limitations in our study. Firstly, although we used a pretested food frequency questionnaire based on a 7-day estimated food record method, it is liable to erroneous portion size estimation, dietary misreporting, and incorrect food description. However, it has lower respondent burden than weighed food diaries. Secondly, we could not report about mothers' sun exposure that could have influenced the vitamin D levels. Moreover, we used a convenience and small sample, so generalizability of our study is limited. Furthermore, mothers (majority) of our sample came from urban city, so the prevalence of phenomenon could be underestimated if compared to that regarding mothers from rural areas.

CONCLUSIONS

We have reported a high rate of vitamin D insufficiency/deficiency in our cohort of pregnant women, constituted mainly by housewives who came from urban areas of Sri Lanka, of whom only nearly half with low educational and socioeconomic levels. It is evident that diet alone may not be enough to achieve a good vitamin D status during pregnancy. These findings, together with existing data, emphasises the need for further evaluation of vitamin D status, re-defining the cut-off levels in pregnancy and evaluating the requirement of routine vitamin D supplementation, especially for more disadvantaged groups of pregnant women in Sri Lanka.

Acknowledgements

Our study received financial assistance by university research grant (Grant No: ASP/01/RE/MED/2015/40).

References

1. Fischer PR, Thacher TD, Pettifor JM. Pediatric vitamin D and calcium nutrition in developing countries. Rev Endocr Metab Disord. 2008;9(3):181. doi: 10.1007/s11154-008-9085-1.
2. Jain V, Gupta N, Kalaivani M, Jain A, Sinha A, Agarwal R. Vitamin D deficiency in healthy breastfed term infants at 3 months & their mothers in India: seasonal variation & determinants. Indian J Med Res. 2011;133:267–273.
3. Spiro A, Buttriss JL. Vitamin D: An overview of vitamin D status and intake in Europe. Nutr Bull. 2014;39(4):322–350.
4. Feizabad E, Hossein-Nezhad A, Maghbooli Z, Ramezani M, Hashemian R, Moattari S. Impact of air pollution on vitamin D deficiency and bone health in adolescents. Arch Osteoporos. 2017 Dec 1;12(1):34. doi: 10.1007/s11657-017-0323-6.
5. Við Streym S, Moller KU, Rejnmark L, Heickendorff L, Mosekilde L, Vestergaard P. Maternal and infant vitamin D status during the first 9 months of infant life-a cohort study. Eur J Clin Nutr. 2013;67(10):1022–1028. doi: 10.1038/ejcn.2013.152.
6. Pehlivan I, Hatun S, Aydoğan M, Babaoğlu K, Gökalp AS. Maternal vitamin D deficiency and vitamin D supplementation in healthy infants. Turk J Pediatr. 2003;45(4):315–320. doi: 10.3109/14767058.2014.924103.
7. Specker BL. Does vitamin D during pregnancy impact offspring growth and bone? Proc Nutr Soc. 2012;71(1):38–45.
8. Li N, Wu HM, Hang F, Zhang YS, Li MJ. Women with recurrent spontaneous abortion have decreased 25(OH) vitamin D and VDR at the fetal-maternal interface. Braz J Med Biol Res. 2017;50(11):e6527. http://doi.org/10.1590/1414-431X20176527.
9. Tabatabaei N, Auger N, Herba CM, Wei S, Allard C, Fink GD, et al. Maternal Vitamin D Insufficiency Early in Pregnancy Is Associated with Increased Risk of Preterm Birth in Ethnic Minority Women in Canada. J Nutr. 2017;147(60):1145–1151. doi: 10.3945/jn.116.241216.
10. Amegah AK, Klevor MK, Wagner CL. Maternal vitamin D insufficiency and risk of adverse pregnancy and birth outcomes. A systematic review and meta-analysis of longitudinal studies. PLoS One. 2017:12(3):e0173605. http://doi.org/10.1371/journal.pone.0173605.
11. Gale CR, Robinson SM, Harvey NC, Javaid MK, Jiang B, Martyn CN, et al. Maternal vitamin D status during pregnancy and child outcomes. Eur J Clin Nutr. 2008;62(1):68.
12. Sachan A, Gupta R, Das V, Agarwal A, Awasthi PK, Bhatia V. High prevalence of vitamin D deficiency among pregnant women and their newborns in northern India. Am J Clin Nutr. 2005;81(5):1060–1064.
13. Meyers LD, Suitor CW. Dietary reference intakes research synthesis. Workshop summary. Washington, DC: National Academies Press; 2007.
14. Hollis BW, Johnson D, Hulsey TC, Ebeling M, Wagner CL. Vitamin D supplementation during pregnancy. Double-blind randomized clinical trial of safety and effectiveness. J Bone Miner Res. 2011;26(10):2341–2357. doi: 10.1002/jbmr.463.
15. Aghajafari F, Field CJ, Kaplan BJ, Rabi DM, Maggiore JA, O'Beirne M, et al. The Current Recommended Vitamin D Intake Guideline for Diet and Supplements During Pregnancy Is Not Adequate to Achieve Vitamin D Sufficiency for Most Pregnant Women. PloS One. 2016;11(7):e0157262. doi: 10.1371/journal.pone.0157262.
16. Dawodu A, Saadi HF, Bekdache G, Javed Y, Altaye M, Hollis BW. Randomized controlled trial of vitamin D supplementation in pregnancy in a population with endemic vitamin D deficiency. J Clin Endocrinol Metab. 2013;98(6):2337–2346. doi: 10.1210/jc.2013-1154.
17. World Health Organization. Guideline: calcium supplementation in pregnant women. Geneva: WHO; 2013.
18. Mithal A, Kalra S. Vitamin D supplementation in pregnancy. J Clin Endocrinol Metab. 2014;18(5):593–

596. doi: 10.4103/2230-8210.139204.

19. Calvo MS, Whiting SJ, Barton CN. Vitamin D intake: a global perspective of current status. J Nutr. 2005;135(2):310–316.
20. Sahu MT, Sahu M. Vitamin D replacement in pregnant women in developing countries. In: Watson R.R. (eds) Handbook of vitamin D in human health. Human Health Handbooks, vol 4. Wageningen: Wageningen Academic Publishers; 2013.
21. Nutrition Surveys and Calculations. Guidelines, Software and additional Information [cited 2017 Jan 05]. Available from: http://www.nutrisurvey.de/.
22. Jayawardena R, Byrne NM, Soares MJ, Katulanda P, Hills AP. Validity of a food frequency questionnaire to assess nutritional intake among Sri Lankan adults. Springerplus. 2016;5(1):162. doi: 10.1186/s40064-016-1837-x.
23. Tran HA. Biochemical tests in pregnancy. Aust Presc. 2005;28:136–291.
24. Edirisinghe E, Perera W, Bamunuarachchi A. Nutritional evaluation of some small coastal fish in Sri Lanka. NARA. 2000;36:47–53.
25. De-Regil LM, Palacios C, Ansary A, Kulier R, Peña-Rosas JP. Vitamin D supplementation for women during pregnancy. Cochrane Database Syst Rev. 2012; 15;(2):CD008873. doi: 10.1002/14651858.CD008873.pub2.
26. Al-Mahroos FT, Al-Sahlawi HS, Al-Amer E, Mahmood NA, Sandhu AK, Sharida H, et al. Prevalence and risk factors for vitamin D deficiency among mothers in labour and their newborns. Bahrain Med Bull. 2013;35:60.
27. Karim SA, Nusrat U, Aziz S. Vitamin D deficiency in pregnant women and their newborns as seen at a tertiary-care center in Karachi, Pakistan. Int J Gynecol Obstet. 2011;112:59–62.
28. Mehboobali N, Iqbal SP, Iqbal MP. High prevalence of vitamin D deficiency and insufficiency in a low-income peri-urban community in Karachi. J Pak Med Assoc. 2015;65(9):946–949.